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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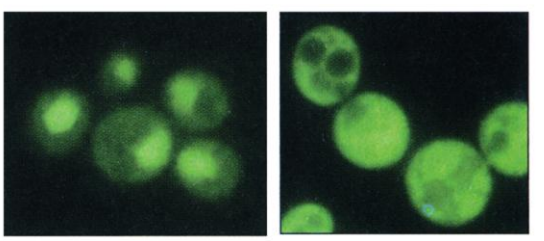
NOTA BENE: CELL BIOLOGY

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.

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