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Structural and Functional Analysis of the C-terminal Domain of Nup358/RanBP2

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Abstract

The nuclear pore complex (NPC) is the sole mediator of bi-directional transport between the nucleus and cytoplasm. Nup358 is a metazoan-specific nucleoporin that localizes to the cytoplasmic filaments and provides several binding sites for the mobile nucleocytoplasmic transport machinery. Here we present the crystal structure of the C-terminal domain (CTD) of Nup358 at 1.75 Å resolution. The structure reveals that the CTD adopts a cyclophilin-like fold with a non-canonical active site configuration. We determined biochemically that the CTD possesses weak peptidyl-prolyl isomerase activity and show that the active site cavity mediates a weak association with the HIV-1 capsid protein, supporting its role in viral infection. Overall, the surface is evolutionarily conserved, suggesting that the CTD serves as a protein-protein interaction platform. However, we demonstrate that the CTD is dispensable for nuclear envelope localization of Nup358, suggesting that the CTD does not interact with other nucleoporins.

Keywords

X-ray crystallography; Peptidyl-prolyl isomerase; Fluorescence localization microscopy; Nuclear Pore Complex (NPC); Human immunodeficiency virus (HIV-1)

Introduction

Nuclear pore complexes (NPCs) are large, proteinaceous transport organelles that perforate the nuclear envelope and are the sole mediator of bi-directional nucleocytoplasmic transport.¹ The human NPC is composed of 34 distinct proteins – 31 soluble nucleoporins (nups) and 3 integral membrane proteins of the pore membrane domain (poms). Each NPC protein occurs in multiple copies to generate a highly symmetrical structure with about 1,000 individual polypeptide chains and an estimated molecular mass of approximately 120 MDa.¹ Electron microscopy structures of the NPC have shown a doughnut-shaped symmetric core that is embedded in ~100 nm pores perforating the nuclear envelope. The symmetric NPC core is decorated with different nucleoporin subsets that form distinct filamentous structures protruding from the nucleoplasmic and cytoplasmic faces.¹ On the

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Accession numbers

The atomic coordinates and structure factors of Nup358^{CTD} have been deposited in the Protein Data Bank under accession code 4I9Y.

nucleoplasmic face, the filaments bundle into a basket-like structure and provide binding sites for various components of other macromolecular machineries that carry out diverse cellular processes at the nuclear envelope, including chromatin remodeling, transcription, and DNA repair.¹ On the cytoplasmic face, the filaments are extended and reach far into the cytoplasm, providing binding sites for a plethora of transport complexes and the mRNA export machinery.¹

In humans, the cytoplasmic filaments are primarily composed of three proteins: Nup88, Nup214/CAN, and Nup358/RANBP2 (referred to as Nup358 in the following text).¹ Nup358 is a unique component of the metazoan NPC and is the largest known nucleoporin, composed of 3,224 residues in humans.^{2,3} Nup358 can be divided into several domains: an N-terminal TPR domain, an α -helical region, four Ran-binding domains, eight tandem zinc fingers, a SUMO E3 ligase domain, and a C-terminal domain that displays sequence homology to cyclophilins (Fig. 1a).^{2,3} Thus far, only the SUMO E3 ligase domain, two Ran-binding domains and the N-terminal TPR domain have been structurally characterized.⁴⁻⁷ Nup358 provides key interaction sites for various components of the nucleocytoplasmic transport machinery including Ran, SUMOylated RanGAP1, the NXF1•p15 mRNA export heterodimer, and the export karyopherin CRM1.^{1,7} Additionally, we have recently shown that the N-terminal domain binds to single-stranded RNA.⁸

Apart from its essential function in nucleocytoplasmic transport, Nup358 is involved in a many other important cellular processes. During mitosis, Nup358 localizes to kinetochores and facilitates spindle formation as well as chromosome congression and segregation.^{9,10} A striking link exists between the cytoplasmic filament nups, their binding partners, and neoplastic disease. The expression of Nup358 is upregulated in plasma cells from patients with multiple myeloma, and mutations and fusions of the Nup358 gene have been associated with inflammatory myofibroblastic tumors.^{11,12} Moreover, mutations in Nup358 have been linked to heightened susceptibility of otherwise healthy children to acute necrotizing encephalopathy following common viral infections such as influenza.¹³ Recently, Nup358 has also been implicated in the delivery and integration of the genomic material of HIV-1 to the genome of terminally differentiated non-dividing cells.¹⁴⁻¹⁷

In order to gain insight into structure and function of Nup358, we have determined the crystal structure of the C-terminal domain (CTD) at 1.75 Å resolution. Nup358^{CTD} adopts a canonical cyclophilin fold, but displays substantial alterations of the active site cavity. Surprisingly, we find biochemically that the observed structural changes fail to render the enzyme inactive and that the active site cavity retains weak peptidyl-prolyl isomerase activity. Moreover, we demonstrate that the active site cavity also facilitates binding to the HIV-1 capsid protein, as previously shown for cyclophilin A. In addition to the active site cavity, Nup358^{CTD} features three extensive evolutionarily conserved surface patches, suggesting that the domain also functions as a protein-protein interaction platform. However, we determined by immunofluorescence microscopy that the CTD is dispensable for nuclear envelope localization of Nup358 *in vivo*, suggesting that it does not interact with other nucleoporins. Together, these data suggest that Nup358^{CTD} is exposed at the tips of the cytoplasmic filaments of the NPC to facilitate binding to physiological substrates and could

potentially be hijacked by the pre-integration complex of HIV-1 to facilitate nuclear import and genome delivery.

Results

Structure determination

Based on a sequence alignment between human Nup358 and cyclophilin A (CypA), we generated an expression construct encompassing residues 3062 to 3224 that we termed the Nup358 C-terminal domain (CTD). Nup358^{CTD} is monomeric in solution as determined by multiangle light scattering coupled to size exclusion chromatography with a measured molecular mass of 17.9 kDa (theoretical 18.4 kDa) (Fig. S1). Nup358^{CTD} crystallized in the monoclinic space group P2₁ with six molecules in the asymmetric unit. The structure of Nup358^{CTD} was solved by molecular replacement and the final model was refined to 1.75 Å resolution with R_{work} and R_{free} values of 12.2 % and 15.8 %, respectively. For details of the data collection and refinement statistics see Table 1.

Structural overview

Overall, Nup358^{CTD} is a globular domain with a diameter of approximately 30 Å. Nup358^{CTD} displays the canonical cyclophilin fold of an 8-stranded β-barrel (β1 to β8) that is flanked on three sides by an α-helix (αA to αC), as first observed in CypA (Fig. 1b,c).¹⁸ The core of the domain is extensively decorated by several long and well-ordered loops that generate a large surface area. An ~20 Å long and ~10 Å wide surface groove is formed at the intersection between β-strands β3, β4, β5, and β6, the α-helix αB, and the loop linking β5 and β6. This groove is utilized for substrate binding and catalysis in CypA and other cyclophilins.¹⁸

In order to identify surfaces of Nup358^{CTD} that might be functionally important, we aligned sequences of Nup358^{CTD} from a diverse set of species (Fig. S2), and mapped the sequence conservation onto the surface of Nup358^{CTD}. A comparison of the conservation and electrostatic potential surfaces revealed that Nup358^{CTD} possesses three evolutionarily conserved surface areas: (1) A groove in the surface that forms the active site in other cyclophilins, (2) an extensive hydrophobic patch on the back of the domain that is generated by hydrophobic substitutions which are not present in other cyclophilins, and (3) an acidic patch on the top of the domain (Fig. 2).

The active site of Nup358^{CTD}

The 17 cyclophilin-like domains present in human proteins are very well conserved in both sequence and structure, especially at the active site, where there are only a handful of substitutions at residues that are catalytically important.¹⁹ In CypA, the model enzyme for studying cyclophilin peptidyl-prolyl isomerization activity, the active site cavity is generated by approximately fifteen residues, which in turn accommodate four to six residues of a linear peptide.¹⁸ The mechanism of peptidyl-prolyl isomerization by cyclophilins remains unclear, but there are several features that are conserved across family members. Hydrophobic residues form the bottom of the cavity that accommodates a substrate *trans*-proline and several residues in the cavity form polar contacts with the peptide backbone of

the substrate peptide.¹⁹ Specifically, a critical arginine (Arg55 in CypA) that is invariant across cyclophilins forms key hydrogen bonds with the isomerized amide bond and the C-terminal backbone carbonyl.¹⁹

Nup358^{CTD} shares ~60 % sequence identity with human CypA and the crystal structures of CypA and Nup358^{CTD} superimpose with a root-mean-square deviation of ~0.4 Å over 130 Cα atoms (Fig. 3).²⁰ Of the fifteen residues that contribute to the putative active site in Nup358^{CTD}, nine are identical with their corresponding positions in CypA (Fig. 3). In particular, the residues that form the majority of the contacts between CypA and its substrates, His3186, Leu3182, Gln3171, and Phe3120, as well as the critical arginine, Arg3115, are conserved. However, other critical residues in the CypA active site that make key contacts with the substrate (Trp121, Phe113, Met61, and Ala103) are altered in the putative Nup358^{CTD} active site (His3181, Val3173, Val3121, and Gln3163). Specifically, the imidazole ring of His3181 in Nup358^{CTD} is still positioned to act as a hydrogen bond donor for the substrate backbone, analogous to Trp121 in CypA, but would be unable to form the additional hydrophobic contacts. Moreover, the substitutions of the bulky hydrophobic side chains Phe113 and Met61 for the smaller side chains Val3173 and Val3121 substantially reduce the extent to which Nup358^{CTD} can form hydrophobic contacts with substrates, and the substitution of Gln3163 for Ala103 introduces a larger side chain where the substrate normally packs closely to the β-carbon of Ala103 (Fig. 3).

Our conservation analysis reveals that most of the putative Nup358^{CTD} active site residues are nearly invariant across various vertebrates. However, whereas Nup358^{CTD} possesses a cyclophilin-like fold, the putative active site of Nup358^{CTD} displays marked differences from canonical cyclophilins, raising the possibility that Nup358^{CTD} either has an altered peptidyl-prolyl isomerase substrate specificity or is catalytically inactive.

Catalytic activity

In order to determine whether Nup358^{CTD} is an active peptidyl-prolyl isomerase, we used a chymotrypsin-coupled spectrophotometric assay on the isomerization of Suc-Ala-Ala-Pro-Phe-2,4-difluoroanilide, a synthetic peptide that mimics CypA substrates.²¹ We found that Nup358^{CTD} indeed possessed peptidyl-prolyl isomerase activity, but with a much lower efficiency than CypA. The Michaelis-Menten constants K_m and k_{cat} of Nup358^{CTD} were determined to be 304.6 μM and 72 s⁻¹, compared to 309.9 μM and 119,913 s⁻¹ for CypA, respectively (Fig. 4a,b).

To confirm that the weak enzymatic activity of Nup358^{CTD} was in fact mediated by the putative active site, we generated the V3173W mutant that introduces the bulky aromatic residue tryptophan directly into the active site cavity. The behavior of the Nup358^{CTD} V3173W on a gel filtration column was indistinguishable from that of the wild-type protein, indicating that the mutation did not affect proper protein folding. We found that the Nup358^{CTD} V3173W mutant failed to accelerate *cis/trans* isomerization over the background rate (Fig. 4c), consistent with previous results that the analogous F113W mutation in CypA dramatically reduces enzymatic activity.²² Our data establish that Nup358^{CTD} is indeed an active peptidyl-prolyl isomerase, but with substantially reduced enzymatic activity, supporting the results of our structural analysis of the active site cavity.

HIV-1 capsid protein interaction

Interactions between cyclophilins and the HIV-1 capsid protein are a critical part of the viral life cycle. CypA binds to the HIV-1 capsid protein (HIV-1^{CA}) and is specifically incorporated into the viral capsid, preventing Ref-1 mediated restriction of HIV-1 infection, and thus is essential for HIV-1 infectivity.²³⁻²⁵ The crystal structure of the CypA•HIV-1^{CA} heterodimer revealed that CypA recognizes and binds a proline-rich loop of HIV-1^{CA} with its active site (Fig. 5).²⁶ Recent studies have proposed that Nup358 plays a role in the delivery and genomic integration of the genetic material of HIV-1 to the nucleus by facilitating an interaction to HIV-1^{CA} via its C-terminal domain.¹⁵⁻¹⁷

To characterize the interaction between HIV-1^{CA} and Nup358^{CTD}, we employed a size exclusion chromatography binding assay. Whereas HIV-1^{CA} and CypA formed a stable stoichiometric 1:1 complex (Fig. 6a), we found the interaction between HIV-1^{CA} and Nup358^{CTD} to be weaker (Fig. 6b). To determine whether the interaction was mediated by the Nup358^{CTD} active site, we tested whether the V3173W mutant is capable for forming a complex with HIV-1^{CA}. Strikingly, the weak interaction observed between Nup358^{CTD} and HIV-1^{CA} was completely abolished in the Nup358^{CTD} V3173W mutant (Fig. 6c). To confirm the weak interaction detected by size exclusion chromatography, we additionally employed isothermal titration calorimetry. Although the interaction strength is at the limit for reliable quantification of thermodynamic parameters ($K_d > 200$), we were able to observe a significant interaction between Nup358^{CTD} and HIV-1^{CA}. In agreement with our size exclusion chromatography assay, we did not observe any interaction between Nup358^{CTD} V3173W and HIV-1^{CA} (Fig. S3).

The results of the isothermal titration calorimetry experiments are consistent with the relatively minor peak shift observed for the interaction between HIV-1^{CA} and Nup358^{CTD} compared to the shift observed for the complex formed by HIV-1^{CA} and CypA. Thus, Nup358^{CTD} binds the HIV-1 capsid protein with its active site, but it does so with a reduced binding affinity, consistent with the observed structural alterations of the active site.

In vivo localization assay

Nup358 localizes to kinetochores after the breakdown of the nuclear envelope, but it is a stable component of the cytoplasmic filaments during interphase.^{2,3,9} To determine whether Nup358^{CTD} is involved in the attachment of Nup358 to NPCs, we performed a series of immunofluorescence localization experiments in HEK293 cells. Both the HA-tagged full-length Nup358 and a C-terminal truncation that lacks the CTD (Nup358^{CTD}) yielded nuclear envelope rim staining that coincides with staining by mAb414, an antibody that recognizes a group of phenylalanine-glycine (FG)-repeat-containing nucleoporins at the nuclear rim.²⁷ In contrast, the staining of a construct containing only the HA-tagged Nup358^{CTD} failed to enrich at the nuclear rim (Fig. 7). These data establish that the CTD is not required for nuclear envelope localization and dispensable for Nup358 anchoring to the cytoplasmic face of the NPC.

Conclusions

We determined the crystal structure of the C-terminal domain of Nup358, a unique component of the metazoan NPC, and established biochemically that the domain possesses peptidyl-prolyl isomerase activity in addition to its previously described E3 ligase activity.²⁸ We show biochemically that Nup358^{CTD} interacts with the HIV-1 capsid protein. Additionally, analysis of the evolutionary conservation of the Nup358^{CTD} surface suggests that the domain also functions as a protein-protein interaction platform. However, it is unlikely that the physiological binding partners are other nucleoporins as Nup358^{CTD} does not localize to the nuclear envelope and is dispensable for nuclear envelope localization of Nup358 *in vivo*.

Despite many years of research on cyclophilins, the precise catalytic mechanism by which these enzymes catalyze proline isomerization remains poorly understood. Deeper mechanistic insights remain elusive partly because of sequence variation within the active site and a lack of physiological substrates. The active site cavity of Nup358^{CTD} displays similarity to those found in the cyclophilin-like domains of cyclophilin G and NK-tumor recognition protein, distinct from “classical” cyclophilin active sites.²⁹ Further experiments with this subset of cyclophilins are required to see whether their distinct active site configuration recognizes a unique set of substrates.

What role could a peptidyl-prolyl isomerase have at the periphery of the NPC? Besides a role in protein folding, peptidyl-prolyl isomerases also participate in a wide range of signaling pathways and Nup358^{CTD} may act in a similar way on an unknown substrate. Alternatively, it is possible that Nup358^{CTD} may also function as a protein-protein interaction module at the tips of the cytoplasmic filaments, perhaps serving as a surveillance domain to attract cargo molecules far away from the transport channel of the NPC. We speculate that this functionality may also serve to specifically recognize a thus unknown binding partner, leading to increased concentration at the cytoplasmic face of the NPC, similar to the Ran-binding domains of Nup358. Given the increased local concentration of Nup358 that is generated by its localization to the NPC, even relatively weak interactions may play physiologically important roles. Viral proteins such as the HIV-1 capsid protein may hijack such functionality to facilitate their nuclear import potentially by promoting uncoating or interactions with transport machinery as suggested previously.¹⁷ Our studies provide a structural and biochemical platform to dissect these interactions.

Methods

Protein expression and purification

DNA fragments encoding residues 3062-3224 from human Nup358, residues 1-165 of human Cyclophilin A, and residues 1-146 of the HIV-1 capsid protein were cloned into a modified pET28a vector, containing an N-terminal hexahistidine-tag followed by a PreScission protease cleavage site using NdeI and NotI restriction sites.³⁰ Nup358^{CTD} V3173W was generated by site directed mutagenesis and confirmed by DNA sequencing.

All proteins were expressed in *E. coli* BL21-CodonPlus(DE3)-RIL cells (Stratagene) in LB media. Expression was induced at an OD₆₀₀ of approximately 0.6 with 0.5 mM IPTG at

18 °C for 16 hours. Cells were harvested by centrifugation and resuspended in a buffer containing 25 mM TRIS, pH 8.0, 500 mM NaCl, 20 mM imidazole, 5 mM β -mercaptoethanol (β -ME) and complete EDTA-free protease inhibitor cocktail (Roche).

For purification, the cells were lysed with a cell disruptor (Avestin) and DNase I (Roche) was added to the lysate before centrifugation at 30,000 x g for 1 hour. The supernatant was filtered with a 0.45 μ m filter (Millipore) and loaded onto a Ni-NTA column (GE Healthcare) equilibrated in buffer A (20 mM TRIS, pH 8.0, 500 mM NaCl, 20 mM imidazole, and 5 mM β -ME). Protein was eluted with a linear gradient of buffer B (20 mM TRIS, pH 8.0, 500 mM NaCl, 500 mM imidazole, and 5 mM β -ME). Fractions were pooled and loaded onto a HiPrep Desalting column (GE Healthcare) equilibrated in 20 mM TRIS, pH 8.0, 100 mM NaCl, and 5 mM β -ME. PreScission protease was added to the eluate and the protein was cleaved at 4 °C for 16 hours. Digested protein was loaded onto a Ni-NTA column and collected in the flowthrough, concentrated in a centrifugal filter (Millipore), and loaded on a Superdex 75 16/60 column (GE Healthcare) equilibrated in buffer containing 20 mM TRIS, pH 8.0, 100 mM NaCl, and 10 mM DTT. Protein-containing fractions were pooled and concentrated to 65 mg/ml for crystallization studies and 30 mg/ml for biochemistry studies.

Protein crystallization and data collection

Protein crystallization was carried out at 21 °C in hanging drops consisting of 1.0 μ l protein solution and 1.0 μ l reservoir solution. Crystals were grown in 0.1 M TRIS-HCl, pH 8.3, 0.2 M NaCl, and 0.9 M K/Na tartrate with a protein concentration of 65 mg/ml. Crystals were cryoprotected in the reservoir solution supplemented with 20 % glycerol and flash frozen in liquid nitrogen. X-ray diffraction data was collected at 100 K at Beamline 12-2 at the Stanford Synchrotron Radiation Lightsource (SSRL).

Structure determination and refinement

X-ray diffraction data was processed with the HKL-2000 denzo/scalepack package. A model of human Cyclophilin A, PDB code 19MC was used as a search model for molecular replacement with Phaser.^{20,31} Subsequent model building and refinement using anisotropic B-factors were performed with Coot and PHENIX, respectively.^{32,33} The final model contains residues 3,062 to 3,224 and was refined to 1.75 Å resolution with R_{work} and R_{free} values of 12.2 % and 15.8 %, respectively. The stereochemical quality of the model was assessed with PROCHECK and MolProbity.^{34,35} For details of the data collection and refinement statistics see Table 1.

Kinetic analysis

Enzyme kinetics were analyzed following a previously established chymotrypsin-coupled spectrophotometric assay.²¹ Peptidyl-prolyl isomerase activity was measured with a spectrophotometer (Shimadzu) at 9 °C using the cleavage of Suc-Ala-Ala-Pro-Phe-2,4-difluoroanilide (Bachem), which led to an increase of absorbance at 290 nm. CypA and Nup358^{CTD} were assayed at final concentrations of 20 nM and 2 μ M, respectively. The final concentration of α -chymotrypsin (Sigma) was 150 μ g/ml. Suc-Ala-Ala-Pro-Phe-2,4-difluoroanilide (Bachem) was dissolved in a solution of Tetrafluoroethylene (TFE) and 500 mM LiCl and the reaction was started by the addition of Suc-Ala-Ala-Pro-Phe-2,4-

difluoroanilide to the cuvette for final concentrations that ranged from 50 μ M to 500 μ M. The reaction was carried out in 20 mM TRIS, pH 8.0, 100 mM NaCl, and 5 mM β -ME.

Analytical size-exclusion chromatography

HIV-1^{CA} was mixed in approximately equimolar amounts with Nup358^{CTD}, Nup358^{CTD}, or CypA and incubated on ice for approximately 30 minutes before injection onto a Superdex 75 10/300 GL gel-filtration column (GE Healthcare) equilibrated in 20 mM TRIS, pH 8.0, 100 mM NaCl, and 5 mM β -ME. Complex formation was monitored by the shift of the respective protein peaks in the chromatogram and by SDS-PAGE of the protein-containing fractions.

ITC measurements

ITC measurements were performed at 23 °C using an ITC200 calorimeter (GE Healthcare). Protein samples were stored in a buffer containing 20 mM TRIS, pH 8.0, 100 mM NaCl, and 4 mM β -ME. To perform the titration, 2.5 μ L of 3.7 mM HIV-1^{CA} was injected into 200 μ L of 350 μ M Nup358^{CTD} or 310 μ M Nup358^{CTD} V3173W every 240 s. The heat generated from dilution was subtracted for baseline correction. Baseline corrected data were analyzed with Origin 7.0 software with MicroCal add-ons. All experiments were performed at least three times.

Immunofluorescence microscopy

For immunofluorescence, Nup358 and Nup358 fragments (residues 1-3224, 1-145, and 146-3224) were inserted into the pCMV-HA vector (Clontech) using SalI and NotI restriction sites. The resulting Nup358 fragments contain an N-terminal HA-tag. HEK293T cells were grown on poly-L-lysine coated slides (Sigma) until ~50 % confluency. Transfection was performed with TransIT-LT1 transfection reagent (Mirus) according to the manufacturer's instructions. After 48 hours, the medium was removed and cells were washed in phosphate buffered saline (PBS) and fixed in PBS, supplemented with 2 % (w/v) formaldehyde for 5 min at room temperature. After two washes with PBS, the cells were permeabilized with PBS containing 0.1 % (v/v) TritonX-100 (Sigma-Aldrich) for 10 minutes at room temperature. The cells were then washed in PBS and blocked in PBS, supplemented with 10 % (v/v) Fetal Calf Serum (FCS) for 20 min at room temperature. For nuclear envelope staining, the cells were incubated with a 1:500 dilution of the monoclonal antibody mAb414 (Abcam) in PBS buffer, supplemented with 0.1 % (w/v) saponin and 10 % (v/v) FCS for 16 hours at 4 °C. Secondary antibody incubation was performed with a 1:3000 dilution of Alexa Fluor 568 goat anti mouse (Invitrogen) in PBS, supplemented with 0.1 % (w/v) saponin and 10 % (v/v) FCS for 1 hour at room temperature, followed by three washes with PBS. For detection of the HA-tagged proteins, the cells were incubated with a monoclonal antibody anti-HA Fluor488 conjugate antibody (Invitrogen) for 1 hour at room temperature, washed three times with PBS, and mounted onto coverslips with ProLong Gold Antifade reagent with DAPI (Invitrogen). Slides were examined with an inverted fluorescence microscopy on a Carl Zeiss AxioImagerZ.1 equipped with a Hamamatsu camera.

Illustration and figures

The sequence alignment of Nup358^{CTD} was generated using ClustalX and colored with Alscript.^{36,37} The scientific illustrations were generated using PyMOL (Schrödinger, LLC; www.pymol.org), Igor (WaveMetrics), and Prism (GraphPad). The electrostatic potential was calculated with Adaptive Poisson-Boltzmann Solver (APBS).³⁸

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

1. Crystal structure of the C-terminal domain of Nup358
2. Nup358 C-terminal domain has peptidyl-prolyl isomerase activity
3. Nup358 C-terminal domain has weak binding affinity for HIV-1 capsid protein
4. Nup358 C-terminal domain is dispensable for nuclear envelope localization

Fig. 1. Domain organization and structure of Nup358^{CTD}. (a) Domain organization of human Nup358. Domain boundaries are indicated by residue numbers. The bar above the domain structure denotes the crystallized fragment. I, II, III, and IV, Ran binding domains; NTD, N-terminal domain; CTD, C-terminal domain; E3, E3 ligase domain. (b) Cartoon representation of Nup358^{CTD} with a view rotated 180° along the vertical axis shown on the right. (c) Cartoon representation of Nup358^{CTD} rotated 90° along the horizontal axis from above. (d) Representative 2|F_o|-|F_c| electron density map contoured at 1.5 σ .

Fig. 2. Surface properties of Nup358^{CTD}. (a) Surface representation of Nup358^{CTD}, with the active site colored in red. (b) Surface representation colored according to sequence identity based on a multi-species sequence alignment (Fig. 2). The identity at each position is mapped onto the surface and is shaded in a color gradient from white (60 % less than 60 % identity) to red (100 % identity). (c) Surface representation colored according to electrostatic potential from -10 k_BT/e (red) to +10 k_BT/e (blue).

Fig. 3. Comparison of Nup358^{CTD} and Cyclophilin A active sites. (a) Detailed view of the Nup358^{CTD} active site. (b) Detailed view of the Cyclophilin A active site (PDB Code 1M9C). (c) Overlay of the active sites from Nup358^{CTD} and Cyclophilin A. Critical active site residues are shown in stick representation, and the Ca-traces are shown in coil representation, according to the coloring scheme in A. The orientation of all active sites is identical.

Fig. 4. Nup358^{CTD} possesses peptidyl-prolyl isomerase activity. (a) Michaelis-Menten plot of the peptidyl-prolyl isomerization of Suc-Ala-Ala-Pro-Phe-2,4-difluoroanilide by Cyclophilin A, Nup358^{CTD}, and Nup358^{CTD} V3173W. (b) Michaelis-Menten plot of the peptidyl-prolyl isomerization of Suc-Ala-Ala-Pro-Phe-2,4-difluoroanilide by Nup358^{CTD} and Nup358^{CTD} V3173W. Note the different scale of the y-axis from panel (a). (c) Representative time-course traces of the peptidyl-prolyl isomerization of Suc-Ala-Ala-Pro-Phe-2,4-difluoroanilide by Cyclophilin A, Nup358^{CTD}, Nup358^{CTD} V3173W, and in the absence of an enzyme.

Fig. 5. Structural comparison of Nup358CTD to the Cyclophilin A•HIV-1^{CA} complex. The structure of Nup358^{CTD} overlaid on the structure of the Cyclophilin A•HIV-1^{CA} complex (PDB code 1M9C). The right panel is a close-up view of the interaction with the HIV-1^{CA} loop rotated 90° along the vertical axis from the left panel. Note the clash between the Nup358^{CTD} Q3163 and the HIV-1^{CA} proline-rich loop.

Fig. 6. Nup358^{CTD} binds weakly to the HIV-1 capsid protein. (a-c) Size exclusion chromatography interaction analysis of HIV-1^{CA} with (a) Cyclophilin A, (b) Nup358^{CTD}, and (c) Nup358^{CTD} V3173W. The analyzed fractions are indicated in gel-filtration profile by a grey bar. Notably, experiments were carried out with identical protein concentrations, the different peak height of the wild-type Nup358^{CTD} is a result of a lower absorbance coefficient.

Fig. 7. Nup358^{CTD} is dispensable for nuclear envelope localization. Nup358^{CTD} and Nup358 fragments carrying a N-terminal HA-tag were transiently transfected into HEK293T cells and analyzed by fluorescence microscopy. HA-tagged Nup358 protein localization was detected with an α -HA antibody (green). The monoclonal α -mAb414 antibody (red) and DAPI (blue) were used as a reference for nuclear envelope and nucleus staining, respectively. The right panel shows the merged images.

Table 1**Crystallographic Analysis**

Data collection	
Protein	Nup358 ^{CTD}
Synchrotron	SSRL
Beamline	BL12-2
Space group	P2 ₁
Cell parameters	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	<i>a</i> =104.1, <i>b</i> =97.1, <i>c</i> =108.2
α , β , γ (°)	α =90.0 β =117.4 γ =90.0
Wavelength (Å)	0.9795
Resolution (Å)	50.0 – 1.75
<i>R</i> _{sym} (%) ^{<i>b</i>}	10.5 (98.5)
$\langle I \rangle / \langle \sigma \rangle$ ^{<i>b</i>}	12.8 (2.3)
Completeness (%) ^{<i>b</i>}	97.7 (92.8)
No. observations	1,742,028
No. unique reflections	188,791 (17,824)
Redundancy	9.2 (7.7)
Refinement	
Resolution (Å)	50.0 – 1.75
No. reflections total	188,714
No. reflections test set	9,500 (5.03%)
<i>R</i> _{work} / <i>R</i> _{free}	12.2 / 15.8
No. atoms	17,190
Protein	15,408
Ligand	244
Water	1,538
<i>B</i> -factors	
Protein	22.6
Water	38.7
R.m.s. deviations	
Bond lengths (Å)	0.009
Bond angles (°)	1.2
Ramachandran plot^{<i>c</i>}	
Favored (%)	97.6
Additionally allowed (%)	2.4
Outliers (%)	0.0
MolProbity score	1.19

^a SSRL, Stanford Synchrotron Radiation Lightsource

^b Highest-resolution shell is shown in parentheses

^cAs determined by MolProbity³⁵

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