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New Clues to How RNA Exits the Nucleus

Researchers have developed a new technique that selectively blocks the export of messenger RNA (mRNA) from the nucleus to the cytoplasm of living mammalian cells. The technique, which uses cell-permeable peptides to ferry inhibitory molecules into the cell, offers a new opportunity for researchers to understand the roles of individual proteins.

The researchers, Howard Hughes Medical Institute investigator Joan A. Steitz and Yale University School of Medicine colleague Imed-Eddine Gallouzi, described their findings in the November 30, 2001, issue of *Science*.

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Export of mRNA from the nucleus to the cytoplasm takes place through a pore complex in the membrane of the nucleus. Export is believed to involve the attachment of an adapter protein to the mRNA. The adapter protein binds both the mRNA and a receptor that in turn interacts with pores in the nuclear membrane and thus serves as a shuttle to enable export of mRNA. Steitz and Gallouzi reported that they had selectively blocked the action of several adapter proteins. The function of one such adapter, called HuR, was explored in detail.

"The problem has been that many different proteins have been identified that bind to mRNA in the nucleus and that are known to shuttle to the cytoplasm," said Steitz. "Many of them are very abundant, and bind virtually every message in the cell. Also, every mRNA in the nucleus is coated with all sorts of RNA-binding proteins."

“So, what seems almost miraculous about these results is that by just inhibiting the interactions with the receptor of one or a couple of these adapters, we can keep the particular mRNA entirely in the nucleus. That’s quite remarkable, and something we still don’t understand.”

Steitz and her colleagues had begun studying the HuR protein because it is involved in preventing the degradation of mRNA. However, evidence was also mounting that the RNA-binding protein aided the nuclear export of mRNAs for early response genes – those that encode growth-regulating proteins needed early in development. The evidence also suggested that HuR might use two independent export pathways, one involving a nuclear export receptor called CRM1 and another that appeared to be completely independent of CRM1.

“We were puzzling about how could we begin to tease apart this protein’s function and demonstrate that there were two alternative pathways,” said Steitz. The researchers realized that they could draw on the discovery by other scientists of short pieces of protein, called Trojan peptides or penetratins, that can be attached to other molecules to ferry them into cells. Those scientists had used these cell-permeable peptides to carry molecules into cells to selectively compete with endogenous molecules to block their action. Steitz and Gallouzi decided to apply the technique to selectively block the action of HuR.

They chose as their cargo-carrying, cell-permeable peptide a small piece of a molecule from the fruit fly, called antennapedia transcription factor. They produced three inhibitory molecules by attaching to the fly peptide short segments of three adapter proteins known to be critical for export. Using these synthetic peptides to selectively block the pathways, the scientists proved that HuR could, indeed transport exit the nucleus via either a CRM1-dependent or CRM1-independent pathway.

Steitz and Gallouzi also used their selective blocking method to show that both pathways were important in transporting mRNA for the early response gene *c-fos*. And, they revealed the functioning of the CRM1-independent pathway by showing that it relied on another nuclear receptor, called transportin-2 – whose function had until then not been known. According to Steitz, the evidence for HuR’s involvement in two export pathways still does not reveal the full story of its function.

“HuR is a strange molecule,” she said. “And whether it’s really doing multiple things both in stability and in export, I’m not sure yet. What’s important is our demonstration that there are multiple pathways of export and that somehow single adapters seem to govern these pathways, even though there are all these other RNA binding proteins on mRNA. And that’s something that we need to understand next.” According to Steitz, one of the keys to understanding mRNA export is discovering why cells appear to need alternative export pathways.

“Our guess is that cells need multiple pathways to be able to adapt to different conditions,” said Steitz. “For example, in this paper, we show that

under conditions of heat shock, HuR does shift pathways. But that phenomenon needs to be explored under other conditions of stress.”

According to Steitz, further studies will include using large-scale genetic analysis to discover which mRNAs use various export pathways. Steitz and her colleagues will also use cell-permeable peptides to carry blockers of other candidate adapter proteins, in an attempt to reveal their role in yet other export pathways.

HHMI investigator Melissa Moore at Brandeis University emphasized that cell-penetrating peptides should have broad applicability. “The more I’ve learned about these peptides, the more excited I’ve become, because they have the promise of being incredibly useful,” said Moore, who authored a Perspective article in *Science* with HHMI investigator Michael Rosbash, also at Brandeis. Moore said that the cell-penetrating peptides could prove important both as basic research tools and in developing targeted clinical treatments.

“Since these peptides can carry a variety of molecular cargoes, including proteins and nucleic acids, they could potentially carry just about anything required to experimentally affect some change in the cell,” she said. “And clinically, they could be used to target drugs that induce, for example cell death, to specific cell types.” Moore added that such drugs could be engineered to hitchhike on cell-type-specific peptides into cells. Or, the drug molecule could be attached to a more general cell-penetrating peptide such that it would only be released, and thus activated, by an enzyme found inside a certain cell type.