The Nucleus's Revolving Door

Many different transport systems carry molecules in and out of the nucleus, but a small protein called Ran seems to act as the dispatcher, coordinating the direction of transport

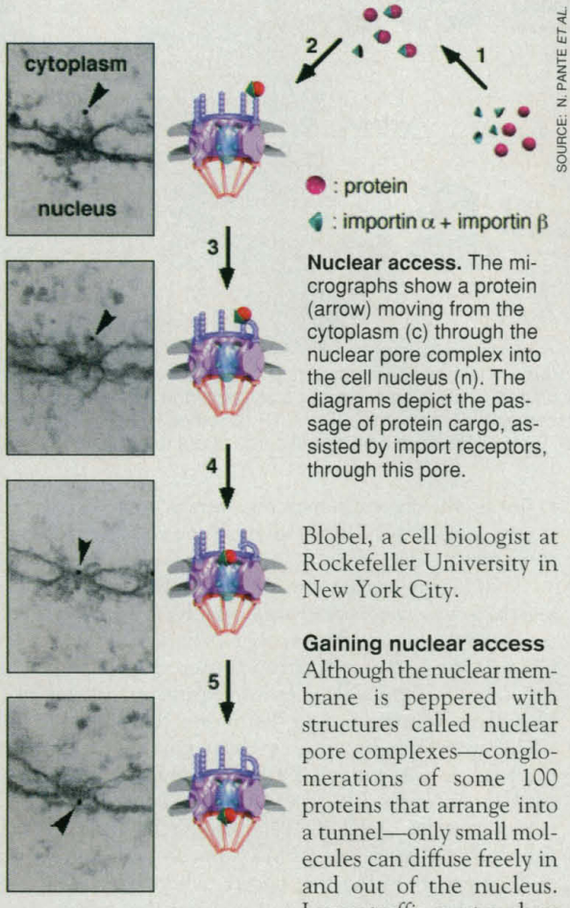
Like any military headquarters, the cell's command center—the nucleus—needs to be in constant communication with its troops to keep the cell running smoothly. Not only does it have to send out its orders, such as the messenger RNAs (mRNAs) that provide the instructions for making proteins, but it also has to receive intelligence from the far-flung reaches of the cell, often in the form of proteins that help regulate the activity of genes in the nucleus. But despite the importance of these communication channels, cell biologists have had little information about just how they operate—until recently, that is.

New work, much of it done over the past 2 years, shows that cells have evolved a highly complex system for transporting large molecules such as proteins and mRNAs into and out of the nucleus. Researchers have identified a host of different, although structurally related, proteins that escort molecules through the nuclear membrane. "There are a larger number of [nuclear transport] pathways than we thought," says cell biologist Stephen Adam of Northwestern University in Evanston, Illinois.

This diversity of transport proteins opens the way to an intricate division of labor. Some move RNA out of the nucleus, for example, while others work only to get proteins in. "You can regulate any particular [pathway] without shutting down [transport] globally," says Mary Dasso, a cell biologist at the National Institute of Child Health and Human Development (NICHD).

Besides identifying the transporter proteins that do the heavy labor of carrying molecules across the membrane, researchers have caught sight of one protein that seems to act as a foreman. Known as Ran, it may oversee the various nuclear transport pathways. It also seems to link them with critical events in the life and death of the cell. While coordinating nuclear transport, for example, Ran interacts with other molecules that help determine when cells divide or whether they commit suicide in so-called programmed cell death, which rids the body of cells with damaged DNA.

Such findings are causing more and more researchers to sit and up and take notice of the pivotal role nuclear transport plays in the life of the cell. Because critical processes such as cell division and protein synthesis depend on molecules that have to be carried into or out of the nucleus at exactly the right time, nuclear transport is "central to understanding cell regulation," says Günter Blobel, a cell biologist at Rockefeller University in New York City.



SOURCE: N. PANTE *ET AL.*

Nuclear access. The micrographs show a protein (arrow) moving from the cytoplasm (c) through the nuclear pore complex into the cell nucleus (n). The diagrams depict the passage of protein cargo, assisted by import receptors, through this pore.

Gaining nuclear access

Although the nuclear membrane is peppered with structures called nuclear pore complexes—conglomerations of some 100 proteins that arrange into a tunnel—only small molecules can diffuse freely in and out of the nucleus. Larger traffic must push or be pushed through. Yet every second, RNAs are leaving, typically complexed with certain proteins. At the same time, thousands of proteins are coming in.

Cell biologists began getting their first clues to how all these molecules make it through the nuclear membrane about 12 years ago when they noticed that certain proteins that need to get into the nucleus all contain a similar stretch of amino acids. Researchers speculated that the stretch might be some kind of shipping tag, marking these proteins for transport across the nuclear membrane. That idea was confirmed about 6 years later, when researchers identified a protein called importin α that recognizes and binds to the sequence. Once that binding occurs, a third protein, importin β, joins the other two, and the resulting complex of molecules makes its way through the nuclear pores.

This early picture of nuclear transport seemed simple—two or three proteins joining together to escort another into the nucleus. But then several groups began to realize that other proteins might also serve as escorts into—and perhaps out of—nuclear pores. Rather than call them importins, a few began to think of these transporter proteins as karyopherins, from the Greek words meaning "nucleus" and "carry."

In 1995, cell biologist Gideon Dreyfuss and his colleagues at the University of Pennsylvania got a clue that helped them locate a new karyopherin when they found a protein with a different nuclear localization sequence. They made the discovery while studying a protein called hnRNP A1 that somehow accompanies mRNAs out of the nucleus and then quickly shuttles back in again. Yet, it lacks the localization sequence found in the other proteins destined for the nucleus.

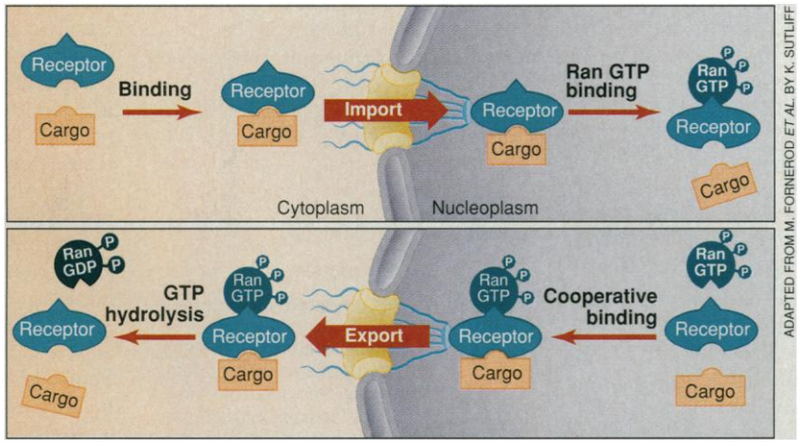
By making a systematic series of changes in the hnRNP A1 protein and testing to see whether the altered proteins were still imported, Dreyfuss and his colleagues homed in on a new import password, called M9, that consists of 38 amino acids. "This was the second signal defined for import," notes biochemist Iain Mattaj of the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany. The Pennsylvania researchers later showed that hnRNP A1 is carried through nuclear pores not by the two importins, but by a different escort protein, which they named transportin. Transportin does, however, resemble importin β; their amino acid sequences are about 24% identical.

Dreyfuss discovered this alternate pathway in human cells grown in the laboratory. But human cells aren't unique in having multiple import routes. While Dreyfuss was tracking down transportin, Blobel's team at Rockefeller showed that the yeast version of transportin, karyopherin β2, also

helps RNA-binding proteins get into the nucleus. Since then, the Rockefeller group has found additional yeast escort proteins.

They were aided in their quest by the sequencing of the yeast genome, completed in 1996. They already knew that yeast has an importin β counterpart, a protein called karyopherin β1, and they identified several sequences in the yeast genome that seemed to code for similar proteins. Meanwhile, another research team searched through the newly acquired database of yeast genes and came up with 13 possible importin relatives.

In the past year, Blobel's group has determined the roles of several of these proteins. Two of them, karyopherin β4 (Kap123) and karyopherin β3 (Kap121), seem to shepherd into the nucleus proteins destined to become part of the protein factories called ribosomes. Ribosomes are assembled inside a nuclear body called the nucleolus before being shipped out again to the cytoplasm, where protein synthesis takes place. Just 3 months ago, the Rockefeller group announced two more karyopherins, one that imports a protein involved in the processing of transfer RNA (tRNA) and another that imports mRNA binding proteins. "The prediction is that there are as many [transport] pathways as there are factors," notes Dirk Görlich, a biochemist with the Center for Molecular Biology at the University of Heidelberg in Germany.



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Ins and outs. Ran's role in nuclear transport is still controversial, but in one model, RanGTP helps unload newly imported molecular cargo and then becomes part of an outgoing cargo complex. Once outside, RanGTP becomes RanGDP to unload the exported cargo. RanGDP may also help load cargo about to enter the nucleus.

Taking leave

But while researchers unraveled more and more of the intricacies of transport into the nucleus, they had only the barest details about how molecules leave the nucleus. The first details came out of labs independently studying the AIDS virus, HIV. In 1995, Susan Taylor's team at the University of California, San Diego, and Reinhard Lührman's team at the Institute for Molecular Biology and Tumor Research in Marburg, Germany, showed that the mRNA made by the virus leaves the nucleus in association with a viral protein called Rev. Rev, they discovered, contains a stretch of eight amino acids, including four leucines, that tags it for export out of the nucleus with its RNA cargo.

Other proteins that need to be transported out of the nucleus bear similar eight-amino-acid sequences, Taylor's team and others found. The leucine-rich sequence, it appears, is an export tag that enables Rev and the other proteins to link up with their escorts, just as the import signal enables molecules to attach to proteins that would allow them access into the nucleus.

But no one was able to tease out the export transporters that recognize the leucine-rich password. Then about a year ago, researchers searching for new AIDS drugs made a discovery that helped unlock the secret. Barbara Wolff and her colleagues at the Sandoz Research Institute in Vienna, Austria, found that an experimental antibiotic called leptomycin B blocks both Rev activity and also all export of other proteins bearing a leucine-rich password. Because other work had shown that in the yeast *Schizosaccharomyces pombe*, this antibiotic interacts with a protein called CRM1, whose only known function was helping to maintain the chromosomes, researchers began to suspect that CRM1 might be an elusive export escort.

That finding caught the attention of researchers who were already hot on CRM1's trail because it resembles importin β. Earlier, Gerard Grosveld and Maarten Fornerod at St. Jude Children's Research Hospital in Memphis, Tennessee, had discovered that CRM1 interacts with a protein that is part of the nuclear pore complex. On learning of the Sandoz results, EMBL's Mattaj and Fornerod, now also at EMBL, immediately began looking at leptomycin's effects on nuclear export in the frog oocyte, where a large nucleus makes it easy to monitor the comings and goings of proteins and RNA.

They found that, as in the mammalian cells studied by Wolff, leptomycin inhibits export of Rev and of certain small RNAs that need to go into the cytoplasm to pick up proteins that help them process mRNA when they return to the nucleus. In contrast, adding excess CRM1 to the nucleus speeds up the export of these substances. And finally, the group demonstrated that CRM1 binds to the leucine-rich export tag, except in the presence of leptomycin. At the same time, several other groups, including those of Karsten Weis of the University of California, San Francisco, Catherine Dargemont of the Pasteur Institute in Paris, and Eisuke Nishida at Kyoto University in Japan, were coming to the same conclusion about CRM1.

The resemblance between CRM1 and importin β suggested that there might be other links between import and export. That expectation was borne out when Görlich and his colleagues set out to pin down how importin α gets back out of the nucleus once it has carried in its protein cargo. They found that importin α leaves the nucleus with the help of a protein called CAS, whose amino acid sequence shows that it is an importin β relative.

Finding all these links between nuclear export and import is "conceptually very important," says Weis. It not only means that much of the knowledge about import receptors can be applied to export receptors, but it also begins to point to ways in which transport into and out of the nucleus might be coordinated. If similar proteins—or even the same proteins—are responsible for transport in both directions, the molecules that they interact with, and are presumably controlled by, are probably similar. Researchers are now boring in on the protein Ran as central to this coordination.

Ran as dispatcher

One clue to Ran's key role is that it turns up consistently in both the import and export complexes. Görlich's group found, for example, that a form of Ran has to bind to a complex of importin α and CAS for the importin to be transported out of the nucleus. And cell biologist Ian Macara and his colleagues at the University of Virginia, Charlottesville, made a similar finding, showing that Rev and other proteins can't be exported from the nucleus unless this form of Ran is present. Another hint of Ran's coordinating role is that it exists in two different forms, one in the nucleus and one in the cytoplasm. Imbalances between those forms might serve to specify the transport direction.

In the form found in the nucleus, Ran appears to be linked to the energy-carrying molecule GTP, while in the cytoplasm it's mainly bound to GDP, the low-energy, spent form of the molecule. That's because Ran is a

GTPase, an enzyme that splits GTP into GDP and phosphate, and the cytoplasm contains two other proteins that trigger Ran to split GTP. In contrast, the nucleus contains a protein that helps Ran shed GDP and recharge itself with fresh GTP.

Presumably, the distribution of these Ran-related proteins results in higher concentrations of GTP inside the nucleus than out of it, and, as Görlich suggested in 1996, that may be key. "The asymmetry in RanGTP and RanGDP may be very relevant in [determining] the direction of transport," says Blobel. Larry Gerace, a cell biologist at The Scripps Research Institute in La Jolla, California, agrees: "It's clearly a central player. But," he adds, "how it functions is highly controversial."

Some researchers have contended that RanGTP provides energy needed to move molecules in and out of the nucleus, but most now think it is just that the chemical form of Ran determines whether molecules are imported into the nucleus or exported out. As a direction sensor, it would keep newly imported proteins from slipping immediately back out again by helping them break away from their transport proteins. And for export complexes in the nucleus, binding with RanGTP signals that it's time to move out.

Once outside, though, the RanGTP may be converted to RanGDP. Gerace notes that RanGAP1 and RanBP2, the two proteins that activate Ran's GTP-splitting activity, accumulate right outside the nucleus near the pores—just the right spot to ensure that any RanGTP quickly becomes RanGDP. The latter may then help proteins seeking to enter the nucleus rendezvous with their importin escorts. After the RanGDP accompanies the import complex through the nuclear membrane, the whole process can be repeated when RanGDP becomes RanGTP or is displaced by a RanGTP.

Although this role for Ran is still conjecture, it's helping to define the direction of the research. "If [Ran] works one way in import and the opposite way [in export], one can make predictions," Görlich explains. "It makes it much easier to design experiments."

The recognition of Ran's central role is also drawing attention to other molecules that interact with Ran or its associated proteins. These molecules don't take part directly in import and export, but, like officials at a border crossing, they can't be ignored. One may help to stabilize import complexes; another may help these complexes accumulate at the entrance of the nuclear pore. With these proteins, the cell may fine-tune protein import and export. For example, NICHD's Dasso says, "our current feeling is that [one such molecule] is coordinating nuclear transport with other nuclear events," such as mitosis or gene activation.

That fine-tuning can be critical to a cell's well-being, says Jonathan Pines, a cell biologist at the Wellcome CRC Institute in Cambridge, England. For example, a protein complex containing two enzymes called cyclin B and CDC2 appears to help coordinate activity in the nucleus and the cytoplasm during cell division. Once mitosis begins, the complex seems to accumulate in the nucleus. But until then, it "is constantly being imported and exported," says Pines. Exactly how is unclear, but by shuttling back and forth, the complex is in constant communication with the nucleus and cytoplasm. "The transport of cyclin B is going to be very important for the regulation of the cell cycle," Dasso says.

Similarly, a cell might regulate its protein productivity by controlling how much mRNA gets out of the nucleus to the ribosomes, where proteins are put together, and how fast this transport takes place. Because the nuclear membrane separates the place where genetic information is encoded and transcribed into RNA from where the RNA message is translated into a protein's amino acid sequence, "you can introduce a great number of regulatory steps," Görlich explains.

This realization is fueling a flurry of activity among cell biologists eager to describe the other import and export pathways. As they discover which molecules travel these many roads, they are rapidly drawing in researchers who once never gave much thought to transport within the cell. Now these researchers are discovering that the journey can be as important as what happens after a molecule arrives. "The field is going to explode in the next year or two," Dasso predicts. "I'm bracing for it to go crazy."

–Elizabeth Pennisi

THE WORLD OF NUCLEAR TRANSPORTERS

Yeast Protein	Homologs	Function
Karyopherin α	Importin α (Kap α)	Helps import proteins with NLS sequences
Karyopherin β1 (Kap95)	Importin β (Kap β1)	Imports NLS proteins, complexed with karyopherin α (importin α)
Karyopherin β2 (Kap104)	transportin (Kap β2)	Imports mRNA-binding proteins, such as hnRNPA1
Karyopherin β3 (PSE1, Kap121)	Kap β3	Imports ribosomal proteins
Karyopherin β4 (Kap123)		Imports ribosomal proteins
*SXM1 (Kap108)		Imports proteins involved in processing tRNA and possibly mRNA
*MTR10 (Kap111)		Imports and possibly exports mRNA-binding proteins
*CSE1	CAS	Exports karyopherin α (importin α)
*NMD5	Ran BP7, Ran BP8	Unknown
*LOS1		Possibly transports tRNA
*MSN5		Unknown
*CRM1, XPO1 (Kap124)	Exportin	Exports proteins with leucine-rich export sequences
*PDR6		Unknown
*YGL241W		Unknown
*YPL125W		Unknown

*Members of the karyopherin β4 family

Additional Reading

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