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Mapping out the nuclear pore complex

In 2000, a first-of-its-kind study by Rout et al. provided a comprehensive survey of the nuclear pore's composition and architecture.

The nuclear pore complex (NPC) mediates transport between the nucleus and cytoplasm. By the late 1990s, components of the NPC, known as nucleoporins, were being discovered at a steady rate, but researchers believed that many more might remain to be identified. After all, a ribosome consists of ∼75 proteins, and an NPC, with a molecular weight of ∼50 MDa, is more than ten times as large. Still less was understood about the mechanisms underlying the NPC's function. Though proteins and RNA were known to be escorted through NPCs by dedicated transport factors, how the NPCs facilitated their passage was unclear. Did the pores dilate to permit cargo transport? Was cargo moved through the pores by motor proteins?

Mike Rout and his colleagues Brian Chait and John Aitchison realized that it was time to draw a comprehensive map of the nucleocytoplasmic transport route. As the researchers would later write in their 2000 *JCB* paper describing the NPC's composition and architecture (1), "The functions of the NPC arise from the complex overlapping contributions of individual [nucleoporins]; hence, a comprehensive approach is essential to understanding the mechanism of nucleocytoplasmic transport." "Looking back," says Aitchison, who now works at the Seattle Biomedical Research Institute and the Institute for Systems Biology, "this represented an early example of a systems biology approach."

"It was the coming together of several new technologies that allowed us to take on this behemoth of a project," Rout, from The Rockefeller University in New York, recalls. "It would have been untenable just a few years before." One of those innovations was the ability to purify large amounts of yeast NPCs using a method that Rout had developed as a postdoc in Günter Blobel's laboratory (2), adapted from nuclear fractionation methods worked out with John Kilmartin (3). Improved mass spectrometry techniques, pioneered by many researchers including Rout's Rockefeller University colleague Brian Chait, were also crucial to identifying the proteins in the purified NPCs. "Mass spectrometry is taken for granted now," says Rout, "but it took us well over a year and a half to complete the analysis." "We spent night after night in front of the computer, going through spectra and teasing out everything in there," adds Chait.

Finally, using the genomic tagging techniques that Rout had developed in collaboration with Aitchison, the researchers determined which of the candidate proteins identified by mass spectrometry were indeed nucleoporins and where they localized within the NPC. "There were some significant surprises," says Rout. "It turned out that the field had already found most nucleoporins. You don't need more than around 30 proteins to make an NPC." This relatively small number of components can assemble into such a large complex because multiple copies of individual nucleoporins are symmetrically arranged throughout the structure.

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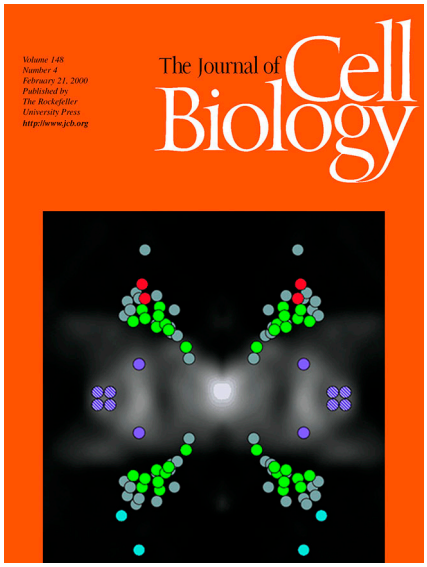
Importantly, none of the components were motor proteins or anything similar, indicating that the NPC isn't an active mechanical gate that opens up to permit the passage of nucleocytoplasmic cargo and that this cargo isn't pushed or pulled through the pore by an active mechanism. Instead, Rout et al.'s map showed that the NPC's central channel is filled and flanked by a large number of nucleoporins containing phenylalanine-glycine (FG) repeats, which had been previously shown to act as docking sites for nucleocytoplasmic transport factors. "The model suggested that the NPC is a virtual, rather than a mechanical, gate," Rout explains. "The FG nucleoporins densely fill the channel and prevent passive diffusion. But transport factors that can bind to the FG repeats are drawn into the NPC, which offsets the difficulties they have in passing through." Rout says that the concept of a virtual gate is now generally accepted, although the field still requires atomic-scale information about the dynamic behavior of FG repeats and their interactions with transport factors in order to understand how the NPC works at the molecular level.



Rout et al.'s 2000 paper provided a comprehensive map of the composition and architecture of yeast nuclear pore complexes.

To this end, Rout and colleagues have continued to refine their maps of the NPC, incorporating functional and structural data to build a detailed picture of the complex's inner workings (4–6). But the field, in turn, continues to enter uncharted territory. "It's emerged that the NPC does a lot more than nucleocytoplasmic transport," says Rout. "It's the nexus of a huge number of activities, including gene regulation and transcript processing." Accordingly, nucleoporins are differentially expressed in numerous cancers and are specifically targeted by invading pathogens.

"Everyone involved thinks that this is likely still the tip of the iceberg," Rout explains. "It's much more complicated than we anticipated in 2000. There's still plenty to explore." And it's a journey that will no doubt be easier with maps in hand.

1. Rout, M.P., et al. 2000. *J. Cell Biol.* 148:635–651.
2. Rout, M.P., and G. Blobel. 1993. *J. Cell Biol.* 123:771–783.
3. Rout, M.P., and J.V. Kilmartin. 1990. *J. Cell Biol.* 111:1913–1927.
4. Alber, F., et al. 2007. *Nature*. 450:683–694.
5. Alber, F., et al. 2007. *Nature*. 450:695–701.
6. Fernandez-Martinez, J., et al. 2012. *J. Cell Biol.* 196:419–434.