Outbound Traffic

Scientists identify proteins that move stuff out of the nucleus

By JOHN TRAVIS

If you think the hundreds of thousands of cars and people entering and leaving Manhattan on any given day is impressive, consider the magnitude of the molecular traffic speeding in and out of a nucleus, the membrane-bound organelle that houses most of the genes in plant and animal cells.

Through several thousand gateways called nuclear pores, vast numbers of proteins and strands of ribonucleic acid, or RNA, travel between the interior of a nucleus and the cellular environment outside it, the cytoplasm. "We're talking about hundreds of molecules per minute per pore," says Karsten Weis of the University of California, San Francisco.

A cell's life depends on this massive transport. Newly synthesized DNA must wrap around proteins called histones, which the nucleus can import at the rate of a million every few minutes. Other proteins streaming into the nucleus help copy a cell's DNA or turn genes on or off.

As for nuclear export, the creation of proteins requires it. RNA must leave the nucleus to convey the protein-making instructions from a gene to the cytoplasmic factories, or ribosomes, that actually build the proteins.

While scientists have made great strides in understanding how molecules gain entry into a nucleus, the study of nuclear export has lagged until very recently. Now, in a flurry of papers, several research groups have identified molecules that seem to help carry proteins and RNA out of the nucleus. "Things are moving rapidly. It's an exciting, exploding area," says nuclear export investigator Thomas Hope of the Salk Institute for Biological Studies in La Jolla.

In addition to highlighting the importance of nuclear export to cells, this explosion of new information may ultimately reveal how a nucleus first arose and may even point to novel ways of fighting the AIDS virus, say investigators.

It's been almost 2 decades since scientists unearthed their first major lead concerning how cells transport molecules in or out of the nucleus. They found that some proteins carry a nuclear localization signal, a short sequence of amino acids that serves as a ticket into a nucleus. Equip a cytoplasmic protein with this signal, and the protein quickly makes the nucleus its new home.

While this localization signal provides an entrée to the nucleus, the protein needs an escort to get there. In the early 1990s, investigators realized that specialized proteins, given such names as transportin, importin-alpha, and importin-beta, usher proteins with a nuclear localization signal to and through nuclear pores.

For a variety of reasons, among them the difficulty of creating test-tube models, "it's been harder to study export. People had to get a lucky break," says Douglass J. Forbes of the University of California, San Diego.

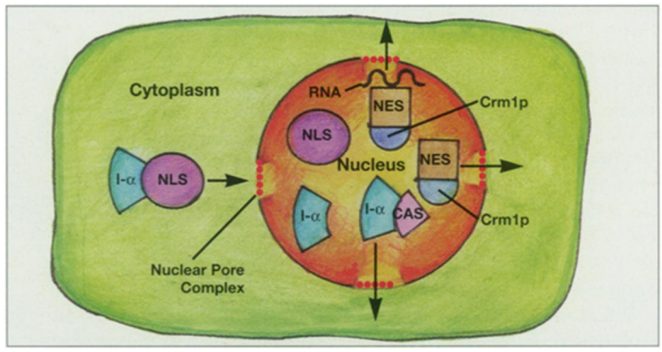
That break came indirectly from the tragedy of AIDS. Seeking chinks in the armor of HIV, the AIDS-causing virus, scientists discovered several years ago that one of its proteins, Rev, makes HIV replication possible by quickly ferrying viral RNA out of an infected cell's nucleus.

As expected, investigators observed that Rev contained an RNA-binding site. More important, they noticed that the protein required another short sequence of amino acids in order to export its attached RNA. This region, now called a nuclear export signal, has shown up in several cellular proteins as well. Rev apparently co-opted an existing export pathway.

Based on their growing knowledge of the import story, scientists speculated that Rev and other proteins bearing different export signals, sometimes along with bound RNA strands, could leave a nucleus only if they were joined to specialized export molecules. Reports in the Sept. 19 CELL, Oct. 3 SCIENCE, and October CURRENT BIOLOGY now describe two proteins that fill the bill.

One of the proteins, Crm1p, was fingered independently by research teams led by Weis, Catherine Dargemont of the Curie Institute in Paris, Iain W. Mattaj of the European Molecular Biology Laboratory in Heidelberg, Germany, and Michael Rosbash of the Howard Hughes Medical Institute at Brandeis University in Waltham, Mass.

Several clues had pointed to Crm1p's involvement in export. Suspecting that a cell draws upon a single family of proteins for both export and import, scientists became intrigued with Crm1p when they noticed that its amino acid sequence resembles that of importin-beta and some other proteins involved in import.



In this simplified diagram of key players in nuclear import and export, importin-alpha (I-α) escorts proteins bearing a nuclear localization signal (NLS) through the nuclear pore complex and into the nucleus. There, a protein called CAS binds to importin-alpha and recycles it back to the cytoplasm. Also inside the nucleus, Crm1p binds to proteins with a nuclear export signal (NES) and moves them and any cargo, such as RNA, out through the pores.

Another line of evidence centered on an experimental antifungal agent called leptomycin B. Research on yeast cells had revealed that this compound kills cells by binding to Crm1p. Scientists linked leptomycin B, and thus Crm1p, to nuclear export by observing that it can prevent Rev from transporting itself and its viral RNA out of the nucleus.

In the new studies of Crm1p, all four research groups established that the protein binds to Rev or its nuclear export signal. Beyond that fundamental step, each group took a slightly different tack to confirm Crm1p's role in nuclear export.

In an experiment with yeast cells, Weis and his colleagues created an artificial protein with three components: a nuclear localization signal, Rev's nuclear export signal, and a fluorescent protein. This protein ordinarily ping-ponged between the nucleus and the cytoplasm. In a strain of yeast with a mutant Crm1p, however, the fluorescent protein accumulated in the nucleus, indicating that Crm1p is required for export.

Working with frog egg cells, Mattaj and his colleagues demonstrated that by

increasing the production of Crm1p in a cell, they could stimulate the nuclear export of Rev and certain kinds of RNA. Moreover, leptomycin B blocked this process and could break apart complexes formed by Crm1p and Rev.

Dargemont's group, experimenting with laboratory-grown cancer cells, also turned to leptomycin B for proof of Crm1p's job. They showed, for example, that the drug prevented the export of proteins bearing the nuclear export signal.

By providing evidence that Crm1p binds to certain proteins that form part of the nuclear pore, Rosbash and his colleagues added another chapter to the Crm1p story. Previous research had linked Rev to these pore proteins, but scientists had been unable to show that the molecules could join together directly. The new data suggest that the pore proteins and the Crm1p proteins bearing the nuclear export signal form a complex involved in export.

The work "indicates that Crm1p interacts with these nuclear pore components and delivers its cargo—Rev, for example—to the pore," concludes Rosbash.

While the identification of Crm1p as the protein used by Rev for exporting HIV's RNA represents a major advance in the nuclear export field, several key questions concerning Crm1p remain unsettled.

First, what material in a healthy cell's nucleus does Crm1p usually export? It clearly helps proteins bearing an export signal to leave the nucleus, but the scientists don't know whether those proteins are the ones that transport the cell's various forms of RNA.

In addition to the RNA that conveys protein-making instructions, the so-called messenger RNA, at least four other classes of RNA exist. Some of the recent experiments suggest that Crm1p exports the cell's messenger RNA, whereas some others indicate that it exports other classes of RNA.

"I think it's a little bit up in the air for the moment," says Rosbash.

"This is still an open question," agrees Weis, whose own data point to Crm1p as an exporter of messenger RNA.

It's possible that the cell's messenger RNA export occurs without Crm1p. Considering the size of the importin-beta protein family, scientists expect ultimately to identify several additional transport proteins. Each form of RNA may have its own export molecule.

One research group has already identified a specialized exporter. In the Sept. 19 CELL, Dirk Görlich of the University of Heidelberg and his colleagues describe a protein called CAS that recycles importin-alpha to the cytoplasm after the import molecule has released its cargo inside the nucleus. CAS is also a member of the importin-beta family of proteins, bolstering the notion that all such molecules are dedicated to either nuclear import or export.

As researchers eventually uncover the full roster of proteins driving import and export, they hope to grasp how cells manipulate nuclear transport to perform specific functions, such as regulating gene activity with proteins called transcription factors. "By changing the localization between cytoplasm and nucleus, you can make a transcription work or not work," notes Weis.

Studies of nuclear export may also help scientists design variations of leptomycin B that prevent Rev from exporting HIV's RNA but allow cells to continue normal nuclear export. "This could be another way of fighting HIV," speculates Forbes.

Finally, investigators note that the development of a nucleus to sequester DNA is the most important difference between eukaryotes, such as plants and animals, and prokaryotes, such as bacteria, whose DNA floats freely in the cell. Consequently, piecing together the molecules behind nuclear import and export could provide insight into how eukaryotes achieved that major evolutionary step. □

Chemistry

Biosensors respond with colored light

When the color of autumn leaves changes from green to red, orange, and gold, it's easy to tell that winter is afoot. Similarly, sensors that change color provide a clear way to discern the presence of chemical substances. Recently, two research groups have demonstrated new schemes for such devices.

One design consists of a specially prepared silicon wafer that signals the presence of organic molecules, DNA, and proteins through subtle changes in color. The device is "exquisitely sensitive," says Michael J. Sailor of the University of California, San Diego in La Jolla, detecting concentrations one-hundredth the size of those currently observable. Sailor, M. Reza Ghadiri of the Scripps Research Institute in La Jolla, and their colleagues describe the sensor in the Oct. 31 SCIENCE.

Etching produces a forest of tiny vertical channels in the thin silicon wafer, making the surface layer porous. This changes the optical properties of the normally silver-colored silicon, giving it an iridescent sheen. The specific colors produced depend on the thickness of the porous layer and the geometry of the channels.

The researchers then attach to the porous silicon a number of molecules that recognize and bind to the substance they want to detect. For example, to detect a particular sequence of DNA, the team attaches many complementary single strands of DNA to the wafer. As a solution containing the target sequence diffuses into the pores and the matching DNA strands bind to each other, the color of the silicon changes. By monitoring that color change with a spectrometer, the researchers can directly confirm the presence of the DNA.

Another sensor, developed by researchers at the University of Pittsburgh, takes advantage of the properties of colloidal crystals, or arrays of tiny polymer spheres (SN: 4/12/97, p. 224). Colloidal crystals diffract visible light and produce different colors, depending on the spacing of the spheres.


Sailor/UCSD

Silicon etched to five different depths displays a variety of colors.

John H. Holtz and Sanford A. Asher embed a colloidal crystal in a gel that can be made to swell when it encounters substances such as glucose or metal ions. As the gel increases in volume, it spreads the polymer spheres apart, thus changing the color of the diffracted light. The team reports its findings in the Oct. 23 NATURE. —*C.W.*

Single enzymes twist and twitch over time

Researchers at the Ames (Iowa) Laboratory have observed the behavior of individual enzyme molecules as they catalyze reactions. Weihong Tan and Edward S. Yeung trapped single molecules of the enzyme lactate dehydrogenase in two different types of vials: the pores of a membrane sold commercially as a filter and holes drilled into a quartz slide. Each vial held just one-thousandth of a trillionth of a liter of liquid.

By monitoring the fluorescent products of the enzyme reaction, the team saw that a single enzyme molecule can change its behavior over the course of a couple of hours. "One molecule can suddenly switch to a higher reaction rate, then go back to what it was before," Yeung says.

The switching probably results when the enzyme twists into different conformations as it interacts with the walls of the vial, he explains. Drugs designed to target these other forms might show greater efficacy. The report appears in the Oct. 15 ANALYTICAL CHEMISTRY. —*C.W.*