an overall rate of between 0.8 and 2% each year—even the rate is uncertain—we desperately need ambitious projects, such as the Tropical Forest Science project on which this work is based, to ensure that effective conservation action is taken.

References and Notes

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PERSPECTIVES: MICROBIOLOGY

Action at a Distance—Bacterial Flagellar Assembly

Robert M. Macnab

The rapid rotation of a long helical filament called the flagellum propels bacteria such as *Escherichia coli* and *Salmonella*. The flagellum is composed of a reversible rotary motor comprising several different proteins; this is connected to a long filament (the propeller) assembled from subunits of the protein flagellin (*1*). The flagellar motor uses the proton electrical potential across the bacterial membrane as an energy source instead of hydrolyzing the energy-rich molecule adenosine triphosphate, which powers many other molecular motors. A molecular switch, set by incoming sensory information from the environment, determines the direction of motor rotation so that bacteria move toward positive stimuli such as food and move away from negative stimuli such as toxins. The flagellum extends far from the bacterial surface, presenting the bacterium with something of a problem—how are individual flagellin monomers added to the distal tip of the growing filament? In a report on page 2148 of this issue (*2*), Yonekura *et al.* describe the beautiful and subtle process of flagellin monomer assembly. By analyzing the tips of individual filaments under the electron microscope, they were able to observe how flagellin monomers that had diffused down the hollow interior of the flagellum became added to the filament tip with the help of a pentameric protein complex called the cap.

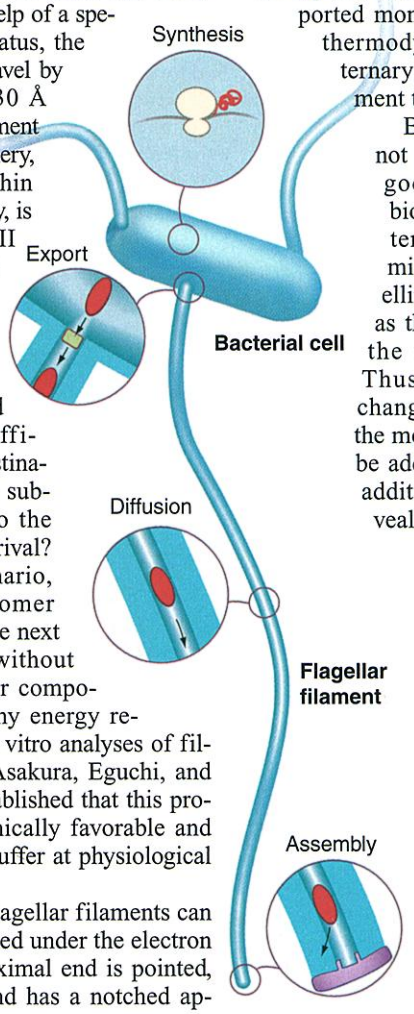
Assembly of the flagellum begins with components, such as the rotary motor, that are closest to the bacterial surface and ends with the filament, the most distal substructure. The filament is extremely long and slender and is composed of tens of thousands of flagellin subunits (*3, 4*) that are synthesized in the cytoplasm and must be exported to the assembly site (see the figure). But how do the flagellin subunits arrive at their destination without getting lost? After translocation across the bacterial inner and outer membranes with the help of a specialized export apparatus, the flagellin monomers travel by diffusion down a ~30 Å channel inside the filament (*5*). The export machinery, probably located within the flagellar basal body, is similar to the type III protein complex that enables pathogenic bacteria to secrete virulence factors (*6*).

The channel ensures that exported flagellin subunits efficiently reach their destination, but how do the subunits get attached to the filament tip upon arrival? In the simplest scenario, each flagellin monomer would just settle in the next available position, without assistance from other components and without any energy requirement. Indeed, in vitro analyses of filament assembly by Asakura, Eguchi, and Iino (*7*) long ago established that this process is thermodynamically favorable and proceeds rapidly in buffer at physiological pH and temperature.

The two ends of flagellar filaments can be readily distinguished under the electron microscope: The proximal end is pointed, whereas the distal end has a notched appearance. However, when filaments grow in vivo, the notched shape of the distal end appears as a thin flat line, suggesting that there is a disklike structure that caps the filament tip (*8*). So, the assembly of flagellar filaments in vitro and in vivo is clearly different.

During the 1980s, biochemical analyses determined that the filament cap is a distinct structure made from the protein FliD (*9*); *fliD* mutant bacteria export flagellin subunits, but these are not added to the filament tip and instead pass out through the channel and are lost (*10*). This finding was interpreted to mean that the cap is a kinetic trap that gives the exported monomers time to settle into thermodynamically favored quaternary interactions with the filament tip.

But things turn out to be not quite that simple. There is good evidence (steric and biochemical) that the amino-terminal and carboxyl-terminal sequences of the flagellin monomers are unfolded as the monomers travel down the filament channel (*11*). Thus, large conformational changes would be required in the monomers before they could be added to the filament tip. In addition, structural studies reveal a mismatch in symmetry



When the cap fits. Monomers of flagellin (red) are synthesized by ribosomes (pale yellow) within the bacterial cell and are then exported by a specialized apparatus (green) at the base of the flagellum into a channel in the growing filament. The flagellin monomers diffuse down the filament channel toward its far end, where they assemble at the filament tip under the guidance of a pentameric cap (purple).

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between the flagellin subunits of the filament and the filament cap. Flagellin subunits are laid down on a local helical lattice with close to 5.5 subunits per turn (*12*). In contrast, under the electron microscope, the filament cap appears as a back-to-back pair of pentameric disks, with no helical symmetry whatsoever (*13*). Although the back-to-back arrangement turns out to be an artifact of isolating the cap in the absence of the filament, the question still remains how a helical structure (the filament tip) can "mate" with a ring structure (the cap).

Which brings us to the work of Yonekura *et al.* (*2*). They analyzed cap-filament complexes by cryoelectron microscopy and three-dimensional image reconstruction, providing remarkable new insights into how the cap orchestrates filament assembly. They argue that, when associated with the tip of the filament, the cap is a flat, pentameric, disklike structure. Each of the five flat segments of the pentamer has a leglike extension that points down toward the growth region of the filament tip. The narrow filament channel suddenly breaks into a much wider chamber just as it reaches the cap region. The fivefold ring symmetry of the cap and the ~5.5 subunits per turn of the filament lattice guarantee symmetry mismatches both of type (ring versus helical) and of azimuthal degree (5 versus 5.5). When one protein of the cap pentamer is at the "dislocation position" (think of a split washer), it will be in a very different environment from the other four members of the pentamer. And, indeed, in five images of the pentameric cap spaced 72° apart (360°/5), one pentameric protein displays a differently shaped opening. The authors propose that this opening is the site where the next flagellin monomer becomes added to the filament structure. The cap then rotates, and another flagellin monomer is ready to enter the next opening. The marked conformational flexibility of the legs (particularly evident in the back-to-back arrangement of isolated double disks), together with the major conformational changes in the flagellin monomers required for their assembly, lead the authors to make the reasonable assumption that the filament cap is a processive chaperone. They propose that the cap chaperone not only prevents loss of flagellin monomers out of the end of the channel, but also actively catalyzes their folding and insertion into the filament lattice. If, indeed, Yonekura and colleagues are correct, then "action at a distance"—the assembly of a structure at a location far away from the bacterial cell—is a much more sophisticated process than any of us could ever have envisaged.

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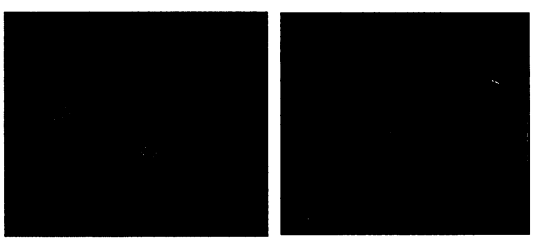
NOTA BENE: CELL BIOLOGY

The Export Business

Cells are faced with something of a problem when it comes to assembling ribosomes, the rotund two-tiered factories that produce proteins. Although mature ribosomes reside in the cytoplasm, the large 60*S* and small 40*S* subunits of which they are composed are assembled from ribosomal RNAs and proteins in a region of the nucleus called the nucleolus. To address the problem of making ribosomes in one place but needing them in another, cells have organized an intricate transport system to ensure that ribosomal proteins are moved into the nucleus, and ribosomal subunits are exported from the nucleus to the cytoplasm. Like traffic wardens directing cars at a busy intersection, a legion of proteins keep the stream of macromolecular traffic—proteins, RNAs, ribosomal subunits—flowing freely through pores in the nuclear membrane, the boundary between nucleus and cytoplasm. According to Johnson and colleagues (*1*), there are at least two traffic warden proteins involved in the nuclear export of the 60*S* ribosomal subunit: Nmd3p, which carries a putative leucine-rich nuclear export signal, and Crm1p, an export receptor that recognizes this signal.

Having established that Nmd3p binds tightly to the yeast 60*S* ribosomal subunit in the final stages of its assembly (*2*), the Johnson laboratory engineered yeast expressing mutant Nmd3p that lacked the carboxyl terminus containing the nuclear export signal. By tagging the large ribosomal subunit protein L25 with a green fluorescent marker, the authors could track the location of the 60*S* subunit in these yeast mutants. In yeast lacking the nuclear export signal, L25 became sequestered in the nucleus (see left figure), whereas in yeast with fully functional Nmd3p, L25 moved into the cytoplasm (see right figure). Thus, 60*S* ribosomal subunits continued to be exported from the nucleus as long as Nmd3p retained its nuclear export signal. The authors surmise that Nmd3p is an adapter protein that binds to the 60*S* subunit, providing it with a nuclear export signal (such signals are necessary for any protein to leave the nucleus).

The Crm1p export receptor transports protein cargo carrying a leucine-rich nuclear export signal through nuclear pores by interacting with proteins in the nuclear pore complex. When they inactivated yeast Crm1p with the antibiotic leptomycin B, Johnson and co-workers found that Nmd3p and fluorescent L25 were retained in the nucleus, whereas in leptomycin-insensitive yeast both Nmd3p and L25 moved freely into the cytoplasm. They propose that the 60*S* subunit is exported from the nucleus by Crm1p, which binds to the nuclear export signal provided by Nmd3p.



Just as traffic on a freeway must pay a toll to pass through a toll booth, nuclear pores exact an energy payment as cargo proteins exit the nucleus. This payment takes the form of conversion of energy-rich guanosine triphosphate (GTP) to guanosine diphosphate through the activity of the small GTPase, Ran. Ran has been identified in many different complexes of cargo proteins and their associated export receptors. In an in vitro assay, the Johnson group has found that Nmd3p, Crm1p and Ran associate together in a complex (*3*). The next step will be to examine how this complex interacts with the 60*S* ribosomal subunit and what structural rearrangements of the nuclear pore complex are required to enable this large conglomerate to squeeze through the nuclear pores.

—ORLA SMITH

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