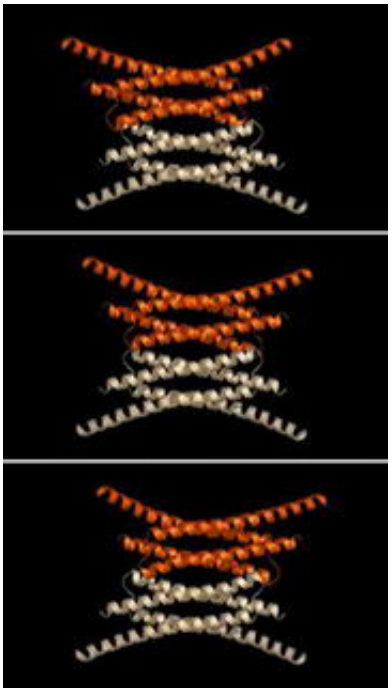
Building the nuclear pore piece by piece

April 16 2007



New depictions of proteins in the nuclear pore complex, which controls how materials enter and leave the nucleus, suggests that they move along a sliding track. The arrangement may help the nuclear pore accommodate cargo in a range of sizes.

The nuclear pore complexes are the sole gatekeepers for the cell's nucleus — proteins, RNA, viruses, anything that passes between the nucleus and the rest of the cell has to use one of these giant protein assemblies. But exactly how each of the almost 2,000 pores that are embedded in the nuclear membrane control this transport has so far remained largely mysterious. It's a critical gap in our knowledge; because

the nuclear pore is the only way in or out of the nucleus, the cell is in dire straits when the pore malfunctions, as in forms of leukemia where nuclear pore complex proteins are mutated.

Two new structural renderings of nuclear pore complex proteins, however, created in the Nobel Prize-winning lab that has worked for nearly 40 years on understanding how proteins are transported within cells, are beginning to shed light on this puzzling assembly.

Each nuclear pore is a portal running between the nucleus, which holds all of the cell's DNA, and the rest of the cell, which contains much of the cell's machinery and can communicate with the outside environment. The core of the structure is a symmetrical tunnel, each end of which is studded with an assortment of "gatekeeper" proteins. These gatekeeper proteins — there are about 30 different kinds found in various combinations — join to form some of the largest protein complexes within any cell. The sheer size of the nuclear pore makes the task of isolating its components, and studying their functions, daunting.

To tackle this problem, members of Günter Blobel's Laboratory of Cell Biology have designed an approach where the nuclear pore complex is broken into smaller, more manageable pieces whose structures can be solved using x-ray crystallography. The entire atomic model of the nuclear pore complex could be built, like a puzzle, by fitting the smaller structures together.

However, when Ivo Melcák, a research associate, and André Hoelz, a postdoc, looked carefully at the crystal structure of two of these components, mammalian proteins called Nup58 and Nup45, they were surprised by what they found: the two proteins in four different conformations. Crystallography usually shows a protein, or proteins, in their most common state; so how could there be four?

The researchers took a closer look at the amino acids that were interacting on each of the two proteins. On each side of the two proteins they saw a long line of charged residues that could interact like a series of magnets. The two proteins could loosely associate with each other in any of a variety of ways, with no one way more common than any other.

“Crystallization is like taking photographs,” says Hoelz, “trapping the protein in a single state. Our crystals show these proteins being very dynamic and moving around.” In fact, when they arranged the different snapshots one after another, like a flipbook, they saw that the Nup58/45 proteins were actually sliding back and forth along each other. The four conformations they had seen were different positions along the slide.

The scientists calculated that one pair of proteins could slide a large distance. Nup58 and Nup45 are also two of the most abundant proteins in the nuclear pore complex — they line much of the central channel. If they each slide a long way, it would suggest that the nuclear pore could change the size of its central channel drastically, like a camera aperture. In this way, the pore could accommodate both small and large cargo passing through the channel. It is the first time this type of movement has ever been documented.

During this time, Hoelz was also working with Johanna Napetschnig, a graduate student in Blobel’s lab, to crystallize a different nuclear pore complex protein, Nup214. The two researchers compared their structure of mammalian Nup214 to the structure of the same protein in yeast and saw for the first time how different these homologous proteins can be in how their core is decorated. The different decorations of the protein allow for many types of regulation in mammalian cells that would not be required in yeast, such as during cell division, where the mammalian, but not yeast, nuclear pore complex disassembles and then reassembles at the completion of the cell division.

The scientists hope that their understanding of the Nup214 structure will eventually shed light on its functional role in the pore and in cancer — particularly acute myeloid leukemia, which is linked to mutations in Nup214. “If you don’t know the structures of these proteins,” says Blobel, a Howard Hughes Medical Institute investigator and the university’s John D. Rockefeller Jr. Professor, “you will never be able to figure out which regions are important for regulation. These structures will provide crucial mechanistic insight into transport between the cytosol and the nucleus, shedding light on the role of specific proteins in diseases like cancer.”

Citations:
Science 315(5819): 1729-1732 (March 23, 2007)
Proceedings of the National Academy of Sciences 104(6): 1783-1788 (February 6, 2007)

Source: Rockefeller University