BACTERIAL MOTILITY AND CHEMOTAXIS: THE MOLECULAR BIOLOGY OF A BEHAVIORAL SYSTEM

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I. INTRODUCTION

The remarkable swimming ability of bacteria, simple unicellular prokaryotic organisms, has been noted since the beginnings of microscopy in the 17th century. The fact that bacterial motility is not of a purely random or dispersive nature has been known since the late 19th century as a result of the pioneering works of Engelmann, Pfeffer, and others, who demonstrated migratory responses to light, oxygen, and various chemicals (Figure 1). The phenomena of bacterial motility and "chemotaxis" (as the migratory response to chemicals is called) have been vigorously explored in recent years by a number of workers. Why the resurgence of interest? It cannot be argued that it is based on immediate practical importance, although a number of applications, e.g., plant infestation and marine fouling, are under investigation. Rather, in this author's opinion, it is the exciting opportunity that bacterial motility and chemotaxis provide for studying, in its entirety, a system of behavior which, although quite complex in its interactions, offers some possibility of being understood in molecular detail because of modern developments in genetics, biochemistry, and instrumentation. Figure 2 gives some indication of the complexity of the system as we presently perceive it; undoubtedly the complexity will ultimately prove to be considerably greater. Within this one aspect of the

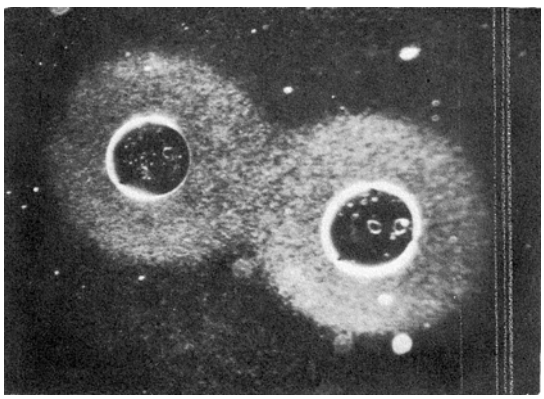

FIGURE 1. A simple demonstration of tactic responses in bacteria. A crowded culture of cells utilizes oxygen more rapidly than it can diffuse from a gaseous source, in this case, air bubbles under a coverslip. Cells migrate up the oxygen gradient generated by diffusion and by their metabolism. Dark field.

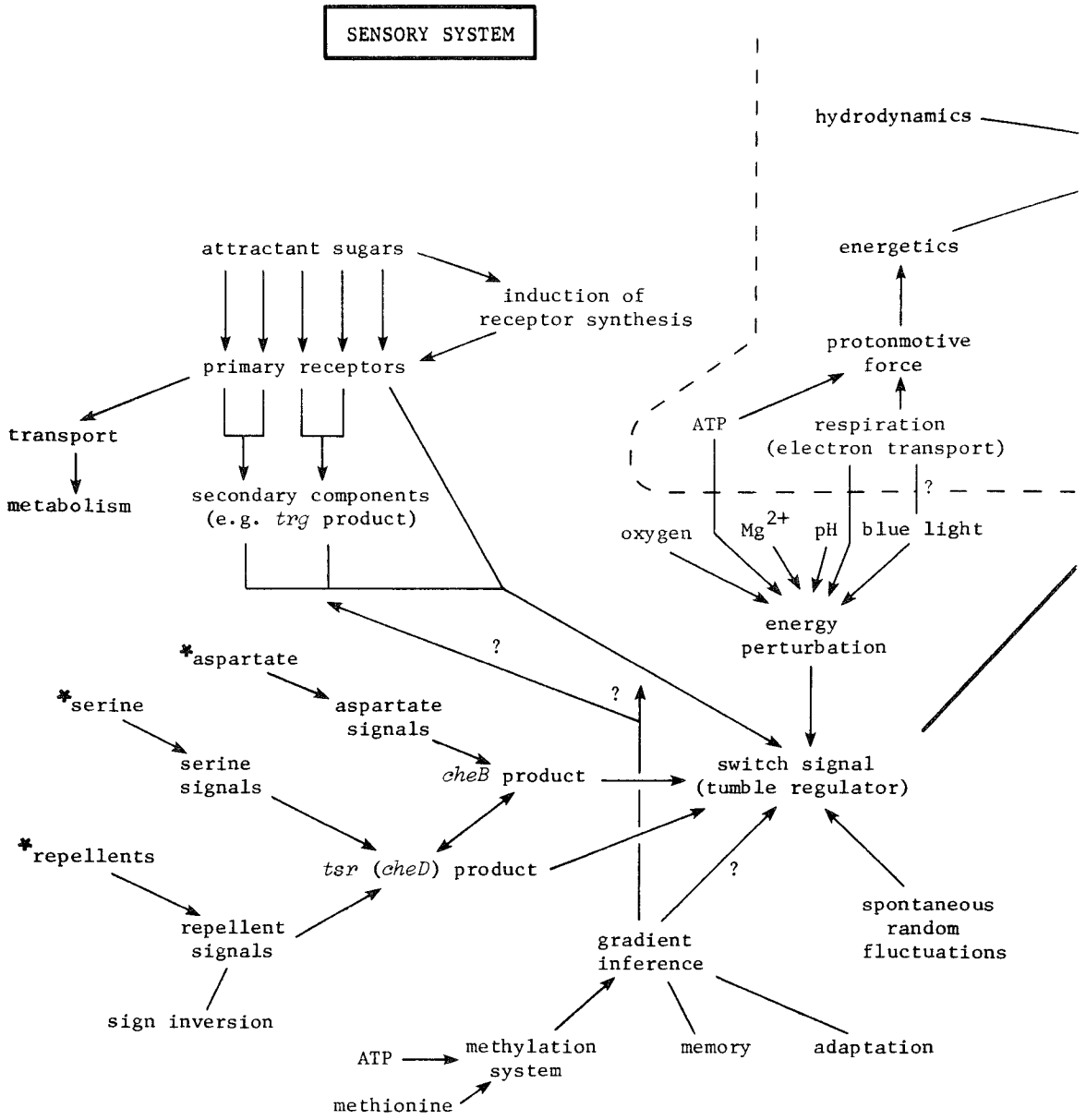

FIGURE 2. A schematic illustration of the complexities of the behavioral system of bacterial motility and chemotaxis, with some of the interrelationships as they are presently understood. Arrowheads imply a causal relationship or a direction of information flow. All chemotactic stimuli (asterisks) eventually feed into a central switch signal which operates (heavy arrow) the switch on the motor to reverse its rotation and effect a tactic response (dagger). Structural and assembly facets of the system are also shown.

total repertoire of the bacterium lie fascinating questions regarding genetic regulation, macromolecular structure and assembly, bioenergetics, molecular mechanics, hydrodynamics, sensory reception, information processing, and motor control. The evolution of a system of this complexity indicates that, in spite of the bacterial cell's numerous adaptive responses to different environments, its prospects of survival are further enhanced by the ability to select a favorable environment by migration.

A brief survey of the principal features of the subject of bacterial motility and taxis is necessary to provide a framework for the main body

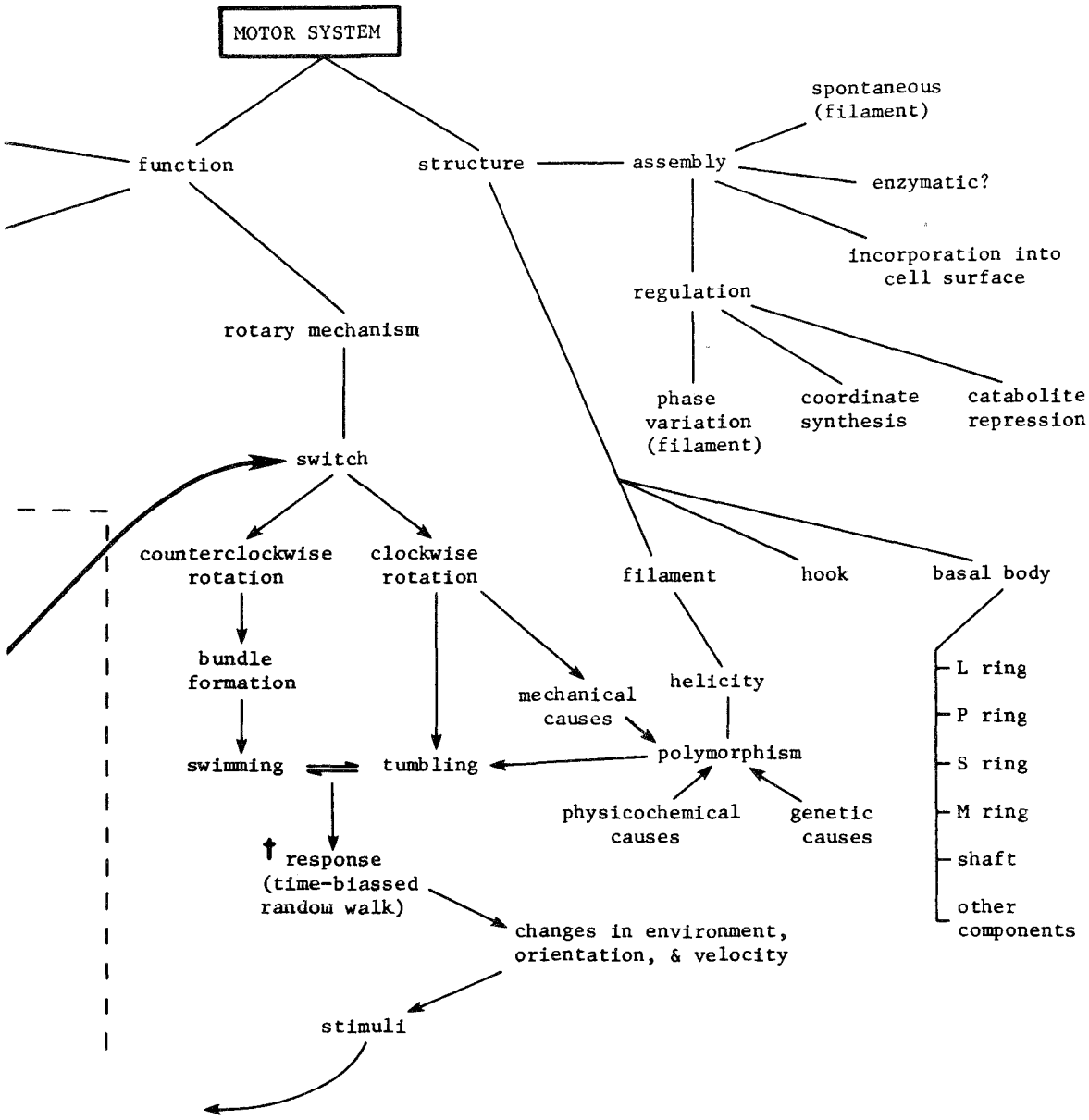

FIGURE 2 (continued)

of the review. Bacteria swim quite rapidly on their scale (approximately ten body lengths per second) by means of organelles called "flagella"; the flagellum consists of a simple self-assembling filament attached to the cell by a more complex basal body whose synthesis and assembly are under sophisticated genetic control. The filament is a passive device, rotated by a mechanism in the basal body, which utilizes as its energy source the protonmotive force across the cell membrane. Thrust is generated from this rotation by virtue of the helicity of the flagella (or, in the Spirilla, of the cell body). The motor is reversible; use of both senses of rotation generates translational movement and directional changes which can be modulated by environmental stimuli. The concentrations of a variety of chemicals in the environment are monitored continuously by highly specific receptors which are, in many instances, known to be proteins on the cell surface. In an unknown manner, secondary components extract information re-

garding changes in the occupancy of receptor sites, i.e., temporal gradient information. Further processing combines some signals but not others, inverts the sign of repellent signals with respect to the sign of attractant signals, and finally combines chemotactic signals with various direct energy perturbations into an overall gradient signal. These processes appear to regulate the methylation of membrane-associated proteins, whose methylated/demethylated status is at least one input to the reversibility of the motor. An overall gradient signal interpreted as "favorable" causes suppression of spontaneous motor reversals, and hence net translational movement in the favorable direction. Thus, chemotactic migration proceeds by means of a time-biased random walk.

This review will describe the current state of knowledge regarding bacterial motility and chemotaxis, viewed as an integrated system, in *Escherichia*, *Salmonella*, and *Bacillus*, the genera which have been subjected to the most intensive study in recent years. Other types of bacterial motility,[1] notably spirillar,[2] spirochetal,[3] and nonflagellar,[4] are therefore excluded. The review will consider first the motor apparatus from the point of view of its structure, assembly, energetics, and mechanism. Next, chemotactic behavior will be examined at a phenomenological level. This section will lead to the analysis of the sensory system, its structure and biochemistry. The enormous contribution which genetic studies have made will be evident throughout; a detailed consideration of the many intricacies of the genetics of motility and taxis will not be attempted, but, for convenience, current versions of the genetic maps for motility and chemotaxis in *E. coli* and *Salmonella* are presented in Figure 3, and summaries of gene functions are given in Tables 1 and 2. These may be referred to whenever genes are alluded to in the text.

An attempt has been made to provide extensive but not exhaustive referencing. Sometimes, in cases where earlier results have contributed to a now more complete picture, only the more recent references are given; from these, the interested reader should be able to extract the other relevant references. A number of other reviews and articles have been written about bacterial motility and chemotaxis,[1,5-14] including ones with special emphasis on historical aspects,[15-17] genetics,[18-20c] flagella,[21-25] relationship to higher systems,[26-29] and ecological implications.[30]

II. MOTOR APPARATUS

The term "flagellum" (from the Latin word for "whip") is applied to the external organelle(s) by which free-swimming bacteria move in liquid media. It is also, rather confusingly, used for the motor organelles of free-swimming eukaryotic cells such as spermatozoa, in spite of the fact that the two types of organelles are quite unrelated structurally, energetically, or mechanistically.[31] The term accurately describes the eukaryotic organelle, which actively bends, but not the bacterial one, which rotates. (Confusion would be removed if the use of "flagellum" were confined to eukaryotes and a new, more informative term "helicum"?] created for the bacterial organelle. *Fla* genes would become *hel* genes, and the structural protein would become "helicin.")

Considerable variation exists in the gross morphology of flagellation;[32] the bacteria under consideration in this review are characterized by peritrichous flagellation, in which the flagella are arbitrary in number and originate randomly on the surface of the cell (Figure 4a). The flagellum has an intrinsic helical waveform, which, when propagated by rotation, generates thrust. The individual flagella form a bundle at the cell pole to cause smooth translational movement (termed "swimming" or "running") of the cell through its environment (Figure 4b). Swimming is disrupted at frequent intervals by chaotic cell motion, i.e., "tumbling", which occurs as a result of flagellar reversal. As will be seen later, both motility modes are essential for the expression of tactic responses.

A. Structure

1. Flagella in the Intact Cell

The most striking features of a bacterial flagellum are its extreme thinness and its regular periodic waveform, which may be seen either by electron microscopy (Figure 5) or by high-intensity dark-field light microscopy.[15,33,34] In the latter technique, flattening artifacts are absent, and, since the two-dimensional projection in the focal plane is invariably observed to be sinusoidal and of constant amplitude, it appears that the true (three-dimensional) wave-

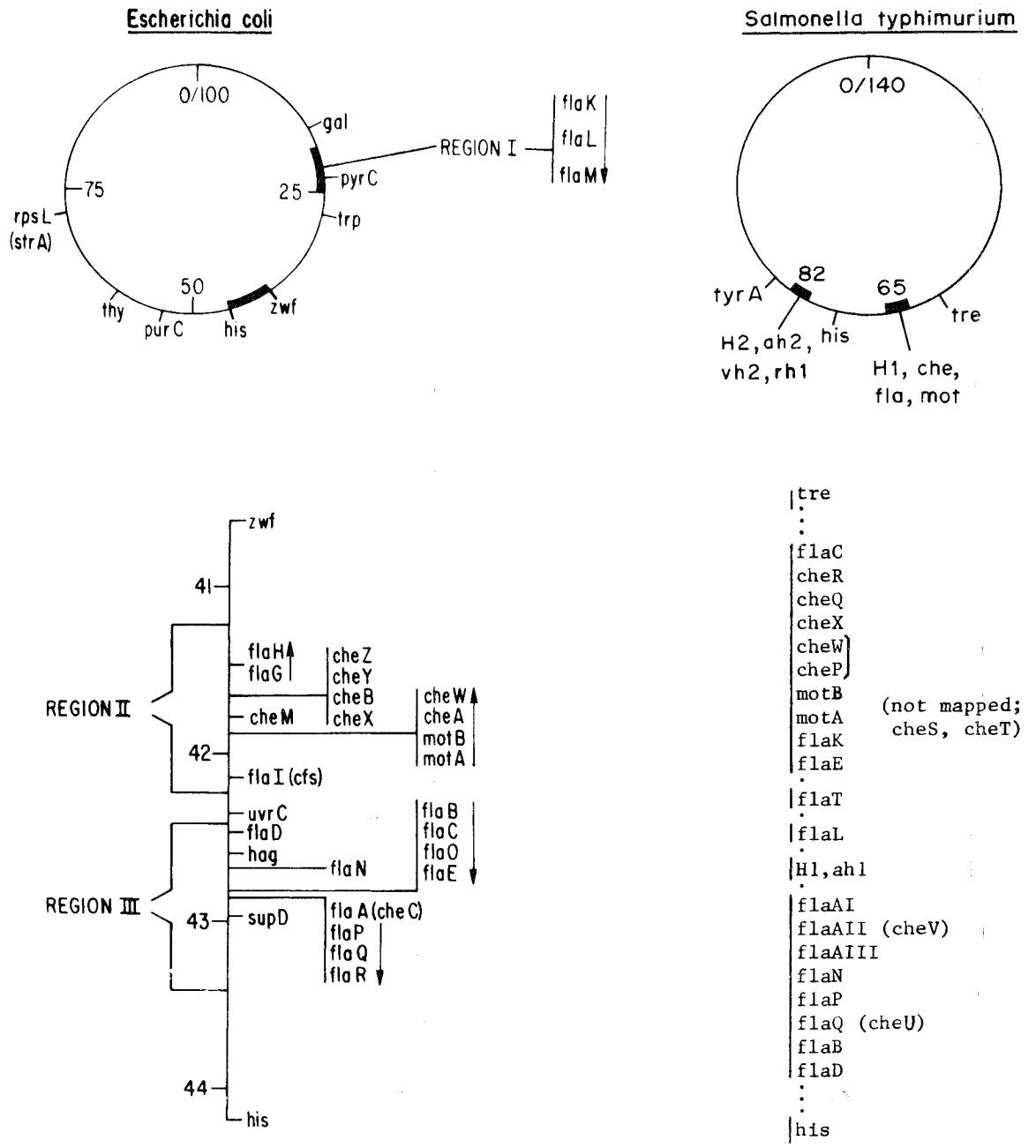

FIGURE 3. Genetic maps of *E. coli* and *Salmonella*, with the portions pertinent to motility and taxis given in expanded form. (See text and Tables 1 and 2 for gene functions where known; for general reviews of the genetic maps of *E. coli* and *Salmonella*, see References 273 and 136, respectively.) Gene symbols such as *flaE* are not necessarily for homologous genes in the two species. Note the massive cluster in the *his* region of both maps and some obvious similarities between them, although in most cases homologies have not been established. There is no region in *E. coli* homologous to the alternate flagellin synthesis region *(H2)* at 82 min on the *Salmonella* map, nor has a region been found in *Salmonella* homologous to Region I on the *E. coli* map. Specific receptor genes, which map in the corresponding transport loci, the *E. coli cheD* gene, and other genes which do not lie in the main clusters, have been omitted. (*E. coli* map from Silverman, M. and Simon, M. I., *J. Bacteriol.* , 130, 1317, 1977; *Salmonella* map modified from Warrick, H. M. , Taylor, B. L., and Koshland, D. E., Jr., *J. Bacteriol.*, 130, 223, 1977. With permission.)

TABLE 1

Motility and Taxis Genes in *E. Coli*[a]

Gene symbol	Map position	Mutant phenotype	Gene product or function	Reference
hag (flaF)	III	Nonflagellate or abnormal filament wave form	Flagellin (filament subunit) structural gene	53—55
flaA (cheC)	III	Nonflagellate or generally defective in chemotaxis	Particpates in flagellar structure, and in signaling; may code for basal body component	53—55, 241, 242
flaB, C, D,N, O, P, Q, R	III	Nonflagellate		54, 55
flaE	III	Polyhook	Controls hook assembly	54, 55, 104
flaG	II	Nonflagellate		54,55
flaH	II	Nonflagellate	Can suppress effect of *galU* mutation; may code for basal body component in outer membrane	54,55,122
flaI	II	Nonflagellate	Overall regulation of flagellar synthesis; under *cfs* control	54, 55, 112
cfs	II	Constitutive for flagellar synthesis	Cyclic AMP dependent promoter of *flaI*	112
flaK, L, M	I	Nonflagellate	Hook synthesis; one gene codes for hook protein; others may be regulatory	102, 102a
motA, B (flaJ)	II	Paralyzed	Membrane-bound proteins necessary for motility; not parts of basal body; genes form *mocha* operon with *cheA*	53—55, 107,109
cheA, W (formerly *cheA)*	II	Generally nonchemotactic; smooth swimming	Enable tumbling but do not control it; *cheA* codes for 2 polypeptides	53, 106, 107, 109, 110, 241, 242, 236b
cheB, X, Y, Z (formerly *cheB)*	II	Generally nonchemotactic; smooth swimming or tumbling	Enables and controls tumbling; interacts with *cheD*	53, 106, 107, 109, 110, 241, 242, 236b
cheC (see *flaA)*				
cheD (tsr)	99′	Generally nonchemotactic or specifically nonchemotactic to serine and certain repellents	Target for serine and repellent signals; interacts with *cheB*. Methyl-accepting chemotactic proteins (MCPI)	241, 242, 236a, 236b

TABLE 1 (continued)

Motility and Taxis Genes in *E. Coli*[a]

Gene symbol	Map position	Mutant phenotype	Gene product or function	Reference
cheM	II	Defective in taxis to aspartate, maltose, and certain repellents	Methyl-accepting chemotactic proteins (MCPII)	106, 251, 236a, 236b
trg	37′	Defective in ribose and galactose taxis	Secondary signaling component	214, 222, 223

[a] See also Figure 3 and, for general reviews of the genetic maps of *E. coli* and *Salmonella*, References 273 and 136, respectively.

TABLE 2

Motility And Taxis Genes In *Salmonella*

Gene symbol	Map position	Mutant phenotype	Gene product or function	Reference
H1	65′	Nonflagellate or abnormal filament waveform, when in Phase 1	Phase 1 flagellin structural gene	133—135
ah1	65′	Nonflagellate in Phase 1	Activates *H1*	129, 130
H2	82′	Nonflagellate or abnormal filament waveform, when in Phase 2	Phase 2 flagellin structural gene	133—135
ah2	82′	Stable in Phase 1	Activates *H2* and *rh1*	129,130
rh1	82′	Stable in Phase 1	Represses *H1*	131
vh2	82′	State of Phase 2 locked in condition at time of mutation	Oscillating activation of *H2*	132
flaAI, AIII, B, C, D, E, K, L, N, P	65′	Nonflagellate		138, 272
flaAII (cheV), flaQ (cheU)	65′	Nonflagellate or generally defective in chemotaxis	Participate in flagellar structure and in signaling; may code for basal body component	111, 138, 272, 116, 117
flaF	Between 52′ & 65′	Nonflagellate		134
flaG	65′	Nonflagellate	Synonymous with *flaAII;* term *flaG* no longer in use	111, 138
flaM	Close to 65′	Nonflagellate	Not known; does not cotransduce with *H1*	272
flaT	65′	Nonflagellate	Overall regulation of flagellar synthesis; under *cfs* control	115
cfs	65′	Constitutive for flagellar synthesis	Cyclic AMP-dependent promoter of *flaT*	115

TABLE 2 (continued)

Motility And Taxis Genes In *Salmonella*

Gene symbol	Map position	Mutant phenotype	Gene product or function	Reference
motA, B	65′	Paralyzed	see corresponding entry in Table 1	135, 137, 138
cheP, Q, S, W	65′ (except *cheS*)	Generally nonchemotactic, smooth		116, 117, 274
cheT, X	65′	Generally nonchemotactic, tumbling		116, 117, 274
cheR	65′	Generally nonchemotactic, smooth; very low methylation level of MCP	Chemotactic methyltransferase; methylates MCP	116, 117, 245, 274
cheU, V (see *flaAII, flaQ*)				

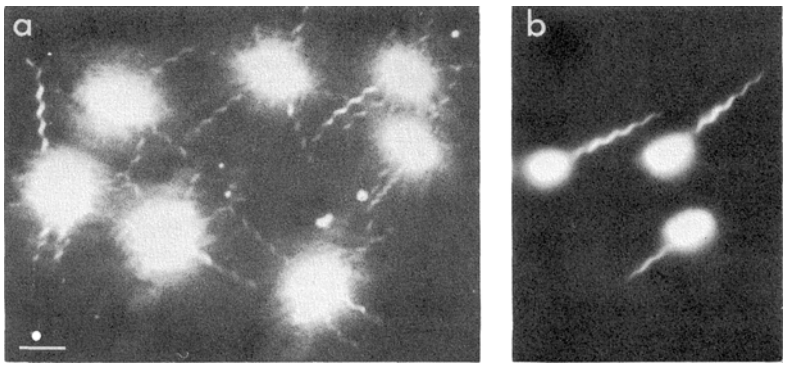

FIGURE 4. (a) Peritrichous flagellation exemplified by *Salmonella*. Cells were rendered nonmotile to permit the filaments to drift apart, revealing their random origin around the cell body. (b) *Salmonella* in swimming mode, with flagellar filaments coalesced into a propulsive bundle; the individual filaments can not be resolved by light microscopy. Dark field; bar equals 5 μm. (From Macnab, R. M., *J. Clin. Microbiol.*, 4, 258, 1976. With permission.)

form must be helical. This conclusion is substantiated by careful observation of the image during progressive adjustment of the focal plane[33,35] and also by selective illumination of different planes.[36]

In contrast to the well-defined helical parameters of wavelength and amplitude, the length, number, and location of filaments are arbitrary. Indeterminate length is a reflection of unidimensional crystalline growth (Section II.A.3). Factors determining the number of flagella synthesized by a cell are still not well understood but appear to stem from regulation at the transcriptional level, in response to cyclic AMP levels (Section II.A.6). The random location of flagella around the cell has not been rigorously demonstrated, but neither in light not in electron microscopy is there any suggestion of preferred regions of origin. How ordered cell motion occurs in spite of this randomness is discussed in Section II.E.1.

The nature of the attachment of the flagellum to the cell is completely obscured by scattering in light microscopy. Even electron micrographs of intact cells are not especially informative in this regard, although a slight enlargement of filament diameter (the "hook") may be seen in the vicinity of the cell surface. In lysed cell preparations, a bulbing or clumping at the point of attachment is evident, and,

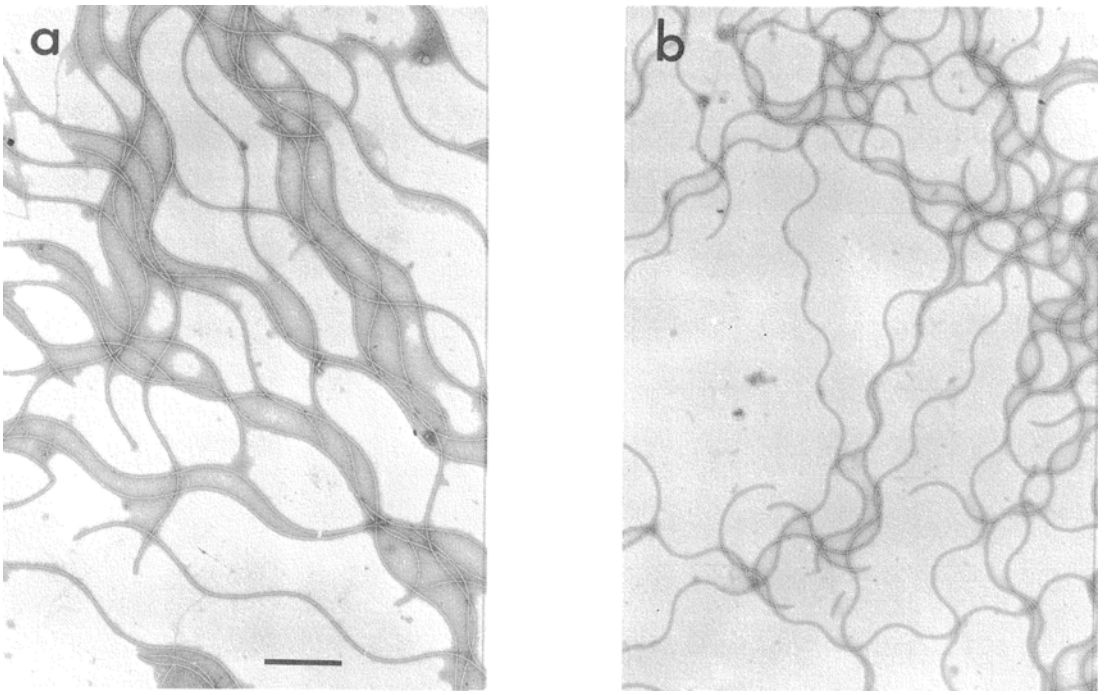

FIGURE 5. Flagellar filaments from *Salmonella* negatively stained with phosphotungstate. Note thinness (19 nm) compared with overall length (of order 5 to 10 μm) and the regular waveform, which in three dimensions is helical. The normal waveform (a) is lefthanded, while other forms, such as curly (b), are righthanded. Conversion from normal to curly in these micrographs was accomplished by shifting the pH from 7.0 to 4.5; conversion by viscous force is important in tumbling (see Section II.E.2). Bar equals 1 μm. (Micrographs from Kamiya, R. and Asakura, S., *J. Mol. Biol.*, 106, 167, 1976. With permission.)

in favorable preparations, some details of structure in the basal region emerge.[37-40] Smith and Koffler[23] present a detailed review of the numerous studies of flagellar basal structure.

2. Intact Isolated Flagella

Because of the difficulty of interpreting basal structures *in situ,* efforts were directed to obtaining isolated preparations free from cell surface material.[41-43] Pure intact flagella from *B. subtilis* and *E. coli* have been prepared by treatments with lysozyme to digest the cell wall, detergent to solubilize the membranes, and deoxyribonuclease to free the flagella from contaminating DNA. Precipitation by ammonium sulfate, dialysis, and differential centrifugation then yielded a fairly pure preparation of well-defined entities (Figure 6a), which have been termed "intact" flagella. It should, however, be borne in mind that these represent a minimum definition of the flagellum in the intact cell — other elements could have been lost in the process of isolation. The helical filament is typically 5 to 10 μm long (2 to 4 wavelengths). Close to the point of attachment there is a slight enlargement to form a differentiated zone which, because of its marked curvature, has been named the "hook." The hook is connected to a complex structure called the "basal body," idealized in Figure 6b as a central rod and four rings in the case of *E. coli*[44] and other gram-negative bacteria[41] or two rings in the case of *B. subtilis*[43,44] The insertion of these structures into the surface of whole cells is very difficult to study (although attempts have been made, with little success, to elucidate the manner of flagellar insertion by electron microscopy of thin-section preparations),[45,46] and so less direct approaches have had to be used. The conclusions reached are, nevertheless, quite convincing. An *E. coli* fraction, characterized as outer membrane on the basis of density, stability to Triton® X-100/Mg^{2+} and other criteria, had basal bodies associated with it by the

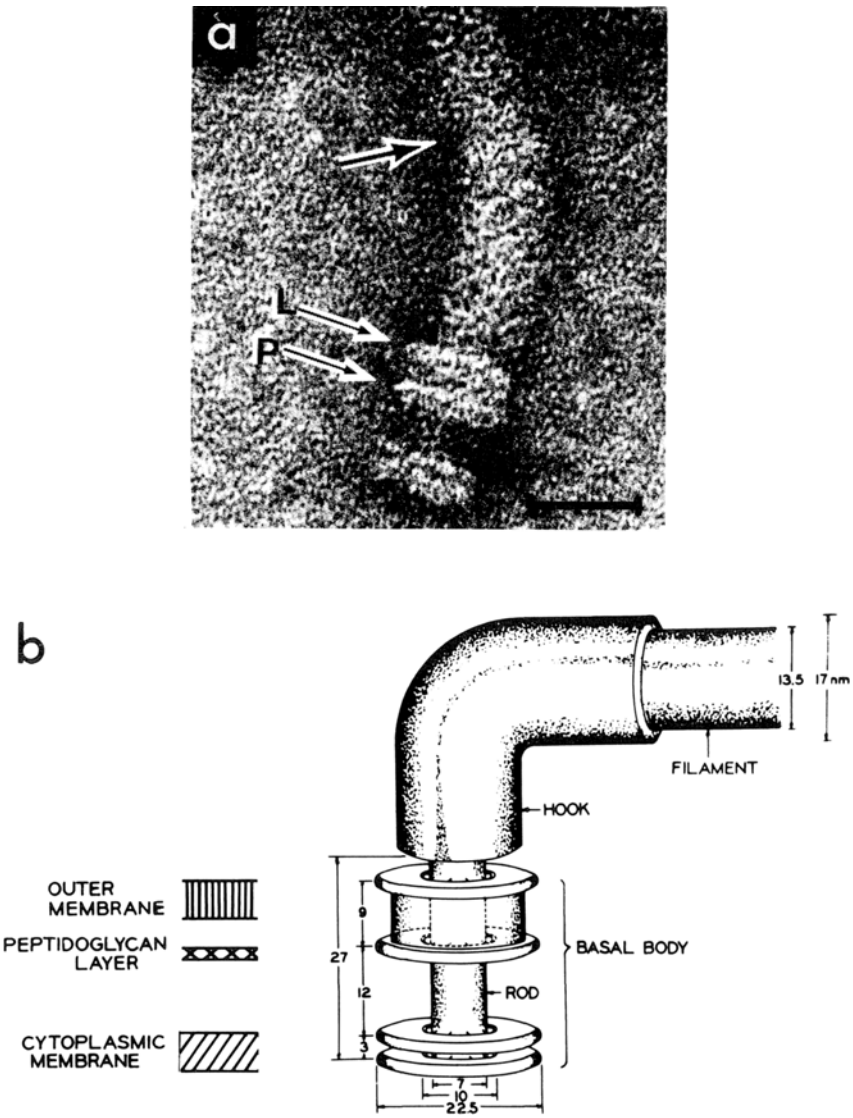

FIGURE 6. (a) Intact isolated flagellum from *E. coli*, negatively stained with uranyl acetate; plain arrow marks the junction of the filament and hook; the outer two rings are marked L and P, indicating that they are imbedded in the lipopolysaccharide (outer membrane) and peptidoglycan layers, respectively; bar equals 20 nm. (b) Drawing, based on (a) and other micrographs of the hook/basal-body complex; also shown is the relationship of the rings in the basal body to the outer membrane, peptidoglycan layer (cell wall), periplasmic space, and cytoplasmic membrane, respectively. The basal body is presumed to be the flagellar motor. (From dePamphilis, M. L. and Adler, J., *J. Bacteriol.*, 105, 384, 1971. With permission.)

outermost (L) ring only. Osmotically lysed spheroplasts from *E. coli* or *B. subtilis* displayed flagella attached to the inner (cytoplasmic) membrane by the innermost (M) ring only; M rings also displayed a tendency to aggregate side by side, as might be expected of a hydrophobic structure. No specific attachments were found for either the S ring (common to gram-positive and gram-negative bacteria) or the P ring of *E. coli*, but, based on distance estimates of the basal body and the cell surface cross section, the S and P rings have been assigned to locations in the periplasmic space and the cell wall, respectively.[47] Negative stain fails

to penetrate between the L and P rings, suggesting that they form a single locked unit, whereas the ready penetration of stain between the S and M rings suggests that they are separate entities, a suggestion which is further substantiated by the frequent loss of the M ring in damaged basal bodies. The conclusion regarding separability of the S and M rings will be important when the rotary mechanism for the motor is discussed later. The absence of L and P rings in gram-positive bacteria is presumably related to the existence of a massive cell wall in which to anchor the structure rather than to any fundamental difference in motor mechanism. Cohen-Bazire and London[41] describe the basal body as protruding into the cytoplasm, but, considering the results of more recent work, this description is probably erroneous and may be a consequence of confusion between outer and cytoplasmic membranes. There does not seem to be any evidence at the present time of flagellar structure extending beyond documented cytoplasmic membrane.

It is interesting to compare some additional features observed in *Spirillum.* For example, the polar region where the flagella are located is devoid of ribosomes and is compartmentalized by a fine "polar membrane."[45] No flagellar basal membrane has been noted in peritrichous bacteria, although detection would be more difficult than in *Spirillum.* A large disc (up to 90 nm) has recently been found by Coulton and Murray[48] surrounding the 22-nm L disc in documented outer membrane. The authors suggest that this may be a stabilizing plate or that it may serve to "exclude lipid phase." Perhaps a simpler explanation, suggested by the close packing of these structures at the polar cap, is that they act to space the individual flagella in the polar tuft — a function not required in peritrichous flagellation. At any rate, no corresponding specializations have been found in the peritrichous bacteria.

In summary, the only morphological features which can be identified with confidence for peritrichous bacteria are the basal bodies and corresponding surface locations, as defined by dePamphilis and Adler.[44,47] The large bulbs observed so often in whole cell micrographs are probably an artifact resulting from draping of the cell surface over the basal body, since no such bulb has been observed in isolated flagellar preparations or in sectioned material.

3. Filament

Although more complex filaments exist in some bacteria, most species, including *Escherichia, Salmonella,* and *Bacillus* have filaments with a relatively simple structure. Flagellar filaments are readily sheared off from cells by high-speed blending and are most conveniently purified by taking advantage of their dissociation and reconstitution properties.[49] The filament structure involves only noncovalent interactions and depolymerizes when subjected to heat (e.g., 65°C for 2 min) or low pH. The monomeric form is a single simple protein (flagellin),[22] as judged by genetic, chromatographic, serological, and chemical evidence.[18,22,50-52] Glycoprotein may be present in the flagella of some species.[23] The structural gene in *E. coli (hag)* maps in region III of the main motility region of the genetic map (Figure 3).[53-55] The more complicated subject of flagellin synthesis in *Salmonella* will be discussed in Section II.A.6. The molecular weight of flagellin shows considerable interspecific variation. For example, *B. subtilis* flagellin has a molecular weight of 33,000 dalton, various *Salmonella* species, 51,000 to 57,000 dalton, and *E. coli,* 60,000 dalton.[51] (Values reported in the literature prior to about 1973 tend to be erroneously low and should be regarded with caution.) The amino acid composition shows considerable interspecific similarity;[23,56] notable features are the absence of cysteine and tryptophan. *Salmonella* flagellin contains an unusual amino acid, ε-*N*-methyllysine,[57] with no evident contribution to structure or function. Flagellin from *B. subtilis* has recently been sequenced by de Lange et al.;[52] it is a 304-residue polypeptide with no obvious regularities or repetitions.* Hydrophobic residues are distributed randomly, but a substantial charge asymmetry ex-

* The sensitivity of enzymes to minor changes in primary structure is well known. Another illustration of this sensitivity of proteins is the drastic change in filament morphology from helical to straight which occurs upon substitution of valine for the alanine residue in position 234.[52] As a counterillustration, Kondoh and Hotani[51] found that *Salmonella* flagellin (51,000 dalton) and *E. coli* flagellin (60,000 dalton) were capable of copolymerization to give filaments of normal waveform.

ists, the N-terminal third of the molecule having a net charge of 6+, the middle third, 9−, and the C-terminal third, 4−. This type of asymmetry may be responsible for the very large dipole moment (860 D) which was measured for *Salmonella* flagellin.[58] Several lines of evidence suggest that flagellin is a decidedly aspherical protein; Bode et al.,[59] studying *Proteus mirabilis* flagellin, concluded from elution behavior on Sephadex® G-100, from sedimentation behavior, and from low-angle X-ray scattering properties that the molecule has a prolate axial ratio of about 7:1 with a long axis dimension of about 16 nm. These conclusions are further supported by various features of the filament structure, to be discussed below. Finally, we may remark that there is nothing about the properties measured so far to lead one to predict, in the present state of knowledge of protein structure vs. function, the remarkable polymerizing and morphological potential of flagellin . Ironically, it is its capacity for unidimensional growth which makes a detailed analysis of structure difficult, by precluding the three-dimensional crystal growth which is needed for high-resolution X-ray crystallographic measurements.

The dissociation of flagellar filaments into monomer is a reversible process.[22,23,60-63] Except at high salt concentration, however, monomer solution is metastable indefinitely unless short seed fragments of flagella are added to initiate growth, which then proceeds until the supply of monomer has been exhausted or until growth terminates spontaneously[64,65] by a process which is not fully understood but which is believed to be caused by faulty insertion of monomer or by insertion of faulty monomer. The reasons why polymerization without seed is such an improbable event are not understood — perhaps only a binding site which provides interaction between more than one subunit simultaneously has sufficient affinity, or it may be that only subunits already incorporated have the appropriate conformation to bind subsequent ones. Filaments are polar,[22,23,66] and crystal growth is a unidirectional process which always proceeds on the end that would, in vivo, be distal to the cell, a point whose significance will be considered in the section on flagellar assembly (Section II.A.6). .[67-69] Spontaneous filament growth is endothermic,[70] the entropy decrease of polymerization apparently being compensated for by a large increase elsewhere, perhaps in water structure; the polymerization process results in a large reduction in the dipole moment per monomer.[58] Dimmitt and Simon[43] found that intact isolated flagella from *B. subtilis* were less readily denatured by heat than were isolated filaments. If, as in *Salmonella*, denaturation proceeds from the distal end,[71] the finding of improved thermal stability in intact flagella suggests that the hook/basal body stabilizes the entire filament, which in turn implies that the structures of the filament in the presence and absence of the hook/basal body must be subtly different.

The salient feature of the filament, namely, its helical form (Figure 5), is an intrinsic characteristic and not a consequence of either active bending or a special template for assembly. This may be deduced immediately from the fact that native and in vitro reconstituted filaments are indistinguishable.[22,23] However, the "normal" helical form is not the only possible one — a variety of other discrete forms exist, as will be described in more detail later. One of these forms, the "straight" form, has proved very useful in studies of subunit arrangement in the filament, for two reasons: (1) it is much less subject to distortion during sample preparation for the electron microscope since there is no helix to flatten, and (2) all subunits are in equivalent sites, a circumstance which cannot occur in helical filaments where only quasi-equivalence is possible.[72]

A number of studies have been made of the subunit structure of flagellar filaments. Czajkowski et al.,[75] because they failed to observe subunit structure in exactly focused electron micrographs of flagellar filaments from *Proteus* and *Bacillus*, erroneously suggest that structures observed by others may be artifacts. While it is true that underfocusing can, by phase-contrast effects, result in a granular appearance, this appearance derives from the scattering properties of the preparation. Therefore, if the pattern of granularity displays periodicity — which it does in the filament electron micrograph, as the optical diffraction patterns clearly demonstrate — the periodicity exists in the structure and is not an artifact.

O'Brien and Bennett[73] examined the structure of a *Salmonella* mutant possessing straight

flagella. Using optical diffraction of electron micrographs, followed by filtering and recombination of the image (Figure 7a,b) they were able to obtain clean one-sided and two-sided images of subunit packing and came to the following conclusions:

1. The diameter of the filament is 19 ± 1 nm; many earlier values, e.g., those of Lowy and Hansen,[74] are smaller than this, but boundary determination of negatively stained material is a notoriously difficult judgment to make, whereas the present value is free from this uncertainty, having been obtained from the repeat distance of a side-by-side aggregate of filaments.
2. Subunits are arranged (Figure 7d) in 11 nearly longitudinal rows (also higher than earlier estimates, which were 8 to 10 rows) separated by 5 nm and are inclined at a small angle (7°) to the filament axis.
3. The close-packed array of subunits defines 5-start and 6-start helices in addition to the longitudinal 11-start helix.
4. The close packing of the 11-, 5-, and 6-start helices defines a nearly hexagonal surface array with approximately 5.6 nm spacing.
5. The basic 1-start helix, which is not close-packed, has 82 subunits in 15 turns.

A study by Kondoh and Yanagida[76] of straight filaments from an *E. coli* mutant reveals the same basic features of an 11-, 5-, 6-start system of subunits as was found in *Salmonella,* but there are differences in detail (Figure 7c); for example, the longitudinal rows are found to be exactly parallel to the filament axis, as opposed to the 7° inclination observed in *Salmonella.*

What is the relevance of studies of straight filaments to the structure of normal filaments? A variety of evidence, such as the ease of interconversion between structures, the constant filament diameter[73,77] (within the accuracy of measurement), and the overall similarity (apart from longitudinal tilt) in surface lattice appearance, suggest that the perturbations which produce quite different structures on a large (micrometer) scale require only minor structural changes at the subunit level. It is, therefore, highly probable that the basic 11-start structure, which determines both filament diameter and internal core diameter, applies to normal as well as to straight filaments and also that the intersubunit distances measured from straight filaments will be essentially unchanged in other structures.

Low-angle X-ray diffraction has produced data on flagellar structure,[77-79] e.g., the axial periodicity, compatible with those obtained by electron microscopy, but it has generally been unable to supply unique new information. If high-angle X-ray diffraction measurements could be carried out, however, these would be extremely informative. Flagellar growth in vivo, to be discussed in Section II.A.6, proceeds from the distal end, presumably by monomer extrusion down the center of the filament. O'Brien and Bennett found very little evidence of central staining in uranyl acetate preparations, and they suggest that when it is observed in phosphotungstate preparations, it may be a result of artifactual swelling. Freeze-etch preparations of *Clostridium* filaments[80] do, however, reveal a central core. Cylindrical packing of spherical subunits would inevitably produce a large core, whereas if the subunits were elongated and stacked radially, the core size would be greatly reduced. Also, if the subunits were stacked in a tilted manner, this would explain both the chevron-shaped head and tail appearance of broken filaments[22,23,66] and certain complexities in the appearance of two-sided filtered images.[73] These conclusions regarding subunit shape are in general agreement with those of Bode et al.,[59] who concluded, from the properties of *Proteus* flagellin discussed above and the chevron-shaped appearance of broken filaments, that the flagellin subunit has an elongated-wedge shape and is tilted at 30° to the filament axis, leaving only a small central core of approximately 3 nm in diameter (Figure 7e). The estimate of diameter should probably be revised upward to approximately 4 nm, because the authors used Lowy and Hansen's earlier model[74] with 8 longitudinal rows rather than the currently accepted model with 11 rows.

The normal and straight forms of flagellar filaments are only two of a much larger number of discrete structures which may be adopted. This structural versatility has been called "polymorphism." Alteration of the primary structure of flagellin,[81,82] in some cases by a single amino acid substitution,[52,83,84] can result in as-

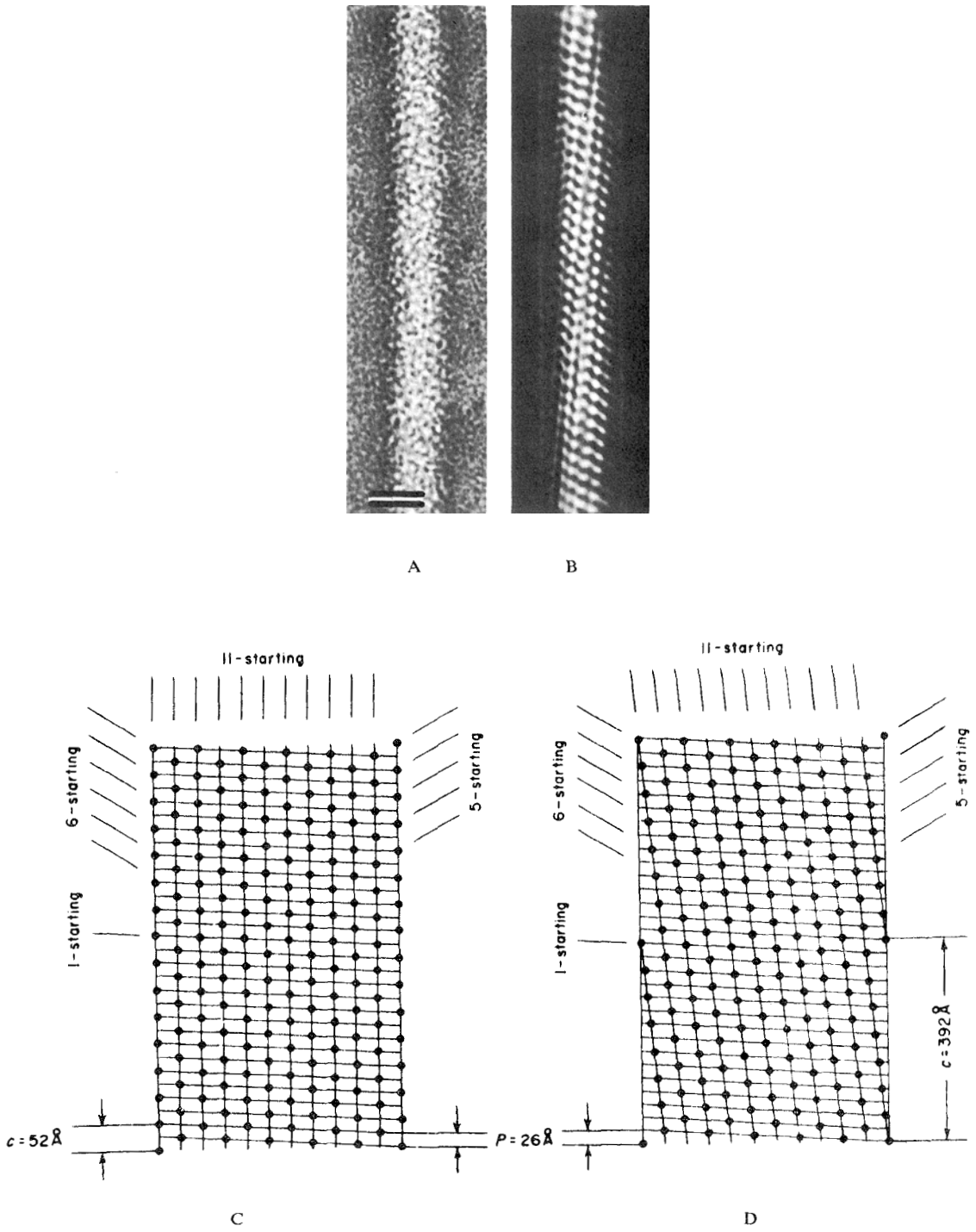

FIGURE 7. (a) Raw and (b) optically filtered electron micrographs of filament from a straight flagellar mutant of *Salmonella*; bar equals 20 nm. (From O'Brien, E. J. and Bennett, P. M., *J. Mol. Biol.*, 70, 133, 1972. With permission.) (c), (d) Lattice structure of straight filaments from *E. coli*[76] and *Salmonella*,[73] respectively. (From Kondoh, H. and Yanigida, M., *J. Mol. Biol.*, 96, 641, 1975. With permission.) (e) Model of flagellar filament, showing wedge shape and tilt of subunits. The number of rows, shown as 8 in the model, is now generally believed to be 11. (From Bode, W., Engel, J., and Winklmair, D., *Eur. J. Biochem.*, 26, 313, 1972. With permission.)

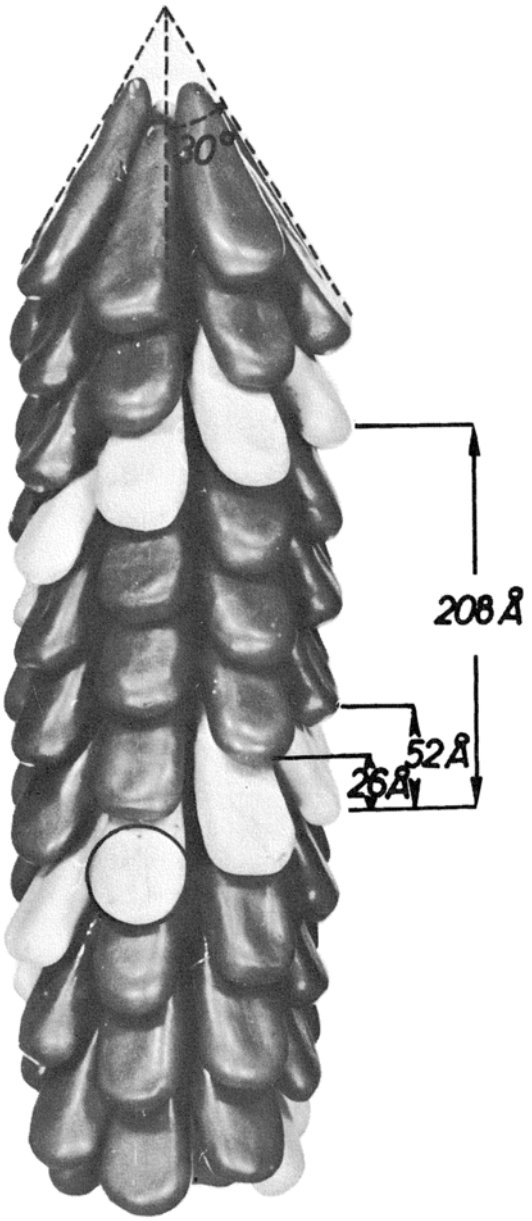

FIGURE 7E

sembly in an abnormal form such as straight'' or ''curly''; incorporation of an amino acid analogue (*p*-fluorophenylalanine) has similar effects.[69,85] Interestingly, mutational sites for determining filament shape are distinct from those determining antigenic specificity.[86] Reconstitution from mixed monomeric solutions also gives discrete forms.[87,88] Recently, extensive studies by Kamiya and Asakura of the effects of pH, temperature, and ionic strength have permitted cycling back and forth between the forms (Figure 5b), and the results have yielded phase diagrams for the equilibria between them.[89,90] Because the same form (curly) can be induced by both high and low pH, the authors conclude that charge effects may be important in determining the structure. This idea fits with the observation by Gerber et al. of reduced dipole moment upon flagellin polymerization.[58] In general, the phase diagrams are still empirical, i.e., there is no theoretical basis for the stability of a given form under a given set of conditions, and this will probably continue to be the case until the details of subunit-subunit interactions have been elucidated. A slight chemical clue to subunit interactions is the inaccessability in the filament of two tyrosine residues per monomer, which are accessible in monomeric solution. Nitration of these groups prevents repolymerization.[91] These residues presumably lie on the surface of the subunit in a region which, in the filament, is involved in interactions with an adjacent subunit.

A general model of subunit stacking has been developed by Calladine,[92,93] which largely explains the observed relationships between twist and curvature of the various helical forms. The accumulated experimental observations[73,76,89,94] reveal that, in a family of polymorphic forms, curvature has a sinusoidal dependence on twist, with members uniformly spaced along the twist axis. This is explained by Calladine as follows: If all 11 rows (the 11-start helices) are in the same conformation, the structure has equivalence throughout and will be straight. Suppose rows are successively converted into an alternate conformation (Figure 8). Each conversion is presumed to introduce a constant twist increment and also to result in an attempted shortening, by a fixed amount, of the line of connections between adjacent rows. The shortening tendency will be counteracted by those rows which are still in the original conformation, and a compromise will be reached in which rows systematically vary in length around the filament, so that the structure is now only quasi-equivalent and will be helical instead of straight.[72] Classical elastic theory predicts that when the strain energy is minimized for each conversion, the curvature should vary sinusoidally from form to form; this, together with

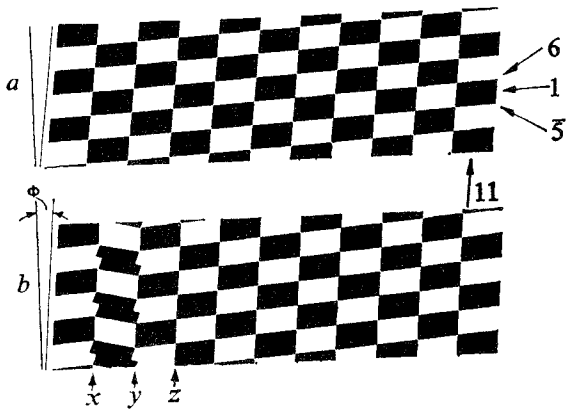

FIGURE 8. Geometrical basis of the model for polymorphism. (a) Connections along the 5- and 6-start helices are identical for all rows (11-start helices) in the straight polymorphic form. (b) Tilting of successive rows and shortening of corresponding lines of connections, e.g., along *x*, introduces twist and curvature to the structure, and so a whole family of polymorphs can be produced. (From Calladine, C. R., *Nature* (London), 255, 121, 1975. With permission.)

the constant twist increments, yields the observed curvature/twist variation for the polymorphic family. Of the 12 predicted forms (0 to 11 in the alternate conformation) for *Salmonella* filaments, 7 have so far been observed experimentally. The general bonding scheme invoked by Calladine has each subunit interacting with four nearest neighbors (on the 5- and 6-start helices) in a bistable manner; all subunits on a given longitudinal row (11-start helix) act cooperatively and must be in the same tertiary and quaternary conformation. To account for the closing of the lattice on itself to form a filament and also the selection of a particular waveform from the total family under any given set of conditions, he invokes a further set of connections on an inner diameter of the filament. Inevitably, a model such as this, even if it predicts the correct relationships between waveforms, raises questions of uniqueness. A possible objection to the model is that the surface lattice in electron micrographs looks packed and pseudohexagonal so that intersubunit distances on the 11-, 5-, and 6-start helices look about the same; the assumption of direct interactions on the 5- and 6-start helices, but not the 11-start helix, therefore seems somewhat arbitrary. The assumption that longitudinal interactions in the filament are highly cooperative has some experimental justification: A mutant isolated by Iino et al.[94] has flagella in which several forms appear to be isoenergetic, yet individual isolated filaments adopt one form exclusively. Another indication of the strength of longitudinal interactions is the high flexural rigidity of the filament, as measured by quasi-elastic light scattering.[95]

We have not yet discussed the handedness of the helical filament; the normal waveform is left-handed,[33,35,36] as has been determined by through-plane focusing and by selective illumination. This is consistent with the observed positive wave propagation and forward propulsion under conditions when the rotation of the flagella is known[96] to be counterclockwise. (Figure 9 presents the pertinent conventions.) However, the family of polymorphic forms in *Salmonella* includes both left-handed and right-handed members.[36,89,97,98]

This brings us to the question of the biological significance of polymorphism. If helical wave propagation were accomplished by a cyclic conformational change, as has frequently been suggested in the past, one could imagine that the various polymorphs are static manifestations of a conformational versatility which in some manner could be used dynamically to trigger phase change (i.e., wave propagation) in the normal form. All available evidence, however, indicates a rotational mechanism, not a conformational one, for flagellar function; the evidence for this type of mechanism and an evaluation of it are presented in Section II.C. A rotational mechanism requires no structural changes in the filament, and one might therefore conclude that polymorphism, though interesting from a structural viewpoint, is irrelevant to cell motility. This assessment has proved to be incorrect. Polymorphism does play an important role in motility, but an unexpected one, involving mechanically induced transitions. Viscous force, acting circumferentially during flagellar rotation, causes transitions between the normal and curly waveforms. These transitions are a key element in tumbling and hence taxis; we will return to this aspect in Section II.E.2.

4. Hook

The hook is a short, curved connecting structure between the filament and the basal body.

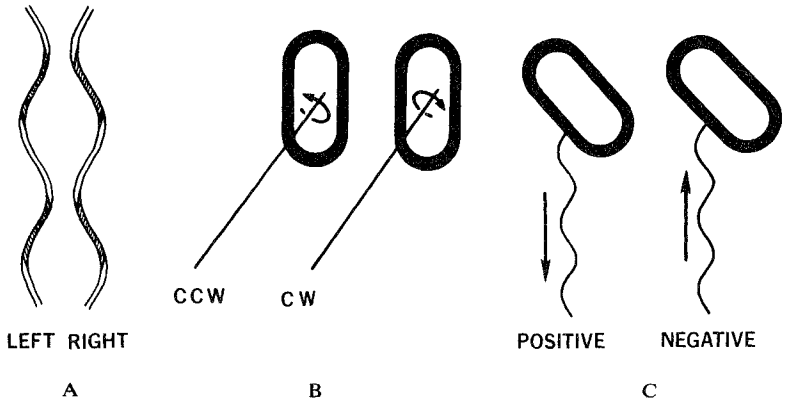

FIGURE 9. Conventions for (a) helical sense, (b) sense of rotation, and (c) sense of wave propagation. Note that rotation is defined for an observer looking along the filament into the cell. (From Macnab, R. M. and Ornston, M. K., *J. Mol. Biol.*, 112, 1, 1977. With permission.)

Unlike the filament, it has a defined length of approximately 70 nm[25] (see, e.g., Figure 1 of Kagawa et al.[99]). It is built from a single protein, antigenically distinct from flagellin,[100] with a molecular weight of approximately 42,000 dalton.[19,25,101] The hook structural gene in *E. coli* has recently been cloned by a $ColE_1$ hybrid episome technique and identified as *flaK*, within the Region I cluster.[102,102a] The function of the hook has not been established. It is not a bearing, since it actively rotates even in *hag* mutants.[103] Its marked curvature suggests a function in orienting the filament. A plausible orienting function would be to flexibly couple the filament to the cell, so that a filament originating laterally could nevertheless readily operate in a bundle on the long axis of the cell. A measurement of the flexural rigidity of the structure would be a useful piece of information in assessing this hypotehsis. (This would most easily be made on polyhooks (Section II.A.6),[104] but the result would be ambiguous, because it is not clear whether a polyhook is an indefinite hook structure analogous to flagellar filaments or whether it is a poly-hook, i.e., an end-to-end assembly of single hook structures, in which case the flexural rigidity might reflect the properties of the hook-hook junctions rather than of the hook itself.)

5. Basal Body

The general structure of basal bodies and their relationship to cell surface layers have already been described. Purified basal bodies when subjected to sodium dodecyl sulfate electrophoresis yield a minimum of nine bands (with molecular weights of 9,000 through 60,000 dalton,[25] excluding flagellin and hook protein. The genes coding for the various polypeptides of the basal region have not been identified, but cloning techniques are currently being employed in an attempt to establish these correlations. The correlation between the proteins characterized by electrophoresis and the morphological features of the basal body is also unknown, one obstacle being the failure so far of attempts to isolate mutants with defective basal bodies.

No enzymatic activity has been found in isolated basal bodies.[47] Vaituzis[105] used cytochemical techniques to assay for ATPase activity in the basal region of whole cells of four species. The results are confusing. In *Vibrio metchnikovii* (a gram-negative polar monoflagellate), a distinct cytoplasmic accumulation of lead phosphate was observed at the flagellated pole. *Bacillus licheniformis* (a gram-positive peritrichous multiflagellate) showed cytoplasmic deposits near some basal regions but not others and some deposits not near any basal regions; in the absence of quantitation and statistical analysis, these data are, at best, only suggestive of specific association. *E. coli* and *Spirillum serpens* (a gram-negative bipolar multiflagellate) both showed uniform periplasmic deposition of lead phosphate. The matter of ATPase activity associated with flagella must therefore be regarded as unsettled. Since ATP hydrolysis

is now known not to be the direct energy source for motility (Section II.B), ATPase activity, if it indeed exists specifically in the basal region, must perform some other function, possibly in assembly or in regulation of tumbling.

6. Flagellar Assembly

The assembly and function of an organelle like the bacterial flagellum is an intricate process. As an illustration of this fact, we may note that 11 structural proteins have been identified so far, whereas at least 25 genes participate in assembly and/or function.[106] Dissection of this system is made difficult by its tight coupling, which results in a rather limited range of phenotypes — mutants in 17 different genes (*hag* and *fla*, Figure 3) in *E. coli* can all lead to nonflagellate phenotype! Recently, cloning techniques using λ or ColE$_1$ hybrid episomes have been put to very effective use in unraveling the complex genetics of motility and taxis.[102,106-110a]

Although the failure to assemble a flagellum may in some cases result from lack of a basal component, it more often appears to be a regulatory defect. Thus, Suzuki and Iino[111] found that nine classes of *fla* mutants lacked flagellin m-RNA.

So far, only a few of the regulatory genes have been identified. *FlaI* (unless otherwise stated, gene symbols refer to *E. coli*) plays an important role as the target for control by cyclic AMP. Expression of the gene is necessary for production of hook and other flagellar proteins and only occurs if cyclic AMP levels are high,[112] although mutation at a second locus (*cfs*, thought to be a cyclic AMP-dependent promotor of *flaI*) can relieve *flaI* from cyclic AMP dependence. Thus, *flaI/cfs* are the targets of cyclic AMP-mediated catabolite repression of flagellar synthesis, described by Yokota and Gots;[113] repression can be overcome by exogeneously supplied cyclic AMP.[114] Analogous to the *E. coli flaI* gene is the *Salmonella flaT* gene[115] which is cyclic AMP dependent, even though *Salmonella* is not subject to catabolite repression. Hook length is regulated by the *flaE* gene. When this regulation is lacking, polyhook structures up to 2 μm in length are observed.[104] Some flagellar regulatory genes (*flaA* in *E. coli*,[55] *flaAII* and *flaQ* in *Salmonella*)[116,117] can also affect chemotactic competence and may therefore code for structural elements in the organelle which function in switching of rotational sense (Section II. D).

It is clear that the state of the cell surface is important in regulating flagellar assembly. *Salmonella* spheroplasts are unable to grow flagella in the presence of penicillin,[118,119] and *Proteus vulgaris* fails to regenerate flagella in the presence of penicillin upon shifting from a nonpermissive temperature (42°C) to a permissive temperature (37°C),[120] indicating that cell wall is needed. Deep rough mutants of *Salmonella*, which are known to be defective in lipopolysaccharide structure, are nonflagellate.[121] *GalU* mutants of *E. coli*, defective in lipopolysaccharide synthesis, are also nonflagellate and lack flagellin m-RNA,[122] but the flagellation defect can be overcome by a suppressor mutation in the *flaH* gene. Komeda et al. suggest two possible mechanisms for this: (1) *flaH* is a structural gene for a product which interacts with the outer membrane; (2) *flaH* mutation can cause overproduction of flagellar components to compensate for excretion losses through a deficient outer membrane.[122]

Ubiquinone mutants are nonflagellate, but it is not known whether the cause is regulatory or not.[123] Flagellar assembly can depend on growth conditions; both glucose, by lowering cyclic AMP levels, and elevated temperature, by an unknown regulatory mechanism, inhibit flagellar assembly.[124]

Nothing is presently known regarding the mechanism of assembly of hooks or basal bodies, except that flagellin synthesis is not necessary.[125] Neither structure has been reconstituted in vitro, and so it cannot be judged whether they are self-assembling or whether regulation of assembly occurs as well as regulation of synthesis of the necessary proteins. The site of assembly is not known either. Is the hook, for example, assembled in the cytoplasm and excreted, or is it assembled by addition of subunits to a nascent external structure attached to a preexisting basal body embedded in the membrane, and, if so, how is its length regulated? The theory of basal bodies as "self-reproducing particles,"[39] based on observation of basal body pairs in electron micrographs, seems improbable and is not justified by the evidence.

Much more is known about filament assembly. This is understood to be a spontaneous process, since in vitro reconstitution occurs

without either an energy source or enzymatic mediation.[22,23,60-63] The subunits normally require either a hook or a fragment of filament before they will attach, as has already been discussed. The most remarkable aspect of filament assembly is that it occurs at the distal end only. This has been established beyond doubt by radioactive and antibody label experiments and by the use of amino acid analogues which cause assembly of heteromorphous filaments.[67-69] A particularly striking demonstration of distal growth was the conferral of motility on a sheared straight mutant of *Salmonella* by filament assembly from normal flagellin supplied exogeneously in the medium at high concentration.[126] Since bulk excretion would be prodigal in the extreme and is not supported by experiments with mixed cultures of different flagellar phenotypes, when copolymerized filaments might be expected,[67] one is forced to assume that distal growth is achieved by extrusion of monomer down the filament; this, however, has never been demonstrated directly. Extrusion of flagellin in its native conformation must be a tight squeeze if the core diameter of 3 nm (or, in this author's revised estimate, 4 nm) and limiting subunit width of 5 nm[59] are correct — perhaps a different subunit conformation is used during the extrusion process. When merodiploid strains of *E. coli* were constructed, with two distinct flagellin genes being expressed simultaneously, homogeneous copolymeric filaments resulted, suggesting that a freely mixed pool of the gene products is available for extrusion.[127]

The complicated regulation of flagellin synthesis in *Salmonella* has been studied extensively. *Salmonella* possesses two structural genes, *H1* and *H2*, which are alternately expressed (the phenonemon of "phase variation"[128] and code for antigenically distinct flagellin molecules. Expression of both genes is under positive control by *ah1* and *ah2* genes, respectively.[129,130] A repressor gene, *rh1*, coregulated with *H2*,[131] ensures that if *H2* is being expressed, *H1* is not. Yet another gene, *vh2*,[132] causes oscillating expression of *ah2* at a frequency of about 10^{-3} per cell division.[18,128,133] As a result, *H2* and, inversely, *H1* are alternately expressed. The alternation occurs at the mRNA level, as has been demonstrated by in vitro synthesis.[50] The *ah1-H1* and *vh2-rh1-ah2-H2* clusters are widely separated on the genetic map,[133-135] at 65 and 82 min, respectively; the latter location also contains the *fla* and *che* genes associated with flagellar assembly and chemotaxis.[136] Zieg et al.[136a] have recently used cloning techniques to prepare segments of DNA around the *H2* locus. Heteroduplex analysis of this DNA shows a bubble which corresponds with phase state; they postulate an inversion in this sequence as the event responsible for regulating the expression of the *H2* gene. This remarkable regulation system, which does not exist in *E. coli*, has no known function — could it be a short-term counterdefense against the immune system?

Operation of the assembled flagellum is dependent on the expression of two genes, *motA and motB*.[53,107,109,135,137,138] *Mot* mutants are paralyzed but possess, by present criteria, morphologically and biochemically normal flagella. The *motA* and *motB* gene products are not among the polypeptides which have been identified in the basal body but are membrane associated, with molecular weights of 31,000 and 39,000 dalton, respectively.[107,109,138a] An extrachromosomal genetic control of motility has recently been reported;[139] a naturally occurring conjugative plasmid results in paralysis of *Salmonella*, but not in loss of flagellation.

B. Energy Source

A number of early workers, basing their efforts on analogy with eukaryotic flagella and muscle systems, searched unsuccessfully for evidence of ATPase activity associated with the bacterial flagellum and, in particular, with the external filament.[137,140,141] In retrospect, the simplicity of the bacterial filament should have been a clue that it was unlikely to contain an enzymatic apparatus capable of chemomechanical energy transduction. At any rate, when emphasis shifted to the basal body, no ATPase activity was found there either[47] (see, however, the discussion of Vaituzis' study in Section II.A.5).[105] Finally, a fundamental difference between bacterial flagella and other motor systems emerged — neither ATP nor any other "high-energy compound" is the primary energy source for bacterial motility. Instead, the flagella are driven by an energized membrane state which can derive from either respiration or ATP hydrolysis.

The compelling evidence for this conclusion is summarized here. Thipayathasana and Valentine[142] showed that infection of *E. coli* by the flagellotropic phage χ, which can only infect motile cells, occurred under anaerobic conditions with wild-type cells, but not with mutants blocked in the membrane-bound ATPase, unless nitrate (a terminal electron receptor for the respiratory chain) was added. In more extensive studies involving direct observations of motility, Larsen et al.[143] demonstrated with *E. coli* and *Salmonella* that anaerobic motility is possible if and only if ATP levels are high and the membrane-bound ATPase is present and functioning; ATP depletion by arsenate treatment or as a result of genetic defects in the ATPase caused paralysis. In contrast, aerobic motility is possible regardless of the state of the ATPase or the ATP pool. The proton ionophore carbonylcyanide *m*-chlorophenylhydrazone (CCCP) abolished motility, while leaving ATP levels high. Taken collectively, these observations rule out ATP and support the intermediate of oxidative phosphorylation as being the primary energy source of bacterial motility. The role of ATP in taxis will be discussed later.

That the energized membrane state driving motility is in fact a proton electrochemical potential (protonmotive force), as expected from the chemiosmotic theory of Mitchell[144] or the proton channel theory of Williams,[145] has recently been shown by Manson et al.[146] and Matsuura et al.[146a] The protocol for these experiments calls for removal of endogenous energy sources, but attempts by various workers to achieve this with *E. coli* or *Salmonella* had been unsuccessful because the lengthy starvation procedures required apparently had adverse regulatory effects on the cell, so that motility could not be readily restored. These difficulties were cleverly circumvented by employing an unusual strain of *Streptococcus* which had been discovered to possess motility.[147] Since *Streptococcus* lacks endogenous energy sources, it can be rapidly and completely starved by removal of the exogenous source, such as glucose. When starved cells were placed in K^+-free medium containing the K^+ ionophore valinomycin, they recovered motility transiently for about 45 sec as a consequence of the K^+ inside the cell diffusing out, down its chemical potential gradient, to generate an electrical potential, negative inside (measured by voltage-sensitive dyes). It is difficult to prove conclusively that inward diffusion of protons, as opposed to some other ionic species, is responsible for the motility, but some obvious alternatives (Na^+, Ca^{++}, and Mg^{++}) were ruled out by excluding them from the medium; also, an imposed ΔpH was effective in transiently restoring motility. The protonmotive force generated by K^+ diffusion generates a burst of ATP, but, since arsenate-treated cells, which have a negligible ability to synthesize ATP, still gave a motile response to valinomycin, it is protonmotive force per se, and not ATP, which restores motility. Although the general conclusion from these experiments is clear, namely, that the protonmotive force is responsible for bacterial motility, there are some puzzling features. Motility ceases even when a substantial protonmotive force (corresponding to an electrochemical potential of -75 mV) remains. Also, the motility consists of tumbling only, with virtually no swimming, unless an attractant is added simultaneously. These complications may be a consequence of the pH changes which inevitably occur in this type of experiment, since it is well known that moderate pH changes induce tactic responses[148] and larger changes destroy motility.[124] Confirmatory observations of valinomycin-activated artificial motility in *B. subtilis* have since been published.[146a]

One genetic clue regarding motility is the fact that the *mot* gene products are located in the membrane, but not in direct association with the basal body.[107,138a] Defective or missing *mot* products may, therefore, cause paralysis, not by jamming the motor, but by failure in some physically remote function, possibly as an ion gate or as an enzyme responsible for production of a diffusable substance which is needed to interact with the motor.

Finally, we mention a well-known, but not well-understood, fact regarding motility: Various heavy metal ions inhibit rapidly and completely, even at quite low concentrations, e.g., Cu^{++} at 10^{-8} *M*.[124,149] (The inhibition is routinely avoided by inclusion of chelating agents in the medium.) Whether the effect is specific to the motor apparatus or whether it is affecting some general physiological parameter is not really known, although it was shown that the viability

of *E. coli* cells after a 4-hr exposure to $10^{-6}M$ Cu^{++} was normal.

C. Mechanism of Wave Propagation

The regular helical waveform of flagellar filaments has already been described. When motile cells are observed by dark-field light microscopy, this waveform maintains a constant shape and propagates positively, i.e., outward from the point of attachment at the cell.[15,33,150] However, since only the contour of the filament can be discerned (details of the filament lattice being far below the resolution limit of the light microscope), such observations do not permit one to distinguish between a mechanism in which the filament is forced to undergo true rotation at the base and one in which it is driven through cyclic conformational changes. (The two mechanisms can be demonstrated by turning a corkscrew while holding onto a piece of elastic tubing sleeved over it; both the corkscrew and the tubing propagate helical waves, but the former does so by a rotational mechanism, whereas the latter does so by a conformational mechanism.)[11a] A plausible conformational model would be along the following lines: Each of the 11-start helices (rows) is, in a helical system, necessarily in a slightly different configuration. (Regarding the concept of quasi-equivalence, see Section II.A.3). The fact that a given row is in a given position, say, on the inside, i.e., closest to the filament helical axis, is arbitrary in an isolated filament; but in an intact flagellum, the position is dictated by a constraint in the basal region. If this constraint is permuted cyclically by means of some cellular chemomechanical transducer, it can, provided coupling is tight enough, cause rows 1 through 11 to successively occupy the inside position. Thus, the filament will have retained its helical form but will have undergone a phase change of 2π as a result of 11 successive conformational shifts. This type of model, although intuitively appealing to many,[72,151,152] has no evidence to support it. The rotational model, i.e., the idea of a "biological wheel," although discomfiting to some, has by now considerable, perhaps conclusive, evidence to support it. For a summary of earlier opinions, see Berg and Anderson,[153] who argue that several published observations support rotation, notably, (1) paralysis of single cells of multiflagellate species, but not monoflagellate species, by bivalent antifilament antibody,[154,155] and (2) paralysis of multiflagellate species by flagellotropic phage χ, even if the latter were previously inactivated by radiation.[156] They also emphasize the conclusions of Coakley and Holwill[157] that, hydrodynamically, the two mechanisms are virtually indistinguishable, since propulsion is achieved by lateral filament displacement, which occurs to the same extent whether the filament is moving conformationally or is rotating (Section II. F).

An experiment purporting to prove flagellar rotation[158] involved observation of cells of *Spirillum* rotating in thin preparations, under conditions where the flagellar tufts were "stationary." The observation is suggestive, but (as Harris[151] has pointed out) not conclusive. Rotation of the flagella could conceivably be occurring at the cell velocity, but about their local axes, so that the waveform does not move.

More direct and convincing evidence for rotation comes from experiments with cells tethered by antibody either to each other, to microscope slides, or to latex bead markers[96,103,159] (Figure 10). Objections have been raised that, because antibody bonds are relatively weak, in the case of a cell tethered to glass the cell and filament might be rotating as a unit with respect to the glass as a result of conformational wave propagation in the filament. Because of such objections, the experiments with straight flagellar mutants take on special significance. A straight filament possesses no — or at least an immeasurably small — helical amplitude and can therefore do no conformational work. If rotating on its axis, it can do negligible work against a viscous medium, because the shear velocity ωr is so small, unless it is rigidly anchored to a larger object. This is in agreement with the observation that independent cells of the straight mutant display no motility but spin rapidly if they are tethered to a slide or to another cell. A related line of evidence is the fact that tethered nonflagellate polyhook mutant cells display cell rotation. Further support for the rotational model comes from observations of clusters of latex beads attached to the straight filaments of mutant cells. These beads were observed to rotate about the inferred axis of the (invisible) filament. In spite of all of these observations, it must be admitted that a

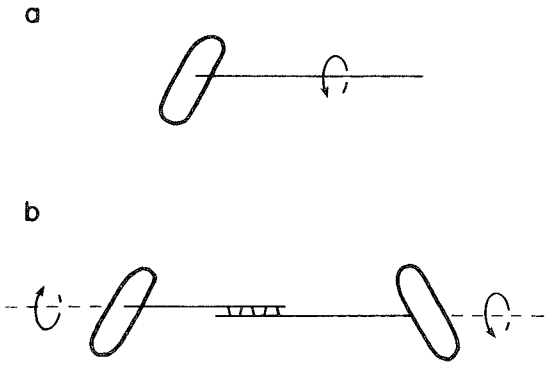

FIGURE 10. Evidence that the flagellar motor is a rotary, not a conformational, device. In the case of a straight flagellar filament, a conformational mechanism can under no circumstances perform work. A rotary mechanism can perform negligible work in a free cell (a), because viscous resistance to rotation is so small, but if two cells are joined to each other by their filaments by means of antibody (b), torque should be transmitted from one cell body to another, and they should mutually rotate; this is observed to be the case.[103] There are a number of other related lines of evidence in support of a rotary mechanism.

direct demonstration of flagellar rotation is still lacking and would be reassuring to have.

Although the rotational mechanism can be considered established, there is no information yet as to how it operates, nor is it obvious how to set about obtaining such information. A major step would be to establish which parts of the basal region are rotating and which are stationary and also where between rotating and stationary parts the torque is developed. If motor mutants could be found mapping in structural genes for the basal body, they might provide answers to these questions. The all-or-none assembly of the basal body (Section II.A.6) is easier to understand than the failure to find a mutant which has an assembled but nonfunctional motor. It is obvious that function is highly dependent on structure; it appears that the reverse is also true. Because the M ring is in the cytoplasmic membrane, across which the protonmotive force exists, and the S ring lies just above the membrane, the suggestion has been made[159] that they are the two elements between which torque is generated. Other elements, e.g., the inner cylindrical surface of the M ring and the outer surface of the axial rod, would also be plausible candidates. The M ring is believed, from rotational symmetry analysis, to have 16 subunits,[44] and one might therefore suppose that if it is the rotor, the subunits would define some elementary step in the rotational process. Attempts by Berg[160] to detect elementary steps in the rotation have so far been unsuccessful.

It should be noted that although it has been established that a protonmotive force is necessary for motility, this does not necessarily mean that it is traded directly for mechanical energy without passing through an intermediate form. If proton translocation is used directly to perform useful work, a plausible mechanism might involve a transmembrane chain of hydrogen bonding interactions (see ref. 161 for a review) in a geometrical situation which requires rotational advance accompanying the translocation of protons from bonding site to bonding site. Such a mechanism has recently been discussed by Lauger.[161a] Adam[162] suggests a mechanism — and calculates it to be feasible energetically — whereby viscous streaming of the cytoplasmic membrane applies drive to the motor; but he does not indicate how protonmotive force can generate the streaming in the first place.

D. Reversibility

Tethering experiments demonstrating rotation also quickly revealed that motor rotation can occur in either a counterclockwise or a clockwise sense[103,159] and that this capability is crucial to chemotactic competence.*[96] The relationship between reversibility and taxis will be discussed later; for the present, we are concerned with the mechanical aspect, and, as there is little experimental evidence yet available on the subject, this section can only outline concepts and speculate on mechanisms.

A conceptual division can be made between the device which permits the motor to operate in either rotational sense (the "switch") and the device which places it in one sense or the other (the "switch signal"). (Examples of the two devices would be the forward/reverse gear system of an automobile and the hand of the driver.) The switch might be either a device which permits reversal of the sense of primary energy

* As an indication of the importance of reversibility to the cell, we may note that it is regulated as a function of growth temperature, in such a way as to maximize tactic responses at that temperature.[163,164] (Responses were observed below the membrane phase transition, in contrast to a previous report.[165])

flow or a gear which, with the same sense of primary energy flow, utilizes it to provide force in either of two rotational senses. (Examples would be a turbine subject to gas flow in either direction and a turbine with blades of reversible pitch.) In view of the many transport processes which are dependent on protonmotive force, it does not seem likely that the switch is a reversal of the global protonmotive force. Conceivably, however, a local (compartmented) protonmotive force coupled to some other energy storage mechanism, e.g., a specialized ATPase* — in which case the parameter determining the local ATP concentration would be the switch signal — might be capable of reversal. If the primary energy source retains its sense, the switch might be a macromolecular assembly in the basal body which, by conformational change, reverses handedness. Coincidentally, in a closely related topic, an interesting precedent exists for a biological macromolecular assembly with bistable handedness, namely, the normal-to-curly conversion of the flagellar filament, discussed in Section II.E.2. A tempting hypothesis for the switch signal would be some aspect of the chemotactic methylation system (Section III.E.2).

E. Motility Modes

Peritrichous bacteria display two principal modes of motility, swimming and tumbling. How these participate in taxis will be considered in Section III. A, but in this section we will consider organelle function per se.

1. Swimming

The flagella of peritrichous bacteria are left-handed helices which, therefore, when rotated counterclockwise, exert a pushing force on the cell. As can be readily observed by dark-field light microscopy (Figure 4b), this results in formation of a helical bundle aligned more or less exactly with the long axis of the cell. The first question which comes to mind on seeing coordinated function like this is whether it derives from a controlling mechanism in the cell. As Anderson[167] has described, active control is not needed;[168] individual filaments, flexibly attached to a cell, will have a spontaneous tendency to come into alignment (Figure 11), and, as this occurs, intertwisting of filaments as a consequence of body rotation and inward radial replacement of the fluid being pumped away axially will further enhance the tendency of the filaments to come into a common domain.

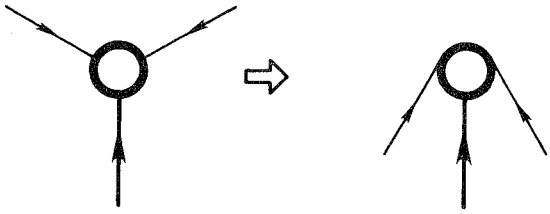

FIGURE 11. Initiation of bundle formation, as described by Anderson.[167] (a) Dispersed flagella, flexibly coupled to the cell and pushing it, will in general be slightly unbalanced so that (b) displacement of the cell will sweep flagella round into progressively more concerted action. (From Macnab, R. M. and Ornston, M. K., *J. Mol. Biol.*, 112, 1, 1977. With permission.)

It is not clear whether the tendency which flagellar filaments have to aggregate side by side, particularly at moderate to high ionic strength, plays any role in bundle stabilization. Gerber et al.[169] demonstrated by electrical birefringence measurements that aggregates can be induced by applied fields, and they postulate this as a mechanism of controlling bundle formation, but the time scale (minutes) of the birefringence effect seems much too long for this postulate to be correct.

When a conformational model for flagellar function was in vogue, operation of flagella in a bundle seemed straightforward enough — the individual filaments could be thought of as strands in a flexing rope. Individually rotating filaments, however, originating at various points around the cell yet operating as a single bundle, are harder to visualize, and indeed this may be one reason that the rotational mechanism was regarded with suspicion. By geometrical analysis and by use of a working model, Macnab established,[168] as had previously been suggested,[21,23] that no problem of entanglement exists, provided the direction of wave propagation is away from the attachment point. Collisions between the filaments merely act to bring them closer into phase and onto a common axis. Hydrodynamic calculations suggest that

* In this context, a re-examination of the question of ATPase activity in the basal region[105] would seem worthwhile. The Mg^{++} requirement for tumble suppression in *B. subtilis*[166] (see Section III.F.1) might be for ATPase function.

right-handed wrapping may occur. In a left-handed helical system, this necessarily involves elastic distortion, which will be minimized in a compact, in-phase bundle; indefinite stable operation of a bundle in such a geometry was demonstrated on the model.

The disposition of the flagella in the prebundle region is not known. Light microscopic observation of this region is extremely difficult because of intense scattering from the cell.

2. Tumbling

Peritrichous bacteria alternate between swimming periods and periods of chaotic cell motion called "tumbling"; tumbling is clearly important, since mutants which are incapable of this motion are found to be chemotactically defective (Sections III.A and III.E.1). Early observations simply describe the bundle as disappearing during a tumble, but improvements in light microscopy which permit individual flagella to be seen[33,34] showed that the flagella actively fly apart, a conclusion also reached from cell angular velocity measurements.[170]

Following the tethered cell demonstration of cell rotation, Larsen et al.[96] showed that smooth-swimming mutants rotate counterclockwise and tumbling mutants rotate clockwise, while wild-type alternate between the two modes. Chemotactic stimulation experiments confirmed the correlation of swimming with counterclockwise rotation and of tumbling with clockwise rotation. As a corollary of the study of bundle formation during counterclockwise rotation, it was shown[168] that during clockwise rotation, bundle jamming should occur, whereas experimentally bundle dispersal is observed.

In detailed microscopic studies, Macnab and Ornston[98] found that reversal of flagellar rotation causes a polymorphic transition from the normal left-handed waveform to a curly right-handed one (Figure 12). Transient curly bundles were noted by Pijper[150] many years ago (the phenomenon of "biplicity"), but their significance was uncertain at that time. The phenomenon of normal-to-curly transitions can be explained in mechanical terms. Viscous force will influence the stability of any structure; if the structure happens to be a left-handed helix and is being rotated clockwise, it is placed under right-handed torsion by the circumferential viscous resistance of the medium, and its stability is thereby reduced.

The susceptibility of flagellar filaments to mechanically induced transitions is intrinsic to their structure and should, therefore, not be dependent on the biology of the cell to which they are attached. For example, if a filament is subjected to axial flow away from an attachment point, it will, regardless of handedness, experience a destabilizing (unwinding) torsion, which will be maximized at the attachment point. In a remarkable verification of this, Hotani has observed and documented with a high-sensitivity television system indefinite cycling between various forms when filaments in vitro are attached to glass at one end and are subjected to axial stress by flow of a viscous fluid.[275] The transition at any instant is confined to a quite local region and propagates positively, i.e., away from the point of attachment.

On tumbling cells, the transition is initiated at the proximal end and proceeds rapidly outward. A filament in transition is in a heteromorphous state and consists of a proximal curly segment, a distal normal segment, and a very localized transition region. The two segments present discontinuous change in axial direction by an angle which is the algebraic difference of the pitch angles of the two forms;[171] the structure of these heteromorphous filaments produced mechanically appears to be essentially the same as those produced by genetic or biochemical manipulations.[171] The angled configuration of a heteromorphous filament presents a large resistance to rotation, and with five or more rotating filaments, in various stages of transition and with mixed handedness, it is not surprising that the resultant cell motion is chaotic. A videotape demonstrating the participation of polymorphic transitions in tumbling has recently been made by Macnab and Ornston, using a high-sensitivity television camera similar to that of Hotani. (It is interesting to note that, if all flagella were fully converted to the right-handed form and were all rotating clockwise, forward propulsion should ensue; this has been observed under special circumstances.[98]

F. Hydrodynamics

As Coakley and Holwill[157] point out, in addition to intrinsic interest,

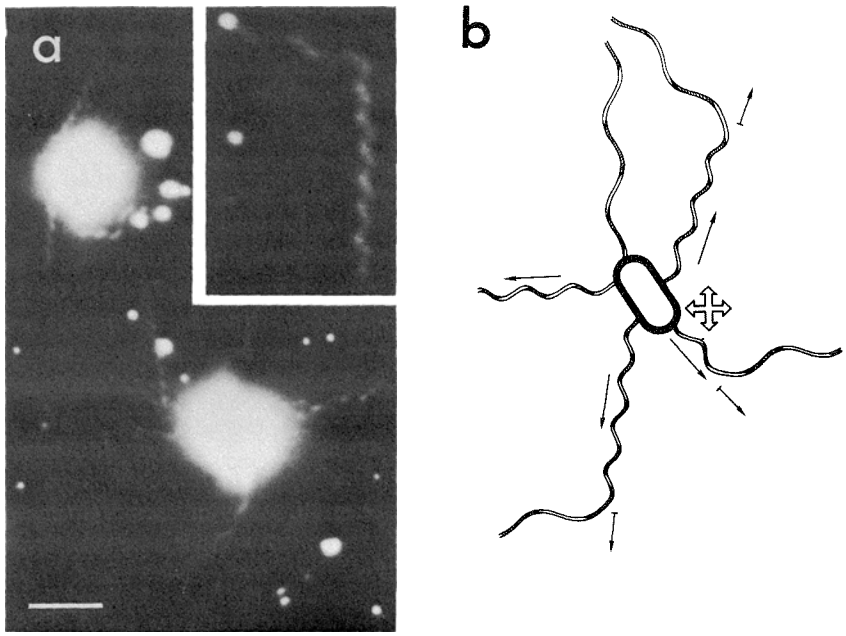

FIGURE 12. Polymorphic transition during tumbling. In clockwise rotation, filaments are placed under right-handed torsion by viscous resistance and undergo transition from the normal, left-handed to the curly, right-handed form, the transition proceeding outward from the proximal end. (a) Dark field micrograph of *Salmonella* in tumbling mode; (inset) stable heteromorphous filament produced by special growth conditions (*p*-fluorophenylalanine) shows the same discontinuity in the axial direction of the normal and curly portions as is observed on heteromorphous filaments generated during tumbling. Bar equals 5 μm(2.5 μm on inset). (b) Drawing illustrating positive wave propagation (arrows) as a result of clockwise rotation of filaments which have been converted to the curly, right-handed form, positive propagation of transition points (arrows with backbars), and chaotic cell movement (open crossed arrow). (From Macnab, R. M. and Ornston, M. K., *J. Mol. Biol.*, 112, 1, 1977. With permission.)

the importance of accurate hydrodynamic analyses of flagellar movement lies in their use to evaluate the power expended by the flagellum against external forces. This power, together with estimates of the energy dissipated within the flagellum (against, for example, elastic forces) can be used to obtain a minimum value for the energy which must be supplied by the chemical reactions which are responsible for bending the flagellum.

In light of recent information concerning the bacterial motor, we may largely ignore internal elastic forces — which, at any rate, were not amenable to calculation — and should replace "bending" by "rotation," but the sense of the statement is still valid.

The present state of hydrodynamic analysis is quite satisfactory, at least for the simple case of a microorganism with a spherical head and a thin filament propagating a uniform helical wave.[157,172,173] No inertial forces enter into the analysis, since only viscous forces are significant at this size scale.[174,175] In fact, swimming velocity is lower in water than in media with twice the viscosity;[176] in a situation where inertial forces predominated, this would be impossible. The equations of Gray and Hancock[177] for the longitudinal and normal frictional coefficients of a thin filament are used, along with the classical coefficients of rotational and translational friction for the spherical head. The filament terms for forces and moments are integrated (which for a uniform helical wave is straightforward), algebraically summed with the force and moment on the head, and the sum then set to zero at steady state. In these analyses, the couple on the filament carries a negligible contribution from rotation about the local filament axis, so that in this regard rotational and conformational models are energetically

equivalent, as was mentioned in Section II. C.

The power dissipation of a swimming monoflagellate bacterium estimated in this way is of order 10^{-9} erg/sec. Typical respiration rates are approximately 1.4×10^{-18} mol O_2/sec.[178] If we assume this is used for glucose combustion ($\Delta G = -5 \times 10^{12}$ erg/ mol O_2) to produce energy useful to the cell with an efficiency of order 10%, the power expended in motility is a small fraction (of order 10^{-3}) of the total useful power dissipation of the cell. Estimates of power dissipation in a peritrichously flagellated cell are more difficult, since it is highly dependent on how tightly the flagella are bundled; provided the bundle is loose, it is not nearly as much as the power which would be dissipated if the same number of flagella were operating in totally separate domains. An indication that friction within the bundle may be considerable is the lower swimming velocity of peritrichous bacteria (approximately 25 μm/sec) compared with *Pseudomonas*, a polar monoflagellate (approximately 50 μm/sec),[34,176] although obviously other factors might be responsible for the difference.

As Chwang and Wu[173] note, it is interesting that the body/filament diameter ratio (approximately 25) and wavelength/helical-amplitude ratio (about 12) approximate the theoretical optima for efficient propulsion. Analyses of cinemicrographic records of bacteria swimming in high viscosity media yield values for the velocities of translation, body rotation, and wave propagation which are in reasonable agreement with the values predicted by the theoretical analysis.[179,180]

III. CONTROL BY SENSORY STIMULI

A. Chemotactic Migration

The most obvious manifestations of chemotactic responses occur when bacteria, cultured on soft agar plates, migrate* in bands, in response to gradients generated by the cells' catabolism.[8a] The cells confine themselves to boundary zones between regions where a particular chemotactically effective nutrient has been depleted and regions where the nutrient is still at a uniformly high level, so that, as consumption proceeds, the band advances. Sharp band formation occurs whenever the steepness of the gradient and the chemotactic sensitivity of the cells are sufficiently great to create a directed velocity which substantially exceeds the random velocity. Various mathematical analyses have been carried out on such band shapes and movements,[181-187] but, as the approach of this review is more at the molecular level, this aspect will not be discussed further.

1. Nongradient Behavior

Observation of individual motile cells in the light microscope reveals the two motility modes of swimming and tumbling described in Section II.E. Information regarding motility has also been obtained by intensity correlation spectroscopy measured on populations;[188-193] difficulties in interpretation, however, make this approach less useful than direct observation on single cells.

The swimming intervals ("runs"), at translational velocities of approximately 25 μm/sec, have mean durations of a few seconds, whereas the tumbling intervals are about an order of magnitude shorter. The endless alternating sequence of swimming and tumbling produces a three-dimensional zigzag trajectory. Berg and Brown,[170] in quantitative measurements of individual cells in an automated tracking microscope, showed that both runs and tumbles terminate with constant probability, i.e., their durations conform to a Poissonian distribution. This applies, strictly speaking, to a single cell only; considerable individuality in tumble frequency exists from cell to cell, but, if each cell is scaled to its mean performance, the normalized data for a population are Poissonian. Such individuality is confirmed and discussed at length in a paper by Spudich and Koshland.[194]

This pattern has given rise to the term "random tumble generator." A less prejudicial term might be, in the terminology of Section II. D, "random inverting switch signal," to emphasize the fact that the termination of a tumble generates a run, as well as the other way around. Such a device would place the motor in counterclockwise or clockwise modes according to whether a randomly fluctuating parameter lies below or above (or vice versa) a critical

* The author would like to plead against the use of the nonverb "chemotax" which appears from time to time in the literature.

value. It should be noted that its mean value must be below the critical value to account for run/tumble asymmetry.[170] There is some evidence from chemotaxis mutants (Section III.E.l). that the basic or default mode of motility is counterclockwise; if this proves to be the case, "tumble generator" will be a semantically appropriate term for a device which has to intervene to switch the motor from its default mode. The role of chemotactic signals then becomes one of inhibition of the tumble generator.

The directional change achieved by tumbling is random azimuthally but possesses a slight forward bias, the mean value being 62 rather than 90°.[170] This forward bias indicates that, on the average, bundle dispersal is not quite complete, a conclusion supported by visual impressions of the flagella in the microscope. At any rate, this forward bias does not seem to play a significant role in the chemotactic response.

2. Behavior in Temporal and Spatial Gradients

Chemotactic agents at steady isotropic concentrations are not found to modify the motility pattern which has just been described,[170,195] unless they approach toxic levels or are needed to provide energy in an otherwise energy limiting situation. This statement does not seem to apply to *B. subtilis*: De Jong et al.[196] found that amino acids stimulate motility without affecting respiration rates or ATP levels. However, effect as attractants did not correlate with effect in stimulating motility. If concentration per se is not a tactic stimulus, a gradient-sensing mechanism must be operative, i.e., the cells must be capable of measuring concentration difference in some way. This implies a comparative measurement, yet the extreme smallness of a bacterium and its ability to detect quite shallow gradients suggest that a spatial comparator across its cell body is an unlikely mechanism. The motion of the cell, however, provides the basis for another mechanism based on temporal comparison, even though only spatial gradients may be present. A cell swimming in a spatial gradient maps a temporal gradient in its own frame of reference; a device capable of making a temporal comparison can then, if it operates on a time scale appreciably longer than the time to swim through one body length, utilize a commensurately large concentration difference. The temporal gradient-sensing hypothesis was clearly validated by Macnab and Koshland[195] in experiments in which cells were subjected to abrupt jumps from one isotropic attractant concentration to another; a concentration increase was found to suppress tumbling and, to a lesser extent, a concentration decrease to stimulate it. "C jump" experiments of this sort have since been widely used as a rapid and sensitive chemotactic assay. A quantitative version has also been described.[197] A crucial feature of the response is the transience of the departure from unstimulated tumbling frequencies — abnormal behavior in the new isotropic medium lasts only while a memory of the old isotropic medium persists. (The term "memory" is used here without apology, to connote the retention by the cell of information about the past to be used in determining current behavior.) Similar, but inverse, responses to concentration jumps of repellents were subsequently demonstrated by Tsang et al.[198] Shallow temporal gradients, generated enzymatically, of isotropic attractants and repellents modulate mean run length in a comparable, though less extreme, manner.[199]

A device for measuring temporal gradients of attractant concentrations must have three characteristic features: (1) an element responding rapidly to attractant concentration, (2) an element responding more slowly to attractant concentration and hence providing memory, and (3) an element for comparing the status of (1) and (2). In order to account for asymmetry, Berg and Tedesco[200] assume that element (2) responds slowly to increases in attractant concentration but rapidly to decreases in attractant concentration.

The means by which tumble modulation produces migration in spatial gradients is quite simple; a cell which happens to be experiencing a positive temporal gradient of attractant because of its current direction of swimming in the spatial gradient has a lowered probability of changing direction by tumbling, so that up-gradient swimming tends to be more prolonged than down-gradient swimming. One should note that there is no specificity in the direction taken after a tumble; only the probability of tumbling has been modulated. This contrasts with orientation responses in higher animals. The trajectories of individual cells in spatial

gradients, studied in most detail by Berg and Brown,[170] conform to this description of a time-biased random trajectory. A "two steps forward — one step back" mechanism of this sort is relatively inefficient. For example, the migration velocity of *Salmonella* in a particular serine gradient was measured to be 2.8 μm/sec, only 10% of the instantaneous swimming velocity.[201] The technique used for this and similar measurements involved placing cells in a stable, defined unidimensional spatial gradient and monitoring population density along the gradient axis by means of light scattering. These data on cell populations are in good agreement with mean data on cells tracked individually.[27] A detailed analysis of chemotaxis in unidimensional spatial gradients has been made by Lovely et al.;[202,203] the simplicity of the gradient form has led to extensive modeling based on classical diffusion theory, with a bias component.[204-208] Interestingly, the time spent in a tumble — when no translational progress is being made — is kept to the minimum value consistent with effective randomization.

Whereas short tumbling responses to "unfavorable" stimuli, defined as attractant decreases or repellent increases, were observed in the concentration jump experiments, the probability of tumbling for a cell swimming in an unfavorable spatial gradient direction was found to equal the nongradient value. These results do not contradict each other but simply reflect the much more severe stimulus levels in the temporal gradient experiments. Both types of experiments indicate a major asymmetry in the gradient-sensing mechanism, or at least in its expression at the motor level. The asymmetry was particularly evident in experiments with tethered cells, subjected to various regimens of concentration increase and decrease of attractants and repellents.[200] Concentration increase of an attractant suppressed clockwise rotation (actually measured as counterclockwise rotation because of the experimental geometry). Such a response, which would correspond to smooth swimming in a free cell, continued until the cell adapted — this was a fairly slow process, lasting approximately 100 sec. (The authors use the term "transition time" for this measurement. The term "adaptation time" would seem to be more descriptive and to conform better with usage in sensory physiology.) If, during this adaptation interval, the concentration was briefly pulsed down to the original level, the response dated from that point, not the point when the first stimulus was given. In other words, the adaptation system was rapidly reset by the downward pulse.

An interesting unanswered question concerns the reset mechanism: Does reset occur upon tumbling or upon negative gradient sensing? Put another way, what is the adaptation state of a cell which has experienced a positive gradient, tumbled, and is once again experiencing a positive gradient? The time scale of the reset mechanism was of the order of 2 sec for large negative concentration jumps; it is probably safe to assume, therefore, that a cell swimming in shallow spatial gradients is fully adapted whenever it is experiencing negative stimulation. Slow adaptation to positive stimuli and virtually instantaneous adaptation to negative stimuli constitute a very clever mechanism: The former aspect permits accumulation of concentration difference signals of considerable size, while the latter aspect prevents averaging of positive gradient information into a "decision" for a cell swimming in a negative gradient direction and vice versa. The accumulation process, of course, must be a trade-off of signal relevance for signal size. In an ideal situation, the cell would have absolutely current information about its status in the gradient; in reality, it utilizes a smoothed value on a time scale which is still unclear but which probably lies between 1 and 10 sec. The persistence, i.e., maintenance of a positive velocity component, of *Salmonella* up a unidirectional spatial gradient of serine lasts approximately 20 sec,[276] which is 8 sec longer than the mean nongradient value.[209] (Persistence times, incidentally, are found to conform to Poissonian statistics in agreement with Reference 170.) This suggests that a time scale of up to tens of seconds for data accumulation is available, although of course it does not prove that the gradient-sensing device actually has such a time constant. Brown and Berg[199] tested smoothing functions on data for cells in enzymatically generated gradients but could find no improvement in fit (compared with unsmoothed data) for time constants of up to several seconds; they observed a deterioration in fit for longer time constants. We must, therefore, regard this question as open. It is in-

teresting to note than an effective time scale of, say 3 sec for concentration difference measurement would give a 30-fold larger signal than a spatial device could achieve.

What function of concentration determines the magnitude of the chemotactic response? Under severe stimulation conditions, such that the stimulus was complete before the response, both Spudich and Koshland in a quantitative version of the original temporal gradient technique[197] and Berg and Tedesco with temporally stimulated tethered cells[200] found that the magnitude of the concentration change (Δc), and not the rate of change (dc/dt), was the factor determining response time. (This applies even in fairly shallow temporal gradients where the time interval to accomplish the concentration change approaches that of the response, according to an unpublished study by Macnab and Koshland.[279] The observation that, in spatial gradients and in temporal gradients too shallow to totally suppress tumbling,[199] run length is dependent on gradient steepness seems to point to a different conclusion. In an attempt to reconcile the two results, Spudich and Koshland state that, if response is measured as the integral of all tumbles suppressed as a consequence of stimulation, the dependence on Δc rather than dc/dt still holds. This statement, while plausible, has not been experimentally substantiated.

The functional dependence of response on concentration change must be determined at least in part by the characteristics of the concentration measuring device, which need not be linear. In fact, devices which have been identified so far in chemotaxis are all proteins which, of course, have nonlinear binding properties. It has now been demonstrated in several instances that chemotactic response has a Michaelis-Menten dependence on the magnitude of the concentration stimulus.[197,199,200,210] If response, e.g., accumulation in a capillary or adaptation time in a concentration jump experiment, is denoted by R and the concentration difference which is the stimulus by $\Delta c = c_2 - c_1$, then R is is found experimentally to be proportional to $c_2/(K + c_2) - c_1/(K + c_1)$, where K is an empirical constant. Where the comparison can be made, the "chemotaxis Michaelis-Menten constant" K is found to be in good agreement with the dissociation constant measured on the isolated receptor.[197,210]

B. Chemotactic Agents

1. Chemical Characteristics

An extensive survey of chemicals for their effectiveness as bacterial chemotactic agents for *E. coli* has been made by Adler and co-workers. Detailed tabulated data on the compounds surveyed can be found in References 148, 211, and 212. Almost all attractants are either sugars or amino acids. The sugars which are powerful attractants are all either *D*-pentoses or *D*-hexoses (ribose, galactose, glucose, mannose, and fructose) or compounds derived from them, such as amino sugars (*N*-acetylglucosamine), sugar alcohols (galactitol, sorbitol, and mannitol), phosphoryl sugars (glucose-1-phosphate), alkyl sugars (methylgalactoside and methylglucoside), and disaccharides (maltose and trehalose).[212] (Unlike man, *E. coli* is not tricked by saccharin or cyclamates.)

Eight of the common protein amino acids are attractants to *E. coli*: alanine, asparagine, aspartate, cysteine, glutamine, glycine, serine, and (less potent) methionine.[211] The corresponding *D*-amino acids are also fairly strong attractants, as are some α- and β-methyl derivatives. Removal or blocking of the amino group greatly reduces potency; the importance of this group is illustrated by the fact that ammonium ion itself is an attractant, utilizing the serine receptor.

In contrast to chemical signaling in higher systems, cyclic AMP and other nucleotides are ineffective as chemotactic agents for *E. coli*.[212] Although cyclic AMP is not an attractant, the possibility that it might participate in signal processing has not been ruled out. So far, however, the only role in chemotaxis that has been demonstrated is the rather drastic one of repression of flagellar synthesis when cyclic AMP levels are low.

Salmonella shows responses to many of the attractants mentioned above, such as ribose,[213] galactose, glucose,[214] aspartate,[195] and serine.[201] Citrate also is an attractant for *Salmonella* (Macnab and Koshland, unpublished observations).[280]

In *B. subtilis*, the attractants which have been studied in detail are amino acids. (Mannitol as an attractant is mentioned cursorily in Reference 215.) Van der Drift and de Jong[216] found that 16 of the 20 common amino acids are attractants, as defined by a measurable response to 10^{-4} *M* or lower, whereas Ordal and

Gibson,[217] as well as confirming the 16, discovered the remaining amino acids, arginine, lysine, aspartate, and glutamate, to be attractants also. The cause of this discrepancy is not clear, but, at any rate, both reports indicate a much more extensive set of responses to amino acids than were found for *E. coli* or *Salmonella*.

Sex-specific attraction of Hfr cells of *Salmonella* to F^- cells has been reported,[218] but the attractant molecular species is not identified.

In an extensive search, Tso and Adler[148] found that the following compounds are repellents for *E. coli*: short-chain fatty acids (acetate through hexanoate); short-chain unsaturated fatty acids; benzoate; leucine, isoleucine, and valine; indole and analogues; salicylate and thiosalicylate. In addition to these, a number of other compounds displayed mildly repellent characteristics.

Salmonella, unlike *E. coli*,[219] is repelled by phenol as well as by the fatty acids, the aliphatic amino acids, and indole.[198] In *B. subtilis*, a number of membrane-active compounds (ionophores, permeant anions, anesthetics) are operationally repellents,[220,221] but there are a number of reasons for believing that they do not operate through the conventional chemotactic machinery.

In these surveys of attractants and repellents, competition experiments frequently revealed that elevated uniform concentration of one compound, e.g., galactose, inhibited the response to a gradient of a second analogous compound, e.g., fucose. Thus, compounds could be divided into classes, and from this the concept of receptors has emerged. The establishment by Adler and his co-workers that specific sensory receptors exist was probably responsible, more than any other single factor, for generating interest in the subject of bacterial chemotaxis. The physical nature of receptors will be discussed in Section III.C.

Specificity of chemotactic agents for the receptors is comparable to substrate/enzyme specificities and seems to be less related to chemical reactivity than to steric factors, which is perhaps not surprising since the function of the receptors is simply to recognize chemicals, not to modify them. As illustrations of the degree of specificity, the taxis threshold of 2-deoxy-*D*-galactose is 10^{-3} *M*, 1000-fold higher than that of the primary substrate, galactose;[212] for β-methylaspartate and aspartate, the thresholds are 5×10^{-6} and 6×10^{-8} *M*, respectively.[211]

Why have positive or negative responses developed for certain compounds? Only a rough and somewhat unsatisfactory rationale can be presented. The advantage to a cell of migration to a sugar like glucose or an amino acid like aspartate is obvious. Yet many excellent carbon sources such as glycerol and many of the protein amino acids are not attractants. Perhaps a representative selection of substrates can normally act as an adequate signal that a biological source of nutrition is nearby. For example, aspartate is probably an indication of hydrolyzing protein and hence the availability of the other amino acids. The presence or absence of tactic responses may also reflect different characteristics of the corresponding transport systems, since it seems very likely, at least for attractants, that chemotaxis developed by adaptation of existing transport machinery. A satisfactory rationale for repellents is even harder to provide than it was for attractants. The fact that, with the exception of indole, they only operate at considerably higher concentrations than attractants (but still well below toxic levels) makes good sense. Repulsion of bacteria from trace quantities of leucine, for example, might literally mislead them away from a nutrient source. The potential adverse affect of known repellents is rather speculative.[198] Fatty acids are excretion products, so high concentrations might be an indication of overcrowding, in which case the chemotactic agent is signaling adverse conditions rather than constituting them. The aliphatic amino acids are subject to cross-inhibition of biosynthesis, which can result in reduced growth. Phenol is toxic — but why, since it is equally so to *E. coli* and *Salmonella*, does it only repel the latter? Also, why do other equally toxic materials, including ones which inhibit motility, not cause tactic responses? On surveying the teleology of chemotaxis in this way, one is tempted to question the validity of the traditional dictum that "nature knows best."

2. Mode of Action

By analogy with phototaxis and aerotaxis, one might suppose that chemotaxis is a consequence of some aspect of the metabolism of chemotactic agents. Adler and co-workers,

however, showed that tactic responses were not a consequence of metabolism,[6] from the following results:

1. Nonmetabolizable analogues, e.g., α-methylaspartate, of the primary substrate can be attractants.
2. Mutants unable to metabolize the substrate are still chemotactically competent.
3. Mutants which are chemotactically defective to a chemical can be capable of metabolizing it.
4. Many metabolizable compounds, e.g., glycerol and pyruvate, including metabolic products of attractants, e.g., glucose-6-phosphate, are not attractants.

In the case of attractant sugars, it has been shown that transport is not necessary either, although a connection between transport and chemotaxis does exist in that the chemoreceptors which have been identified so far are also elements of the transport systems for the chemical they recognize; there is, however, a separability of function, at least in the case of the galactose-binding protein (*mglB* gene product), since loss of function as a galactose chemoreceptor did not necessarily cause loss of galactose transport function or vice versa.[222,223] The role of attractant sugars is, therefore, confined to the act of being recognized by binding to a surface receptor. They do not have to be transported or metabolized to provide the stimulus, although of course both of these processes do normally accompany recognition.

The data on amino acids allow one to conclude, as was done for sugars, that specific recognition does take place, but that metabolism need not. However, there is no information available which proves that transport is unnecessary or even that the chemoreceptor is at the cell surface (see Section III.C).

C. Initial Signal Processing — The Chemoreceptors

The concept of chemoreceptors[6] derives primarily from the competition studies (described in Section III.B),;, which clearly indicate the existence of specific recognition devices. In some instances, these have been shown to be proteins at the cell surface. However, such isolation and characterization of the receptors is still confined to just two types, the periplasmic and phosphotransferase sugar-binding proteins.

Sugar receptors for galactose, ribose, and maltose* are inducible periplasmic binding proteins, loosely associated with the outer surface of the cytoplasmic membrane and contained by the cell wall.[213,214,224-226] They can be released by osmotic shock,** the number of copies released per cell ranging from 10^3 to 6×10^4, provided the system has been fully induced. They have molecular weights in the range of 30,000 to 40,000 dalton, are soluble as monomers in aqueous buffers, and are heat stable. The amino acid composition is unremarkable except for the lack of cysteine. Initial reports[227] that the galactose-binding protein of *E. coli* has two binding sites for galactose — a feature which could have had mechanistic implications with regard to a temporal comparison — have now been shown to be incorrect.[228] The galactose-[214] and ribose-[213] binding proteins of *Salmonella* also have only a single site. Dissociation constants of periplasmic receptors are in the range of 10^{-7} to 10^{-6} *M*. Affinities for the primary substrate and various analogues are in agreement with their relative effectiveness as chemotactic agents. This, together with the fact that mutants lacking the binding protein lack the corresponding tactic response, is the basis of their identification as receptors in the following systems: galactose/*E. coli*,[222,223,229,230] galactose/*Salmonella*,[214] ribose/*E. coli*,[225] ribose/*Salmonella*,[213,231] and maltose/*E. coli*.[232] The receptor proteins were also found to be components of the transport systems for the corresponding substrate, as was mentioned earlier.

The other class of chemoreceptor which has been described is the sugar-specific enzyme IIA

* In addition to the *malE* gene product, which is the periplasmic maltose-binding protein and the true chemoreceptor, the *lamB* gene product — an outer membrane receptor for phage λ — also affects maltose taxis because it participates in periplasmic accumulation of maltose.[232]

** One report exists[229] of recovery of tactic competence by combining shocked and therefore chemotactically incompetent cells with concentrated shock fluid, but since difficulty has been experienced in repeating this result, it should be regarded with caution.

moiety of the phosphotransferase system.[233] The protein is a hydrophilic, but membrane-bound, component of the transport system; its function appears to be the recognition of substrate prior to its phosphorylation and transport. Specific enzymes for glucose, fructose, mannose,[233] and three hexitols[234] have been described and correlated with tactic responses. The separability of function in taxis and transport is less clear-cut than in the case of the periplasmic proteins described above. Mutants defective in a given enzyme IIA protein, e.g., mannose, are defective in mannose transport and taxis. So far, however, no mutants comparable to the *mglB* mutant described above have been found, in which the lesion affects only one function; also, mutants lacking nonspecific components of the transport system (the HPr and enzyme I proteins) are chemotactically defective. However, it is likely that the latter result reflects the need for the enzyme II to be in association with a stable complex of proteins in order to function properly. A similar situation exists in the galactose transport system. The *mglA* and *mglC* genes of the galactose transport system are not the chemoreceptor genes but can affect galactose taxis.[223] A strong indication that transport and metabolism are not required for taxis of the phosphotransferase system sugars is the fact that the phosphorylated forms, e.g., glucose-6-phosphate, which are actually transported by the system are poor attractants.

No receptor for an amino acid attractant or for any repellent has been characterized (specific taxis mutants have been found in some cases, without identification of the corresponding receptor),[12,148,211] and various known binding activities fail to correlate with tactic activities.[148,235] It remains to be seen whether the receptors for substrates other than sugars have similar properties to those described above. There are, in fact, several major differences between sugar taxis and amino acid taxis, which suggest that the receptors may operate differently:

1. Responses to amino acids are much stronger.[210]
2. Serine can affect steady-state motility.[170]
3. It is effective as an attractant over a very wide concentration range.[201]
4. It shares a signaling component with repellents.[148,236-236b]
5. Aspartate taxis mutants map in a che gene,[12,211,236a,236b] whereas sugar taxis mutants map in the corresponding transport system loci.[213,222,232-234]
6. No transport-defective serine or aspartate taxis mutants are known.[211]

Whether these features of amino acid taxis reflect differences at the receptor level or in subsequent signal processing is still unclear.

The occupancy status of a chemoreceptor must be communicated in some way to subsequent elements in the chemotactic machinery, and, as metabolism of the chemotactic agent has been shown to be unnecessary, one is left with a change in the receptor itself as the means of conveying the information. Because the periplasmic binding proteins are so easily released from cells, there is good reason to believe their properties in vitro will be essentially the same as they would be in the intact cell; isolated, purified periplasmic binding proteins, therefore, provide the most suitable system for studying receptor changes upon binding. The significance of any such changes is, of course, dependent on where they are occurring in the protein; a change observed at the substrate binding site may not in itself be the information conveyed to a subsequent element — it is more probable,indeed, that a conformational change at an allosteric site is utilized.

An extensive examination of the *E. coli* galactose-binding protein has been carried out by Boos and co-workers. (See References 237 and 238 for summaries of this work.) Changes in electrophoretic mobility occur upon substrate binding, and this is interpreted as a result of alteration of surface charge. Tryptophan absorption and fluorescence are altered also. In the latter case, glucose and galactose effect slightly different changes; since the effects are not additive, they cannot be ascribed to two different binding sites, and from this observation, the authors conclude that they reflect slightly different consequences of binding at the same site. This makes it likely, but not certain, that the tryptophan residue which is responsible is at the binding site, on the hypothesis that conformational change remote from the binding

site would probably not reflect subtle details of the initiating event.

Direct evidence for an allosteric conformational change comes from work by Zukin et al.[239] with the corresponding galactose-binding protein from *Salmonella*; it has only one tryptophan residue, whose fluorescence is altered by galactose in a similar manner to that observed in *E. coli.* A second fluorophor (5-iodoacetamidofluorescein) was covalently attached, and it was also found to be sensitive to galactose binding. Next, fluorescence of the extrinsic dye upon excitation of the intrinsic tryptophan was measured; the results, treated by the energy transfer theory of Forster, yielded a minimum distance of 3 nm between the two chromophores, clearly showing that both cannot be near the binding site and, therefore, that long-range conformational change must be responsible for the spectral shifts of at least one of them. The significance of these results in terms of an allosteric site actually used in the chemotactic apparatus awaits the identification of the other component to which the receptor presumably binds.

D. Intermediate Processing

A reasonable hypothesis regarding the receptor changes described at the end of the last section would be that the receptor transmits information regarding its conformation by direct association with another macromolecular component in the membrane. No direct evidence has yet been obtained for other cellular components in physical association with the chemoreceptor, but certain examples of competition and of mutants displaying semispecific chemotactic deficiencies cannot be explained at the level of the primary chemoreceptor. Both *E. coli* and *Salmonella* have different receptors for ribose and galactose, yet high uniform concentration of one substrate reduces or eliminates tactic responses to the other.[212,214] A mutant lacking the ribose-binding protein failed to inhibit galactose taxis, thus eliminating the possibility that ribose interacts directly with the galactose-binding protein. (It is interesting historically to note that the original, correct description of separate receptors for galactose and ribose might not have been made except for the fact that assays were tried on cultures induced only for the substrate under test, so that the competing receptor was absent.) Similar effects have been observed between the sugar-specific enzyme IIA receptors of the phosphotransferase system[212,234] and among many amino acids in *B. subtilis* (although the receptors are not known in this case).[240] The pattern of inhibition of amino acid taxis in *B. subtilis* is exceedingly complex; an unexpected feature is the lack of reciprocity in many cases. As a result of the discovery of a gene *(trg)*[222,223] which affects ribose and galactose taxis but not taxis generally, it seems safe to conclude that competition is occurring at that level rather than as a result of direct interactions between the receptors themselves. Strange and Koshland[214] assume that the primary receptors bind to the *trg* product, and that the latter is in the membrane. However, the *trg* product has not been isolated, nor is its location in the cell known, and, therefore, possibilities such as cytoplasmic galactose- and ribose-specific signals interacting with a soluble protein cannot be ruled out.

If a direct interaction is taking place, what is its nature? One possibility would be that a stable complex of the galactose and ribose binding proteins with the *trg* product exists and that conformation information regarding state of occupancy is transmitted within this complex, although stoichiometric objections to this model can be raised because of the inducible character of the receptors. An alternative model has been described[214] in which galactose and ribose receptors, when occupied, compete for the same site on the *trg* protein. A rare type of mutant observed in *E. coli*[223] may be pertinent to this question; this type of mutant has normal ribose taxis and defective galactose taxis but maps in the *trg* gene. This suggests either that different binding sites for the galactose and ribose receptors exist on the *trg* protein or that a single site exists which is more critical in its requirements for binding the galactose-binding protein. Isolation of the *trg* product, estimation of its abundance in the cell, and study of its interactions with the receptors would all clearly be useful, as would be a better general characterization of periplasmic binding proteins with regard to their mobility and degree of association with the cytoplasmic membrane. Assuming that the *trg* protein is not in physical association with the transport systems (it is hard to see how it could be so to both the

galactose and ribose systems simultaneously), then the *trg* protein ought to be in competition with the transport complex for binding of substrate-bound receptor. This competition would seem strange from a teleological point of view; also, it should result in elevated transport rates in *trg* mutants, yet these are observed to be normal. Perhaps the *trg* protein does compete, but to an extent which makes negligible impact on the transport system.

Other examples of a shared component are the *tsr* and *tar* gene products[148,236-236b] (see also section III.E.2). Lesions in the *tsr* gene abolish responses to a variety of repellents and also to the attractant serine, whose unusual characteristics were noted in Section III.B. The *tsr* product must have a close association with the central mechanism of chemotaxis and therefore presumably be further down the "information chain" than the *trg* product, since some *tsr* mutants (also described as *che*) exhibit general chemotactic deficiency.[20,241,242] In these mutants, because the phenotype is one of smooth swimming, chemotactic responses of any sort are impossible. *Tar*$^-$ mutants are defective in aspartate, maltose, and certain repellent tactic responses. A more extensive discussion of the function of the *tsr* and *tar* gene products is given in Section III. E. 2. The sharing of a signaling component by two receptor classes means that low-concentration information from one class will be ignored in the presence of high-concentration information from the other. While this seems a useful device for two chemically similar nutrients such as galactose and ribose, it does not seem at all so for say, serine and repellents.

E. Final Processing

We consider now the point at which information from all receptors funnels into a central system to generate the signal which determines the sense of rotation of the motor. The conclusion that simultaneous access to a common signaling system is possible is based on the findings of Tsang et al.[198] and of Adler and Tso[243] that, if more than one stimulus is given to a bacterial population simultaneously, the response is approximately the algebraic sum of the responses to the stimuli given individually; these results were later demonstrated in a more quantitative manner by Spudich and Koshland[197] and by Berg and Tedesco.[200]

1. General Chemotaxis Mutants

Numerous mutants specifically defective to particular chemotactic agents have been isolated (see Section III.C), and, where the chemoreceptor is known, it is found to be the site of the defect. The tumbling frequency of such mutants is normal, i.e., the defect exists only in the sensory mechanism and is not transmitted to the motor apparatus. There are also examples of defects in secondary (semispecific) components, as was described in Section III.D. A third type of chemotaxis mutant, first described by Armstrong et al.,[244] is characterized by an inability to give migratory responses to any physiologically normal chemotactic stimulus; such mutants have been termed general chemotaxis mutants and given the symbol *che*.

Che mutants have normal flagellation and are motile, but careful examination has, in all cases so far, revealed that their motility is not normal. Tumbling is either rare (smooth-swimming mutants) or unusually frequent (tumbling mutants). For example, of 172 independent mutants isolated by Parkinson, 146 were smooth, and 26 tumbling;[20] similarly, Aswad and Koshland[274] isolated 72 *che* mutants, 68 of which were smooth swimming and 4 of which were tumbling. That mutants with these phenotypes are chemotactically defective is not surprising, because taxis proceeds by selective modulation of tumbling, and modulation of a parameter requires a suitably poised value in the absence of the modulating stimulus. What is interesting, though, is that they are the only types of *che* mutants which have been found. (Null mutants in the *cheD* gene are a special case; they do have wild-type motility, but are of *tsr*, not *che*, phenotype [see below].) This is not a consequence of the selection methods. In Parkinson's protocol, for example, a mutagenized culture is incubated at high dilution in a semisolid agar plate. Wild-type cells give rise to large colonies because they migrate rapidly outward in response to self-generated gradients; nonmotile cells give very compact colonies; and *che* mutants, whether smooth or tumbling, give rise to colonies of intermediate size, because, although motile, they move randomly, not selectively, outward. If *che* mutants with wild-type tumbling frequency existed, they would also be expected to give intermediate-size colonies. Aswad and Koshland's liquid gradient selection technique[274] likewise should have been able to

detect *che* mutants possessing normal motility.

The failure to find such mutants indicates that there is no protein in the chemotaxis apparatus which receives information from all receptor systems and transmits it to the tumble regulation system but which is not itself a part of the tumble regulation. A remote possibility is that such products do exist, but for some reason defects in them are lethal.

At least nine *che* genes exist in both *E. coli*[53,106,242] and *Salmonella*,[116, 117] including *fla* genes capable of yielding *che* phenotype. The correspondence between genes in the two species has not yet been established, and, unless otherwise indicated, the genes below refer to *E. coli*. No examples of null mutants have been found to have the tumbling phenotype,[242] suggesting that the basic mode of the motor is counterclockwise, with additional features being necessary to switch it to the clockwise mode.

***CheA* and *W* genes** — Mutants in the *E. coli* *cheA* (old)* genes are all of smooth phenotype, so that these genes seem to enable tumbling but not to control it. The genes are cotranscribed with the genes which enable motility in the first place — *motA, B, cheA*, and *W* constitute the *mocha* operon recently described by Silverman and Simon.[109] Another interesting feature of the *cheA* (new) gene is that it codes for two polypeptides, a phenomenon which may result from processing a single polypeptide or from transcription from different initiation sites within the same gene. A similar phenomenon exists in *cheM* (see Section III.E.2).

***CheX, B, Y*, and *Z* genes** — *CheB* (old) genes also enable tumbling (the null phenotype being smooth), but, since examples of tumbling mutants have been found to map in these genes, we may assume they control tumbling also. A further comment may be made about the role of *cheB* in tumble regulation. One assumption would be that *cheB* determines the steady state level of tumbling but that the chemotactic signaling system perturbs that level without going through the *cheB* product. However, because different tumbling *cheB* mutants have been found to display different responses to severe stimulation with aspartate and serine, it seems more likely that the chemotaxis signals eventually feed through the *cheB* product, in which case it serves not only to regulate tumbling but to modify the regulation according to environmental stimulation.

***CheC* gene** — Whether *cheC* enables tumbling or controls it cannot be judged from the fact that the small number (three) of motile *cheC* mutants which have been isolated are smooth; the *cheC* gene product is an example, analogous to ones in *Salmonella*, of a component which is so intimately associated with the motor apparatus that null mutants cannot assemble flagella (*flaA* mutants).

***CheD* gene** — The *cheD* gene is a special case (see also III.E.2). It is not needed for tumbling or for its control (the null phenotype has a normal tumbling frequency) , but it serves to relay information regarding serine and repellents to the regulating system. It must, on the other hand, be in intimate association with the regulating system, because some *cheD* defects totally block tumbling and hence chemotaxis.[241] In other words, if a normal *cheD* gene product is present, the whole of the chemotaxis apparatus works normally; if the *cheD* gene product is missing entirely, the chemotaxis apparatus works normally for all agents except serine and repellents; and if a faulty *cheD* product is incorporated, the apparatus does not work at all. There are several pieces of evidence to suggest that the *cheD* product interacts with the *cheB* product, perhaps the clearest being the fact that some *cheD* suppressor mutations map in the *cheB* gene.[241] Parkinson[241] has drawn up a schematic model of data processing based on present knowledge regarding the *cheA* to D genes. A number of *che* genes which have been characterized recently participate in a reversible methylation reaction. Knowledge of this topic is developing rapidly at the present time and is sufficiently detailed to merit consideration in a separate section.

2. The Chemotaxis Methylation System

For many years, it has been known that methionine, though a mediocre attractant, plays a crucial general role in chemotaxis.[215,246,247] Cells unable to synthesize methionine, whether because they are auxotrophic for methionine or because biosynthesis has been repressed, swim

* Until recently, only four *che* complementation groups were described, *cheA, B, C*,[53] and *cheD*.[242] Two of these have now been further resolved: *cheA* (old) is now known to consist of two genes (*cheA* and *W*) and *cheB* (old) of four genes (*cheX, B, Y*, and *Z*).[106] A reconsideration of observations on mutants in old genes *cheA* and *cheB* is needed.

smoothly unless exogeneous methionine is supplied at around 10^{-6} *M* or higher. The effects are reversible on a time scale of minutes. The methionine is required for the synthesis of *S*-adenosylmethionine, using ATP as the adenosyl donor.[248-250]

To locate the target of the presumptive methylation reaction for which *S*-adenosylmethionine is the substrate, Kort et al.[251] pulse-labeled *E. coli* cells with [methyl-^{3}H] methionine and, after fractionation, identified on electrophoretic gels a labeled component in the cytoplasmic membrane fraction. Because of correlations with chemotaxis to be described below and because only the methyl group of methionine is incorporated, the protein has been termed "methyl-accepting chemotaxis protein" (MCP). It was found to have a molecular weight of 62,000 dalton and to exist in at least 700 copies per cell.

Whether the protein is specific to regulation of chemotaxis or fulfills any more general cellular role is not known. The implication of the protein in chemotaxis was based on several lines of evidence:

1. General chemotaxis mutants (*che*$^-$) had abnormal methylation levels, and reversion to wild-type tactic behavior was accompanied by reversion to normal methylation levels.
2. Chemotactic stimulation caused an increase in methylation levels.
3. Methylation levels in various *fla* mutants were extremely low.

The results from the mutants were too complex to be interpreted. In particular, none of the mutations seemed to be in genes central to the methylation process, since the levels of methylation bore no systematic relationship to phenotype, even when occurring in the same gene. For example, two different *cheB* mutants, both smooth swimming, had methylation levels of 0 and 2.7 times the wild-type level, respectively. This, however, applies to the old categorization of *che* mutants in *E. coli* — perhaps a more comprehensible pattern may emerge now that additional genes have been recognized (Section III.E.1). The significance of levels above normal is questionable, considering the reproducibility of the assay.

The low methylation levels in *fla* mutants indicates that flagellar assembly is required for a methylation reaction, but this does not necessarily imply that MCP is a component of the flagellar structure. In fact, its electrophoretic mobility does not match that of any of the polypeptides in the basal body.[110] Its abundance in the cell is also inconsistent with a structural role in the flagellum.

It is now clear that there is not just one MCP. W. Springer and Koshland[245] found two MCP proteins in *Salmonella,* with molecular weights of 63,000 and 65,000 dalton respectively. Silverman and Simon[106] resolved the *E. coli* MCP peak into three bands (molecular weights of 60,000, 61,000, and 63,000 dalton). With improved gel resolution, it now appears that there may be as many as 3 genes and 11 MCP products from them. Thus, M. Springer et al.[236a] describe 2 *E. coli* genes, *tsr* which codes for 6 MCPs (collectively called MCP I) in the molecular weight range of 56,000 to 65,000 dalton, and *tar* which codes for another four (MCP II). Silverman and Simon[236b] find 3 *E. coli* genes, *cheD* (which they regard as identical to *tsr*) coding for at least two proteins (MCP I), *cheM (tar)* coding for 3 proteins (MCPII), and a third gene, *cheZ,* coding for a smaller, 24,000-dalton protein (MCP III), which, unlike the others, is cytoplasmic rather than membrane bound. Methylation of MCP I occurs in response to stimulation by the attractant α-aminoisobutyrate (a serine analogue) and the repellents acetate, benzoate, indole, and leucine, whereas methylation of MCP II occurs in response to stimulation by the attractants aspartate and maltose and the repellents Ni(II) and Co(II). Serine itself and ribose and galactose cause methylation of both MCP I and II.[236a] These findings are in agreement with the specificities of chemotactic behavioral lesions in *tsr*$^-$ and *tar*$^-$ mutants. *Tsr/tar* double mutants are totally chemotactically defective. It is interesting that strains lacking MCP I show enhanced methylation of MCP II and vice versa, suggesting an interlocking regulation.

From these results, it appears that chemotactic signals flow through the methylation reactions of MCP I, MCP II, or both. The role of MCP III is still unknown.

Variation of the extent of methylation occurs among bands within, e.g., MCP I. Quantitative information on this aspect is not yet available,

but it is tempting to suggest that differential extent of methylation could be a device for gradient inference. Several different genes seem to be required for methylation to proceed. Thus, *cheX* and *cheW* are both needed for methylation of either MCP I or II,[236b] and *cheB* also affects methylation levels.[236a]

The enzyme responsible for methylation of MCP, termed the "chemotaxis methyl transferase" (CMT), has recently been identified in *Salmonella* by W. Springer and Koshland[245] and found to map in the *Salmonella cheR* gene. (The corresponding *E. coli* gene is probably *cheX*.[281] The CMT enzyme was demonstrated to be cytoplasmic because, in an in vitro assay, cytoplasmic membranes from wild-type and *cheR* mutant cells were both methylated to similar levels by wild-type cytoplasm, whereas wild-type cytoplasmic membrane was poorly methylated by cytoplasm from the *cheR* mutant. The enzyme was purified and found to have a molecular weight of 38,000 dalton. The enzyme is quite substrate specific — it does not appreciably methylate a variety of proteins or membranes which were tested, but, interestingly, homology is sufficient to permit methylation of *E. coli* MCP under the control of *Salmonella* CMT. The site of methylation within the substrate is also specific; the methylated form of MCP contains a γ-glutamyl ester.[252,253]

There is currently disagreement as to whether the methylation system is an absolute requirement for tumbling or whether it is a regulatory input only; also, it is not clear whether the chemotaxis input to tumble regulation operates directly via the methylation system or in some more complex manner.

Because *cheX* mutants, which are unable to methylate MCP, are capable of smooth and tumbling responses, but not of terminating them, it is assumed that methylation participates in the adaptation process only, not in the initial excitation.[253a]

The effects of methionine starvation on tumbling are highly dependent on phenotype. Some tumbling mutants of both *Salmonella* and *E. coli* respond like wild-type cells by becoming smooth swimming, whereas others continue to tumble.[247,254] Methionine does have an effect on the tumbling capability of some of the latter strains, however, since their smooth response to positive concentration jumps of attractant is extended. One of the mutants which continued to tumble in the absence of methionine could be converted to smooth-swimming phenotype by the presence of constant levels of attractant. Wild-type cells also reflect the same phenomenon by converting more rapidly to smooth swimming upon starvation in the presence of attractant, suggesting perhaps that low levels of endogeneous methionine from protein breakdown, normally sufficient to maintain methylation and tumbling, were being depleted as a consequence of the attractant.

The finding is an important one, because it is the first evidence of a dynamic process relating to chemotaxis and yet occurring under nongradient conditions; there is no paradox involved, because although the tumbling level, as well as the parameter determining it, is constant, this does not necessarily imply that the system is at equilibrium, only that it is in a steady state. Indeed, an equilibrium device will not work. Consider this fact in the context of the model originally proposed by Macnab and Koshland.[195] If we have $W \rightarrow X \rightarrow Y$, where X is the tumble regulator and the reaction rates are controlled by attractant, there is a flow of reactants through the system even though the pool size of X may be constant. The only way to avoid such a flow would be to have $W \rightleftharpoons X$, in which case the pool size of X will be constant at equilibrium. However, if the same enzyme were mediating both forward and reverse reactions, no shift of equilibrium and therefore no signal could be achieved by a chemotactic stimulus. We can invoke separate enzymatic paths, but it is contrary to the second law of thermodynamics to have a cyclic flow $W \circlearrowright X$ which leaves no net change in the system as a whole. Therefore, something must be flowing through the gradient-sensing device even under nongradient conditions. Segel[255] has devised an equilibrium mechanism which is thermodynamically sound but which, since it depends on a shift in equilibrium as a function of attractant concentration, is incompatible with the experimental observation that tumbling frequency is independent of attractant concentration.

One mutant resisted all attempts at methionine starvation but, like the rest, became smooth swimming upon arsenate treatment. (Arsenate depletes ATP levels, and therefore

presumably prevents *S*-adenosylmethionine formation and MCP methylation.) One explanation of all these results would be that steady state methylation is required for tumbling, but quite drastic measures are necessary in some strains to overcome endogeneous methionine supply. Alternatively, one could conclude that the methylation reaction is not absolutely required for tumbling and that the arsenate effect is a manifestation of some other role of ATP in motility. To complicate matters further, phenol is found to cause tumbling in arsenate-treated cells and in *Salmonella cheR* (methyl transferase) mutants.[245] This strongly suggests that, at least in the case of repellents, the methylation system can be totally bypassed by a sufficiently strong chemotactic stimulus.

M. Springer et al.[256] found that *E. coli* requires methionine only for the process of adaptation to a new (higher) attractant concentration, not for maintenance of the adapted state or deadaptation to the original state. The result that maintenance does not require methionine would seem to contradict the observations that constant attractant levels cause rapid methionine utilization;[254] methionine starvation should then result in net demethylation and collapse of the adapted state, which behaviorally it does not. Therefore, the authors make an additional assumption that demethylation aspects of methyl turnover also require the presence of methionine, presumably in a regulatory role. It should be emphasized that the experiments just described are behavioral. The model put forward to explain the results shows steady state methylation levels increasing with steady state attractant concentration. This is in conflict with the authors' earlier experimental data, where only transient increase in methylation levels was observed to occur in response to stimulation with attractant, with the level after adaptation falling to the prestimulus level. This drop was subsequently shown[251a] to stem from the use of modest amounts of metabolizable attractants. Nonmetabolizable attractants or metabolizable attractants at sufficiently high concentrations result in maintenance of the new methylation level. An overall conclusion, especially clear from the results of Kort et al. with mutants,[251] must be that the level of methylation of MCP is not the sole determinant of tumbling frequency. In fact, either stimulation of methylation levels by attractant jump or depression of them by methionine starvation can have the same effect, namely, suppression of tumbling. Therefore, although the methylation system plays an essential role in chemotaxis, the methylation level of MCP cannot be the final signal to the motor.

Several models have been proposed for the role of the methylation reaction in gradient sensing. Aswad and Koshland[250] suggested that *S*-adenosylmethionine was needed for the degradation of a tumble inhibitor. In the version of M. Springer et al.,[254] the methylation reaction is on the sole metabolic pathway to the tumble-controlling compound (a tumble enabler). Frere[257] places it on the sole pathway from the tumble-controlling compound (a tumble inhibitor). W. Springer and Koshland[245] place the methylation reaction in a regulatory role over the enzymes mediating the metabolic pathway of the tumble-controlling compound (a tumble inhibitor). These models are, of course, still quite speculative, and objections can be raised against them. With as many as 11 different proteins undergoing methylation, a quite complicated scheme may be necessary. In this regard, it is perhaps necessary to emphasize that there is nothing sacrosanct about the linear W → X → Y format, which was originally used by Macnab and Koshland[195] solely as an illustration of the logical features of gradient sensing. Multiple pathways to and from X may very well exist in reality.

3. Gradient Inference

Given a multiplicity of receptors, a motor response to their changing occupancy, and an undetermined number of intermediate steps of data processing, we are faced with the question as to where in the whole scheme gradient inference (memory, temporal comparison) takes place. Does the information that is relayed to the tumble-generating mechanism by the receptors, either alone or in association with other specific components, describe occupancy or temporal change in occupancy? The observation that response times to multiple stimuli are additive, rather than reflecting simply the longest component, has been used to suggest that the mechanism of gradient inference must be central.[12] This seems unwarranted. Additivity of responses simply implies additivity of stimuli, but it does not discriminate between additivity before and after gradient inference. In

crude terms, $d(c_1 + c_2)/dt$ is the same as $dc_1/dt + dc_2/dt$. A nonadditive mechanism seems inherently implausible, anyway. Thousands of copies of a given specific receptor, e.g., the galactose receptor, exist per cell, and there is nothing to suggest that they operate as a coordinated system. The signal from any one receptor molecule is presumably very small and therefore must be added to signals from the other receptor molecules; additivity of signals from all galactose receptors and additivity of signals from galactose receptors and other receptors are thus seen to be the same process.

Inhibition of tactic responses to ribose by galactose or vice versa occurs at constant concentration of the inhibiting species,[212,214] and, therefore, the information which the receptors convey to the *trg* protein must concern their state of occupancy, not how it is changing. It should be noted, however, that this result does not preclude the possibility that the receptor/*trg* protein complex might be capable of generating gradient information.

F. The Signal to the Motor

It is well established that chemotactic signals cause cell migration by modulating the natural frequency of motor reversals. What is known about how modulation is effected? Before considering this issue in the context of chemotactic signals, it will be useful to review a number of other ways in which the reversibility of the motor can be modulated. With the exception of methionine starvation (discussed in Section III.E.2), these can all be related to a theme of energy perturbation in the cell.

1. Energy Perturbation as a Signal

Aerotaxis — Taxis to oxygen is extremely common among aerobic bacteria and has been recognized since the late 19th century. Increased oxygen concentration suppresses tumbling, while decreased oxygen concentration enhances it; oxygen participates in respiration and hence the production of protonmotive force and ATP. Other electron acceptors such as fumarate and nitrate also elicit tactic responses.[258]

Phototaxis — Photosynthetic bacteria undergo reversals with high frequency when subjected to sudden decreases in light intensity.[259] Harayama and Iino[260] showed that a transient decrease in membrane potential, as measured by the voltage-dependent absorption spectrum of endogeneous carotenoids, occurs in *Rhodospirillum rubrum* under these conditions. Electron transport is involved, since antimycin was found to inhibit both light-induced reversals and the transient absorption shift, but not motility; both inhibitions could be relieved by phenazinemethosulfate. Valinomycin plus potassium had the same effect as antimycin.

Proton ionophores — Addition of moderate concentrations of a proton ionophore such as *m*-chlorocarbonylcyanide phenylhydrazone (CCCP) causes transient tumbling.[261,262] The protonmotive force across the cell membrane is, of course, diminished by such ionophores.

High-intensity blue light — Rather surprisingly, nonphotosynthetic bacteria like *Salmonella* display motility responses to visible light.[33] Although the intensities required are far above those which would be experienced naturally, the phenomenon provides some useful insight into the mechanism of bacterial motility. The response to a brief high-intensity pulse is a marked increase in tumbling frequency, followed by a relaxation to normal behavior. Strong positive chemotactic stimuli override the tumbling response, suggesting a common mechanism of interaction with the motor. The action spectrum, as yet a very crude one, shows that light between 350 and 450 nm is most effective and that light beyond 530 nm is totally ineffective. Of the chromophores present in the bacterial cell, only flavoproteins are compatible with these data. The effect is intrinsic,[263] rather than being a photodynamic response to extrinsic dyes of the sort first noticed by Raab.[264] Although the exact consequences of flavin excitation are unclear, it seems highly likely that energy stemming from electron transport will be perturbed thereby. More prolonged illumination causes smooth swimming and, finally, paralysis.[263]

Divalent cations — Magnesium, calcium, manganese, and zinc ions all act as attractants to *Salmonella* and *E. coli*.[265] They are not effective in mutants lacking the membrane-bound ATPase, which is a necessary link in oxidative phosphorylation, or in cells in which the ATPase has been inhibited by *N,N*^{1}-dicyclohexylcarbodiimide (DCCD). In *B. subtilis*, depletion of Mg^{++} by chelation in the presence of the ionophore A23187 (specific for divalent cat-

ions) causes permanent tumbling, suggesting a specific requirement for Mg^{++} as well as the general phenomenon of energy perturbation.[166] In a wild-type cell, a sudden increase in Mg^{++} would be expected to shift the ADP/ATP equilibrium and perturb the energetic state of the cell.[266]

Applied electric fields — *Spirillum volutans*, a large bipolarly flagellated bacterium, can be stimulated to reverse by the sudden application or cancellation of electric fields.[267] Such field changes would be expected to operate primarily at the region of highest electrical resistance, namely, the cell membrane.

These observations on aerotaxis, phototaxis, protein ionophores, high-intensity blue light, divalent cations, and applied electric fields have suggested to a number of workers [7,17,33,221,245,261,268,269] that energy perturbation and, more particularly, perturbation of membrane potential, modify tumbling, although it should be noted that, in most instances, membrane potential was not actually measured. A reasonable hypothesis would be that any stimulus which suddenly decreases membrane potential will cause tumbling and any stimulus which suddenly increases membrane potential will suppress tumbling. This is similar to the Links-Clayton hypothesis for the phototactic and aerotactic responses of photosynthetic bacteria.[259]

2. The Chemotactic Signal to the Motor

Returning now to stimulation by attractants or repellents, where neither transport nor metabolism need occur, a central question arises: Do chemotactic stimuli operate by perturbing the energy state of the cell, or do they operate by a different mechanism, but one whose effect on the flagella is the same as that achieved by energy perturbation?

This question has been addressed by several groups recently, but the answers obtained appear to be in conflict. Szmelcman and Adler[268] studied the membrane potential of cells as a function of chemotactic stimulation. For measurement of membrane potential, they used tritiated triphenylmethylphosphonium ion ($TPMP^+$) which, being permeant to membranes, distributes itself at equilibrium, according to the Nernst equation. After equilibration of $TPMP^+$, it was found that the sudden addition of an attractant caused a rapid uptake (Phase A) followed by a release (Phase B) to below prestimulus levels, then a slow uptake (Phase C) before final relaxation to the prestimulus equilibrium value. Assuming that these changes are true indications of membrane potential, the results could be described as

1. Phase A, a fast hyperpolarization on a time scale of 10 sec or less
2. Phase B, depolarization to a minimum value at about 30 sec
3. Phase C, a hyperpolarizing maximum at about 3 min

On the basis of measurements with nonattractants and specific chemotaxis mutants and of the effect of methionine starvation, Phase A was linked to chemotaxis; mutants with general chemotaxis defects, nevertheless gave this phase, but *fla* and *mot* mutants did not. Phases B and C were linked, as a result of experiments with mutants and substrate analogues, to transport and metabolism, respectively. (The polarity changes can be rationalized in terms of a sudden energy drain for transport and an energy boost from metabolism.) Repellents, as well as attractants, were found to give an initial hyperpolarization. Since chemotactically they exert opposite effects, the production of a similar effect on membrane potential is surprising. The authors suggest that the ionic species responsible for the potential change may be different in the two cases and that the motor reversal mechanism is capable of distinguishing between them. The failure of *mot* mutants to give Phase A could be explained if the *mot* product was a gate responsible for ion fluxes that are necessary both as a power source and as a control of sense of rotation. The more obvious explanation of the *mot* (and *fla*) results — namely, that potential changes are a consequence, not a cause, of reversals (and reversals obviously cannot take place in the absence of motor function) — would seem to be ruled out by the fact that *che* mutants gave the hyperpolarization response but not the behavioral response. The requirement for methionine for Phase A is construed as a control of ion flux by the methylation system.

Miller and Koshland[262] used $TPMP^+$ and the voltage-sensitive fluorescence of a cyanine dye

to measure the state of the membrane of *B. subtilis* under a variety of stimulus conditions, with results which differ from those just described for *E. coli*. The effects on membrane potential, as measured by dye fluorescence, of oxygen depletion and restoration, of CCCP, and of valinomycin at a variety of external potassium levels, were all as expected; also, motility patterns conformed to the concept, outlined above, of energy perturbation as a regulatory input to the motor. Addition of the attractant alanine, on the other hand, had no effect on fluorescence, and if alanine and CCCP were added simultaneously at appropriate concentrations, a smooth-swimming response and membrane depolarization could be observed simultaneously. From these observations, the authors reject transient perturbation of membrane potential as the mechanism by which chemotactic signals are expressed. Minor changes in cellular $TPMP^+$ levels upon addition of attractant or even buffer were noted, but these changes did not match, in either magnitude or sign, the changes found in the *E. coli* experiments. Miller and Koshland point out that $TPMP^+$ is a poor indicator of rapid potential changes and that the extremely high cell densities required for the $TPMP^+$ technique (approximately 10^{10}/ml) render it prone to oxygen artifacts. Although both these criticisms are valid, they cannot explain why the effects observed by Szmelcman and Adler occurred only upon chemotactic stimulation.

Manson et al.,[146] in the course of their experiments with *Streptococcus faecalis* which demonstrated protonmotive force as the energy source for motility (Section II.B), found that the attractant leucine was effective in producing smooth swimming during the motility burst induced by valinomycin or gramicidin D and, noting that the membrane potential should be clamped under these conditions, concluded that it would be surprising if membrane potential was an essential feature of chemotactic signaling.

It is to be hoped that these discrepancies in the current literature will be resolved shortly. If membrane potential proves not to be the chemotactic signal to the motor, a chemical transmitter becomes a prime candidate, but, aside from a role of methylation reactions somewhere in the scheme, there is little information regarding its identity. Ordal[269a] has recently reported that Ca^{2+} ion must be present in the cytoplasm at $10^{-7}M$ or greater to enable tumbling in *B. subtilis*, and suggests that it may operate on the flagellar switch.[269b] If linked to chemotactic stimulation, it might also be the chemical transmitter from the sensory apparatus.

The effect on the flagellar motor of reduced steady state energy levels, aside from the obvious effect of reducing speed, is unclear at present. Both Ordal and Goldman[261] and Miller and Koshland[262] find that tumbling frequency is normal in *B. subtilis* when energy is at a reduced steady state level, as a consequence of addition of uncouplers of oxidative phosphorylation. In *Salmonella*, however, prolonged blue light exposure,[263] oxygen deprivation, and treatment with DNP[277] all result in suppression of tumbling; in fact, slow reversed rotation of individual flagella has not been observed under any circumstances so far. Whether a major difference in this regard exists between gram-positive and gram-negative bacteria and whether tumble suppression in *Salmonella* at low metabolic levels is related to reduced protonmotive force, reduced ATP levels, or both, awaits further investigation. Reduced ATP levels would be expected to interfere with the methylation system but might have additional consequences as well.

3. Topological Considerations

Finally, we may consider the topology of the signaling systems. The chemoreceptors (at least for sugars, Section III.C), the methyl-accepting chemotaxis proteins (MCP), the *mot* gene products, and the flagella are all either outside the membrane (extracellular or periplasmic) or are membrane bound. A number of the *che* and *fla* gene products[106,138a] including the methyltransferase,[245] are cytoplasmic but may have transient associations with the membrane. Is the signal to the motor membrane-associated or is it cytoplasmic? Is it a cell-wide parameter or a local one? Different answers to these questions might apply depending on whether by signal we mean the fluctuating parameter causing spontaneous tumbles or the "driven" parameter deriving from tactic stimuli — these parameters may or may not be the same.

Attempts to measure the degree of synchrony of spontaneous flagellar reversals by direct vis-

ualization have been unsuccessful so far, being hampered by the fact that reversals only take place when the motors are rotating rapidly (Section III.F.2). It can, however, be stated that cessation of function, e.g., under high-intensity blue light stimulation, is not synchronous,[278] which suggests that some degree of individuality or compartmentalization of flagella exists.

It would be of great interest to know what relationship the site of tactic stimulation bears to the site of response. A complete "sensory-motor unit," with stimulation eliciting response only from those flagella in the immediate vicinity of the stimulus, seems unlikely, since there are far too many receptors (6×10^4 copies for the galactose-binding protein, for instance). Since there are even too many MCPs (at least 700 copies per cell) to be in intimate association with the half-dozen or so flagella, we would therefore anticipate that, following local stimulation, a signal would travel throughout the cell and eventually impinge on all flagella. The kinetics of signal spread might differentiate between electrical potential transmission and small molecule chemical transmission, but no experiments of this sort have yet been described. The degree of synchrony (approximately 20 msec)[270] of the two polar tufts of the giant bacterium *S. volutans* (40 μm long) is too great to derive from small-molecule chemical transmission from one end to the other. Since, however, there is no indication that spontaneous reversal at one end requires a signal from the other end, this does not really rule out chemical signaling; the reversal could result simply from a cell-wide chemical signal reaching a critical value and affecting both tufts simultaneously. Although *S. volutans* is outside the scope of this review, it is perhaps worth noting that in this organism the flagella are acting antisynchronously because, from the point of view of the membrane, the flagella in the two tufts are rotating in opposite directions. This may turn out to have general implications regarding both the mechanism for driving flagella and for signaling reversals.

IV. CONCLUSIONS

In the introduction to this review, the point was made that bacterial chemotaxis as a behavioral system is sufficiently complex to be interesting yet is amenable to detailed molecular study. This view is borne out by the rapid developments which have taken place in the field in the last 10 years or so. These include:

1. Complementation analysis and mapping of many (probably most) of the relevant genes
2. Elucidation of a number of features of genetic regulation of assembly and function in motility and chemotaxis
3. A phenomenological description of motor function in the presence and absence of chemotactic stimulation, at the level of both the free-swimming cell and the individual motor organelle
4. Verification that a gradient-sensing mechanism exists and that it is based on temporal comparison
5. An ultrastructural description of the motor organelle
6. Demonstration that protonmotive force is the energy source for motility and that the mechanism of propulsion is rotation of a rigid helix
7. Isolation and characterization of a number of receptors and demonstration of conformational changes upon binding of substrate
8. Identification of some intermediate components of the signaling system, phenotypically and genetically
9. A partial biochemical characterization of the mechanism of signaling (the reversible methylation of membrane-bound proteins)

These findings constitute an impressive increase in our knowledge of bacterial motility and taxis, but the subject is still very far from being fully understood. There are some aspects in which the future direction for research is relatively clear — for example, identification and localization of the *trg* product and study of its interaction with primary receptors; identification of the demethylase; systematic study of the methylation levels of the various methyl-accepting proteins, under carefully defined stimulus levels; and clarification of the methylation levels of different *che* mutants. There are, on the other hand, aspects for which it is difficult at present even to define a feasible experimental approach. Two central examples are the process of flagellar assembly and the molecular mecha-

nism of flagellar function. Flagellar assembly, to say nothing of the integration of the organelle into the cell surface, is difficult to study because of its highly coordinated character, which may derive both from close genetic regulation and from features of cooperative stabilization of the structure. (It is interesting to compare this situation with that of the T-even phage, which is an even more complicated structure but one which, because it is produced in high yield per bacterium and is capable of partial assembly, has been dissected in detail.[271]) The fact that the flagellum is a small independent unit makes it very difficult to devise a strategy for studying its operation, particularly since it apparently must be embedded in the membrane. As in the case of assembly, genetic approaches are less useful than one would hope, because, for unknown reasons, mutants with functionally defective basal bodies have not been found. Perhaps, with these two illustrative examples in mind, it would be prudent to restate our expectations by saying that bacterial chemotaxis and motility comprise a system in which a number of aspects, particularly those associated with signaling, are amenable to study at the molecular level but that other aspects may remain elusive for some considerable time.

At this point, it seems appropriate to assess what has been learned from this system regarding principles of biological organization and mechanism. One such principle is that cell behavior can be influenced by an external chemical signal without the molecule itself participating in the cellular biochemistry; the molecule binds at a specific surface receptor and provides information only. This phenomenon, whose existence in higher systems, e.g., acetylcholine or insulin reception, is well known, is thus seen to have developed at a much earlier evolutionary stage. Memory and adaptation to external stimuli are also shown to be features which emerged early in evolution, albeit in a very rudimentary form. It is significant to consider the organization and integration of sensory input as well. As noted in Section III.B, only a limited number of substances are chemotactically active; this, plus the fact of utilization of transport components for chemotaxis, is indicative of economy of design. Another organizational feature which emerges is the funneling of inputs at an intermediate stage; this process, as discussed in Reference 214, permits high sensitivity for each class of inputs, e.g., ribose, when it is occupied alone but limits the overall signal size should several related classes be stimulated simultaneously. A similar type of organization obtains in neuronal systems, where a multiplicity of input fibers converge on a single neuron. Specific and reversible protein modification (the methylation system) is demonstrated as a biochemical regulatory mechanism — it will be interesting to see whether the methylation reaction exerts its effect in chemotaxis by modifying the chemical character of the methyl-accepting protein, e.g., as a substrate or as an enzyme modified at its active site, or by modifying its conformation. The existence of a rotary motor in bacteria, while interesting (and deflating to man's ego), need not involve any novel biological principles. One could, for example, imagine an actomyosin type of chemomechanical transducer. The fact that the bacterial flagellum utilizes protonmotive force does, however, place it in a category distinct from other chemomechanical transducers which have so far been described, but it also serves to emphasize the generality of proton translocation as a biological device for transducing energy, by adding bacterial motility to its already known functions in transport and ATP synthesis.

As a final comment, one can only marvel at the intricacy, in a simple bacterium, of the total motor and sensory system which has been the subject of this review and remark that our concept of evolution by selective advantage must surely be an oversimplification. What advantage could derive, for example, from a "preflagellum" (meaning a subset of its components), and yet what is the probability of "simultaneous" development of the organelle at a level where it becomes advantageous?

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