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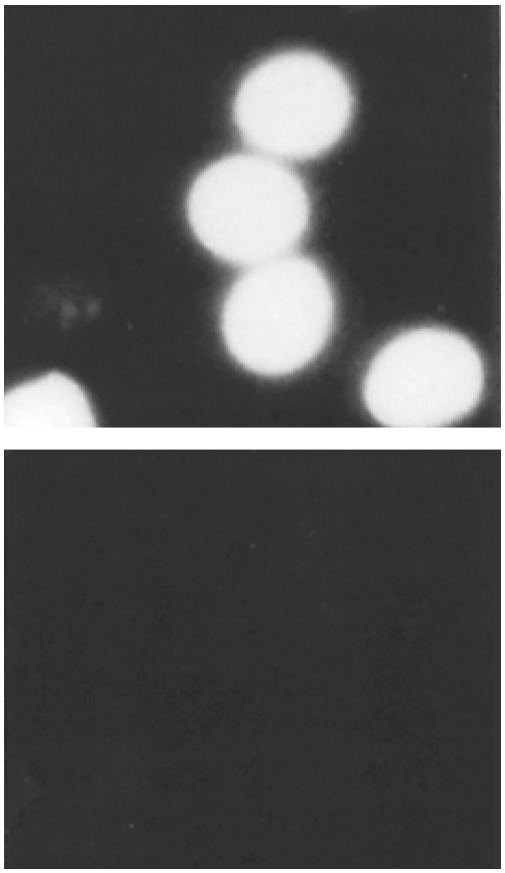
In vitro nuclear import

The nuclear pore serves as the gateway to the nucleus, allowing molecules to slip in and out. A technique devised by Larry Gerace (Scripps Research Institute in La Jolla, California) and colleagues helped researchers ferret out the biochemical details of nuclear import and export, identifying the driving force as the protein Ran. The same system helped scientists pinpoint the proteins that ferry cargo into the nucleus.

In the early 1990s, cell biologists knew that proteins need the right credentials to gain admission to the nucleus—a short string of amino acids called the nuclear localization sequence (NLS). But the mechanism that shuttled cargo through the pore remained mysterious. To study the question, Gerace and colleagues created a new system. The only available procedure, which used *Xenopus* egg extracts, yielded a nuclear envelope with functional pores but didn't allow users to pinpoint the transport proteins, says Gerace. It wasn't possible to discern them from the factors needed for reassembling the nucleus. As an alternative, his group bathed cells in digitonin, a detergent that attacks cholesterol and punches holes in the cell membrane, leaving the nuclear membrane intact. The nuclei of these "permeabilized" cells would take up proteins bearing an NLS, as long as the researchers added cytosol and ATP (Adam et al., 1990), suggesting that something in the cytoplasm helped usher molecules through the pore.



GERACE

In vitro nuclear transport (top) works only with lysate and ATP.

When Gerace's group fractionated the cytoplasm of HeLa cells and added each fraction to permeabilized cells, they found that a fraction with small proteins spurred nuclear transport (Melchior et al., 1993). From previous work, the researchers suspected the involvement of GTPases, protein switches that can toggle between GDP- and GTP-carrying versions. So they tested two GTPases lurking in the low molecular weight fraction and saw that one, Ran, promoted nuclear uptake of proteins sporting an NLS. About the same time, Günter Blobel's lab at Rockefeller University in New York City made a similar discovery (Moore and Blobel, 1993). Experiments on permeabilized cells also revealed the importin proteins responsible for transporting molecules into the nucleus (Adam and Gerace, 1991; Adam and Adam, 1994; Gorlich et al., 1994; Radu et al., 1995).

Further probing has allowed researchers to build an intricate model of nuclear transport. What drives molecular movement is a Ran asymmetry across the membrane. Thanks to a Ran exchange protein with an affinity for chromosomes, RanGTP abounds inside the nucleus, while RanGDP predominates in the cytoplasm. Researchers have observed the gradient (Kalab et al., 2002), and they've shown that tampering with it stalls nuclear import and export (Izaurralde et al., 1997), whereas flip-flopping it reverses the direction of transport (Nachury and Weis, 1999). The low concentration of RanGTP on the cytoplasmic side triggers the importins to latch onto molecules with an NLS and haul them through the pore. Inside the nucleus, RanGTP spurs the importins to dump their load. Trucking molecules out of the nucleus, a job performed by the exportins, also depends on Ran (Richards et al., 1997). The protein helps to set up the mitotic spindle and to rebuild the nuclear membrane after mitosis, so the big question now, says Gerace, is What else does this busy and versatile molecule do? **ML**

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