# CHAPTER 2

# Integral Proteins of the Nuclear Pore Membrane

**Merav Cohen, Katherine L. Wilson and Yosef Gruenbaum**

The nuclear envelope contains three distinct membrane domains. The outer nuclear membrane faces the cytoplasm and is continuous with the rough endoplasmic reticulum (ER). Like the rough ER, the nuclear outer membrane is covered with ribosomes engaged in translating secreted and integral membrane proteins. The inner nuclear membrane faces the nucleoplasm, has its own unique protein composition and interacts with the fibrous meshwork of the nuclear lamina (reviewed in ref. 6). The inner and outer nuclear membranes fuse to form the third membrane domain, termed the pore membrane domain. Nuclear pore complexes (NPCs) are anchored at the pore membrane domain and mediate both passive diffusion and active nucleocytoplasmic transport. Active transport requires signals on the imported or exported macromolecules, termed nuclear localization signals (NLS) and nuclear export signals (NES), respectively. Transport is mediated by soluble NLS and NES receptors (termed importins/exportins/karyopherins/transportins), whose direction of movement is determined by Ran, a small GTP-binding protein (reviewed in refs. 22, 48 and 50). NPC structure includes soluble proteins, termed nucleoporins (nups) and integral membrane proteins, termed POMs. The NPC is anchored to the pore membrane by binding to POMs.[37] POMs are also proposed to have roles in nuclear pore assembly, nucleocytoplasmic transport and NPC organization (see below).

The protein composition of the yeast NPC has been determined.[38] Yeast NPCs consist of multiple copies of at least thirty distinct proteins, with a total estimated mass of 50 MDa. The size and complexity of the NPC appears to have increased during evolution. For example, the vertebrate NPC has an estimated maximum mass of 120 MDa, with an estimated forty different proteins;[35] (reviewed in ref. 48). Many vertebrate nucleoporins have orthologs or functional homologs in yeast and plants. Overall, NPCs are significantly conserved in both structure and protein composition between yeast and humans. One possible exception to this trend are the POMs, which have no obvious similarity between yeast and vertebrates.

## Yeast POMs

Five integral membrane proteins have been localized to the pore membrane domain in the yeast *Saccharomyces cerevisiae* (reviewed in ref. 7). These five POMs are named Snl1,[29] Pom152,[55] Ndc1,[5] Pom34[38] and Brr6.[11] Yeast POMs are discussed below.

*SNL1* was identified in a genetic screen for high copy suppressors of the lethal phenotype caused by over-expression of the carboxy-terminal 200 residues of Nup116 (*NUP116-C*), in the *nup116* null background. Loss of *NUP116* function causes the nuclear membranes to

*Nuclear Import and Export in Plants and Animals*, edited by Tzvi Tzfira and Vitaly Citovsky.

herniate and cover the NPCs. Over-expression of *SNL1* also suppresses the temperature-sensitive phenotypes of mutations in two other genes:[29] *gle2*, which is essential for NPC assembly,[36] and *nic96*, which is involved in the transport of polyadenylated RNA and possibly in protein transport.[21] Cells that lack *SNL1* expression are viable, and so are double-null mutants for *snl1* plus a second POM named *pom152*. Lack of synthetic lethality between *SNL1* and *POM152* suggests a possible functional redundancy with other, possibly unidentified, POMs.[29]

A fraction of Snl1 is localized to the ER,[29] which suggests that Snl1 might shuttle between the pore membrane domain and ER. It would be interesting to compare the diffusional mobility of Snl1 at the NPC versus the ER, to determine if Snl1 proteins in the ER are actively exchanging with pore-localized Snl1.

Pom152 was identified as a glycoprotein with N-linked high mannose oligosaccharide modifications,[55] which localized to NPCs.[55] Pom152 is an integral membrane protein that spans the pore membrane once, using only one of its two predicted transmembrane domains. Its short amino tail (175 residues) faces the NPC, and its long carboxy-tail (1141 residues) is localized in the lumen space between the inner and outer nuclear membranes.[46] *S. cerevisiae* strains that lack the *POM152* gene are viable. However, mutations in *pom152* are lethal in combination with mutations in other genes, including *NUP188* and *NUP170*,[1] which are both involved in establishing the functional diameter of the NPC.[39] It is not clear why mutations in *POM152* are 'synthetically lethal' in combination with mutations in *NUP188* or *NUP170*, but these findings suggest that Pom152 might also be involved in determining the diameter of the pore or NPC. Over-expression of Pom152 reduces the growth rate of cells, for reasons not yet understood.[55]

When *POM152* is ectopically expressed in mammalian cells, it is correctly localized to the pore membrane domain.[55] This result indicates a functional conservation of the pore membrane domain between yeast and mammals. Though there is very little homology between the yeast *POM152* and the vertebrate *GP210* (see below), several characteristics are shared between these pore membrane proteins. Notably, both proteins have a predicted hydrophobic region that is not embedded in the membrane. It would be interesting to test whether *GP210* can functionally complement *pom152* mutations.

*NDC1* was originally discovered as an essential gene, which is required at a late stage of spindle pole body (microtubule organizing center) duplication.[53] Yeast spindle pole bodies are embedded in the nuclear envelope, like NPCs, and Ndc1 localizes at both types of structure. This may indicate a functional or assembly-related link between these two nuclear membrane-embedded organelles.[5] In *ndc1*-null cells, the spindle pole body fails to be inserted into the nuclear envelope, but NPCs are positioned and function normally. The lack of a nuclear transport phenotype in cells with mutations in *pom152, ndc1* or both, suggests functional redundancy between these POMs and other nucleoporins. Interestingly, in *pom152*-null cells with defective Ndc1 protein, the spindle pole bodies are again inserted into the nuclear envelope, suggesting that *pom152* mutations suppress *ndc1* mutations. Though it is not known if Ndc1 and Pom152 interact directly, it was proposed that the lack of Pom152 releases 'defective' Ndc1 molecules from the NPC, allowing them to function (weakly, but in higher numbers) at spindle pole bodies.[5]

Pom34 was recently identified by mass spectrometry as a component of biochemically-purified yeast NPCs, and localized to the pore membrane domain.[38] Biochemical extraction also revealed that *POM34* encodes an integral membrane protein. Pom34 has a predicted leucine zipper motif and two putative transmembrane domains.[38] Neither the topology of Pom34 in the pore membrane domain, nor its requirement for cell growth or viability, have been determined.

*BRR6* was identified by complementation of the *brr6* cold sensitive nuclear transport mutant.[11] *BRR6* encodes a 22.8 kDa integral membrane protein located at the nuclear rim in a