

Chapter 5

Evolution of the Bacterial Flagellum

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The bacterial flagellum is an organelle that looks strikingly similar to a machine constructed by humans. This similarity has led to claims that it is a construct, rather than a product of evolution. Indeed, the bacterial flagellum has become the mascot of the intelligent-design movement; it is one of only two examples of alleged design considered in any depth, graces the cover of William Dembski's book (2002), and features in a recently made video promoting intelligent design. Yet there is no "the" bacterial flagellum.

The image that graces Dembski's book is the flagellum of eubacteria, one of the two fundamental subdivisions of prokaryotes (bacteria in general). Archaeobacteria, the other fundamental prokaryote group, has a flagellum that is superficially similar but does not look like a human-constructed machine. Given the importance that the eubacterial flagellum has assumed in debates over intelligent design, it is worthwhile looking at "the" flagellum in more detail.

In this article I will outline the construction and function of eubacterial and archaeobacterial flagella and their relationship to other systems. I will discuss some of Dembski's objections to current accounts of flagellar evolution and end with a speculative scenario for the evolution of eubacterial flagellum.

Michael Behe has listed the eubacterial flagellum as one of the systems that he believes is irreducibly complex and unable (or unlikely) to be produced by evolution (Behe 1996). Building on Michael Behe's claims, William Dembski (2002) has made the eubacterial flagellum a central point of his key chapter, "Doing the Calculation" (289), and has produced an analysis of the flagellum by assuming that all elements of the flagellum arose randomly. He claims that his analysis supports the intelligent design of the flagellum.

Kenneth Miller (2003) and David Ussery (Chapter 4) have addressed key aspects of our understanding of the flagellum and its evolution. Dembski (2003) has not found such accounts convincing. (1) Dembski does not seem to understand that the eubacterial flagellum is only one of a range of motility systems in bacteria, systems moreover that revolve around a common thread, and that motility is but one function of the flagellum. Further, (2) Dembski artificially categorizes the eubacterial flagellum as a machine. By viewing the eubacterial flagellum as an isolated "outboard motor," rather than a multifunctional organelle with no explicit, human-constructed analog, Dembski makes the problem of flagellar evolution artificially and misleadingly difficult.

Is the Flagellum Evolvable?

One of the issues with Dembski's method for deciding whether systems are designed is that his calculation first requires elimination of systems assembled by natural laws such as natural selection. Given our finite state of knowledge,

there is always the possibility that if we currently do not have an explanation due to natural laws, we may find one in the future. In order to avoid this problem, Dembski attempts to provide a proscriptive generalization that will eliminate any explanation based on natural law and then also allow him to eliminate chance hypotheses.

To provide this proscriptive generalization, Dembski accepts Michael Behe's (1996) description of the flagellum as irreducibly complex (IC) and claims that the flagellum, considered as a system of motor, shaft, and propeller, cannot be built sequentially. He thus claims that describing the flagellum as IC eliminates natural selection as a possible mechanism and proceeds to his calculations to eliminate chance. There are, however, two problems with using the alleged IC nature of the flagellum that are not covered in his book.

First, while Behe has allegedly eliminated directly evolved systems (but see Chapter 5), Behe (1996) himself points out that these systems may evolve indirectly:

Even if a system is irreducibly complex (and thus cannot have been produced directly), however, one can not definitely rule out the possibility of an indirect, circuitous route. As the complexity of an interacting system increases, though, the likelihood of such an indirect route drops precipitously (40).

IC by itself does not provide the proscriptive generalization that Dembski requires.

Second, the specification of “an outboard motor,” which provided the IC system description of motor, shaft, and propeller, is a flawed human analogy to the actual flagellar system. Thinking in terms of human design has misled Dembski. Indeed, in terms of Dembski’s modification of Behe’s original definition,

A system performing a given basic function is irreducibly complex if it includes a set of well-matched, mutually interacting, non-arbitrarily individuated parts such that each part in the set is indispensable to maintaining the system's basic, and therefore original, function. The set of these indispensable parts is known as the irreducible core of the system (285, emphasis added).

The flagellum is probably not IC at all, as the original function of the eubacterial flagellum, which survives massive pruning of the flagellar components, is almost certainly secretion, not motility (Hueck 1998; Berry and Armitage 1999; Aizawa 2001). To explain this claim, I first will examine the variety of motility systems found in bacteria and explain how the eubacterial flagellum functions as far more than just a motility system.

Motility Systems in Prokaryotes

In discussions of the eubacterial flagellum, the impression is often given that the eubacterial flagellum is the only motility mechanism in bacteria. However, not all prokaryotes move, and not all motile prokaryotes use flagella. Furthermore, the motility methods in the two domains of prokaryotes, eubacteria and archaeobacteria, are very different, even though they look superficially similar [Figure 5.1]. Within the eubacteria themselves, there are a range of motility systems. Entire groups have no flagella but still manage effective swimming; others use gliding motility across surfaces. I will briefly summarize the basic mechanisms used in gliding and swimming motility; as we will see later, they throw light on the origin of the flagellum.

Table 5.1 shows different motility systems in prokaryotes. It is by no means exhaustive, and there are probably undiscovered motility systems. What is clear from this table, however, is the extensive role of secretion in motility. This role is more extensive than at first glance, as many systems that look unrelated to secretion are in fact rooted in secretion.

In gliding motility, almost all the mechanisms are related to secretion, where the bacteria glide along a trail of secreted material in a manner reminiscent of slugs. Further, the slime-secretion systems in gliding cyanobacteria bear a strong resemblance to type-III secretory systems (Spormann 1999), and, in many gliding eubacteria, the secretory systems rotate

(Pate and Chang 1979) and are driven by proton-motive force, as are the eubacterial flagella (Pate and Chang 1979).

The apparent exception is the motility produced by the type-IV pilus [Figure 5.1 D], where a long whiplike filament is used to pull the bacterium along as the pilus attaches to a surface, contracts, then releases and extends to attach to a surface again (this mechanism is also called twitching motility). This exception is only apparent, as the type-IV pilus is related to the type-II secretory systems (Thomas et al. 2001). They use the same motor systems, and the pilus seems to be an elaboration of the protein-transport apparatus. The type-II system uses an extension–retraction system to export proteins across the membrane [Figure 5.1 C].

Let us now look at two of the swimming systems, cyanobacterial nonflagellar swimming and the archaeobacterial flagellum. Cyanobacterial swimming is a very simple system consisting of at most one, possibly two components. Cyanobacterial swimming is due to coordinated movement of a semirigid calcium-binding filament in the outer surface of the cyanobacterial coat. Interestingly, the semirigid filaments that function as oars appear to be modifications of the protein that guides slime from the slime nozzles of gliding cyanobacteria (Samuel et al. 2001).

Archaeobacterial flagella are instructive, in that they look superficially similar to eubacterial flagella. However, they are constructed in an entirely different manner and are significantly simpler (Thomas et al. 2001) [Figure 5.1

B, E]. Unlike the eubacterial flagellum which can be described as a “motor, shaft, and propeller,” the archaebacterial flagellum consists of a motor and combined shaft–propeller. This shows that the alleged “three-part” IC system of the flagellum can indeed be simplified. Currently only 8-10 archaebacterial flagellar proteins are known, although it is likely that more remain to be discovered. There is no homology between the flagellar proteins of the eubacteria and archaebacteria (Thomas et al. 2001). The archaebacterial flagellum is, however, homologous to the type-IV pilus, which is responsible for twitching motility [Figure 5.1, D,E]. They use similar motor proteins, similar assembly proteins, and similar chemical-sensing pathways (Thomas et al. 2001). Thus there is a path in the development of the archaebacterial flagellum, from a secretory system to a rotatory swimming motility organelle through a functional intermediate.

There is a clear link between secretory systems and motility, from simple gliding systems to more-complex swimming systems. This link is important in understanding the eubacterial flagellum, as it too, is a secretory system.

Structure of the Eubacterial Flagellum

Just as there is no “the” bacterial flagellum, there is no “the” eubacterial flagellum. Within the eubacteria there are at least two, possibly three, flagellar systems (Asai et al. 1999; Berry and Armitage 1999), based on whether their

motor systems run on protons or sodium and the complexity of the flagellar whip. Within these groupings, the structure of the flagellar elements varies; however, there is a common structure [Figure 5.1 B]. The eubacterial flagellum has a helical filament (propeller), a hook (universal joint), a rod (drive shaft), an S-P ring (bushing around the rod, but only in Gram-negative bacteria), and the SMC ring complex, which is the “motor,” includes the stator and the rotor [FIGURE 5.1 HERE]. The entire assembly is hollow, including the actual filament. The significance of this fact will become apparent later.

The rotor, hook, and filament are made of (nonidentical) helical proteins that self-assemble to give hollow, cylindrical structures. The filament cylinder is helical so that it acts as a “screw propeller” when it rotates. Many eubacteria can switch the direction of rotation of the propeller (and hence the direction of travel), and the switch mechanism appears to be part of the motor complex. Eubacteria also have a chemical-sensing system which regulates the activity of the flagellum so that they swim toward or away from a chemical stimulus.

Between 30 and 50 genes are involved in the construction and regulation of the canonical eubacterial flagellum (44 in the case of the Salmonella typhimurium and Escherichia coli flagella, but only 27 in the case of Campylobacter jejuni); only around 18-20 form the actual motor–switch–shaft–propeller complex.

Homologies with the Type-III Secretory System and Other Systems

As we have seen, there is a deep link between secretory systems and bacterial motility; there is a strong link between the eubacterial flagellum and the type-III secretory system. Bacteria have multiple secretory systems, and we have already encountered the type-II system. Type-III secretory systems are primarily involved in secreting proteins that allow bacteria to attack and invade eukaryotic cells.

The type-III secretory system forms a “rivet” structure identical to the rod and SMC ring complex of the flagellum (Hueck 1998; Berry and Armitage 1999; Macnab 1999)[Figure 5.1 A]. Proteins exported by this system are shunted through the hollow SMC ring and through the rod to the outside of the cell (Hueck 1998; Berry and Armitage 1999; Macnab 1999). (See Fig. 5.1.) In flagellum assembly, flagellins and hook proteins are shunted to the outside of the cell via the rod and ring complex. The proteins attach to the outer rim of the rod and self assemble into a tubular structure that will become the hook and filament, and flagellar proteins pass through this tube as it grows (Hueck 1998; Macnab 1999). Thus the flagellum and the type-III secretory system share the same structure and function. This is no mere resemblance. Homology studies show that many of the flagellar proteins are related to parts of the type-III protein-secretion system (Hueck 1998; Berry and Armitage 1999; Macnab 1999), and the majority of the homology is in the rivet structure and the secretory apparatus [Figure 5.1 A,B].

Furthermore, the genes for the rivet rod and SMC ring complex form a single transcription unit in both the type-III secretory systems and the flagellum. The orientation and order of these genes in the transcription unit are very similar between the type-III secretory systems and the flagellum (Hueck 1998; Berry and Armitage 1999; Macnab 1999). Finally, phylogenetic studies suggest that type-III systems share a common ancestor (Aizawa 2001).

Importantly, the switching/torque generation system of the flagellum has homologs in virtually every type-III secretory system examined so far (Hueck 1998; Berry and Armitage 1999; Macnab 1999). Intriguingly, several type-III secretory systems have tubular structures attached to the rod. *E. coli* has a filamentous structure attached to one of its type-III secretory systems which has significant similarity to the flagellar filament (Sekiya et al. 2001). While secretion in the flagellum is closely linked to flagellar assembly, it also plays a wider role. For example, pathogenic *E. coli* use the flagellar system to secrete enzymes that attack cell walls (Young et al. 1999).

While there is no apparent homolog of the motor (MotAB) in type-III secretory systems, the motor is homologous to the motor of the Tol-Pal and Exb-TonB secretory systems (Cascales et al. 2001). This homology links MotAB and the flagellum to a wide range of secretory systems, including those such as the carbohydrate-secretory systems used in gliding motility. Like those systems, deletion of MotAB not only paralyses the flagellum, but also significantly reduces secretion through the flagellum, emphasising the dual role

of the system (Young et al. 1999). Intriguingly, aglU, a major component of some of the gliding secretory systems, is also related to the one of the components of the Tol-Pal secretory system (White and Hartzell 2000).

While the type-III secretory system does not have a chemical sensing system like the eubacterial flagellum, close homologs of this system are present in the type-IV twitching motility system and gliding motility systems (Spormann 1999; Thomas et al. 2001).

Thus, there are deep links between the structure of the eubacterial flagellum and secretory systems. Flagella share the same basic structure as secretory systems; motors that power secretory systems power them; and they are regulated by chemical-sensing systems that regulate other secretory systems.

The Eubacterial Flagellum in Context

Looking at the context of the bacterial flagellum gives us further insight into how the flagellum arose. Flagella are often thought of exclusively as a swimming motility organelle, yet they have a wide range of other functions. First and foremost is secretion. As we have seen, the flagellum secretes the subunits that form the hook and filament parts of the flagellum. But the flagellum also secretes nonflagellar proteins of importance to bacteria (Young et al. 1999).

The next function is adhesion. The flagellum attaches bacteria to surfaces; this is important for forming biofilms (Watnick et al. 2001), which allow cells to exploit resources on surfaces. Indeed, the ability of the flagellum to bind to cells is critical for pathogenic bacteria to attach to their host cells to attack them (Giron et al. 2002). Even nonmotile pathogenic bacteria express flagella that are crippled in terms of swimming (Andrade et al. 2002), presumably due to the role of flagella in adhesion and invasion of host cells. Importantly, flagella are important in organizing bacteria into a mass to produce a nonswimming form of motility called swarming (Kirov et al. 2002).

Dembski has said that the specification for the eubacterial flagellum is “an outboard motor,” but as we can see the flagellum is also and at the same time a bilge pump and an anchor (to continue the nautical theme). If we view this organelle simply as an outboard motor, we have a distorted view of what it is and what it does.

When viewed as a swimming structure, the flagellum is IC. Remove the motor, it stops functioning; remove the hook (universal joint), it stops functioning; remove the filament, it stops functioning (although in some bacteria removal of the filament results in weak motility). Viewing the flagellum as an outboard motor—and an IC motor at that—provides no insights into the origin or functioning of this structure.

But view it as a secretory structure, and it is not IC. Remove the filament and it still works; remove the hook and it still works; remove the

motor and it still works, not as well as with the motor, but it still works. But which, in Dembski's terms, is the original function? Secretion plays a crucial role in this organelle, and you can't make flagella without secretion—so secretion must be the original function. This conclusion is backed up by the crucial role that secretion plays in other motility systems. Indeed, secretion is a common thread in all motility systems described so far. This is because one of the fundamental problems that a swimming system has is how to get the structures that will be used as oars or propellers through the cell wall. Secretory systems, which are fundamental to the functioning of bacteria, have already solved this problem, and would be needed to get the swimming structures across the cell wall. Therefore it is understandable that evolution would build motility systems on top of existing secretory systems. Thus, Dembski's analysis is deeply flawed.

Dembski and Type-III Secretory Systems

When Dembski wrote his chapter, "Doing the Calculation," he was unaware of the proposal that eubacterial flagellum were related to type-III secretory systems. Kenneth Miller (2003) has written a critique of Dembski's chapter based on the flagellum's relation to the type-III secretory system Dembski (2003) has written a response to this critique and dismissed the link between type-III secretory systems and the flagellum. Among other things he says:

Miller doesn't like my number 10^{-1170} , which is one improbability that I calculate for the flagellum. Fine. But in pointing out that a third of the proteins in the flagellum are closely related to components of the TTSS [type-III secretory system], Miller tacitly admits that two-thirds of the proteins in the flagellum are unique. In fact they are (indeed, if they weren't, Miller would be sure to point us to where the homologues could be found).

In fact, they are not. While Miller emphasized the type-III secretory system, we now know that the majority of the eubacterial flagellar proteins have homologs. As I have pointed out, the motor proteins and the chemical-sensing system have homologs in other secretory systems. Other flagellar proteins such as the sigma factors have homologs as well (Chapter 5). In the end there is not much unique left in the flagellum.

Dembski also writes,

But let's suppose we found several molecular systems like the TTSS that jointly took into account all the flagellar proteins (assume for simplicity no shared or extraneous proteins). Those proteins would be similar but, in all likelihood, not identical to the flagellar proteins (strict identity would itself be vastly improbable). But that then raises the question how those several molecular machines can come together so

that proteins from one molecular machine adapt to proteins from another molecular machine to form an integrated functional system like the flagellum.

The answer, which Dembski has missed, is that the flagellum arises in stages, rather than (as he implies) a whole range of subsystems coming together at once. As we have seen for the archaeobacterial flagellum, a flagellum is not suddenly assembled from a secretory system and other bits lying around into a swimming flagellum in one go.

The archaeobacterial flagellum passed from being a secretory structure, to a gliding motility system, to a rotatory swimming system. At each point there was time for substructures to adapt to each other before the next stage.

Dembski is also dismissive of type-III secretory systems for another reason. Modern type-III systems are specialized for attacking eukaryotes. As eukaryotes are supposed to have arisen after flagella, he claims the type-III systems cannot be ancestral to flagella. But no one has suggested that eubacterial flagella arose from modern type-III systems. Dembski seems unable to contemplate a generalist ancestral type-III secretory system which later specialized into motility and predation systems. Furthermore, many eubacterial flagella are also specialized for attacking eukaryotes, and we do not suppose this means that they arose after the eukaryotes did. Interestingly, predation may be a very old adaptation, some bacteria prey on other bacteria using a hollow

pilus not unlike the flagellar filament, so type-III systems may have been involved in predation long before the rise of eukaryotes (Guerrero, R. et al. 1986).

Proposed Evolutionary Pathway

A possible scenario for the evolution of the eubacterial flagellum is as follows: a secretory system arose first, based around the SMC rod–pore-forming complex, which was the common ancestor of the type-III secretory system and the flagellar system. Association of an ion pump (which later became the motor protein) to this structure improved secretion. Even today, the motor proteins, part of a family of secretion-driving proteins, can freely dissociate and reassociate with the flagellar structure. The rod–pore-forming complex may even have rotated at this stage, as it does in some gliding motility systems. The protoflagellar filament arose next as part of the protein secretion structure (cf the *Pseudomonas* pilus, the *Salmonella* filamentous appendages, and the *E. coli* filamentous structures). Gliding/twitching motility arises at this stage or later and is then refined into swimming motility. Regulation and switching can be added on later as there are modern eubacteria that lack these and function well in their environments (Shah and Sockett 1995). At every stage there is a benefit to the changes in the structure.

Dembski may deride this scenario as a just-so story, but we have evidence for it in the form of a variety of intermediates which function well. We

have simple secretory systems, rotating secretory systems, and nonrotatory secretory systems with flagellumlike whips on them. We have the examples of the gliding and swimming cyanobacteria that use modified versions of the same systems. Finally, the links between type-II secretion, type-IV gliding motility, and archaeobacterial flagellar swimming motility are guides to our understanding of how eubacterial flagella arose.

Dembski (2003) scathingly says that, in the six years since Behe first claimed the eubacterial flagellum was IC, researchers have no more than the type-III secretory system to point to. As we have seen, this claim is wholly incorrect. In these years, we have identified yet more homologies, including the critical motor proteins, understood that the archaeobacterial and eubacterial flagella are entirely different, and uncovered the deep linkages between secretion and motility. Given that it has taken around 200 years to even begin to understand motility in bacteria, it is amusing that Dembski can declare evolutionary description of the eubacterial flagella a “failed project” because it has not provided an account that he regards as sufficiently detailed in a mere six years.

Conclusions

Dembski has claimed that, as the eubacterial flagellum is irreducibly complex, he can eliminate explanations based on natural law for the origin of the flagellum. This conclusion is wrong for two reasons: (1) Being IC does not

eliminate indirect evolutionary explanations, and flagella can evolve from simpler systems through a series of functional intermediates. Further, (2) eubacterial flagella are not the “outboard motors” that Dembski envisages, but rather organelles that are involved in swimming, gliding motility, attachment, and secretion. They occupy one end of a range of secretion-based motility systems in bacteria of varying complexity, and several existing intermediate stages show how the flagellum could well have arisen by evolution and natural selection.

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