
The Structure and Composition of the Yeast NPC

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1 Introduction

The double-membraned nuclear envelope (NE) behaves as a selective barrier that segregates the genome from all cytosolic processes. A highly regulated exchange system between these two compartments is essential for proper cell growth, progression through the cell cycle, accurate responses to developmental and extracellular signals and to maintain the functional integrity of the nucleus. The sole mediators of controlled nucleocytoplasmic transport are the nuclear pore complexes (NPCs), large proteinaceous machineries embedded within specialized circular pores that traverse the NE. In actively growing cells it is estimated that every minute hundreds of proteins and ribonucleoprotein particles (RNPs) traverse each NPC in both directions. The basic mechanisms of nuclear transport appear to be highly conserved across distantly related species (reviewed in Nigg 1997; Mattaj and Englmeier 1998; Gorlich and Kutay 1999; Wentz 2000). Although metabolites, water, ions and small macromolecules can freely diffuse through aqueous channels of 10 nm in the NPC, large macromolecular particles with a diameter of up to 30 nm are selectively transported across the NPC via a highly regulated energy-dependent process. Active transport requires specific soluble transport factors that recognize individual substrates both inside and outside the nucleus and mediate their interaction with the stationary phase of the NPC translocation machinery. Specifically, the translocation of transport substrates is known to require the docking of the transport complex to the NPC, the active translocation of the docked complexes across the NPC and the release of the substrate into the target compartment. Various models have been proposed to explain how this docked complex is actively translocated across the 50–60 nm long NPC transporter and then subsequently released into the nucleoplasm, and the matter is still highly controversial (see below). All models agree in attributing a crucial importance to the protein Ran in maintaining vectorial cargo transport and regulating the binding and release steps that take place during translocation. As a member of the Ras superfamily of small GTPases, Ran exists within the cell in a GDP-

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bound and in a GTP-bound form. The balance between these two forms is regulated by a variety of Ran cofactors that are asymmetrically distributed within the cell. As a consequence cytoplasmic Ran is thought to exist prevalently in the GDP-bound form, while Ran-GTP is thought to predominate in the nucleus. This differential distribution of Ran-GTP versus Ran-GDP would establish directional transport by ensuring that transport complexes are formed in one compartment and disassembled in the other (reviewed in Cole and Hammell 1998; Mattaj and Englmeier 1998; Pemberton et al. 1998; Wozniak et al. 1998; Gorlich and Kutay 1999). Understanding this regulated transport demands an understanding of the detailed three-dimensional map of the NPC and of the interactions and relationships between the soluble and stationary phases of nuclear transport.

NPCs are present in all eukaryotic cells, and despite interesting differences in details, their morphology is remarkably conserved among evolutionary divergent phyla (Maul 1977; Yang et al. 1998). This makes studies using model organisms relevant to all eukaryotes. In particular, several characteristics make the yeast *Saccharomyces cerevisiae* an excellent model system to investigate the structure and composition of NPCs, and their role in nucleocytoplasmic transport. *S. cerevisiae* undergoes a closed mitosis such that the NE remains intact during cell division allowing the isolation and study of NPC components from all stages of the cell cycle. Furthermore, like many other aspects of yeast cell biology, the process of nucleocytoplasmic exchange appears to lack many of the complicated elaborations present in metazoans rendering it a much simpler model system. The genetics and molecular biology of yeast are better understood than those of any other eukaryote, and the genome is fully sequenced greatly speeding the process of identification and characterization of unknown proteins. Finally, many sophisticated cell biological and biochemical analysis techniques have been developed for this organism.

2 Overview of the Yeast NPC Structure

NPCs from all eukaryotes share a common architecture, and many NPC proteins (collectively named nucleoporins or nups) are conserved across phyla. In metazoans, the core of the NPC consists of a cylinder with a plane of pseudo-mirror symmetry running parallel to the NE, composed of eight inter-connecting spoke-like structures symmetrically arranged around a “central transporter” (or central channel; Unwin and Milligan 1982; Hinshaw et al. 1992; Akey and Radermacher 1993). Electron microscopic images of the central transporter suggest that it is a centrally tapered hollow tube that spans the entire width of the NPC (Akey 1990; Akey and Radermacher 1993; Goldberg and Allen 1996; Kiseleva et al. 1998). Functional studies employing colloidal gold particles attached to nuclear-targeted proteins indicate that the NPC has a central hole with a functional diameter of ~9 nm that allows the free diffusion of small molecules but restricts passive diffusion of macromolecules

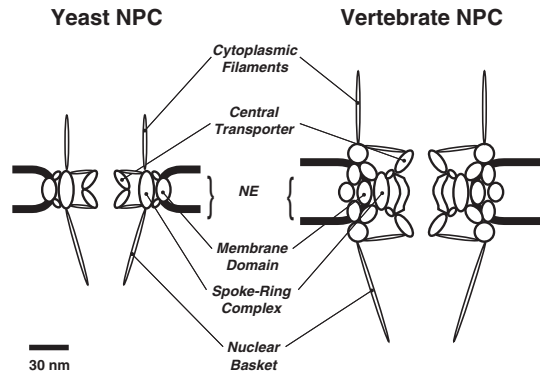


Fig. 1. Scale diagram of an idealized vertical section from a yeast NPC and a vertebrate NPC. Protein domains visualized in electron micrographs are shown as *ovals*. (Adapted from Yang et al. 1998)

through the pores. However, during active transport the central transporter can accommodate the passage of massive substrates, such as ribosomal subunits and pre-mRNPs, with a diameter up to 26 nm (Paine et al. 1975; Feldherr et al. 1984; Dworetzky and Feldherr 1988; Feldherr and Akin 1994a,b, 1997; Feldherr et al. 1998; Kiseleva et al. 1998). Peripherally associated nuclear and cytoplasmic filaments project from the core and are distinctly asymmetrical. Although the cytoplasmic filaments spread outwards perpendicularly to the central plane of the NE, the nuclear filaments conjoin to form the “nuclear fishtrap” or “nuclear basket” (Ris 1991; Goldberg and Allen 1992). Transport substrates dock to these peripheral filaments and translocate through the transporter on their way in and out of the nucleus.

The NPCs of *Saccharomyces* share many common features with their vertebrates counterparts, although they are significantly smaller both in mass and in volume (Fig. 1; Fahrenkrog et al. 1998; Yang et al. 1998). Interestingly, the differences in NPC size and mass between yeast and vertebrates can be accounted for by a concomitant simplification of the structure. In substance, the data are consistent with the hypothesis that the yeast NPC comprises only the central core of the vertebrate NPC and lacks many of the peripheral attachments including the luminal spoke ring, the nuclear ring, and the cytoplasmic ring with its attached cytoplasmic particles. Likewise, the cytoplasmic fibers and nuclear basket appear to be conserved but are anchored to more central domains of the spoke-ring assembly. The central transporter is also smaller and appears to be missing a central cylinder, which gives the vertebrate transporter its hour-glass shape (Rout and Blobel 1993; Yang et al. 1998). The results of these comparative studies suggest that the architecture of an NPC can vary considerably and still be functional. Thus, the yeast NPC is likely to have retained or recapitulated the features that characterize what a streamlined NPC might look like. Accordingly, the yeast NPC is able to ensure the efficient

exchange of material between the nucleus and the cytoplasm but apparently lacks the higher order structures necessary in multi-cellular organisms.

3 Yeast Nucleoporins: What's NUP, What's Not

A variety of immunological, biochemical and genetic techniques have been successfully employed in the past few years to identify yeast NPC components (reviewed in Rout and Wentz 1994; Doye and Hurt 1997; Fabre and Hurt 1997). Of course, given the highly dynamic nature both of NPCs and of their interactions (see below), it is not always possible to establish what constitutes a "complete NPC". On first approximation, this problem can be generally resolved by adopting an operational definition such as considering bona fide nucleoporins to be those proteins that are stably associated with the NPC. Thus, the candidate nucleoporin should immunolocalize to the NPC by immunofluorescence (IF) microscopy or better by immunoelectron microscopy (IEM), should cofractionate with the NPC in subcellular fractionation procedures, and should interact genetically and biochemically with other known nucleoporins.

3.1 General Characteristics of Yeast Nucleoporins

Recent work has allowed us to set an upper limit for the total number of nucleoporins in yeast, and establish a rough map of the distribution of all known nucleoporins in the context of the three-dimensional map of the yeast NPC (Kraemer et al. 1995; Nehrbass et al. 1996; Fahrenkrog et al. 1998; Hurwitz et al. 1998; Marelli et al. 1998; Kosova et al. 1999; Strahm et al. 1999; Bailer et al. 2000; Rout et al. 2000). The total number of bona fide yeast nucleoporins is now estimated to be ~30 (Rout et al. 2000). This is a surprisingly low number for such a massive structure, especially considering that, for example, the much smaller ribosome is composed of ~80 different proteins. This apparent discrepancy between size and composition can be resolved by considering the high level of symmetry displayed by NPCs. First, most nucleoporins are symmetrically distributed with respect to the central plane of the NE. Hence the majority of yeast nucleoporins are present in two to four copies per spoke and are therefore present in 16 to 32 copies per NPC (Rout et al. 2000). Thus, the yeast NPC appears to be composed mainly of 16 copies of a subset of nucleoporins: eight copies facing the nucleus and eight copies facing the cytoplasm. If one considers that the average molecular weight of individual yeast nucleoporins is relatively high (~100kDa), one can calculate that ~30 proteins each present in an average of 16 copies would produce a structure of ~50 MDa, thus completely accounting for the mass of the yeast NPC (measured to be between 55 and 66 MDa; Rout and Blobel 1993; Yang et al. 1998). At least 65% of yeast

S. cerevisiae nucleoporins have direct orthologs in vertebrate genomes attesting once again to the validity of this as a model system for the study of NPCs and in general nucleocytoplasmic transport processes. Yeast nucleoporins can be divided into three partially overlapping classes based on their sequence characteristics and presumed function: FG nucleoporins, non-FG nucleoporins, and pore membrane proteins (POMs).

3.1.1 FG Nucleoporins

Nearly half (12 out of 30) of yeast nucleoporins belong to the “FG nucleoporins” family (reviewed in Rout and Wentz 1994; Fabre et al. 1995). These are characterized by the presence of at least one domain containing multiple GLFG, FXFG, or FG amino acid repeat motifs separated by polar spacer sequences, and are generally thought to be filamentous in nature (Buss et al. 1994). The spacer sequences between the FXFG and the FG repeats are generally highly charged and rich in serine and threonine residues. The GLFG spacers are generally devoid of acidic residues and have a prevalence of asparagine and glutamine residues. The role of these proteins in NPC translocation has been firmly established on the basis of numerous biochemical and genetic analyses (reviewed in Doye and Hurt 1997; Fabre and Hurt 1997; Ohno et al. 1998; Ryan and Wentz 2000).

FG nucleoporins are known to provide the NPC docking sites for soluble transport factors associated to their cognate transport substrates. This was convincingly demonstrated using several methods. In vitro experiments performed using purified components have demonstrated that FG repeat motifs of various nucleoporins interact directly with members of the β -karyopherin family of transport factors and with other soluble transport factors (Rexach and Blobel 1995; Nehrbass and Blobel 1996). In addition, ex vivo biochemical studies have shown that β -karyopherins and other non-karyopherin transport factors interact specifically with FG nucleoporins or with truncated forms of these nucleoporins containing FG repeat motifs (Radu et al. 1995; Aitchison et al. 1996; Iovine and Wentz 1997; Pemberton et al. 1997; Rout et al. 1997; Katahira et al. 1999; Hurt et al. 2000). Finally, in vivo localization analyses of various reporter transport substrates in yeast strains carrying truncated or otherwise non-functional mutant forms of various FG nucleoporins have demonstrated the physiological relevance of these docking interactions to ensure appropriately regulated nucleocytoplasmic transport (reviewed in Doye and Hurt 1997; Fabre and Hurt 1997).

Although the NPCs contain an extremely high number of FG repeat motifs and there is considerable overlap between different transport factors for their ability to bind certain FG nucleoporins, individual transport factors have strong preferences for specific docking sites at the NPC (Rexach and Blobel 1995; Aitchison et al. 1996; Rout et al. 1997; Marelli et al. 1998; Floer and Blobel 1999). This might indicate that there are different transport routes across the



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