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Purification, crystallization and preliminary X-ray analysis of a Nup107–Nup133 heterodimeric nucleoporin complex

Thomas Boehmer and Thomas U. Schwartz*

Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA

Correspondence e-mail: tus@mit.edu

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The nuclear pore complex (NPC), the sole gateway of traffic between the nucleus and the cytoplasm, is built up from multiple copies of about 30 proteins collectively termed nucleoporins (nups). Nups are organized into distinct subcomplexes. Nup107 and Nup133 are members of the essential Nup107–160 subcomplex, a component of the central NPC architecture. A dimeric complex of the C-terminal domains of human Nup107 and Nup133 was expressed from a bicistronic vector in *Escherichia coli*, purified and crystallized in two different crystal forms. Crystals grown in the presence of 18–22% PEG 3350 belong to space group $P2_12_12_1$ and diffracted to 2.9 Å. Native and seleno-L-methionine-derivative crystals grown in the presence of 1.1 *M* sodium malonate belong to space group $C2$ and diffracted to 2.55 and 2.9 Å, respectively. Structure determination of this complex will give the first insights into the protein–protein interactions within a core module of the NPC.

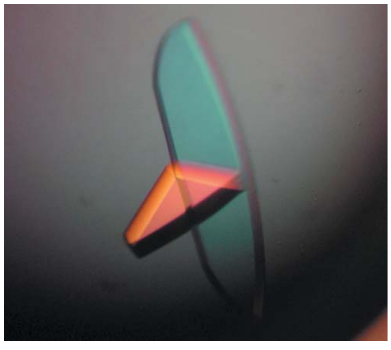
1. Introduction

Nuclear pore complexes (NPCs) are embedded in circular openings of the nuclear envelope and represent the exclusive gateways for macromolecular traffic into and out of the nucleus (Tran & Wente, 2006). With an estimated molecular weight of 60–80 MDa and an inventory of about 30 proteins (called nucleoporins or nups), the NPC stands among the most elaborate macromolecular machines in the cell. Their architecture, an eightfold symmetric framework with fiber-like extensions into the nucleus and the cytoplasm, is generated by the arrangement of nucleoporin subcomplexes around the NPC's twofold and eightfold axes of symmetry (Beck *et al.*, 2004; Schwartz, 2005).

The nonameric Nup107–160 subcomplex in vertebrates (Fontoura *et al.*, 1999; Loiodice *et al.*, 2004) is present in 16 copies per NPC and is located on both its cytoplasmic and nuclear face (Belgareh *et al.*, 2001). It is indispensable for NPC assembly and is therefore thought to play a critical structural role (Boehmer *et al.*, 2003; Harel *et al.*, 2003; Walther *et al.*, 2003). Furthermore, in mitosis a small pool of Nup107–160 subcomplexes colocalize with kinetochores (Belgareh *et al.*, 2001; Loiodice *et al.*, 2004) and the spindle apparatus (Enninga *et al.*, 2003; Orjalo *et al.*, 2006), pointing towards an additional role in mitotic chromosome segregation.

Molecular modeling, largely using the latest structure-prediction tools, has shown that the components of the Nup107–160 subcomplex share a common molecular architecture, being simply composed of a β-propeller, an α-helical repeat domain or a tandem arrangement of both (Devos *et al.*, 2004). This was partially confirmed by the crystal structure of the N-terminal domain of human Nup133, which revealed a seven-bladed β-propeller (Berke *et al.*, 2004).

Nup133 is incorporated into the Nup107–160 subcomplex through its interaction with Nup107 *via* the C-terminal α-helical domains of both proteins (Belgareh *et al.*, 2001; Lutzmann *et al.*, 2002; Berke *et al.*, 2004). Here, we report the crystallization and X-ray diffraction analysis of a 55 kDa complex consisting of C-terminal domains of both human Nup107 and Nup133.

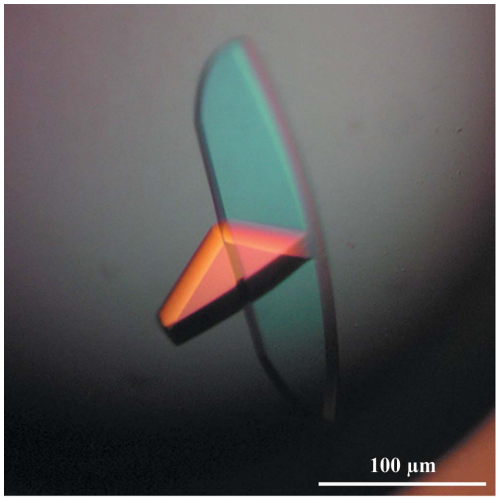


2. Protein expression and purification

The coding regions of human Nup133 (residues 934–1156) and Nup107 (residues 658–925) were cloned into the two multiple cloning sites of the bacterial bicistronic expression vector pET-Duet1 (EMD Biosciences). A sequence coding for a thrombin-cleavable GST tag was added at the 5′-end of the open reading frame for Nup107 and the resulting expression vector was transformed into *Escherichia coli* strain BL21(DE3) RIL (Stratagene). Cells were grown in Luria–Bertani broth medium containing 0.4%(*w*/*v*) glucose, 100 µg ml^{-1} ampicillin and 34 µg ml^{-1} chloramphenicol at 303 K to an OD_{600} of 0.8, induced with 200 µ*M* isopropyl *β*-D-1-thiogalactopyranoside and then grown for 16 h at 296 K.

The cells were harvested by centrifugation at 6000*g* for 8 min, resuspended in 20 ml lysis buffer (137 m*M* NaCl, 2.7 m*M* KCl, 5.4 m*M* Na_2HPO_4, 1.7 m*M* KH_2PO_4, 1 m*M* DTT, pH 7.4) per 1000 ODs (1 l with an OD_{600} of 2.5 corresponds to 2500 ODs) and lysed at 277 K using a French press. The crude lysate was supplemented with 200 µ*M* phenylmethanesulfonyl fluoride and clarified by centrifugation at 20 000*g* for 15 min. The cleared lysate was incubated with 1 ml glutathione Sepharose beads (4 Fast Flow, GE Healthcare) per 1000 ODs for 30 min at 277 K. After three batch washes with six bed volumes of lysis buffer, the resin was loaded onto a disposable column (Pierce) and the Nup107–Nup133 complex was eluted in four bed volumes of 50 m*M* Tris–HCl 8.0, 5 m*M* potassium phosphate pH 8.0, 1 m*M* DTT, 10 m*M* glutathione.

The eluate was dialyzed overnight at 277 K against 10 m*M* Tris–HCl pH 8.0, 150 m*M* NaCl, 5 m*M* potassium phosphate pH 8.0, 0.1 m*M* EDTA, 1 m*M* DTT. 2 h into the dialysis, human thrombin (Calbiochem) was added in a 1:100 (*w*:*w*) ratio to remove the N-terminal GST tag from Nup107. The complex was purified *via* anion-exchange chromatography on a HiTrapQ column (GE Healthcare) using a linear NaCl gradient (150–500 m*M*) and then twice *via* size-exclusion chromatography on a Superdex S75 column (GE Healthcare) in a buffer containing 10 m*M* potassium phosphate pH 7.0, 150 m*M* NaCl, 0.1 m*M* EDTA and 1 m*M* DTT. The protein was concentrated to 10 mg ml^{-1} using a 30 kDa cutoff centrifugal filter device (Millipore), flash-frozen in liquid N_2 and stored at 193 K. Selenomethionine-substituted derivatives were prepared according to published protocols (Doublié, 1997).



(*a*)

(*b*)

Figure 1
Crystals of the Nup107 (residues 658–925)–Nup133 (residues 934–1156) complex. (*a*) Crystals belonging to space group $P2_12_12_1$ grew in 0.1 *M* HEPES pH 7.5, 0.2 *M* NaCl and 18–22% PEG 3350 (native 1). (*b*) Crystals belonging to space group *C*2 grew in 0.1 *M* HEPES pH 7.0, 1.1 *M* sodium malonate pH 7.0 and 1–1.5% PEG MME 5000 (native 2).

3. Crystallization

Crystallization experiments with the purified Nup107–Nup133 complex were set up in 96-well sitting-drop trays using commercially available sparse-matrix screens (Hampton Research, Qiagen). The initially obtained crystals were improved in hanging-drop vapor-diffusion setups using grid screens around the original crystallization conditions.

The Nup107–Nup133 complex crystallized at 293 K after mixing 1.5 µl protein sample with 1.5 µl reservoir solution containing 0.1 *M* HEPES pH 7.5, 0.2 *M* NaCl and 18–22% PEG 3350 (native 1; Fig. 1*a*). The crystals were highly sensitive to the concentration of PEG 3350 and grew in large plates with dimensions of up to 400 × 200 × 30 µm within 7 d. The presence of both Nup107 and Nup133 was confirmed by SDS–PAGE analysis of a dissolved crystal (Fig. 2, lane 2).

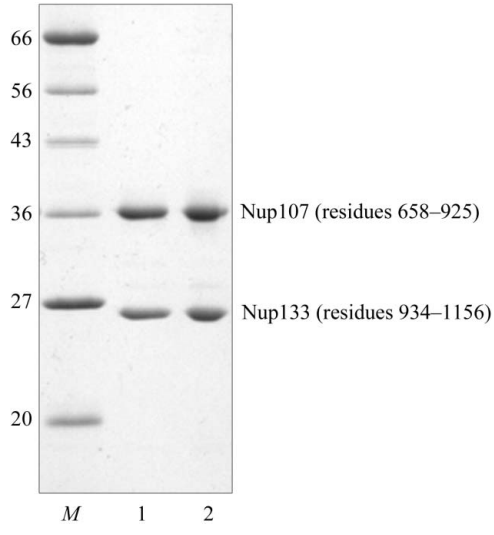


Figure 2
SDS–PAGE analysis of dissolved crystals. A large orthorhombic crystal (native 1) was dissolved in loading buffer and analyzed on a 12.5% SDS–PAGE gel. Both Nup107 and Nup133 are detected in the same ratio in the crystal (lane 2) when compared with the purified Nup107–Nup133 complex (lane 1). Lane *M* contains protein standards; sizes are indicated in kDa. The gel was stained with Coomassie Brilliant Blue R-250.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

Data set	Native 1	Native 2	SeMet
Wavelength (Å)	0.9792	0.9792	0.9792
Resolution (Å)	50–2.9 (3.0–2.9)	40–2.55 (2.64–2.55)	40–2.9 (3.0–2.9)
Space group	$P2_12_12_1$	$C2$	$C2$
Unit-cell parameters			
a (Å)	54.6	51.7	51.9
b (Å)	65.6	127.9	128.8
c (Å)	179.7	152.7	153.4
β (°)		97.36	97.65
Unique reflections	14444	32243	39961
R_{sym}† (%)	8.2 (41.3)	10.5 (48.7)	6.7 (21.4)
Completeness (%)	95.4 (88.6)	99.4 (98.8)	92.0 (52.5)
Redundancy	3.4 (3.1)	4.2 (3.1)	3.4 (1.8)
$I/\sigma(I)$	12.1 (2.0)	16.1 (2.0)	17.8 (2.7)

† $R_{sym} = \sum |I_i - \langle I \rangle| / \sum I_i$, where I_i is the intensity of the ith observation and $\langle I_i \rangle$ is the mean intensity of the reflection.

A second crystal form was obtained with 0.1 *M* HEPES pH 7.0, 1.1 *M* sodium malonate pH 7.0 and 1–1.5% PEG MME 5000 as reservoir solution (native 2; Fig. 1*b*). These crystals grew within 3 d and their size was increased by reducing the protein concentration to 5 mg ml^{-1}. Crystals of selenomethionine-derivatized protein (14 methionines in 492 residues) were obtained under the same conditions.

4. Data collection and processing

For X-ray diffraction studies, crystals grown in the presence of 18–22% PEG 3350 (native 1) were cryoprotected by soaking in 0.1 *M* HEPES pH 7.5, 0.2 *M* NaCl and 30% PEG 3350 for 12–16 h prior to flash-freezing in liquid N_2. Native (native 2) and seleno-L-methionine-derivative crystals grown in the presence of 1.1 *M* sodium malonate were frozen after a quick wash in 0.1 *M* HEPES pH 7.0, 1.1 *M* sodium malonate pH 7.0 and 10% ethylene glycol.

X-ray diffraction data were collected from single frozen crystals at beamline 24IDC, Advanced Photon Source (Argonne, IL, USA) and are summarized in Table 1. The data were processed using the programs *DENZO* and *SCALEPACK* (Otwinowski, 1993). Native 1 crystals had orthorhombic symmetry ($P2_12_12_1$) and diffracted to 2.9 Å. Native 2 and seleno-L-methionine-derivative crystals belonged to the monoclinic space group $C2$ and diffracted to 2.55 and 2.9 Å, respectively. A fluorescence scan indicated the presence of seleno-L-methionine in the SeMet crystals. Assuming the presence of one Nup107–Nup133 complex per asymmetric unit, the solvent content of the orthorhombic crystals was 56.7% and 72.2% for the monoclinic crystals, respectively. Structure determination is currently in progress.

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