Hypothesis

A plausible mechanism for flagellar rotation in bacteria

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A novel model for the action of the flagellar motor of bacteria is presented in which rotational motion is produced by conformational changes in a helically or rotationally symmetric multi-subunit component of the basal body. The model is consistent with the known properties of the motor, including its ability to rotate equally well clockwise and counterclockwise. Formally, the model is similar to mechanisms that have been proposed for other biologic transducers of free energy, such as active transporters.

Bacterial motility *Bacterial flagellum* *Flagellar motor*

Flagellated bacteria swim by rotating their flagellar filaments, which, having an overall helical shape, generate a thrust in a propeller-like manner. The mechanism which produces such rotational motion is unknown although various mechanisms have been proposed [1–9]. Here I propose a mechanism for flagellar rotation which is fundamentally different from all existing models. Although there are few, if any, experimental data favoring any particular model, the one proposed here is in some ways superior to the others. It is potentially more easily testable; it makes use of only well-characterized properties of proteins, especially multi-subunit complexes and proteins involved in free energy transduction; and it accounts for the capacity of bacterial flagella to rotate equally well in clockwise and counterclockwise directions.

The flagellum consists of three parts [10]: a rather rigid, helically shaped filament that is typically several micrometers long; a basal body consisting of a rod (or rods) which supports a system of coaxial rings designated M, S, P, and L; and a short flexible filament known as the hook that couples the filament to the basal body. Both the filament and hook, which are outside the cell envelope, are thought to play a passive role in the actual energy-transducing process. The portion of the basal body containing the M and S rings, which are associated with the inner (cytoplasmic) membrane, is likely to be, at least in part, the energy-transducing apparatus.

The energy source for bacterial flagellar rotation is the transmembrane proton-motive force (pmf) [3,11–15] or, in the case of certain alkalophilic bacteria, a sodium electrochemical potential [16].

In all current models (except that of Adam [2]), flagellar rotation is accomplished by means of an essentially rigid rotor component revolving within a stator component [3–9]. The way torque is produced at the interface between these two components is the major basis for distinguishing among the various models. I propose that torque develops when a multi-subunit component of the basal body, most likely one of the rings or rods, undergoes a change in quaternary structure such that a twist is introduced between the two ends of the component. Presumably the component involved in the transition has either cyclic or helical symmetry.

This type of structural transition is not novel, and many examples have been documented, including some for which the biological function is to perform mechanical work. For example, a

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change in the twist of actin filaments provides the force required for extension of the acrosomal process in *Limulus* sperm [17,18]. A particularly well-characterized example is the contraction of the bacteriophage T4 tail during infection. The helically arranged subunits of the sheath component of the tail undergo a slight rearrangement, which reduces the overall length of the tail by one-third, while the two ends of the tail undergo a relative twisting of 360° [19]. Similar kinds of structural transition have been characterized for bacterial flagellar filaments in vitro [20,21] and in vivo [34].

Multi-subunit complexes having rotational symmetry also undergo transitions in quaternary structure that twist the complex along the direction of the symmetry axis. For example, it has been proposed that gap junctions are opened or closed by a 7.5° tilting of the subunits [22]. At least one allosteric enzyme, aspartate transcarbamylase, undergoes a similar type of change in quaternary structure [23], a twisting motion (9°) along the molecule's three-fold axis. Presumably this twisting is only incidental to the allostery, but one can easily envision a component of the bacterial basal body undergoing a similar transition.

There is nothing unusual or special about structural changes producing a net twist between the ends of complexes in which the subunits are arranged with cyclic or helical symmetry. Almost any arbitrary small change in the relative arrangement of the subunits will suffice. Note that for helical complexes the extent of twisting is proportional to the number of subunits, so that distortions of the order of 1 Å per subunit can produce large net twists, as exemplified by the contraction of the bacteriophage T4 tail mentioned earlier.

Thus far I have shown how a conformational change in a multi-subunit structural protein can yield an increment of rotational motion. However, the bacterial flagellum is capable of continuous unidirectional rotational motion. How can such behavior be generated by structural transitions of the type described?

For concreteness a simple specific model will be described (fig. 1) in which functions are arbitrarily assigned to specific components of the flagellar basal body. The portion of the rod connecting the S and M rings is assumed to be the component that undergoes the conformational change, resulting in a twist between the S and M rings which are assumed not to rotate. We refer to these two conformational states of the rod component as R1 and R2. I assume for clarity of presentation that the rod undergoes a net twist of 90° counterclockwise as viewed from above (outside the cell) in going from R1 to R2.

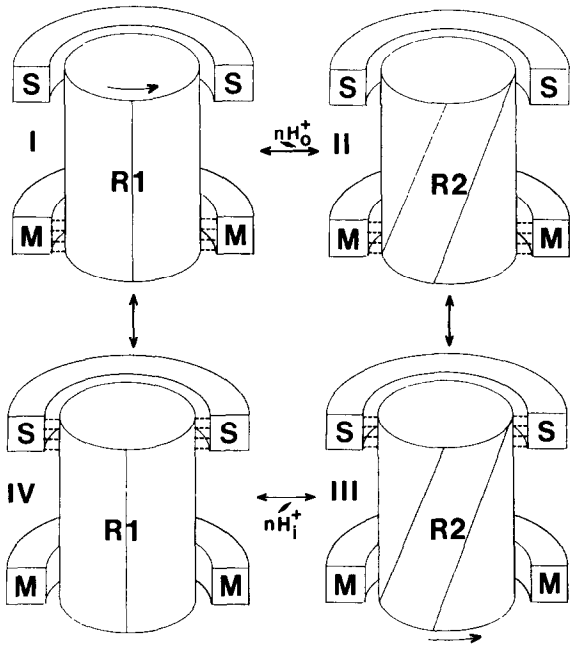

Fig. 1. Schematic model for flagellar rotation. In the portion of the flagellar basal body illustrated, the rings S and M are concentric about the central shaft or rod, R. This portion of the basal body is assumed to span the inner membrane of the cell wall. The rod can exist in two structural states, R1 and R2, which are related by a 90° twist about the long axis of the rod. The twisting is symbolized by the vertical (R1) and angled (R2) striations drawn on the surface of the rod. Dashed lines between a ring and the rod imply strong binding so that rotation of the rod within the ring is forbidden; absence of dashed lines implies that the rod is free to rotate. A tight mechanical linkage between the distal (top) end of the rod and ultimately the flagellar filament is assumed but not shown. H_o^+ and H_i^+ are protons derived from the extracellular and intracellular environments, respectively.

Three ligands are involved in the operation of the motor: protons and the two rings, S and M. Useful work (unidirectional rotation) is assured by the following pattern of ligand binding. In state I the rod binds protons from a site exposed to the extracellular environment and also binds strongly to

the M ring which anchors the rod firmly in the cell membrane. In this state the rod has little or no affinity for the S ring. Upon binding *n* protons the rod switches to conformation R2 and a counterclockwise twist of 90° is introduced at its distal end, the proximal end being held immobile through its interaction with the M ring (state II). Following the transition to R2, the relative affinities of the rod for the S and M rings invert; the rod is now firmly anchored at its distal end by the S ring and free to rotate within the M ring (state III). In this state the previously bound protons are free to dissociate, but now from the cytoplasmic side of the cell membrane. Upon dissociation of the protons the rod reverts to R1, but since the rod is anchored at its distal end, the actual twisting occurs at the cytoplasmic end (state IV). Having returned to its initial conformation and having lost the bound protons to the cell interior, the rod is free to return to its initial state in which it interacts strongly with the M ring. Repetition of the cycle generates successive 90° increments of counterclockwise rotation at the rod's distal (top) end, which is assumed to be coupled to the flagellar filament (not shown in fig. 1).

In more general terms, this model has only two essential features:

(i) A multi-subunit component of the flagellum, most likely one of the rods or rings, undergoes a twist-producing conformational change. I will call the component that undergoes the twisting motion the 'twistor' to distinguish it from the 'rotor' component of other models for flagellar rotation. The actual amount of twisting is probably substantially less than the 90° assumed in the specific model. It is not essential for protons to bind directly to the twistor, as in fig. 1. They could, for example, interact with another component, which in turn induces the conformational change in the twistor. This type of situation could account for the recently observed direct proportionality of motor speed to the amount of *motB* gene product present [24]. The *motB* gene codes for a membrane-associated protein which plays a crucial, although as yet unknown, role in motility [25].

(ii) The twistor is held rigidy in place by binding interactions of its ends with immobile cell-wall components, but not at both ends simultaneously while it is switching between structural states. The relative affinities of the twistor for the two ligands inverts when it switches between structural states. A cyclical series of steps employing these features can produce unidirectional rotational motion, as illustrated by the specific model of fig. 1.

Recently it has become appreciated that a diverse group of biological transducers of free energy (active transporters, proton ATPases, muscle) may function by similar mechanisms [26–28]. Two important features of these mechanisms are that the transducer cycles between two or more conformational states and that the ligand-binding properties of the transducer depend on its conformation. The model I have described for flagellar rotation conforms to this scheme. The nature of the conformational change plays a key role in determining the kind of work performed. For active transporters the conformational change provides a pathway allowing passage of ligands across the membrane, whereas in muscle it provides the mechanical force for contraction. For bacterial flagella conformational change produces an increment of rotational motion. Although it is premature to attribute a single kind of mechanism to all these forms of biological free energy transduction, it is nevertheless intriguing that the possibility appears reasonable.

The capacity of the bacterial flagellum to rotate both clockwise and counterclockwise is essential to chemotaxis [11,29]. One advantage of the model proposed here, as compared with many earlier models, is that this reversibility is easily accounted for. In the model in fig. 1, a change in the sense of rotation can be accomplished by simply reversing the affinities of the twistor for the ring components in its two structural states. This could be accomplished in vivo by an effector molecule (tumble regulator) binding to the rings at specific allosteric sites. Note that there is no need to modify the structural transition undergone by the twistor component when the sense of rotation reverses, thus accounting for the observation that flagella rotate at the same speed in either direction [28]. Reversing the polarity of the pmf would also reverse the sense of rotation, and this too is consistent with experimental observation [12].

As far as I am aware, the model does not contradict any known properties of the motor.

However, Berg and Khan [7,31] have found that for *Streptococcus* strain V4051 the torque generated by the flagellar motor is approximately constant over a temperature range of 4–38°C. Since all known conformational changes in proteins show some dependence of rate on temperature, Berg and Khan concluded that such changes are unlikely to be involved in the operation of the flagellar motor. However, this conclusion is justified only if the conformational change is the rate-limiting step in the overall mechanism, and this was not shown to be true. In fact, under the experimental conditions used, the torque generated by the flagella was directly proportional to the applied pmf – a situation which could be interpreted to mean that the putative conformational change was not rate-limiting. It will be interesting to see whether torque is independent of temperature for bacterial strains in which the flagellar motors can be saturated with respect to pmf.

Consideration of the differences between the model proposed here and previous models suggests several experimental approaches for distinguishing among the models. For twistor-type models torque is generated within the rotating component (twistor), and changes in structure at the quaternary level are essential. It may be possible to induce this same structural transition in vitro by exposing isolated flagellar basal bodies or subcomponents thereof to different environmental conditions. Possibly, the expected change in twist could be detected and quantified by electron microscopy and computerized image-processing techniques, such as have been applied to the flagellar hook [32] and basal body (cited in [9]). Structural transitions of the type expected have been characterized by such methods in other biological systems [17,22]. If the magnitude of the twist found in an isolated component matches the stepping increment of a functioning motor, this observation would constitute strong evidence in favor of a twistor mechanism.

A more thorough study of nonmotile mutants could also provide useful evidence. Referring again to the specific model of fig. 1, a mutation in the rod component such that the relative affinities of the rod for the S and M rings do not invert when the rod changes from R1 to R2 would cause the flagellar filament to simply rotate back and forth by one stepping increment without generating any net rotation. There is no reason to expect such phenotypes for rotor-stator models. A reasonable place to begin searching for such a mutant would be the so-called 'paralyzed' mutants, which are nonmotile but have a morphologically normal flagellum. As far as I am aware, these mutants have not been examined in sufficient detail to distinguish fine motions of the type expected. The discovery and characterization of such a mutant could provide evidence for a twistor type of mechanism, and could also prove useful for measuring the size of the motor's stepping interval in vivo, which remains unknown despite considerable effort by Berg and colleagues [33].

At present no strong evidence favors twistor mechanisms over rotor-stator mechanisms for flagellar rotation in bacteria. The twistor model is, however, a clearly defined, plausible alternative to other proposed mechanisms for flagellar operation, all of which are variations of rotor-stator models.

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