The Structure of a Yeast Dyn2-Nup159 Complex and the Molecular Basis for the Dynein Light Chain – Nuclear Pore Interaction*

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Background: Dyn2 and Nup159 interact to induce oligomerization of the Nup82 cytoplasmic fibril complex.
Results: Dyn2 homodimers symmetrically bind Nup159 target sites through an anti-parallel β-strand interaction.
Conclusions: Dyn2 homodimers bind diverse, arrayed Nup159 target sites, locally directing Nup159 homodimerization.
Significance: Learning how nucleoporins interact to form fibril architecture is crucial for understanding how the nuclear pore complex gates traffic.

SUMMARY
The Nuclear Pore Complex gates nucleocytoplasmic transport through a massive, eight-fold symmetric channel capped by a nucleoplasmic basket and structurally unique, cytoplasmic fibrils whose tentacles bind and regulate asymmetric traffic. The conserved Nup82 complex, composed of Nsp1, Nup82 and Nup159, forms the unique cytoplasmic fibrils that regulate mRNA nuclear export. While the nuclear pore complex plays a fundamental, conserved role in nuclear trafficking, structural information about the cytoplasmic fibrils is limited. Here, we investigate the structural and biochemical interactions between S. cerevisiae Nup159 and the nucleoporin: Dyn2. We find that Dyn2 is predominantly a homodimer and binds arrayed sites on Nup159, promoting Nup159’s parallel homodimerization. We present the first structure of Dyn2, determined at 1.85 Å resolution, complexed with a Nup159 target peptide. Dyn2 resembles homologous metazoan dynein light chains, forming homodimeric composite substrate binding sites that engage two independent 10 residue target motifs, imparting a β-strand structure to each peptide via anti-parallel extension of Dyn2’s core β-sandwich. Dyn2 recognizes a highly conserved QT motif, while allowing sequence plasticity in the peptide’s flanking residues. Isothermal titration calorimetric analysis of Dyn2’s comparative binding to two Nup159 target sites shows similar affinities (18 and 13 µM), but divergent thermal binding modes. Dyn2 homodimers are arrayed in the crystal lattice, likely mimicking Dyn2’s arrayed
architecture on Nup159's multivalent binding sites. Crystallographic inter-dimer interactions potentially reflect a cooperative basis for Dyn2-Nup159 complex formation. Our data highlights the determinants that mediate oligomerization of the Nup82 complex and promote a directed, elongated cytoplasmic fibril architecture.

The S. cerevisiae Nuclear Pore Complex (NPC) is a 66 MDa structure composed of approximately 30 different proteins that embed in the nuclear envelope and facilitate transport across this barrier (1). NPC proteins are highly conserved in function and sequence across eukaryotes and carry out biologically conserved functions: mRNA export into the cytoplasm and gated transport of specific proteins into and out of the nucleus.

The proteins that make up this highly coordinated and complex structure form an eight-fold symmetrical pore from a limited number of structural folds (2). The types of domains in the nuclear pore proteins, or nucleoporins (Nups), are primarily α-solenoids, β-propellers, Phe-Gly (FG) rich repeats, coiled-coil domains, and transmembrane domains (3,4). Transmembrane domains traverse the double nuclear envelope membrane and underlie the NPC core topology and biogenesis (5,6). FG repeats are primarily concentrated in the core interior where they function as a physical or entropic barrier to entering proteins while reversibly binding nuclear transport receptors and selectively allowing their passage (1,7). On either side of the core, asymmetrically distributed elements are positioned to facilitate asymmetric, unidirectional transport. The nucleoplasmic side of the NPC contains proteins tethered into a basket-like structure that protrudes 95 nm into the nucleus, potentially serving as a molecular checkpoint for pre-mRNA before it exits the nucleus (8,9). On the cytoplasmic surface, NPC fibrils stretch 50 nm into the cytoplasm (8). Cytoplasmic fibrils are primarily composed of nucleoporins from the Nup82 complex that bind translation initiation factors and mRNA export machinery (10). The Nup82 complex consists of Nup82, Nup159 and Nsp1 that work with Nup116, Nup42, Gle1, and Nup100, to mediate mRNA export in concert with the mRNA nuclear export receptor Mex67 and the DEAD box RNA helicase Dpb5 (4,11-13).

Nup159 is a prime component of the Nup82 complex and plays a directed role in coordinating nucleoporins involved in mRNA export rather than protein trafficking between the cytoplasm and nucleus (14). Nup159 has an extended multi-component architecture that facilitates its roles in mRNA export, as well as filament localization in the NPC structure (14-17). Nup159’s N-terminal domain constitutes a seven-bladed β-propeller that extends into the cytoplasm and mediates Dpb5 binding (18). Deletion of Nup159’s N-terminal domain results in a temperature-sensitive phenotype, lethal at 37°C and hallmarked by Dpb5 mislocalization and constitutive mRNA export defects at 23°C (14,16). Nup159’s central 700 amino acids form an FG-rich repeat domain. C-terminal to the FG-rich repeats is a 100 amino acid region termed the dynein light chain interacting domain (DID) that uses a pentameric array of dynein light chain binding motifs to bind the yeast dynein light chain Dyn2 (11). C-terminal to the DID, Nup159 contains a predicted helical region (19) that is essential for Nup159’s stability and localization on the NPC, and has recently been shown to form a heterotrimeric structure with Nup82 and Nup116 (14,16,20). Higher-order oligomerization of the Nup82 complex requires both the Nup159 DID region as well as Dyn2 (11). The functional role of a dynein light chain at the nuclear pore is independent of its role in the cytoplasmic dynein microtubule motor complex (11).

The dynein light chain is a promiscuous protein, involved in a diversity of protein target interactions, only a subset of which involve binding to the cytoplasmic dynein microtubule motor complex. In S. cerevisiae, Dyn2 interacts with the dynein intermediate chain, Pac1, through tandem canonical 10-12 residue stretches, each containing a conserved QT motif (21). Similar Dyn2 binding motifs are arrayed in Nup159, where class averaged electron microscopy of Nup159’s DID region saturated with Dyn2 showed a stack of five densities like beads on a string, effectively mediating Nup159’s dimerization (11). As with Dyn2, the higher dynein light chain orthologs, LC8 and DYNLL, bind partners outside of the dynein motor complex including the signaling molecules nNOS and Pak1, the apoptosis regulator Bim/Bmf, and the mRNA localization protein Swallow (22-24). Given the diverse set of
dynein light chain partners, it has been postulated that the dynein light chain functionally serves as a
dimerization machine. This role correlates with the
structures of higher Dyn2 orthologs that show
dynein light chains complexed 2:2 with a variety
of target peptides (23,25-28).

While studies to date have biophysically
characterized dynein light chains from Drosophila,
rat and human, molecular details of the S. cerevisiae
dynein light chain have remained outstanding. S. cerevisiae
is a leading model system for biophysical, biochemical and genetic
investigations of the NPC and the cytoplasmic
dynein motor complex. To further our molecular
understanding of Dyn2 and its functional role in
the NPC, we determined the x-ray crystal structure
of Dyn2 in complex with a Nup159 target site. We
couple structural data with gel filtration, multi-
angle light scattering and isothermal titration
calorimetry to derive a model for the Dyn2-
Nup159 interaction and Dyn2’s role as a
dimerization machine.

EXPERIMENTAL PROCEDURES

Cloning and Expression of Full Length Dyn2
from S. cerevisiae - Full length Dyn2 was cloned
from S. cerevisiae S288c into the pGEX-6P2
expression vector (GE Healthcare) using the
polymerase chain reaction and BamHI and EcoRI
engineered flanking restriction sites. The Dyn2
insert was sequence verified against Genbank
accession NC_001136. pGEX-6P2-Dyn2 was
transformed into E. coli BL21 DE3 (pLysS) and
grown under ampicillin selection in 6 L of LB
media at 37°C. At an optical density of 0.8 (600
nm), GST-Dyn2 expression was induced using 0.1
mM isopropyl-1-thio-]-D-galactopyranoside  for
16 hours at 18°C. Cells were harvested by
centrifugation at 2100 x g for 10 min. at 4°C and
the pellets resuspended in buffer A: 150 mL of 25
mM HEPES, pH 6.8, 300 mM NaCl, and 0.1% β-
mercaptoethanol, and stored at -20°C.

Protein Purification - Resuspended cell pellets
were thawed and lysed by sonication at 4°C. 0.1
mM phenylmethylsulfonyl fluoride was added to
the lysate and cell debris was pelleted by
centrifugation at 23,000 x g for 45 min. Supernatant was loaded onto a 5 ml Glutathione
Sepharose Fast Flow affinity column (GE
Healthcare). GST-tagged Dyn2 was eluted from
the glutathione column with 100 ml of 3 mM
 glutathione pH 8.0 in buffer A. The GST-Dyn2
eluate was exchanged into buffer B (25 mM
HEPES, pH 6.8 and 0.1% β -mercaptoethanol)
using an Amicon Ultra 10 kDa spin concentrator
(Millipore) and incubated for 16 hours with
PreScission protease (GE Healthcare). The cleaved
protein was loaded onto an SP Sepharose Fast
Flow column (GE Healthcare) and eluted over a
linear 0-1 M NaCl gradient in buffer B. Dyn2 peak
fractions were pooled and exchanged into 50 mM
NaCl, 25 mM HEPES, pH 6.8, and 0.1% β -
mercaptoethanol using an Amicon Ultra 3 kDa
spin concentrator (Millipore) and concentrated to 5
mg/mL, snap frozen in liquid nitrogen and stored
at -80°C. All purification procedures were
executed at 4°C. The final, purified Dyn2 protein
contained an N-terminal GPLGS cloning artifact.

Synthesis of Nup159 Peptides - Nup159 pep1
(YSAFDVQTSL, residues 1103-1113), pep2
(NYAESGIQTDL, residues 1116-1126), pep3
(YVKHNSTVTQV, residues 1141-1151), pep4
(YAVDNGLQTEP, residues 1153-1163), and
pep5 (YTCNFSVQTFE, residues 1165-1175) (Fig.
1C) were synthesized at the UNC Microprotein
Sequencing and Peptide Synthesis Facility. Pep 1,
pep3, pep4 and pep5  were designed with an amino
terminal tyrosine in order to quantify the peptide
concentration once solubilized. Lyophilized
peptides were solubilized in 50 mM NaCl, 25 mM
HEPES, pH 6.8 and 0.1% β -mercaptoethanol.

Crystallization - 1.0 mM Dyn2 was incubated with 1.5 mM Nup159 pep2 in 50 mM NaCl,
25M HEPES, pH 6.8, and 0.1% β -mercaptoethanol for 30 minutes on ice.
Crystallization followed the hanging drop protocol using 1 µL of the Dyn2-Nup159 pep2 mixture
and 1 µL of the 1 mL well solution: 0.3 M ammonium acetate, pH 5.5, 5% methyl
pentanediol, and 35% polyethylene glycol 4000.
Crystals grew at 20°C to 200 x 200 x 600 µm
over the course of a week. Crystals were
transferred to fomblin oil (Sigma) for
cryoprotection and flash frozen in liquid nitrogen.

Data Collection, Structure Determination, and
Refinement - Dyn2-Nup159 pep2 crystals were
maintained at 100 K under a cryo-cooled nitrogen
stream and diffraction data collected using a
Rigaku Micromax 007HF x-ray generator (copper
anode, 1.54 Å wavelength), Osmic mirrors and a
Rigaku Saturn 944+ CCD in the UNC Macromolecular X-Ray Crystallography Core Facility. 0.5° oscillations were collected over 160° from a single crystal. Data were indexed, integrated and scaled using HKL2000 (29) (Table 1). The structure was determined using the AutoMR molecular replacement program (PHENIX crystallographic suite (30)) and a modified 2PG1 (26) coordinate file in which a monomeric, apo Drosophila LC8 search model was used. The model was built using AutoBuild (PHENIX) (30) and refined iteratively through manual builds in Coot (31) followed by refinement runs using phenix.refine (PHENIX) (30). Refinement statistics were monitored using a Free R, calculated using 10% of the data, randomly excluded from refinement (32). The final model includes two Dyn2 molecules (chains A and C: residues 7-92), two Nup159 pep2 molecules (chain B: residues 1117-1126; chain D: residues 1116-1126 with N1116 modeled as alanine) and 217 water molecules.

Size Exclusion Chromatography and Multi Angle Light Scattering - 100 µL of 200 µM Dyn2 was injected onto a Wyatt WTC030S5 silicone size exclusion column (for elution of 5,000 - 1,250,000 Da proteins) in 50 mM NaCl, 25 mM HEPES, pH 6.8, 0.1% β-mercaptoethanol, and 0.2 g/L sodium azide, and passed in tandem through a Wyatt DAWN HELEOS II light scattering instrument and a Wyatt Optilab rEX refractometer. The light scattering and refractive index data were used to calculate the weight-averaged molar mass (M_w) and the mass fraction in each peak using the Wyatt Astra V software program (Wyatt Technology Corp.) (33).

Isothermal Titration Microcalorimetry - ITC experiments were carried out at 15°C in buffer C: 50 mM NaCl, 25 mM HEPES, pH 6.8, and 0.1% β-mercaptoethanol on a Microcal AutoITC200 (GE Healthcare). Peptides were exchanged into buffer C using G-25 Sephadex Quick Spin Columns (Roche). 17 x 2 µL injections of 1 mM pep2 or pep4 were automatically injected into 200 µL of 100 µM Dyn2. The resulting binding isotherms (Fig. 6A and B) were analyzed using the Origin 7.0 software package (OriginLab) and were fit to a one-site binding model. Experiments were conducted in triplicate and averaged to determine respective mean K_D values with standard deviations as shown.

Protein Data Bank Accession Number - Coordinates for the Dyn2-Nup159 complex have been deposited in the Research Collaboratory for Structural Bioinformatics PDB under accession code 4DS1.

RESULTS

S. cerevisiae Dyn2 is a member of the conserved dynein light chain family - The dynein light chain, a component of the cytoplasmic dynein motor complex, is highly conserved from yeast to human (Fig. 1A). The dynein light chain is 90% identical across higher eukaryotes ranging from C. elegans to human, with significant identity extending to lower eukaryotes, as exemplified by the 50% identity between S. cerevisiae Dyn2 and D. melanogaster LC8. Across organisms, evidence points to the dynein light chain’s role as a factor that promotes substrate dimerization. While the dynein light chain is a component of the dynein microtubule motor complex, it is not exclusive to this complex. Recent work has shown that approximately 25% of the S. cerevisiae dynein light chain member, Dyn2, is associated with the nuclear pore complex. Dyn2 associates with the Nup82 cytoplasmic fibril complex, binding to pentavalent motifs arrayed in Nup159’s Dynein light chain Interaction Domain (DID) (Fig. 1B-D) (11). The Dyn2 binding motifs share a canonical QT motif with variable flanking components. Similar tandem binding sites have recently been mapped in the Dynein Intermediate Chain, Pac11, and shown to mediate Dyn2 interaction (Fig. 1C, D) (21). To understand the molecular basis of the Dyn2-Nup159 interaction, we cloned Dyn2 from S. cerevisiae (S288c) genomic DNA into the E. coli expression vector pGEX-6P2, expressed and purified Dyn2 to homogeneity, removing the N-terminal GST tag. Nup159 peptides corresponding to the second and fourth Dyn2 DID binding sites (pep2 and pep4) were synthesized, purified by HPLC chromatography and verified by mass spectrometry analysis. Pep4 incorporated an N-terminal tyrosine to facilitate concentration determination while pep2 concentration was determined using its endogenous tyrosine.

The Dyn2 homodimer forms two composite substrate binding sites using a central β-
sandwich and flanking α 2-helices – To elucidate the structural determinants underlying the Dyn2-Nup159 interaction, we screened mixtures of Dyn2 and Nup159 pep2 and pep4 for co-crystallization. We obtained crystals of the Dyn2-Nup159 pep2 (residues 1116-1126) complex using a 1:1.5 molar ratio of Dyn2 and Nup159 pep2 respectively. The crystals diffracted to 1.85 Å resolution and belonged to the space group P2_1,2,2_1. We solved the structure by the molecular replacement method using a peptide-free monomer chain derived from the Drosophila dynein light chain (2PG1) that showed 50% sequence identity with Dyn2 (26). Two Dyn2 chains occupy the asymmetric unit, homodimerized around a non-crystallographic two-fold axis. Clear electron density was evident for two Nup159 pep2 chains, each bound to the Dyn2 homodimer. The R and R_free values for the Dyn2-Nup159 pep2 structure are 15.1% and 18.0% respectively. Crystallographic data and refinement statistics are presented in Table 1.

Dyn2 homodimerizes across a composite central β-sandwich (Fig. 2A). Each β-sheet is composed of five β-strands arranged in an anti-parallel organization: β1-β4-β5-β2-β3’ in which the final β3’ strand is provided by the homodimeric mate. The prime interface between Dyn2 molecules is mediated by the anti-parallel sheets that form the central β-sandwich, the β2-β3’ and β2’-β3 strand interactions encompass the non-crystallographic two-fold operator that relates each Dyn2 molecule. Flanking the central β-sandwich, each Dyn2 molecule contributes an α 1 and an α 2 helix that bridge β1 and β2. The α 1-α 2 helix-turn-helix motifs symmetrically pack against the two β-sheets that form the central β-sandwich. The Dyn2 homodimer symmetrically binds two Nup159 peptides; the basis for the interaction is an extension of each β-sheet through an anti-parallel strand that is stabilized through buttressing interactions with the neighboring α 2-helix.

Dyn2 architecture is homologous to other dynein light chain structures determined to date, with the highest structural homology to the human dynein light chain 8 (LC8) complexed with a peptide from the Protein Inhibitor of Neuronal Nitric Oxide Synthase (PIN) (pdb 1CMI), 0.6 Å Cα rmsd over 87 residues (47% identity, Fig. 2B), and ranged among dynein light chain structures to 2.3 Å Cα rmsd over 81 residues when compared to the dynein light chain structure 1YO3 from Plasmodium falciparum (37% identity) (34,35). The main elements that show structural diversity between Dyn2 and the Drosophila LC8 structure (1CMI) are restricted to loop regions, specifically the α 1-α 2 loop, the β3-β4 loop and the β4-β5 loop. The core secondary structure elements of the domain show little plasticity. Diversity of allowable residues in the target peptide N-terminal to the canonical QT motif, in turn show structural diversity in the target β-strand backbone bound to the dynein light chain, as shown in the overlay of the Dyn2-Nup159 peptide structure with the human LC8-PIN peptide structure (Fig. 2B, Supplemental Fig. 1).

The Dyn2 homodimerization interface involves an extensive hydrophobic and electrostatic interface that buries approximately 940 Å² of solvent accessible surface area on each Dyn2 molecule. Core β-strand-β-strand hydrogen bonding networks extend the anti-parallel sheets across homodimeric mates (β2-β3’ and β2’-β3), augmented through additional backbone-side chain electrostatic interactions as well as van der Waals contacts between side chains (Fig. 3A). Helix α 2’ packs against the β3 strand and buttresses the dimerization interface through the use of charged side chains, primarily E38’ and K46’, that afford van der Waals contacts as well as hydrogen bonding to β3 residues N64 and T70 respectively (Fig. 3A, B). Overall, the homodimerization interface involves a pseudo-symmetric set of reciprocal interactions involving conserved residues (Figs. 1A, 3A).

Dyn2 exists as a multimer in solution – While the crystal structure of the Dyn2-Nup159 pep2 complex showed Dyn2 in a homodimeric state, we wanted to determine whether this dimeric form existed in solution in the absence of bound peptide. To determine Dyn2’s oligomeric state, we utilized size exclusion chromatography coupled with multi-angle light scattering (SEC-MALS). We analyzed the elution and mass profiles of purified Dyn2 injected at an initial concentration of 200 μM. The Dyn2 elution profile contained three main peaks with masses respectively calculated at 25.8 kDa, 50.5 kDa and 87.8 kDa. On average, 87% of the eluted mass fraction was in
the 25.8 kDa peak (Fig. 3C). The theoretical calculated molecular mass of our Dyn2 construct is 10,852 Da. Thus, under the conditions analyzed, peptide-free Dyn2 was found primarily as a homodimer with the remaining population in higher-order oligomeric states.

The Dyn2 homodimer binds parallel Nup159 peptides using a conserved composite binding site – Nup159 contains a pentameric array of Dyn2 binding sites (11). In the structure we present here, the Dyn2 homodimer is complexed with two Nup159 peptides corresponding to the second Dyn2 binding site in the Nup159 DID. The Nup159 peptide binds in a conserved pocket formed at the Dyn2 homodimer interface, consisting of both hydrophobic and charged residues (Fig. 4B). Nup159 pep2 (chain B) buries 911 Å² of solvent accessible surface area while each of the Dyn2 molecules bury 512 and 129 Å² for a collective 641 Å² of solvent accessible surface area buried at a single Nup159 pep2 binding site. 16 of the 25 Dyn2 residues involved in Nup159 peptide binding are 100% invariant across the twelve species shown in Fig 1A, and 22 of the 25 are at least 80% invariant across these species (Fig. 4A). In Nup159, the glutamine (Q1123) and threonine (T1124) that constitute signature dynein light chain binding determinants, bind to the periphery of the peptide binding cleft in an area of high dynein light chain conservation. Analysis of the Dyn2 electrostatic surface shows that the peptide binding cleft is composed of mixed charges near the peptide N-terminal region while positive charges dominate the electrostatic potential at the peptide’s C-terminal region. Key salt bridges in the complex include interactions between the invariant Dyn2 K12 and Nup159 D1125 as well as Dyn2 E38’ and Nup159 Q1123. The Nup159 peptides form a β-strand interaction, extending the central β-sheets formed by Dyn2 homodimerization. The Nup159 β-strand runs anti-parallel to the Dyn2 β3 strand and extends across seven residues, terminating at the glutamine, Q1123, that composes the QT motif (Fig. 4, 5). The Nup159 Q1123 side chain forms a network of hydrogen bonds with the start of the neighboring Dyn2 α2’ helix; capping the end through interactions with the R39’ backbone amine as well as one of the E38’ side chain carboxyl oxygens. In addition, the Q1123 side chain forms a hydrogen bond with the F65 backbone amine on β3. Q1123’s backbone is stabilized through a hydrogen bond to Dyn2’s Y78 hydroxyl group (Fig. 5 A-C). T1124 from the Nup159 QT motif forms extensive contacts with Dyn2 F65, engaging the F65 backbone carboxyl and amine through hydrogen bonds from its own backbone amine and side chain hydroxyl group. The T1124 side chain γC also forms van der Waals contacts with the F65 benzene ring. Preceding the QT motif, the N-terminal six Nup159 residues primarily use an anti-parallel β-strand-β-strand hydrogen bond network as well as van der Waals contacts to bind the conserved Dyn2 groove, indicative of the highly variable composition accepted in dynein light chain targets. Overall, the Nup159 pep2:Dyn2 interface is mediated by extensive hydrogen bonding and van der Waals contacts involving ten residues in the Nup159 peptide. All Nup159 residues modeled contact one Dyn2 protomer and six of these ten residues make additional contacts with the Dyn2’ homodimeric mate, indicating that high-affinity Dyn2-substrate recognition is mediated via Dyn2 dimerization. The Nup159 pep2:Dyn2 interface buries 1565 Å² of solvent accessible surface area at each binding site. The two Nup159 peptides bound to the Dyn2 homodimer run parallel to each other, related by a two-fold symmetry axis, with their mid points separated by approximately 20 Å.

Nup159 DID sites two and four respectively bind Dyn2 with 17.9 and 13.1 μM affinity, using differential thermal binding modes – To determine the affinities between Dyn2 and the five Nup159 Dyn2 binding sites in the DID, we synthesized the respective peptides and performed isothermal titration calorimetry, titrating peptides into the calorimeter cell containing Dyn2. Each Nup159 peptide binding experiment was performed in triplicate and the fitted values were averaged. Each individual binding experiment was best fit to a one-site model (using the Dyn2 monomer concentration) (33). Pep2 showed an endothermic isotherm (Fig. 6A) while pep4 showed an exothermic isotherm (Fig. 6B). The experimentally determined affinities between Dyn2 and Nup159 pep2 and pep4 are shown in Fig. 6 and have K_{D}s equal to 17.9 and 13.1 μM respectively. Pep3 did not show sufficient signal to noise and was not soluble at the concentrations.
needed to determine binding accurately. Pep1 and Pep5 are highly hydrophobic and once solubilized, failed to show binding to Dyn2 as determined using isothermal titration calorimetry (data not shown). This may be due to the weaker binding affinities for these peptides as was qualitatively shown in the aforementioned PepScan assay (11), or due to a folded/aggregated state that precluded Dyn2 from binding.

Translational arrangement of the Dyn2 homodimer facilitates contiguous binding to arrayed QT motifs – The arrangement of delineated QT motifs in Nup159 are nearly contiguous, separated by one or two amino acids except for a tentative QT region linking sites two and three that showed no Dyn2 binding activity in the previously mentioned PepScan assay (11). In the same investigation, electron microscopy of the Dyn2:Nup159 DID complex showed five densities arranged like beads on a string, leading the authors to propose a model in which five Dyn2 dimers bound parallel Nup159 DID arrays (11). Stelter et al. modeled the bound Dyn2 dimers in a translational array. In the P2_121 lattice presented here, we note a translational arrangement of Dyn2 dimers in the crystal lattice, that supports the Stelter et al. Dyn2:Nup159 complex model. As shown in Figure 7, Dyn2:Nup159 pep2 complexes are translationally arranged in the crystal, with a 34 Å translational component approximately collinear to the Nup159 peptide, effectively placing the C-terminus of one Nup159 peptide proximal to the N-terminus of the neighboring Nup159 peptide. Five Dyn2 dimers in this crystal lattice span 170 Å, on par with the 20 nm filaments observed by Stelter et al. in electron micrographs of the Dyn2:Nup159 DID complex.

DISCUSSION

The dynein light chain, while a component of the cytoplasmic dynein motor complex, is promiscuous and has been identified as a component in numerous, diverse complexes. A universal role postulated for the dynein light chain is to serve as a dimerization machine. In S. cerevisiae, 25% of the Dyn2 cytoplasmic pool is found associated with the nuclear pore complex (11). Nup159, a component of the Nup82 complex of the cytoplasmic fibrils, was identified as a Dyn2 binding partner that promotes stable association of the Nup82 complex with the NPC (11). Nup159’s pentameric array of Dyn2 binding sites link the N-terminal FG repeat region with the C-terminal NPC anchor region (20).

Our analysis of Dyn2 homodimer binding to individual Nup159 peptides showed similar affinities for pep2 and pep4, at 17.9 µM and 13.1 µM respectively, while binding for pep1, pep3, and pep5 could not be experimentally determined based on properties of the individual peptides as synthesized. Binding curves fit best to a one-site binding model and are comparable to LC8 binding to peptides of similar size: DYNLL1 binds a seven amino acid peptide from Bmf with a K_D of 1.1 µM and similarly sized nNOS peptide with a K_D of 7.0 µM (36). The affinities determined between Dyn2 and the Nup159 peptides do not take into account potential cooperativity between arrayed Dyn2 homodimers based on interactions we observed in translational symmetry mates in the Dyn2:Nup159 crystal. The affinities and differential thermal binding modes determined for Nup159 pep2 and pep4 reflects plasticity in the Dyn2 binding pocket. The Dyn2 binding site does not have many steric occlusions, and can thereby accommodate sequence diversity as observed with the LC8 family (25,36,37). The Dyn2:Nup159 crystal structure shows that extensive backbone/backbone interactions mediate the anti-parallel beta sheet extension. This backbone-based interaction affords tight binding while simultaneously enabling diversity in the side chains that flank the core, conserved QT binding motif. The QT motif is present in most Dyn2/LC8 binding peptides characterized to date and constitutes the C-terminal flank of the target peptide’s β-strand. The QT motif contributes a network of hydrogen bonds and van der Waals contacts with the dynein light chain’s conserved groove, directly contacting residues from each subunit of the homodimer. The amino acid diversity flanking the QT motif likely underlies the differential affinities and thermal binding modes observed across dynein light chain targets. Pep4 exhibited exothermic binding, indicative of a strong enthalpic, electrostatically-driven interaction, while pep2 exhibited endothermic binding, indicative of a hydrophobic, entropically-driven interaction. Pep2 and pep4 each have electrostatic and hydrophobic residues. A key hydrophobic determinant that may underlie the
endothemic binding observed with Nup159 pep2, is the tyrosine residue at position 1117, 6 residues upstream of the QT motif (i.e. Q-6; see Fig. 1D). The corresponding residue in Nup159 pep4 is an alanine. The Nup159 pep2 Q-6 tyrosine makes numerous van der Waals contacts with the Dyn2 homodimer (Fig. 5C). Peptide-specific exothermic and endothermic binding has been observed with Dyn2 homologs from other species and highlights the sequence diversity within target sites that dynein light chains are capable of accommodating (27,36-40).

Our work represents the first biophysical and structural characterization of the yeast dynein light chain, Dyn2. At physiological conditions, Dyn2 exists predominantly in the homodimeric state. As a homodimer, Dyn2 is positioned to interact with target sites and induce and stabilize parallel dimerization in these target proteins. Dimerization machines can crosslink targets, homo or heterodimerize targets, serve to architecturally extend a target, as well as promote a target’s avidity for binding partners. The Dyn2:Nup159 structure creates a foundation for understanding Dyn2’s role in the NPC as a dimerization machine that can scaffold Nup159 and extend the protein at least 170 Å (5 x 34 Å). Our structural and biophysical investigations of the Dyn2:Nup159 interaction have additional implications for Dyn2’s mode of interaction and function with the dynein intermediate chain, Pac11, its potential role in promoting Pac11 dimerization and aiding in the recruitment of the dynein activation complex, dynactin (21).
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**FOOTNOTES**

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The abbreviations used are: Nuclear Pore Complex (NPC), Nucleoporin (Nup), Dynein light chain Interacting Domain (DID), dynein Light Chain 8 (LC8), Protein Inhibitor of Nitric oxide synthase (PIN), Size Exclusion Chromatography and Multi-Angle Light Scattering (SEC-MALS), Protein Interfaces, Surface and Assemblies (PISA), Glutathione S-Transferase (GST), Phenylalanine-Glycine (FG), Luria Broth (LB), Charge-Coupled Device (CCD), Isothermal Titration Calorimetry (ITC), Root Mean Square Deviation (RMSD)

**FIGURE LEGENDS**

**FIGURE 1.** *S. cerevisiae* Dyn2 is a conserved dynein light chain involved in diverse macromolecular complexes including the nuclear pore complex and the cytoplasmic dynein motor complex. A. Sequence alignment of 12 dynein light chain family members ranging from *S. cerevisiae* to human. Residues aligned with 100% and 80% identity are colored green and yellow respectively. Amino acid numbers and secondary structure elements, based on the *S. cerevisiae* Dyn2 structure are shown above the alignment. Residues involved in Dyn2 dimerization and Dyn2-Nup159 pep2 binding are indicated below the alignment by asterisks based on EMBL-EBI PDBe PISA (Protein Interfaces, Surface and Assemblies); black: Dyn2:Dyn2 chain A:C interactions; red: Dyn2:Nup159_2 chain A:D or chain C:B interactions; blue: Dyn2:Nup159_2 chain A:B or chain C:D interactions. Solvent accessible (SA) surface area for respective Dyn2 chain C residues is indicated below the alignment, calculated in the presence (black) and
Structure of the Dynein LC – Nup159 Complex

absence of the Nup159_2 chain D (gray) using the Accessible Surface Area Analysis tool in CCP4 (41).

B. Cartoon diagram of the Nuclear Pore Complex illustrating the cytoplasmic localization of Nup159 and the Nup82 complex to cytoplasmic fibrils. C. Domain architecture of known Dyn2 binding proteins: Nup159 and Pac11. Nup159 is composed of an N-terminal β-propeller domain involved in Dbp5 binding, central FG-rich repeats common to nucleoporins, a Dyn11 light chain interacting domain (DID) composed of five QT consensus motifs (residues 1103-1177) with Dyn2 binding activity, and a C-terminal region involved in Nup159 anchoring to the Nuclear Pore Complex (11,14,15). Pac11, the yeast dynein intermediate chain, shares architectural similarities with Nup159, composed of an N-terminal coiled-coil domain, tandem Dyn2 QT binding motifs, and a C-terminal WD-40 repeat domain, predicted to be a β-propeller. D. Sequence alignment of the Dyn2 binding motifs from Nup159 and Pac11 highlighting the invariant QT motif. Nup159_2 secondary structure is indicated above the alignment. Nup159_2 residues involved in Dyn2 binding are indicated by asterisks below the alignment, as is SA surface area, calculated in the presence (black) and absence of Dyn2 chains (gray).

FIGURE 2. Structure of the Dyn2-Nup159 pep2 complex shows a quaternary complex composed of a Dyn2 dimer, bound to two Nup159_2 peptides through parallel, composite β-sheets. A. Cartoon diagram of the Dyn2-Nup159 complex. Dyn2 chain A is shown in orange (α-helices) and dark blue (β-strands), Dyn2 chain C is shown in beige (α-helices) and light blue (β-strands). The two-fold non-crystallographic symmetry operator that relates the Dyn2 and Nup159 chains in the asymmetric unit is indicated about the z-axis. Image at right shows the complex after a 90° rotation about the y-axis. B. The complex as shown in the two orientations in A, with Dyn2 chain A superimposed on the human dynein light chain, LC8 (light green) bound to a PIN peptide (yellow) (pdb 1CMI) after a least squares fit with an rmsd of 0.6 Å over 87 aligned Cα atoms (34). Helices are shown in cylindrical format. Structural differences between Dyn2 and human LC8 are indicated by red arrows, and are dominated by loop regions as well as the bound peptides.

FIGURE 3. Dyn2 homodimerizes via an extensive network of van der Waals contacts and hydrogen bonds. A. Interaction matrix, showing the pseudo-symmetric bonding and contact networks formed between Dyn2 protomers A and C in the complex. Secondary structure elements corresponding to the residues of each protomer are indicated along the axes of the matrix. Backbone/backbone, backbone/side chain, side chain/side chain, and van der Waals interactions are indicated in blue, pink, red, and grey respectively and correlate with distances less than or equal to 3.5 Å (hydrogen bonds) and 4.5 Å (van der Waals contacts). Numbers in cells indicate the total number of hydrogen bonds (greater than one) between two residues. B. Diagram of key residues and structural elements involved in the Dyn2-Dyn2 interface. The Dyn2 homodimer is shown as colored in Figure 1. Specific Dyn2 residues mediating homodimerization are shown in stick format. Hydrogen bonds are indicated as dashed lines. The interface involves extensive antiparallel β-strand-β-strand interactions as well as contributions from the α2 helices that flank the central β-sandwich. Inset shows the relative orientation of the complex. C. SEC-MALS analysis of Dyn2, injected at an initial concentration of 200 μM (green) in 100 μL. The Raleigh Ratio elution profile was normalized. Dyn2 predominantly forms a dimer in solution at pH 6.8, with additional, higher-order tetrameric and octameric species detected as well. The Dyn2 construct analyzed has a calculated monomeric molecular weight of 10,852 Da.

FIGURE 4. Dyn2 binds substrates through a highly conserved, positively charged composite groove formed by Dyn2 dimerization. A. Conservation, as highlighted in Figure 1A (green: 100% identity, yellow: 80% identity, as determined across twelve diverse species), mapped on the Dyn2 dimer shown in surface representation. Nup159 pep2 is shown in stick format in purple, inserted in the highly conserved interdimer groove. The highly conserved QT substrate motif (green sticks) is located C-terminal to the Nup159_2 β-strand. Conservation however, is equally distributed across the Dyn2 substrate-binding region. Inset shows the relative orientation of the complex in cartoon format colored as in Figure 2A. B.
Electrostatic surface calculated using APBS to generate solvent accessible surface potentials that are shown in k_BT/e, colored according to the key shown (42). Nup159 pep2 is shown in purple stick format with specific Dyn2 residues involved in hydrogen bond contacts labeled. The conserved QT motif is shown in green stick format. Dyn2:Nup159 pep2 interactions include Y68:E1119, E38':Q1123 and K12:D1125. The complex is oriented as in A.

**FIGURE 5.** The Dyn2:Nup159 pep2 interaction is mediated by an extensive interaction network that recognizes ten contiguous Nup159 residues, dually conferring specificity and substrate plasticity. A and B, Close up of residues involved in the Dyn2:Nup159 pep2 interaction. Secondary structure elements are shown as in Figure 2A, with specific residues that mediate the Dyn2:Nup159 pep2 interaction shown in stick format and their corresponding hydrogen bonding network shown with dashed lines. C, Interaction matrix, showing the contact networks formed between Dyn2 protomers A and C with Nup159. Secondary structure elements and protomer designation are indicated along matrix axes. Backbone/backbone, backbone/side chain, side chain/side chain, and van der Waals interactions are indicated in blue, pink, red and gray respectively and correlate with distances less than or equal to 3.5 Å (hydrogen bonds) and 4.5 Å (van der Waals contacts).

**FIGURE 6.** The Dyn2 interaction with Nup159 pep2 and pep4 occur in a 1:1 stoichiometry, and exhibit similar affinities but differ in their thermal binding modes. A. 17 x 2 μL of 1 mM Nup159 pep2 was injected into 200 μL of 100 μM Dyn2. The thermogram (upper panel) displays μcal/sec over the injection period (min). B. 17 x 2 μL of 1 mM Nup159 pep4 was injected into 200 μL of 100 μM Dyn2. Dyn2 binding to Nup159 pep2 (A) displayed an endothermic binding isotherm, while Dyn2 binding to Nup159 pep4 (B) showed exothermic binding. Thermograms (upper panels) were integrated and the resulting isotherm was fit to a one-site binding model (lower panels) through iterative fitting. K_D values presented (inset, lower panel) are the average of three independent experiments: Dyn2-Nup159 pep2: K_D = 17.9 ± 3.8 μM, ΔH = 2500 cal/mol, ΔS = 31 cal/mol/deg, N = 0.33 sites; Dyn2-Nup159 pep4: K_D = 13.1 ± 1.6 μM, ΔH = -4000 cal/mol, ΔS = 8.6 cal/mol/deg, N = 0.39 sites.

**FIGURE 7.** Crystallographic contacts array Dyn2 dimers linearly in an arrangement that affords polarized binding to arrayed Dyn2 binding motifs. Dyn2-Nup159 crystallographic symmetry mates shown in cartoon representation, colored as in Figure 2A. Dyn2 interdimer interactions coupled with parallel, arrayed binding motifs on Nup159 likely promote linear, cooperative binding activity between Dyn2 dimers and Nup159.
### Table 1: Data Collection and Refinement Statistics

|                          |                 |
|--------------------------|-----------------|
| Wavelength (Å)           | 1.54178         |
| Space group              | P2₁2₁2₁         |
| Cell dimensions (Å)      |                 |
| a                        | 33.9            |
| b                        | 48.0            |
| c                        | 110.7           |
| Resolution (Å)           | 50.0-1.85 (1.92-1.85) |
| Reflections              |                 |
| Measured                 | 80,909          |
| Unique                   | 15,121          |
| Completeness (%)         | 93.9 (62.7)     |
| Mean redundancy          | 5.4 (2.5)       |
| I/σ                      | 24.8 (5.9)      |
| R$_{\text{sym}}$ a       | 0.05 (0.15)     |

### Refinement

| Resolution (Å)           | 29.3-1.85 (1.92-1.85) |
| R$^b$/ R$_{\text{free}}$ (%) c | 15.1 (18.0) / 18.7 (24.7) |
| No. reflections, R/R$_{\text{free}}$ | 13,573/1508 |
| Total atoms              | 1758             |
| Protein/water            | 1541/217         |
| Stereochemical ideality (rmsd) | 0.006/1.02 |
| Bonds/angles (Å/°)       |                 |
| Mean B-factors (Å$^2$)   |                 |
| MC/SC/water              | 13.8/19.9/28.5  |
| B-factor rmsd (Å$^2$)    |                 |
| MC/SC                    | 1.4/2.5         |
| Ramachandran analysis    |                 |
| Favored/allowed (%)      | 98.4/1.6        |

Parentheses list statistics for the high resolution shell

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* $R_{\text{sym}} = \Sigma_n \Sigma_h |I(h)| - <I(h)> |\Sigma_h \Sigma_i I(h)$ where $I(h)$ is the integrated intensity of the $i$th reflection with the Miller Index $h$ and $<I(h)>$ is the average over Friedel and symmetry equivalents.

* R value = $\Sigma (|F_{\text{obs}}| - k|F_{\text{calc}}|)/ \Sigma |F_{\text{obs}}|$.

* $R_{\text{free}}$ is calculated using a 10% subset of the data that are removed randomly from the original data and excluded from refinement.
A. Structure of the Dynein LC – Nup159 Complex

1. Cytoplasmic Fibrils

2. Nuclear Basket

3. N. cerevisiae Nuclear Pore Complex

4. S. cerevisiae

5. A. gossypii

6. C. dubliniensis

7. S. pombe

8. D. discoideum

9. A. laevis

10. X. laevis

11. D. rerio

12. R. norvegicus

13. P. troglodytes

14. C. elegans

15. D. melanogaster

16. H. sapiens

B. Diagram of Nup159 and Pac11 domains

1. N-terminal domain

2. FG repeats

3. DID

4. C-terminal domain

5. Dyn2 binding

6. WD-repeat

7. Coiled-coil

C. Diagram of Nup159 and Pac11 domains

1. N-terminal domain

2. FG repeats

3. DID

4. C-terminal domain

5. Dyn2 binding

6. WD-repeat

7. Coiled-coil

D. Diagram of Nup159 and Dyn2 contacts

1. Nup159 contacts

2. Dyn2 contacts

3. SA surface area

FIGURE 1
FIGURE 2

Structure of the Dynein LC – Nup159 Complex

A

Dyn2' (C)

Nup159 (B)

Dyn2 (A)

β2' - β3 loop

β4 - β5 loop

α1 - α2 loop

B

Dyn2' (C)

Peptide

PIN

Nup159 (B)

LC8

Dyn2 (A)

90°

β3 - β4 loop

β4' - β5' loop

β5

β5'

α1-α2 loop
FIGURE 4

A

B

100% identity 80% identity

Q1123 T1124

Y68 E1119

D1125 K12

-2.0 k_BT/e 2.0 k_BT/e
FIGURE 5

A

B

C

Nup159_2 (B)

Dyn2' (C)

Dyn2' (A)

Interactions

- Backbone/Backbone
- Backbone/Side Chain
- Side Chain/Side Chain
- van der Waals Contacts

Structure of the Dynein LC – Nup159 Complex
FIGURE 6

A  

Dyn2 / Nup159_2 Binding

B  

Dyn2 / Nup159_4 Binding

$K_D = 17.9 \pm 3.8 \mu M$

$K_D = 13.1 \pm 1.6 \mu M$
FIGURE 7

Structure of the Dynein LC – Nup159 Complex

[Image of the structure of the Dynein LC – Nup159 Complex]
The Structure of a yeast Dyn2-Nup159 complex and the molecular basis for the dynein light chain - nuclear pore interaction
Erin M. Romes, Ashutosh Tripathy and Kevin C. Slep

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