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Cell Cycle-Dependent Differences in Nuclear Pore Complex Assembly in Metazoa

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SUMMARY

In metazoa, nuclear pore complexes (NPCs) assemble from disassembled precursors into a reforming nuclear envelope (NE) at the end of mitosis and into growing intact NEs during interphase. Here, we show via RNAi-mediated knockdown that ELYS, a nucleoporin critical for the recruitment of the essential Nup107/160 complex to chromatin, is required for NPC assembly at the end of mitosis but not during interphase. Conversely, the transmembrane nucleoporin POM121 is critical for the incorporation of the Nup107/160 complex into new assembly sites specifically during interphase. Strikingly, recruitment of the Nup107/160 complex to an intact NE involves a membrane curvature-sensing domain of its constituent Nup133, which is not required for postmitotic NPC formation. Our results suggest that in organisms with open mitosis, NPCs assemble via two distinct mechanisms to accommodate cell cycle-dependent differences in NE topology.

INTRODUCTION

NPCs are the exclusive channels of nucleo-cytoplasmic transport in eukaryotic cells. These multiprotein assemblies have an estimated mass of ~60 MD (Hetzer et al., 2005) and are embedded in the double lipid bilayer of the NE. Each NPC assembles from ~30 different nucleoporins (Nups), present in multiple copies, totaling ~500 polypeptides (Alber et al., 2007; Beck et al., 2004; Cronshaw et al., 2002). NPCs consist of a NE-embedded scaffold surrounding the central channel, largely composed of the Nup107/160 and Nup93/Nup205 complexes (Figure 1A). The Nup107/160 complex has been shown to be an early and essential player in NPC formation both in vitro and in vivo (Harel et al., 2003; Walther et al., 2003a). In vertebrates, it consists of nine polypeptides (Nup160, Nup133, Nup107, Nup96, Nup85, Nup43, Nup37, Seh1, and Sec13) assembled in a Y-shaped complex (Lutzmann et al., 2002). Its members are primarily composed of β-propellers and α-solenoids (Brohawn et al., 2009), a protein fold composition shared exclusively with other membrane coating protein complexes including clathrin coats and the COPII coatomer of the ER/Golgi (Alber et al., 2007; Brohawn et al., 2008; Devos et al., 2004). Furthermore, several of the scaffold Nups in yeasts and vertebrates possess an ALPS-like motif shown to target curved membranes in vitro (Drin et al., 2007). Attached to the NPC core are the cytoplasmic and nuclear rings from which eight filaments and the nuclear basket emanate. Many peripheral Nups contain phenylalanine-glycine (FG)-repeats that interact with nuclear transport receptors, providing a selective barrier for diffusion of macromolecules (Weis, 2003).

Relatively little is known about NPC biogenesis in metazoa, which occurs during two different cell cycle phases. The first pathway occurs at the end of mitosis and involves the ordered recruitment of ER membranes and disassembled NPC components to chromatin (Anderson and Hetzer, 2008b; Dultz et al., 2008; Walther et al., 2003b). In vitro studies using *Xenopus* egg extracts revealed NPC assembly during NE reformation is initiated by recruitment of the Nup107/160 complex (Belgareh et al., 2001; Harel et al., 2003; Walther et al., 2003b) to chromatin; a step mediated by the DNA-binding Nup ELYS/Mel28 (Franz et al., 2007; Gillespie et al., 2007; Rasala et al., 2006). This is followed by recruitment of ER membranes, containing the transmembrane Nups POM121 and Ndc1, and subsequent incorporation of Nup155 and Nup53 (Antonin et al., 2008).

The second pathway requires targeting and insertion of newly synthesized Nups to an intact interphase NE and it is unclear if this process is distinct from postmitotic assembly. In mammalian cells, only three transmembrane Nups have been identified: POM121, gp210, and Ndc1 (Chial et al., 1998; Hallberg et al., 1993; Mansfeld et al., 2006; Stavru et al., 2006b). While gp210 is not expressed in all cell types (Eriksson et al., 2004) and thus unlikely to play a role in NPC biogenesis, RNAi-mediated silencing of POM121 and Ndc1 has been shown to negatively affect NPC assembly (Antonin et al., 2005; Mansfeld et al., 2006; Stavru et al., 2006a; Stavru et al., 2006b). Furthermore, the appearance of POM121 has been shown to be an early step in NPC assembly both in vivo and in vitro (Dultz et al., 2008; Rasala et al., 2008), and essential for NE formation in vitro (Antonin et al., 2005). Other studies, however, suggest POM121 might be dispensable for NPC formation (Stavru et al., 2006a). These apparently contradicting studies imply the role of transmembrane Nups in NPC biogenesis, while still undefined, may be redundant.

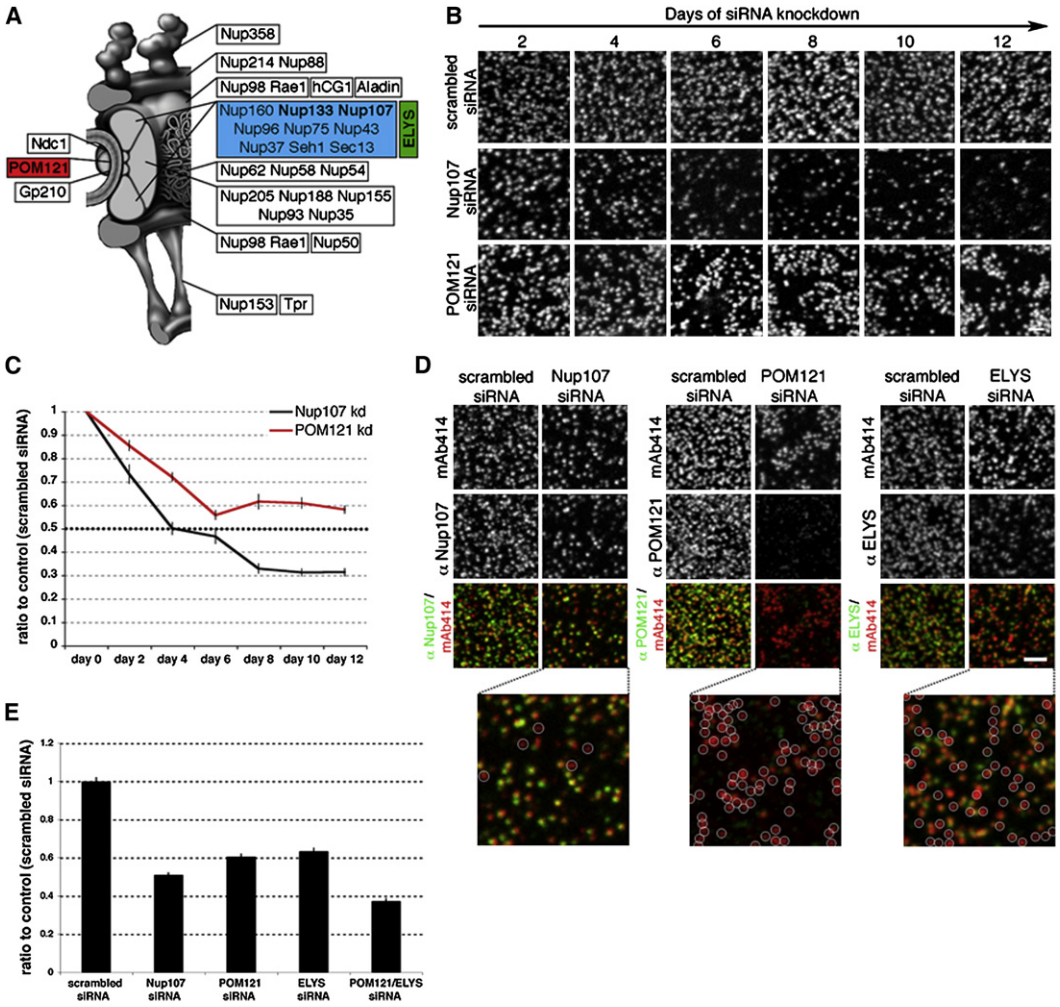

Figure 1. POM121 and ELYS Have Nonredundant Roles in NPC Assembly

(A) Schematic of NPC composition. Nups analyzed in this study are color-coded.

(B) U2OS cells were treated repeatedly with scrambled, POM121 or Nup107 siRNA oligos for 12 days, fixed at indicated time points and stained with mAb414.

(C) Quantification of mAb414 immunofluorescence (representing total NPCs per nucleus) over time, graphed as a ratio to control levels, n > 25 per time point.

(D) Immunofluorescence staining of nuclear surfaces using mAb414 and antibodies against Nup107, POM121 or ELYS in U2OS cells treated with siRNA oligos for 4 days against the indicated Nup. White circles indicate NPCs lacking either Nup107, POM121 or ELYS.

(E) Quantification of mAb414 immunofluorescence in U2OS cells treated with siRNA oligos against indicated Nups, n > 26 nuclei per condition.

All error bars are standard error. Scale bars represent 2 μm. See also Figure S1 and Figure S2.

Here, we show that incorporation of NPC components into an intact NE occurs by a mechanism that specifically requires POM121 and a membrane curvature-sensing domain in Nup133. Neither of these components is required for postmitotic NPC formation. Interestingly, recruitment of the Nup107/160 complex to new assembly sites in interphase does not involve ELYS, which is specifically required for its recruitment to chromatin at the end of mitosis.

RESULTS

The Nucleoporins ELYS and POM121 Have Nonredundant Functions in NPC Assembly

Studying potential differences between postmitotic and interphase NPC assembly is complicated by a subset of extremely long-lived Nups difficult to efficiently deplete by RNA interference (Rabut et al., 2004) such as the essential Nup107/160 complex (Figure 1A) (D'Angelo et al., 2009; Rabut et al., 2004). To improve the extent of Nup107/160 complex depletion, we repeatedly transfected cells with siRNA oligos specific for Nup107 for a total of 12 days. Cells were stained at two day time points with mAb414, an antibody that recognizes Nup358, Nup214, Nup153 and Nup62 (Davis and Blobel, 1987) (Figure 1B). While the overall NPC number in control cells, treated with scrambled oligos, remained constant, Nup107 knockdown resulted in a strong reduction of NPC density (Figure 1B and Figure S2A available online). Measuring the total fluorescence intensity of each nucleus, which accurately reflected the doubling of NPC number in interphase (Maul, 1971) (Figure S1A), we detected a sharp decrease of NPC numbers to ~30% in Nup107-depleted