Journal of Structural Biology 177 (2012) 99–105

Contents lists available at SciVerse ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Analysis of the yeast nucleoporin Nup188 reveals a conserved S-like structure with similarity to karyopherins

Dirk Flemming [a,*,1], Damien P. Devos [b,1], Johannes Schwarz [a], Stefan Amlacher [a], Malik Lutzmann [c], Ed Hurt [a,*,1]

[a] Biochemie-Zentrum der Universität Heidelberg (BZH), Im Neuenheimer Feld 328, D-69120 Heidelberg, Germany
[b] EMBL, Meyerhofstrasse 1, D-69117 Heidelberg, Germany
[c] Institute of Human Genetics, CNRS, Montpellier, France

ARTICLE INFO

Article history:
Available online 22 November 2011

Keywords:
Nuclear pore complex
Nucleoporin
Nup192
Nup188
EM structure
Karyopherin
Thermophile

ABSTRACT

Nuclear pore complexes (NPCs) embedded in the double nuclear membrane mediate the entire nucleocytoplasmic transport between the nucleus and cytoplasm. Each NPC is composed of about 30 different proteins (nucleoporins or Nups), which exist in multiple (8, 16 or 32) copies within the NPC scaffold. Recently, we have identified and characterized the large structural Nups, Nup188 and Nup192, from the thermophilic eukaryote *Chaetomium thermophilum*, which exhibited superior properties for biochemical and structural studies, when compared to their mesophilic orthologs. Here, we study the large structural Nups from the model organism yeast *Saccharomyces cerevisiae*. Our data show that yeast Nup188 like its thermophilic orthologue *ct*Nup188 exhibits a twisted S-like structure, which flexibly binds the linker nucleoporin Nic96 via a short conserved α-helix motif. Using bioinformatic methods, we have generated a pseudo-atomic structural model of Nup188 and its related Nup192, which further strengthens the view that the large α-solenoid structural Nups are related to karyopherins.

1. Introduction

In all eukaryotes nucleocytoplasmic transport occurs solely through nuclear pore complexes (NPCs), highly elaborate supramolecular assemblies within the double nuclear membrane (Fahrenkrog and Aebi, 2003). A set of ~30 conserved nucleoporins (Nups) form the NPC, whose spatial arrangement and structural organization has been extensively studied in the yeast *Saccharomyces cerevisiae* as well as a number of other model organisms (Cronshaw et al., 2002; Franz et al., 2005; Rout and Aitchison, 2000; Tamura et al., 2010). Although the size of the NPC seems to vary between species, the overall scaffold is conserved. Moreover, Nups from diverse species differ markedly in their amino acid sequence, but their fold-types and domain organization as well as the composition and modularity of the NPC seem to be very conserved and to have been already present in the last common eukaryotic ancestor (Degrasse et al., 2009; Devos et al., 2006).

The NPC is build from eight spoke units symmetrically surrounding the center of the central pore, through which active nucleocytoplasmic transport takes place. Translocation of large cargo through the NPC is facilitated by shuttling transport receptors, many of which are members of the karyopherin superfamily. This large group of nuclear transport receptors, 14 members in yeast, and at least 19 members in human (Fried and Kutay, 2003; O'Reilly et al., 2011) share a modest sequence homology but a high similarity in their fold type formed by stacked pairs of antiparallel α-helices (HEAT-repeats), which arrange into a flexible superhelical structure (Conti and Izaurralde, 2001; Gorlich et al., 1997). Short sequence motifs in the cargo (Hoelz and Blobel, 2004) are recognized by these transport receptors directly.

Detection of fold similarity between the Nups forming the core structure of each spoke, that are mainly composed of α-solenoid, β-propeller or both, and of their similarity to membrane-curving complexes like clathrin, COPI and COPII coatomers lead to the proposal of a common origin to both types of complexes (Devos et al., 2004, 2006). This prediction was further strengthened by solving the X-ray structures of some of these Nups (Brohawn et al., 2008).

Recently, the reconstitution of a new module in the core structure of the NPC was possible utilizing the proteins derived from the thermophilic eukaryote *Chaetomium thermophilum*. This inner ring module is composed of the three largest structural Nups (Nup170, Nup188, Nup192) flexibly bridged by short linear motifs provided by linker Nups (Nic96 and Nup53) and has the potential to span the entire width of the NPC from the pore membrane to the transport channel (Amlacher et al., 2011). By negative stain EM analysis

* Corresponding authors. Fax: +49 6221 54 43 69.
E-mail addresses: Dirk.Flemming@bzh.uni-heidelberg.de (D. Flemming), devos@embl.de (D.P. Devos), ed.hurt@bzh.uni-heidelberg.de (E. Hurt).
[1] These authors equally contributed to this work.

1047-8477/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jsb.2011.11.008

the entirely α-helical structures of Nup192 and Nup188 were shown to adopt a similar S-like structure with two twisted half-circles each resembling in shape and curvature the known structures of the karyopherin/importin β superfamily of transport receptors (Amlacher et al., 2011; Petosa et al., 2004).

In this study, we have further characterized the large α-solenoid yeast Nups, Nup188 and Nup192. We could show that *sc*Nup188 binds *sc*Nic96 *in vitro*. Moreover, we analyzed the EM structure of purified *sc*Nup188 and compared it to the EM shape of its *C. thermophilum* (*ct*) orthologue. Finally, we present pseudo-atomic models of the large α-solenoid Nups, which strengthen the hypothesis that these Nups are evolutionary related to the shuttling nuclear transport receptors.

2. Materials and methods

2.1. Strains and plasmids

S. cerevisiae strains used in this study are DS1-2b (Y2197) and dyn2Δ (Y5019) (for genotype description see (Bassler et al., 2001). Plasmids used in this study were generated by standard procedures; *scNUP188* and *scNUP192* were cloned into the pNOPPATA2L 2μ plasmid (Hellmuth et al., 1998) and transformed into yeast strains genomically disrupted for the gene of interest. pNOPPATA2L-ProtA-TEV-DID1-Nup188 was generated by insertion of the DID1 sequence between the ProtA-TEV tag and *NUP188* in the plasmid pNOPPATA2L-ProtA-TEV-Nup188.

2.2. Protein purification

For ProtA affinity-purifications, *S. cerevisiae* strain DS1-2b was transformed with the respective plasmid. Yeast cells were grown over night at 30 °C in SDC-leu medium and harvested at an OD_{600} of 3. Cells were lysed in 25 mL cold NB buffer (20 mM Tris–HCl pH 7.5, 150 mM NaCl, 50 mM K(OAc), 2 mM $Mg(OAc)_2$) supplemented with protease inhibitor mix FY (Serva), 1 mM dithiothreitol (DTT) and 0.1% (v/v) NP40 (lysis buffer) with glass beads using a bead beater (power 500, 4 min, 2 repeats with 1 min break) at 4 °C. Subsequently, the lysate was centrifuged (3300*g* for 10 min and 27000*g* for 30 min at 4 °C) and the ProtA-TEV tagged proteins were purified from the supernatant with 300 μL IgG Sepharose beads suspension (IgG Sepharose™ 6 Fast Flow, Amersham Bioscience). After one batch wash (10 mL lysis buffer for 10 min at 4 °C on a turning wheel) and three washes (3 × 10 mL NB buffer with 0.01% (v/v) NP40 and 1 mM DTT in Poly-Prep® Chromatography Columns, Bio-Rad) by gravity flow, the proteins were eluted by TEV cleavage in 150 μL NB buffer with 0.01% (v/v) NP40 and 1 mM DTT for 90 min at 16 °C in a Mobicol™ minispin column (MoBiTec, Germany).

For single particle EM analysis, ProtA-TEV tagged *sc*Nup188 and *sc*Nup192 were purified as described above. The TEV eluates were further purified by size exclusion chromatography (S200-10/300 GL, GE-Healthcare) on an Äkta-Basic-System (Amersham). Ion exchange chromatography of *sc*Nup188 was performed in NB buffer and NB + 1 M NaCl.

For the DID1–Dyn2 labeling the DID1 sequence was inserted between the ProtA-TEV tag and Nup188 in the plasmid pNOPPATA2L-ProtA-TEV-Nup188. The resulting plasmid pNOPPATA2L-ProtA-TEV-DID1-Nup188 was transformed into a *dyn2Δ* deletion strain to avoid *in vivo* dimerization of the modified protein by endogenous Dyn2. The ProtA-based affinity-purification was performed as described above and the labeling of *sc*Nup188 with the DID–Dyn2 label was performed according to Flemming et al. (2010).

2.3. Electron microscopy and image processing

For negative staining, 5 μL of sample were placed on a freshly glow-discharged, carbon-coated grid and then washed three times with water, stained with uranyl acetate (2% w/v), and dried. Electron microscopy was carried out on a Phillips CM-200 FEG or a JEOL JEM-1400 microscope under low dose conditions both equipped with a 2 K × 2 K Tietz-CCD camera (TVIPS F224) at a nominal magnification of 27,500 (*sc*Nup188) or 20,000 (Nup188-Nic96). The nominal pixel size was 5.19 or 5.6 Å.

For image processing a total of 1944 (for *sc*Nup188) and 891 (for DID-*sc*Nup188) were selected manually using 'Boxer' (Ludtke et al., 1999). All subsequent image processing was carried out in IMAGIC V (van Heel et al., 1996) following the program's standard procedures. Particles were band-pass filtered and normalized in their gray value distribution and mass-centered. Alignment, iterative refinement of class averages and the calculation of the three-dimensional maps followed the procedures described in (Lutzmann et al., 2005). Relative spatial orientations were determined by sinogramm correlation. This process was started several times, beginning with different class averages for the initial determination of Euler angles. Three-dimensional maps were calculated using the exact weighted back projection algorithm. Determination of Euler angles and calculation of three-dimensional maps were repeated until the map converged into a stable shape, from which projections could be generated that were similar to all of the initially observed class averages. A three-dimensional map was further refined by projection matching. This process was repeated several times. The final volumes were visualized using the UCSF Chimera package software and Gaussian filtered (Pettersen et al., 2004).

2.4. Bioinformatic analyses

Homologous sequences were collected by Psi-Blast. Hidden-Markov Model were build and screened against similar models for all proteins of known structure using the HHSearch suite of programs (Soding, 2005). Full-atoms three-dimensional models were build using Modeller (Sali and Blundell, 1993). Secondary structure and coiled-coil predictions were made by PSI_Pred (Jones, 1999).

3. Results

3.1. Affinity-purification of yeast Nup188 and Nup192 and in vitro reconstitution of a scNup188–scNic96 heterodimer

We sought to obtain structural information of the two largest yeast Nups, Nup188 and Nup192, although the yield and stability of these Nups when purified from yeast or recombinantly from *Escherichia coli* were not as high as in the case of the corresponding orthologues from *C. thermophilum* (Amlacher et al., 2011). To obtain sufficiently pure material for EM studies, we overexpressed *sc*Nup188 and *sc*Nup192 in yeast, and subsequently affinity-purified these large Nups, which were tagged with ProtA-TEV, by IgG–Sepharose chromatography (see Section 2). After TEV elution, these Nups were further purified by gel filtration chromatography to obtain sufficiently pure material. SDS–PAGE revealed that isolated *sc*Nup188 eluted in a distinct peak of ~200 kDa and thus behaved like a monomeric protein (Fig. 1A). In contrast, *sc*Nup192 did not behave like this, but eluted in a broad range from ~3 MDa to ~200 kDa from the gel filtration column (Fig. 1B). This finding suggested that purified *sc*Nup192 forms aggregates under the isolation conditions used. Indeed, negative stain EM of the high molecular fractions showed ring-link structures of different size (data not shown), but the physiological meaning of these Nup192 structures