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Nuclear Pore Complex Structure: Unplugged and Dynamic Pores

Nuclear pore complexes (NPCs) are large protein assemblies embedded in the nuclear envelope that act as passageways for transport of molecules into and out of the nucleus. Two new studies, one in *Nature Cell Biology* (Rabut et al., 2004) and one in *Science* (Beck et al., 2004), offer direct evidence that the NPC is a highly dynamic structure.

All molecular traffic between the nucleus and the cytoplasm travels through the nuclear pore complex (NPC). Extensive studies have revealed the protein composition and structure of the NPC. The mammalian NPC is composed of 30 different proteins (nucleoporins) that are arranged in an octagonal structure consisting of a massive membrane embedded ring (the lumenal spoke ring) sandwiched between two thin rings (cytoplasmic and nuclear rings). Eight short filaments protrude from the cytoplasmic ring into the cytosol, and a basket-like structure is attached to the nuclear ring. The lumenal spoke ring contains a central channel of ~50 nm in diameter through which molecular transport occurs. This central channel is often plugged with a particle called the central plug or transporter, which is a controversial component of the NPC. The central plug has been hypothesized to represent material in transit rather than a permanent component of the NPC.

Thanks to the improvements in electron microscopy (EM) methods and instrumentation, the structure of the NPC has been refined over the years. Five different three-dimensional (3D) reconstructions have revealed the fine structure of the NPC (Hinshaw et al., 1992; Akey and Radermacher, 1993; Yang et al., 1998; Stoffler et al., 2003; Beck et al., 2004). While previous 3D reconstructions have been calculated from isolated, detergent-extracted NPCs or from isolated nuclear envelopes, the new 3D reconstruction by Baumeister and coworkers (Beck et al., 2004) has been generated from electron tomography of frozen-hydrated intact *Dictyostelium* nuclei. These isolated nuclei were shown to be transport competent, and they have thereby opened the exciting possibility of determining the structure of the NPC as it is transporting a cargo. In addition, the steps of nuclear transport can be blocked, and cryo-electron tomographic reconstructions can be conducted to reveal transport-related configurations. *Dictyostelium* also has the advantage of allowing one to combine genetic, structural, and functional analyses.

The novel 3D reconstruction by Beck et al. (2004) is consistent with previous reconstructions (Yang et al., 1998; Stoffler et al., 2003) and reveals similar features of the NPC structure. However, due to further improvements of cryo-electron tomography pioneered by the Baumeister laboratory, a higher resolution of 8–9 nm was achieved. These improvements include increasing the angular range and decreasing the tilt increments for the collection of images. They also used anisotropic algorithms and procedures for alignments and averaging that accounted for the missing cone (i.e., images cannot be collected by tilting the specimen more than 63° clockwise or counterclockwise). In addition, Beck et al. (2004) took only identical images for their calculations. This yielded a 3D model of the NPC that is less elongated than previous models. Thus, to go from the tip of the cytoplasmic filament to the distal ring of the nuclear basket, a cargo has to travel only ~120 nm instead of the ~200 nm it has been assumed to travel before.

The new 3D reconstruction revealed the cytoplasmic filaments for the first time. They look extremely kinky but shorter (35 nm instead of 50 nm) and more delicate than it had been assumed before. In addition, Beck et al. (2004) could identify two distinct structural states of the NPC. One is with the cytoplasmic filaments bent toward the central channel of the NPC and with a central particle in the same plane as the tip of the filaments. Thus, the filaments appear to be interacting with this central particle (similar to images that showed filaments ushering cargo into the central channel; Panté and Aebi, 1996). In the second structural state depicted by Beck et al. (2004), the cytoplasmic filaments were more disorganized and probably in an extended configuration. The central particle was located inside the central channel.

Based on these two models and the fact that the size, shape, and position of the central particle was highly variable, Beck et al. (2004) concluded that the central particle most likely represents cargo complexes in transit. Based on their 3D reconstruction and their data using atomic force microscopy, a similar conclusion was draw by Stoffler et al. (2003). NPCs without central plugs will account for images in which large cargo (for example, hepatitis B capsids) is seen within the central channel (Panté and Kann, 2002; Rabe et al., 2003). Thus, we now

have an unplugged and dynamic model of the NPC ready to test the various debated NPC translocation models.

Another recent study has also revealed that the NPC is a highly dynamic structure. Rabut et al. (2004) measured the dissociation rate of nucleoporins from NPC in living cells. For their study, 19 of the 30 nucleoporins were tagged with green fluorescent protein (GFP) and expressed in mammalian cells. Monoclonal cell lines expressing GFP-tagged nucleoporins were then analyzed by inverse fluorescence recovery after photobleaching (iFRAP). For this technique, most of the cells were bleached, and the loss of fluorescence in the unbleached region was recorded over time. Using a simple model of nucleoporin association with the NPC, Rabut et al. (2004) then calculated the rate of dissociation of nucleoporins from the NPC for the 19 nucleoporins. Their analysis yielded three dynamic classes of nucleoporins: scaffold, adaptor, and dynamic nucleoporins. The scaffold nucleoporins (10 of the 19 nups studied) were very stable proteins with extremely low dissociation rates; they correspond to the nucleoporins located in the lumenal spoke ring. The adaptor nucleoporins (6 of the 19 nups studied) were mobile with dissociation rates intermediate between the scaffold and dynamic nucleoporins. The dynamic nucleoporins (3 of the19 nups studied) were highly mobile with high dissociation rates. It was surprising to find the gp210 nucleoporin in the later group. This nucleoporin is located in the lumenal spoke ring of the NPC with most of its mass in the perinuclear space. The iFRAP data for the other two dynamic nucleoporins (Nup50 and Nup153) were consistent with a model in which the NPC has two binding sites for each of the nucleoporins. Having nucleoporins that can dissociate from the NPC would be in favor of a mechanism in which these nucleoporins can bind and escort cargo during nuclear transport.

The work of Beck et al. (2004) and Rabut et al. (2004) demonstrates that the NPC is not a stationary structure. Particular components of the NPC are able to change their morphology and several nucleoporins are mobile. With this new knowledge of the NPC structure and dynamics, scientists will now be able to test models for nuclear transport.

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On the InterAktion between Hexokinase and the Mitochondrion

The protein kinase Akt is now well recognized as a potent inhibitor of apoptosis. Work published by Majewski et al. in the December 3rd issue of *Molecular Cell* indicates that a major pathway by which Akt suppresses cell death is by stimulating the translocation of hexokinase to the mitochondrion. Hexokinase, in turn, antagonizes the release of mitochondrial cytochrome C.

One of the remarkable properties of higher eukaryotic cells is their capacity to command not only elaborate mechanisms for maintaining survival but also equally intricate strategies for committing suicide. This range of cellular actions can be thought of as a continuum of response to stress, in which the cell first uses its resources to adapt to trauma, but failing that, incites a deliberate self-demise. This view predicts an intimate relationship between survival and metabolism such that when the latter is sufficiently disrupted, "programmed" cell death ensues. The mitochondrion serves as the central integrator of metabolism and apoptosis as it continually assesses the cellular energy state or presence of signals influencing survival and acts in response to these cues in a dominant manner. The serine-threonine protein kinase Akt, also known as protein kinase B, is a potent antagonist of cell death but also mediates many of the metabolic actions of the anabolic hormone insulin, implicating similar signaling pathways in the regulation of apoptosis and metabolism (Cho et al., 2001; Datta et al., 1999). In the December 3rd issue of *Molecular Cell*, Majewski et al. provide compelling evidence that a major mechanism by which Akt suppresses apoptosis as initiated by the mitochondrion is by eliciting the translocation of hexokinase to this organelle (Majewski et al., 2004).

Hexokinase catalyzes the initial step in intracellular glucose metabolism. Though often associated with regulation of glycolysis, phosphorylation of glucose by hexokinase also determines flux into pathways leading to glycogen synthesis and the hexose monophosphate shunt (pentose phosphate pathway). Over 40 years ago, it was recognized that a substantial portion of hexokinase exists in the cell associated with mitochondria (Wilson, 2003). In this location, hexokinase preferentially utilizes as substrate ATP produced by oxidative phosphorylation rather than relying on cytoplasmic nucleotide. This makes wonderful sense, since when (for example) hexokinase is needed to initiate the replenishment of depleted energy, its substrate ATP might well be low in the cytoplasm. Hexokinase binds to the outer