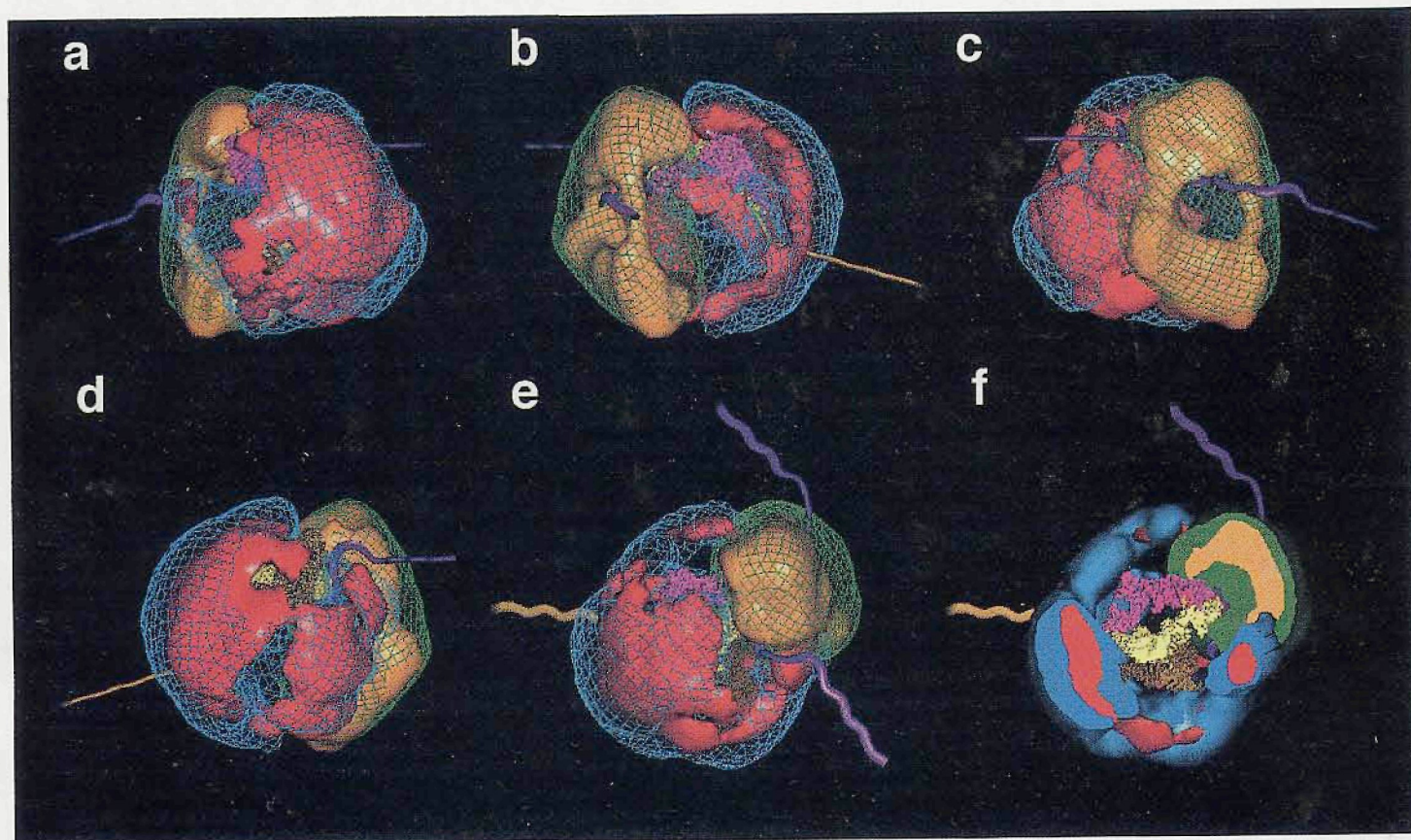


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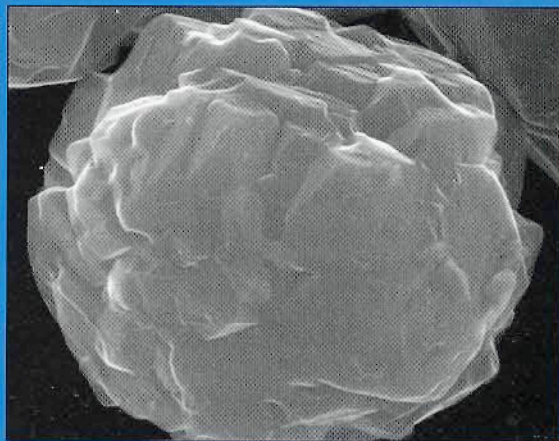
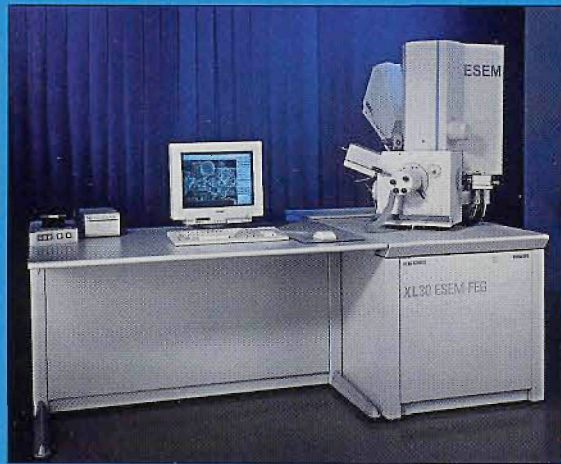
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Plugging A Small Hole

Stephen W. Carmichael,¹ Mayo Clinic

It has long been appreciated that communication between the nucleus and the cytoplasm of a cell occurs through the nuclear pores. Regulation of this communication has remained a mystery. A breakthrough in our understanding of this regulation was recently presented by Carmen Perez-Terzic, Jason Pyle, Marisa Jaconi, Lisa Stehno-Bittel, and David Clapham of Mayo Clinic¹. Using field emission scanning electron microscopy (FESEM), transmission electron microscopy, and atomic force microscopy (AFM), they demonstrated the presence of a small plug within the nuclear pore that was present under certain physiologic circumstances. This "plug" may regulate the movement of molecules through the pore.

Perez-Terzic *et al.* examined oocytes of the African clawed toad (*Xenopus laevis*) under normal physiologic conditions, under conditions that depleted nuclear calcium, and under conditions where nuclear calcium levels had been restored. Under normal conditions, about seven percent of nuclear pores appeared to be plugged when viewed with FESEM. Under calcium-depleting conditions, the vast majority (about 90 percent) of the calcium pores appeared to be plugged. The calcium-depleting conditions included incubation with inositol 1,4,5-trisphosphate [Ins(1,4,5)P₃] (a second messenger that drives the transfer of calcium from the nucleus to the cytoplasm, thereby depleting the nucleus of calcium), incubation in a low calcium-containing medium, and incubation in a medium containing a calcium-chelating agent. The depletion of calcium within the nucleus could be confirmed by laser scanning confocal microscopy and appropriate calcium-sensing dyes. The appearance of the central plug corresponded with the inhibition of the diffusion of molecules with a relative molecular mass of about 10,000 through the nuclear pore complex. When normal

calcium levels were restored, the number of plugged pores dropped to about five percent, demonstrating that this "plugging" event is reversible.

The topography of the nuclear pore and its central plug was examined with AFM. The nuclear pore complex was demonstrated to be an octagonal structure of about 160 nm in diameter with a plug positioned about 10 nm below the cytoplasmic ring of the central pore. The sample scanning was performed in the constant-force mode with a Z range of up to one micron, which demonstrates that the measurement of the pore was not limited by the instrumentation. Under calcium-depleting conditions, the diameter of the complex was increased just slightly and the central depression where the pore had been was only about 2 nm below the surface of the complex. The inner diameter of the pore channel was about 68 nm when calcium was present, but decreased to about 34 nm after treatment with [Ins(1,4,5)P₃]. This conformational change was compared to the iris of the eye. Perez-Terzic *et al.* suggested that diffusion of molecules was further restricted by the appearance of the central plug that they compared to a caged ball valve.

This clearly represents strong morphologic evidence for the physical restriction of molecules diffusing through the nuclear pore. Perez-Terzic *et al.* suggest that this plugging mechanism of the nuclear pore can operate *in vivo* and their evidence is quite compelling. As they point out, the major challenge will be to determine the purpose and physiologic context of this gating mechanism. ■

1 The author gratefully acknowledges David Clapham for reviewing this article. I also thank David for many interesting and educational discussions during our years together at Mayo Clinic, and I extend warm wishes to him for success in his new position at Harvard University.

2. Perez-Terzic, C., J. Pyle, M. Jaconi, L. Stehno-Bittel, and D.E. Clapham, Conformational states of the nuclear pore complex induced by depletion of nuclear CA²⁺ stores, *Science* 273: 1875-1877, 1996.

Front Page Image

The Translating Ribosome: Phosphorus Imaging by EM

Montage of merged reconstructions from electron spectroscopic images of the *Escherichia coli* ribosome showing the putative positions of two aminoacyl tRNAs, the putative path of the mRNA, and the putative path of the nascent polypeptide chain. Panels (a-d) are views of the full complex related by a 90° rotations about the vertical axis. Panels (e,f) are both top views, differing only in that (f) is a cutaway view of the ribosomal reconstruction depicted as a solid surface. Panel (f) thus illustrates the interior cavity in which the tRNAs are contained. The colour scheme is as follows: blue mesh or surface - large subunit, 150±8 eV loss; green mesh or surface - small subunit, 150±8 eV loss; orange surface - small subunit, NetP; red surface - large subunit, NetP; purple ribbon - mRNA; orange ribbon - nascent polypeptide; magenta tRNA - aminoacyl tRNA; yellow tRNA - peptidyl tRNA; brown tRNA - exit tRNA. The ribbons were generated using INSIGHT II user-defined functions, and the tRNA molecules were obtained from the Protein Data Bank (Brookhaven, New York; access code 1TRA).

For further information, review the article *Probing Ribosomal RNA By Electron Spectroscopic Imaging and Three-Dimensional Reconstruction* on page 10 of this issue.

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Don Grimes, Editor