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The Conserved Transmembrane Nucleoporin NDC1 Is Required for Nuclear Pore Complex Assembly in Vertebrate Cells

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Summary

Nuclear pore complexes (NPCs) are large proteinaceous channels embedded in the nuclear envelope (NE), through which exchange of molecules between the nucleus and cytosol occurs. Biogenesis of NPCs is complex and poorly understood. In particular, almost nothing is known about how NPCs are anchored in the NE. Here, we characterize vertebrate NDC1—a transmembrane nucleoporin conserved between yeast and metazoans. We show by RNA interference (RNAi) and biochemical depletion that NDC1 plays an important role in NPC and NE assembly in vivo and in vitro. RNAi experiments suggest a functional link between NDC1 and the soluble nucleoporins Nup93, Nup53, and Nup205. Importantly, NDC1 interacts with Nup53 in vitro. This suggests that NDC1 function involves forming a link between the NE membrane and soluble nucleoporins, thereby anchoring the NPC in the membrane.

Introduction

The NE defines the boundary between the nucleus and cytoplasm of a eukaryotic cell. It is composed of two parallel membrane bilayers—the inner nuclear membrane and the outer nuclear membrane, which is continuous with the endoplasmic reticulum (ER). Both nuclear membranes are fused to form holes occupied by NPCs that mediate nucleocytoplasmic transport.

NPC structure and composition are partly conserved between yeast and vertebrates. An NPC consists of multiple copies of about 30 different nucleoporins, many of which are also members of subcomplexes that can be isolated from cell extracts. The modular organization of the NPC into subcomplexes is reflected in its three-dimensional architecture (for review see Schwartz [2005]). The assembly of nucleoporins into the NPC is a fascinating example of a process involving many different protein interactions that occur in a spatially and temporally defined order. In vertebrates, NPC assembly occurs both after mitosis, when the NE reforms, and during interphase, when NPCs have to be inserted into the closed NE.

Postmitotic NE and NPC assembly have been studied in vitro by using fractionated *Xenopus laevis* egg extracts, which assemble nuclei around sperm chromatin. Depletion experiments in this system revealed pivotal roles for two distinct sets of soluble nucleoporins. Whereas the conserved Nup107-160 subcomplex is recruited to chromatin early during mitosis, Nup155 joins the NPC later. The lack of either results in deficiencies in NPC assembly and, in the case of Nup155, also NE assembly (Harel et al., 2003; Franz et al., 2005; Walther et al., 2003). Nup155 is part of a distinct nucleoporin subcomplex in mammals that also contains Nup205, Nup93, Nup53/35, and likely Nup188 (Grandi et al., 1997; Hawryluk-Gara et al., 2005; Miller et al., 2000). Depletion of Nup53 or Nup93 from cultured somatic cells by RNAi leads to a loss from the NPC of both interacting nucleoporins and the group of mAb414-reactive nucleoporins, suggesting an important role of this nucleoporin subcomplex in NPC assembly (Hawryluk-Gara et al., 2005; Krull et al., 2004).

NPCs are assumed to be anchored in the NE by transmembrane nucleoporins, but the nature of the linkage between soluble and transmembrane nucleoporins is presently unclear. Another puzzling aspect of NPC structure and assembly has been the lack of obvious conservation of the transmembrane nucleoporins in evolution. Three distinct pore membrane proteins (poms), Pom152p, Pom34p, and Ndc1p, have been identified in *Saccharomyces cerevisiae* (Chial et al., 1998; Rout et al., 2000; Wozniak et al., 1994). Whereas Pom152p and Pom34p are not essential, the deletion of *NDC1* or its *S. pombe* homolog *cut11*$^{+}$ is lethal (West et al., 1998; Winey et al., 1993). Both Ndc1p and Cut11p are also found at the spindle pole body (SPB) and play a role in SPB duplication, a function that may contribute to their being required for viability (Thomas and Botstein, 1986; West et al., 1998).

The two previously characterized vertebrate transmembrane nucleoporins POM121 and GP210 have no obvious homologs in yeast. POM121 has been implicated in NPC reassembly after mitosis because its depletion blocked NE and NPC assembly in vitro (Antonin et al., 2005). Studies on the role of GP210 in NPC

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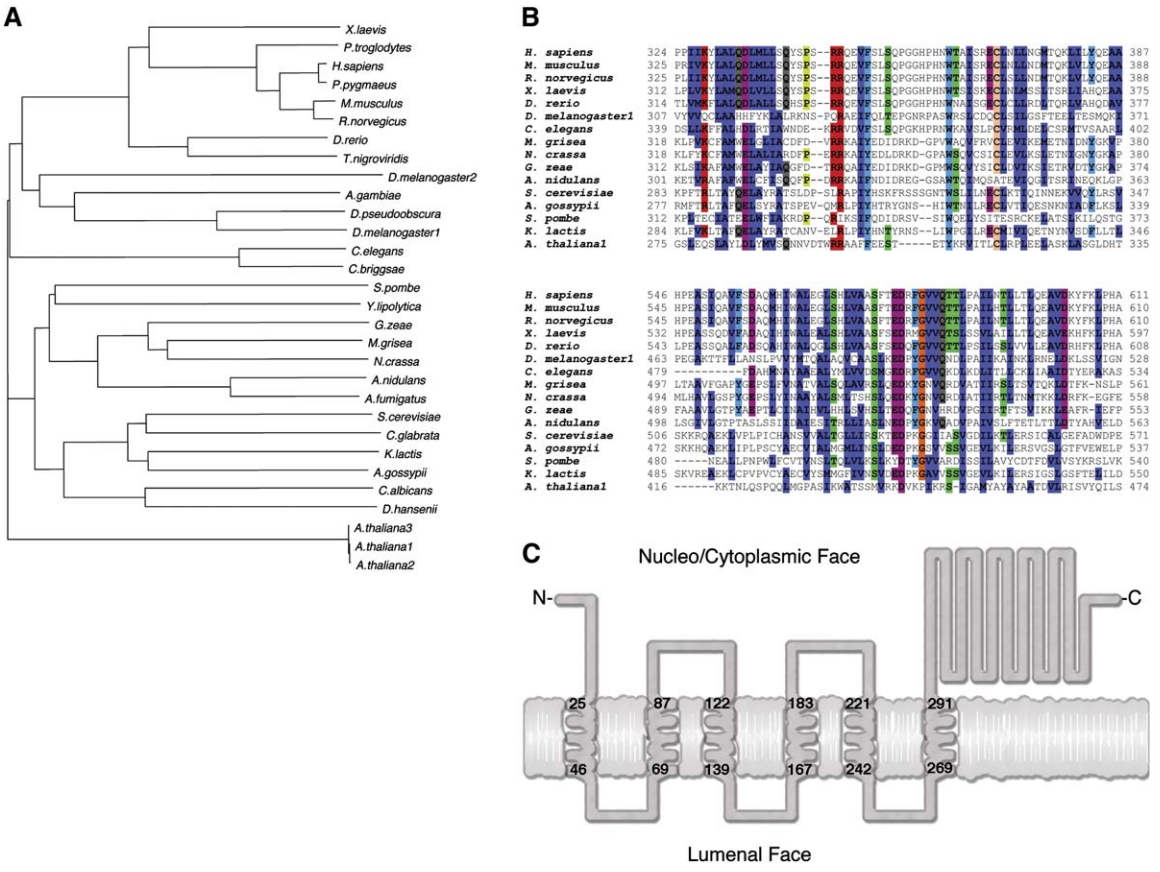

Figure 1. NDC1 Is a Conserved Transmembrane Protein

(A) Phylogenetic tree of NDC1. The phylogenetic relationship between NDC1 homologs from eukaryotic species was derived by using ClustalW.

(B) Multiple sequence alignment of the most conserved parts of the C-terminal domain of hNDC1 from selected eukaryotic species. The alignment was generated by ClustalW. Conserved positions were highlighted in color if at least 50% of the sequences were conserved with respect to the presence of the following groups of amino acids: AILMV, blue; WFY, cyan; RK, red; DE, magenta; ST, green; P, yellow; C, pink; NQ, gray; and G, orange.

(C) Predicted membrane topology of hNDC1. Transmembrane segments were predicted by using the TMPRED software.

biogenesis in different experimental systems have not yielded uniform conclusions ([Cohen et al., 2003; Galy et al., 2003; Antonin et al., 2005]; see Hetzer et al. [2005] for discussion).

We report here on the identification and characterization of vertebrate NDC1, the homolog of the essential yeast integral membrane nucleoporin Ndc1p. Depletion experiments performed by RNAi in cultured somatic cells in vivo and in the NE assembly system in vitro show that NDC1 is required for NPC assembly and NE formation. NDC1 interacts with Nup53 in vitro and may therefore recruit the Nup53-Nup93 nucleoporin subcomplex to the assembling NPC. Our data provide insight into NPC assembly and, in particular, suggest how the central NPC framework is anchored in the NE via a transmembrane nucleoporin.

Results

NDC1 Is Conserved in Evolution

To identify potential homologs of the yeast transmembrane nucleoporins in higher eukaryotes, we performed blast searches with 200 amino acid segments of each protein. Using the C-terminal domain of *S. pombe NDC1* (Cut11p), we were able to identify a *Caenorhabditis elegans* homolog, which in turn allowed us to find potential vertebrate NDC1s (Figure 1A). All these proteins contain an N-terminal segment with several predicted transmembrane anchors, which are poorly conserved in primary sequence, and a C-terminal domain displaying higher conservation (Figures 1B and 1C). Notably, human NDC1 (hNDC1, FLJ10407) was among 67 novel proteins identified in a comprehensive proteomic study of potential NE membrane proteins (Schirmer et al., 2003). A recent comparative genomics study of NPC proteins identified Ndc1p-like proteins in many eukaryotic species (Mans et al., 2004).

Characterization of hNDC1

To gain insight into the subcellular localization of hNDC1, we performed immunofluorescence in HeLa cells by using an antibody raised against the C-terminal 19 amino acids of hNDC1. The antibody was specific by immunoblotting (Figure 2A). hNDC1 localized to the NE in a punctate pattern, indicative of NPC localization. p62, a central component of the NPC, colocalized with hNDC1 at the nuclear rim. Colocalization was not perfect, possibly due to the harsh extraction conditions