Generation of GTP-Ran for Nuclear Protein Import

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The nuclear localization sequence (NLS) (one or two stretches of basic amino acids) marks cytoplasmic proteins for active transport through the nuclear pore (*1*). Nuclear import requires the assistance of four soluble proteins: karyopherin α/importin α (the NLS receptor), karyopherin β/importin β (which mediates binding to the nuclear pore complex), the small guanosine triphosphatase (GTPase) Ran, and p10/NTF2 (a protein with a heretofore mysterious function). In this issue, Nehrbass and Blobel (*2*) examine the interaction of p10 with the other soluble proteins and with the proteins of the nuclear pore complex and begin to dissect how these interactions result in movement of a substrate through the nuclear pore complex.

The new findings indicate that p10 can bind guanosine diphosphate (GDP)–Ran, karyopherin β, and a family of repeat-containing nucleoporins (to which karyopherin β can also bind independently). Perhaps the most important aspect of these results is the welcome light shed on a puzzling riddle about the GTPase cycle of Ran during nuclear import. This riddle is attributable to the fact that Ran's exchange factor (RCC1) is located inside the nucleus, whereas its GTPase-activating protein (Ran-GAP1 or RNA1) is cytoplasmic. Thus, RCC1 generates GTP-Ran in the nucleus, and the Ran GTPase-activating protein (Ran-GAP1) would quickly convert any cytoplasmic Ran to the GDP-bound form. Yet paradoxically, all experimental evidence has indicated that Ran must at some point be in the GTP-bound form on the cytoplasmic side of the nuclear envelope to function in nuclear import. So what is the source of this GTP-Ran?

This question has been complicated by the fact that Ran is required for mRNA export from the nucleus (movement in the opposite direction), a process that appears also to require Ran in the GTP-bound form and hydrolysis of its bound GTP in the cytoplasm (hence the yeast name for the GAP of RNA1). These observations have resulted in a critical question: Does the GTP-Ran generated by RCC1 inside the nucleus diffuse (or get transported) into the cytoplasm and survive the presence of the GAP long enough to facilitate import? Or is there another mechanism for generating Ran-GTP on the cytoplasmic side of the nuclear envelope?

The new results of Nehrbass and Blobel indicate that there is another exchange reaction on the cytoplasmic side of the nuclear envelope—at the nuclear pore complex itself—and that p10 is a major player in this reaction. Their data show that, by the cumulative effects of multiple, relatively low-affinity interactions, a transient complex is formed that consists of a repeat-containing nucleoporin, karyopherin α and β, GDP-Ran, and p10. Complex formation stimulates GDP release and GTP uptake by Ran, thereby forming GTP-Ran at the exact spot at which this "active" Ran is required for dissociation of the complex and forward movement of an import substrate into the nucleus.

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References

1. D. Görlich and I. W. Mattaj, *Science* **271**, 1513 (1996).
2. U. Nehrbass and G. Blobel, *Science* **272**, 120 (1996).

key to future treatment or vaccines, to distinguishing virulent from less virulent strains, or to understanding the mechanism of attenuation. Progress in this direction is presented in this issue of *Science* (*10*).

Ball and colleagues (9) provide evidence that NSP4, a nonstructural protein of rotavirus, may be the first viral enterotoxin. For the virus, NSP4 may play a role in viral assembly, although its function is not completely understood. To the host, NSP4—more specifically, a 22–amino acid peptide in the hydrophilic cytoplasmic domain of the molecule—acts as an enterotoxin and triggers a signal transduction pathway that causes an increase in intracellular Ca^{2+}, which potentiates Cl^- secretion by a Ca^{2+}-dependent signaling pathway and induces diarrhea in mice. NSP4 causes secretory diarrhea in a way similar to that of the heat-labile enterotoxin β of *Escherichia coli*, suggesting that viral and some bacterial enterotoxins may be similar, although the mechanisms by which other enteric viruses such as the caliciviruses, astroviruses, or adenoviruses cause diarrhea are not known. This virulence factor is age dependent, as are natural rotavirus infections, and its existence partially explains the cross protection conferred by some vaccines against heterologous strains of rotaviruses that differ in their outer capsid proteins associated with neutralization but may share NSP4 antigens. NSP4 may define attenuation as well. Antibodies to NSP4 also protect against disease; pups born to dams immunized with the key peptide had decreased incidence and severity of diarrhea. Perhaps NSP4 should be a target for future rotavirus vaccines.

Another murky area in rotavirology is the mechanism of immunity to rotavirus. Because rotavirus only infects the small intestine, protection against disease is believed to be associated with antibody at the mucosal surface, a mechanism important in other respiratory and enteric infections. Unfortunately, local immunity is difficult to measure in children, and antibody levels that rise after infection return to baseline in a few weeks so that static levels cannot be used to predict protection. Therefore, serum antibodies are the standard measure of immunity for most field studies and vaccine trials. Neutralization titers against the two outer capsid proteins of the virus correlate to some degree with protection, and most vaccinated children seroconvert by neutralization assay to at least one of the strains included in the vaccine (*11*). In contrast, antibodies to the inner capsid protein VP6 do not neutralize the virus and are not associated with protection in passive feeding studies.

Recently, improved mouse models for rotavirus have facilitated studies of the mechanism of immune protection against disease (*12*). In this issue, Burns and coworkers (*13*) have raised BALB/c mice with "backpack tumors" that each expressed a different monoclonal antibody against the inner (VP6) or one of the outer (VP4) capsid protein of rotavirus in an effort to identify the viral antigen critical for protection against disease. The tumors that protected the mice best against rotavirus infection were not those that produced neutralizing antibodies to the outer capsid protein VP4. Rather, the most protective backpacks unexpectedly secreted a nonneutralizing immunoglobulin A (IgA) antibody directed at VP6, the abundant inner capsid protein that is not associated with neutralization in vitro. Furthermore, this antibody was not active directly in the gut lumen where secretory IgA is believed to be protective but was active when it reached the gut epithelium from the basolateral side, where it presumably inactivates virus during transcytosis into the lumen.