Review

# The nuclear pore complex

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## Abstract

Nuclear pore complexes, the conduits for information exchange between the nucleus and cytoplasm, appear broadly similar in eukaryotes from yeast to human. Precisely how nuclear pore complexes regulate macromolecular and ionic traffic remains unknown, but recent advances in the identification and characterization of components of the complex by proteomics and genomics have provided new insights.

The nuclear pore complex (NPC) forms the conduit for the exchange of information between the nucleus and cytoplasm. NPC structures of amazingly similar appearance have been identified in all eukaryotes from yeast to human, yet differences in NPCs between species are likely to provide important clues to NPC function. As the exclusive site of macromolecular traffic between the nucleus and cytoplasm, the NPC provides an important control point for the regulation of gene expression. Precisely how the NPC regulates the traffic of ions and macromolecules remains a topic of speculation and has become the primary research focus of a number of laboratories in recent years. Recent advances towards the identification and characterization of all NPC components by proteomics and genomics, as well as studies of NPCs *in vitro,* have propelled the field rapidly forward, and are discussed in this article.

## The structure of the nuclear pore complex

Nuclear pore complexes are proteinaceous structures embedded in the double membrane of the nuclear envelope. In order to understand how the NPC functions, it is useful to examine the similarities and differences between NPCs from the yeast *Saccharomyces cerevisiae* and the frog *Xenopus laevis*. The NPC is a large structure with a molecular weight of approximately 125 MDa in vertebrates and 66 MDa in yeast [1,2]. A vertebrate cell nucleus contains on the order of 2,000 NPCs, whereas the smaller yeast nucleus contains approximately 200. NPCs have eight-fold rotational symmetry through the central axis of the pore and two-fold mirror symmetry through the plane of the nuclear envelope, suggesting assembly as a modular structure, a notion that is supported by structural and biochemical analysis of pore complex assembly *in vitro* [3,4].

In addition to their difference in size, yeast NPCs (yNPCs) and vertebrate NPCs (vNPCs) differ in several fundamental structural features. The main mass of the vNPC is contained in a three-part structure that surrounds and supports a central transporter (see Figure 1a) [1,5,6]; the three-layered structure is composed of thin cytoplasmic and nuclear rings that sandwich a central spoke domain. The spoke domain is itself also composed of an inner ring element that surrounds a structure called the central transporter and an outer lumenal ring that interacts with the nuclear membrane; vertical spoke elements connect the cytoplasmic and nuclear thin rings to the central spoke domain. Extending from the cytoplasmic thin ring are eight filaments, each of 2-3 nm in diameter and approximately 50 nm in length. The nucleoplasmic side of the NPC is comprised of eight 100 nm filaments that join at a smaller ring structure, forming a fish-basket-like structure emanating from the nuclear thin ring (Figure 1a).

The yNPC is much simpler in structure (Figure 1b) [5]. Compared to the vNPC, it lacks the cytoplasmic and nuclear thin rings and the lumenal ring of the central spoke domain. Instead, the central transporter is encircled by an inner spoke ring and a membrane-associated ring. Because they

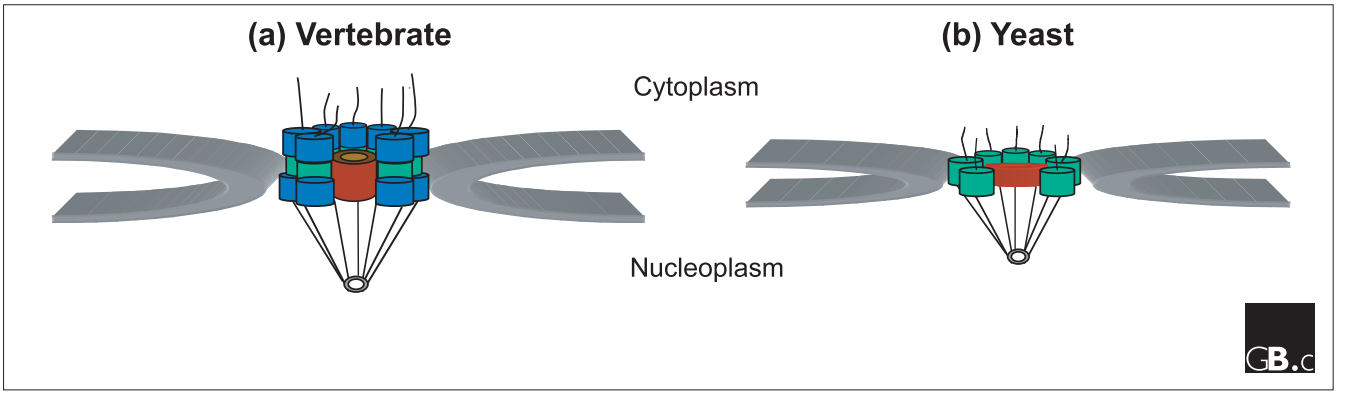


**Figure 1**
A comparison of the structures of **(a)** vertebrate and **(b)** yeast nuclear pore complexes (NPCs). The spoke complex of the vertebrate NPC is composed of three sections: cytoplasmic and nuclear rings (in blue) and the central spoke ring (green; some details of the spoke ring are omitted from the figure, for simplicity, but are described in the text). The yeast NPC lacks the cytoplasmic and nuclear rings. The red structure in the middle of each NPC is the central transporter. The relative sizes of each component are approximate.

lack the outer thin rings, yNPCs also lack parts of the spoke domains that are present in vNPCs. Like vNPCs, yNPCs have filamentous structures emanating from the cytoplasmic and nucleoplasmic faces of the complex, although in yeast these filaments are slightly shorter than in vertebrates [7]. Overall, the yNPC is smaller (96 nm diameter by 35 nm high) than the vNPC (145 nm diameter by 80 nm high), has half the mass of the vNPC and occupies one fifth of the volume.

## Transport through the NPC

Permeability studies have demonstrated that the NPC forms a selectively permeable barrier through the nuclear envelope. Inert polymers and small proteins less than 9 nm in diameter or less than 30-40 kDa in mass can freely diffuse through the NPC [8]. Larger particles traverse the NPC by a facilitated mechanism that is still poorly understood [9,10], and the NPC can accommodate the transport of particles as large as 25 nm in diameter. Cryo-electron microscopy images of NPCs indicate that their structure can expand radially to accommodate the passage of larger particles, suggesting that a gating mechanism may be built into the pore [5,11]. Direct observation of particles in transit through the NPC confirmed that the central transporter structure forms the pathway for macromolecular traffic [12]. Different sites for diffusion channels have been proposed, but no direct observation of diffusion has been made [4]. Although many experiments indicate that the NPC forms an aqueous channel between the nuclear and cytoplasmic compartments, patch-clamp techniques suggest that pore complexes may actually be regulated channels that behave much like ion channels that can open and close [13].

The bulk of macromolecular traffic through the NPC is mediated by a system of mobile transporter proteins, distinct from the NPC itself, that act together to move molecules between the nuclear and cytoplasmic compartments. The mobile transporters that directly bind molecules to be transported are members of a family of proteins that share common features, the founding member of the family having been importin β, also known as karyopherin β [14]. The mobile transporter proteins all have molecular masses between 90 and 130 kDa, with the bulk of each protein folded into a series of helical HEAT repeats. HEAT repeats are tandemly repeating 40-50 amino-acid motifs that form interactive surfaces in a number of proteins [15]. The transporter HEAT repeats form two domains, an amino-terminal half that binds the small GTPase Ran when it is associated with GTP and a carboxy-terminal half with a cargo-binding domain. It is this domain structure that allows each transporter to function as either an import receptor or an export receptor: export receptors bind their cargoes cooperatively with Ran-GTP, while import receptors release their cargoes when bound to Ran-GTP. Ran is thus the key component that defines compartment identities for transport. The nuclear localization of the Ran guanine-nucleotide exchange factor, RanGEF, is believed to maintain nuclear Ran in a GTP-bound form. A Ran GTPase-activating protein (RanGAP) localized to the cytoplasmic face of the nuclear pore ensures that any Ran in the cytoplasm will be bound to GDP. Other small Ran-binding proteins are involved in modulating GTP hydrolysis or the transport of Ran from the cytoplasm to the nucleus. The enrichment of RanGTP-binding mobile transporter proteins in the nucleus has also been suggested to contribute to the nuclear abundance of Ran [16].

A key step in transport is the interaction of the mobile transporter protein with nucleoporins, the structural proteins of the pore complex. These interactions are believed to be the

basis for the selective transport of macromolecules through the pore. Many of the nucleoporins involved directly in transport share repeats containing phenylalanine-glycine dipeptides and are known as FG nucleoporins [17]. The FG repeats interact with specific HEAT repeats of the mobile transporter [18]. Although this is a relatively weak interaction, each of the FG nucleoporins contains multiple FG repeats that could provide multiple interaction sites for a transporter. A number of studies suggest that different transporters follow distinct pathways through the NPC, although some of these pathways have several nucleoporins in common [19-21]. In *S. cerevisiae* 14 transporters have been identified in the genome; 9 have been shown to function as importers and 4 as exporters [14]. At least one mobile transporter, Kap124/Msn5, may have a dual role in import and export, but most are believed to be specific for either import or export [22]. There are at least 22 transporters in human cells, so it is likely that several of these will not have a counterpart in yeast [23].

Which transporters interact with which nucleoporins has been an important question from the beginning. An early model for translocation of proteins through the NPC suggested that the asymmetric arrangement of nucleoporins in the NPC formed an affinity gradient along which a mobile transporter could move by diffusion, with repeated association and dissociation from nucleoporins [24]. A recent examination of the affinity of a transport complex for immobilized nucleoporins lends support to certain aspects of this mechanism [25]. In a more comprehensive proteomics analysis, Rexach and coworkers [19] have identified the transporters, as well as other proteins, that interact with 8 of the 13 yeast FG nucleoporins. The results suggest that some nucleoporins interact with as many as 10 different transporters while other nucleoporins are selective for the Kap95p-Kap60p mobile transporter complex.

## Analysis of nuclear pore complex structure and composition

Early estimates of the protein complement making up the pore complex placed the total number of individual protein species at between 100 and 200. This estimate was based solely on the estimated mass of the pore (125 MDa), the eight-fold radial and two-fold mirror symmetry of the pore, and the estimated average size of a nucleoporin (100 kDa). Since the first structural features of the pore were identified, it has been a goal in the field to identify all of the pore complex components and to determine how individual nucleoporins interact to form the functional structure. Over several years, a number of groups have identified subcomplexes of nucleoporins that could be localized to the central transporter, the cytoplasmic filaments or the nuclear baskets [26].

A major leap forward in the analysis of nucleoporins and NPC structure was the result of a convergence of two lines of research. First, completion of the yeast genome made it possible to identify likely NPC proteins by sequence similarity to known nucleoporins. Second, purification of the yNPC by Rout and colleagues made possible the potential identification of all pore-complex proteins by a proteomics analysis [2,27]. Surprisingly, they found that the yNPC is composed of only about 30 proteins, far fewer than the original estimates, with each protein present in 8 to 56 copies per pore. Localization of all 30 proteins by protein-A tagging or immunolocalization with specific antibodies yielded yet another surprise: the majority of yeast nucleoporins are fairly symmetrically distributed on both the cytoplasmic and nucleoplasmic sides of the pore. The antibody localization techniques used can only identify nucleoporins at the exposed edges of the pore complex, however, and in fact, the bulk of the pore scaffold is not well-represented in this localization study.

Only five nucleoporins were found exclusively on either the nuclear or the cytoplasmic side of the pore complex, while another four nucleoporins have a 'biased' localization, predominating on one side or the other. Notably, seven of these nine nucleoporins contain FG repeats, and their localization to peripheral structures of the NPC places them in ideal positions to interact with the mobile transporters. In a more limited study, Aebi and colleagues [7] used pre-embedding immunogold electron microscopy to localize Nsp1p and its interacting nucleoporins to three distinct subcomplexes within the NPC. Taken together with the study by Rout and colleagues [27], these results would suggest that although individual nucleoporins are distributed throughout the pore complex, they might be present in different complexes with other nucleoporins in each pore-complex substructure, generating a functional asymmetry. On the other hand, localization of several vertebrate nucleoporins suggests that the localization of nucleoporins in the vNPC is much more asymmetrical than in the yNPC [10,17]. It had long been assumed that vectorial nature of import and export was due in part to the asymmetrical distribution of nucleoporins. Whether this asymmetry is necessary only for the docking of transport complexes at the pore or is also required for translocation through the pore remains a matter of speculation.

## Evolutionary conservation of nuclear pore complexes

The vNPC might be expected to be different in many ways from the yNPC. In addition to the structural differences in the NPCs, as discussed above and shown in Figure 1, differences in yNPCs and vNPCs also are likely to be reflected in NPC function. A major difference between yeast cells and those of other eukaryotes is that the yeast nuclear envelope does not have a nuclear lamina, the intermediate-filament-containing structure underlying the nuclear membrane; several lines of evidence suggest an interaction between the NPC and the lamina in other cells [28-30]. Budding yeast

cells do not break down the nuclear envelope in mitosis, nor do the NPCs disassemble as they do in vertebrates. These two structural differences are likely to be reflected in the protein composition of the pore in the two types of cell. Structural analyses of vNPCs and yNPCs suggest that each interacts differently with the nuclear membrane [5]. NPCs are anchored to the nuclear membrane by integral membrane proteins. Four integral membrane proteins are present in the yNPC, while only two have been identified in the vNPC thus far [10,27]; and, significantly, neither of the two vertebrate proteins is related to the yeast proteins. The differences in the way NPCs are anchored to the membranes between yeast and vertebrates seems likely to be related to the dynamics of the NPC during the cell cycle.

The identification of vertebrate nucleoporins has lagged behind that of yeast because of the difficulty in isolating a pure population of NPCs from vertebrate tissues. The close association of the vNPC with the underlying lamina means that many non-nuclear-envelope proteins will be present in any preparation. Efforts to wash or extract isolated nuclear envelopes to remove this contaminating material also remove pore complex proteins, making the purification difficult. Recently, Miller and Forbes [31] have overcome this problem by taking advantage of specialized structures called annulate lamellae that are present in some metazoan cells. Annulate lamellae are flattened sacks of double membranes with tightly packed arrays of pore complexes that are found in differentiating and rapidly growing cells [32]. These structures can be assembled *in vitro* in large quantities from *Xenopus* egg extracts, and can thereby be purified. Two-dimensional gel analysis of such a purified preparation suggested that the majority of vNPCs contain only 40-50 individual protein species, a number remarkably similar to that of the yNPC despite the larger size of the vNPC.

When the known yeast nucleoporins are compared to the vertebrate nucleoporins whose sequences are known, there is surprisingly little homology between the proteins. Only about half of the vertebrate nucleoporins have clear yeast homologs identifiable by sequence, function and localization [10,27]. Several others have weaker or very weak similarity in sequence. In many cases, the only similarity between proteins is the presence of FG repeats. At least two vertebrate nucleoporins, Nup153 and Nup358, contain zinc-finger domains, whereas none of these have been found in yeast nucleoporins [33,34]. Another major difference is the lack of yeast nucleoporins containing Ran-binding domains. Nup2p is the only yeast nucleoporin with a Ran-binding domain, although its role as an authentic nucleoporin has been questioned [27,35]. The vNPCs, on the other hand, contain at least two Ran-binding nucleoporins, Nup358 and Nup153, both of which are implicated in initial or terminal events in import and export [34,36]. Whether this difference represents a fundamental difference in the mechanisms of transport between yeast and vertebrates is unknown. Mass spectrometric analysis of the proteins in the purified *Xenopus* NPC will provide an important body of data in the analysis of NPCs.

A useful approach aimed at identifying functional nucleoporin homologs between yeast and *Xenopus* has been to analyze the composition of subcomplexes of nucleoporins. Such an approach is not only helpful for identifying components of a complex, but when coupled to chemical cross-linking it can also be used to analyze the spatial organization of the complex. In yeast, this approach has been widely used because a tagged version of a nucleoporin gene can substitute for the wild-type untagged gene in the genome. The tagged protein can then be isolated by affinity chromatography from a yeast lysate and co-adsorbing proteins identified from gels by mass spectroscopy. The utility of chemical cross-linking for analyzing complexes was recently demonstrated by Rappsilber and coworkers [37], who found that complex components could be identified by mass spectrometry even after chemical cross-linking to preserve large complexes during extraction. Now that all of the yeast nucleoporins encoded in the genome have been identified, the interactions between them within the NPC can be mapped by biochemical methods.

NPC subcomplexes have been identified in vNPCs by means of either chemical extraction of purified nuclear envelopes or the separation of the subcomplexes formed during NPC breakdown at mitosis [10]. Because it is not feasible to substitute a tagged version of a vertebrate nucleoporin gene into the genome, introduction of a tagged or modified nucleoporin to the NPC has been limited to a nuclear reassembly assay in *Xenopus* egg extracts [38]. Although tagged nucleoporins can be reconstituted into the extracts and assemble into NPCs, the NPC in a reassembled nucleus is attached to the lamina and is contaminated with chromatin and other nuclear components. Recently Forbes and coworkers [39] overcame these difficulties by developing a two-step 'organelle trap' assay. In this assay, soluble proteins from a *Xenopus* egg extract are applied to an affinity column containing a ligand of interest. The bound proteins are eluted, biotin-tagged and reconstituted into the NPCs of annulate lamellae assembled *in vitro*. Since the annulate lamellae do not have a lamina, the NPCs are easier to dissociate. Using this approach, a novel vertebrate nucleoporin, Nup188, was identified, and it was found to be complexed with two other known nucleoporins; both of these have yeast homologs, and both yeast homologs are found to form a complex with yeast Nup188. Although the yNup188 and vNup188 have limited sequence similarity, the presence of the two proteins in similar complexes suggests that they and their associated proteins form a conserved subcomplex within the NPC. This method can now be used to rapidly identify unknown vertebrate nucleoporins and map their interactions.

Given the differences and similarities between the proteins of the yeast and vertebrate NPCs, what evolutionary forces have

driven all eukaryotes to maintain structures of such structural and functional similarity? Answers to this question will come only from complete analysis of pore complexes from a number of species. The structure of the NPC from *Chironomus* has been examined and suggests a strong conservation of structures between vertebrates and invertebrates [40]. Analysis of the NPCs in genetic model organisms such as *Drosophila* and *Caenorhabditis elegans* has to date lagged behind that of mammalian and amphibian NPCs. The main reasons for this are the lack of easy methods for isolating biochemical quantities of nuclear envelopes from these organisms and the lack of cross-reactive immunological probes for nucleoporins. A simple search of the Flybase [41,42] and WormPD [43-45] databases reveals that only about ten nucleoporin homologs have been identified in each organism, either by direct localization or by sequence similarity. Recently, the nucleoporin composition in *C. elegans* was investigated with the monoclonal antibody 414 that primarily recognizes mammalian Nup62p but also recognizes several other nucleoporins [46,47]. The pattern of proteins recognized by western blotting with this antibody suggests that at least Nup62p may be conserved and that some worm nucleoporins may be significantly smaller in size than those of either yeast or vertebrates. Now that the genomic sequences of both *Drosophila* and *C. elegans* are available, these systems are ripe for analysis of the NPC by proteomics; all that will be required is the development of methods for large-scale isolation of NPCs. The ease of genetic manipulation of flies and worms could open up new approaches to study the role of the NPC in metazoans during differentiation and development, bringing new techniques to a field that has already benefited so much from the application of both traditional biochemical techniques and proteomics.

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