The Mtr2-Mex67 NTF2-like Domain Complex

STRUCTURAL INSIGHTS INTO A DUAL ROLE OF Mtr2 FOR YEAST NUCLEAR EXPORT*

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Claire Senay‡, Paul Ferrari§, Corinne Rocher§, Klaus-Jörg Riegèr§, Jacques Winter§, Denis Platé, and Yves Bourne¶

From the AFMB CNRS, UMR 6098, 31 Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France and the Aventis Pharma, Infectious Diseases Group, 102 Route de Noisy, 93235 Romainville Cedex, France

The formation of the Mtr2-Mex67 heterodimer is essential for yeast mRNA export as it constitutes a key nuclear component for shuttling mRNA between the nuclear and cytoplasmic compartments through the nuclear pore complex. We report the crystal structures of apo-Mtr2 from the human pathogen Candida albicans and of its complex with the Mex67 NTF2-like domain. Compared with other members of the NTF2 fold family, Mtr2 displays novel structural features involved in the nuclear export of the large ribosomal subunit and consistent with a dual functional role of Mtr2 during yeast nuclear export events. The structure of the Mtr2-Mex67 NTF2-like domain complex, which overall is similar to those of the human and Saccharomyces cerevisiae homologs, unveils three putative Phe-Gly repeat binding sites, of which one contributes to the heterodimer interface. These structures exemplify an unrecognized adaptability of the NTF2 building block in evolution, identify novel structural determinants associated with key biological functions at the molecular surface of the yeast Mtr2-Mex67 complex, and suggest that the yeast and human mRNA export machineries may differ.

In eukaryotic cells, the nuclear and cytoplasmic compartmentalization requires that a large number of molecules be continuously transported through the nuclear pore complex (NPC), a huge macromolecular structure that spans the nuclear envelope. Nucleoporins represent a subset of the NPC components and form a dense network of proteins that line the channel of the NPC. Transport through the channel requires binding of protein or RNA cargoes to soluble transport receptors and is mediated by the phenylalanine-glycine (FG) repeats that characterize most nucleoporins. The two most common repeats that are found in nucleoporins and are often present in many copies along the whole molecule are based on GLFG or FXFG cores (1–3).

The best characterized pathway of protein import and export involves members of the conserved family of transport receptors called karyopherins/importin-β, also known as exportins/importins. The karyopherin transport factors share a common structural framework and respond to the small GTPase, Ran, to bind or release their cargo within the appropriate cellular compartments. Meanwhile, Ran must also shuttle across the NPC to equilibrate its nuclear level; this is performed via a specific nuclear import factor known as nuclear transport factor 2 (NTF2) (4–8).

Unlike this well established protein transport pathway, no karyopherin family member that would function in general mRNA export has been identified. Instead, recent studies in yeast and metazoans have pointed to several highly conserved proteins that are known as nuclear export factor (NXF) and are specifically required for mRNA export, but do not include karyopherin or NTF2 (9). Among members of the NXF family, the best characterized candidate is the Saccharomyces cerevisiae protein Mex67, whose conserved metazoan counterpart is known as TAP or NXF. Mex67 interacts with both bulk poly(A+)-RNA and nuclear pore complexes (10) and binds ~1150 mRNAs corresponding to ~36% of all transcriptional events in yeast (11); this suggests that Mex67 plays a key role in yeast nuclear mRNA export. The specific association of Mex67 with the small Mtr2 protein was shown to be essential for nuclear mRNA import and this heterodimer also shuttles between the nucleus and the cytoplasm (12, 13). Mex67/TAP is structurally unrelated to other Ran-dependent karyopherins and is composed of three distinct modules: a leucine-rich repeat (LRR) domain, a middle (M) domain that shares a NTF2-like fold and a carboxyl (C) domain with a UBA (ubiquitin-associated) fold (8, 9).

The M and C domains of Mex67/TAP were shown to function in shuttling and contacting the nucleoporins FG repeats that line the pore channel (14–16). This suggests that they compete with the karyopherins for binding to FG nucleoporins and that both types of receptors use the same binding sites for translocation through the NPCs. The crystal structure of the human TAP-p15 heterodimer complex bound to a FG repeat demonstrated that this complex closely resembles the NTF2 homodimer necessary for shuttling Ran (17). In addition, a single FG repeat binding site was identified at the molecular surface of the TAP NTF2-like domain, consistent with genetic and biochemical studies (16).

Overexpression of the human TAP-p15 complex partially restores the growth of the lethal mtr2/mex67 double knockout strain in yeast (18), suggesting the existence of a conserved mRNA export machinery from yeast to humans (8). Indeed, the crystal structure of the S. cerevisiae Mtr2-Mex67 NTF2-like fold domain (Mtr2-Mex67) complex revealed that Mtr2 is a novel member of the NTF2-like family and that its association

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The atomic coordinates and structure factors (code 1Q40, 1Q42) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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¶ To whom correspondence should be addressed. Tel.: 33-4-91-16-45-08; Fax: 33-4-91-16-45-36; E-mail: yves@afmb.cnrs-mrs.fr.

1 The abbreviations used are: NPC, nuclear pore complex; NTF2, nuclear transport factor 2; r.m.s.d., root mean square deviation; Ca, C. albicans.
with Mex67 resembles that of the TAP-p15 complex (19). However, several more recent discordant results remain to be elucidated. First, Mex67 must associate with Mtr2 to efficiently bind to FG repeats (14), whereas TAP can interact with FG nucleoporins in the absence of p15 (16). Second, Mtr2 associates with Nup85, a subunit of the Nup84 complex that is crucial for nuclear mRNA export (13) and both Mex67 and Mtr2 communoprecipitate with the NPF-associated protein SacII (20). Finally, two Mtr2 mutant alleles (mtr2-1 and mtr2-55) work together to inhibit the 60S ribosomal subunit export (21) while Mtr2 associates with the 60S pre-precursor particle Arx1 during ribosomal nuclear export (22), indicating that Mtr2 alone is directly linked to ribosomal nuclear export. Altogether, these data suggest that Mtr2, unlike p15, has gained additional functions in binding non-FG containing proteins within the NPCs, and that Mtr2 and p15 have related but distinct functions for nuclear export of mRNAs.

To address these issues, we have solved the crystal structures of Candida albicans apo Mtr2 and of its complex with the Mex67 NTF2-like domain at 1.75 Å and 1.95 Å resolution, respectively. The structure of Mtr2 unexpectedly shows that it retains its NTF2 fold and does not assemble as a homodimer in contrast to other members of the NTF2 fold family. Moreover, this structure highlights novel features that are unique to the yeast proteins and may explain the additional functions recently reported for yeast Mtr2, compared with its human homolog p15. Finally, the overall structure of the Mtr2-Mex67 complex, which overall resembles those of the human and S. cerevisiae homologs, reveals the presence of three putative FG repeats binding sites respectively located in Mex67, Mtr2 and at the complex interface.

EXPERIMENTAL PROCEDURES

Expression and Purification of the Native and Selenomethionyl Proteins—The nucleotide sequence of C. albicans Mtr2 was subcloned into a pET28 derivative expression vector, resulting in the pSEZ52 expression construct, and transformed into BL21 (DE3) Escherichia coli cells. Expression of N-terminal His-tagged Mtr2 was induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 4 hours at 28 °C according to the high cell density cultivation protocol (23), supplemented with 60 g/liter glycerol and 20 g/liter casamino acids. The recombinant protein was purified by immobilized metal affinity, anion exchange and size exclusion chromatography and dialyzed against 50 mM HEPES pH 7.5, 500 mM NaCl. The final yield was 0.70 mg of pure Mtr2 per g of wet pellet. Purified Mtr2 was concentrated to 3.5 mg/ml and stored at −20 °C.

The nucleotide sequence of the Mex67 NTF2-like domain (residues 294–512) was inserted immediately after the 6-His-tagged Mtr2 termination codon (plus the Shine-Dalgarno sequence) into the pSEZ52 expression vector, resulting in the pSEZ52 expression vector, resulting in the pSEZ52 expression construct. Co-expression in BL21 (DE3) E. coli cells used the same conditions as for Mtr2; the heterodimer complex was purified and dialyzed as described for Mtr2, except that cation exchange was used as the second purification step, concentrated to 9.5 mg/ml and stored at −20 °C. The final yield was 0.29 mg of pure complex per g of wet pellet.

For production of selenomethionyl (Se-Met) Mtr2-Mex67 complex, the pSEZ52-Mdomain plasmid was transferred to a Meta− β180 (DE3) E. coli strain; co-expression of the complex was performed as described above, except that Se-Met was added to the medium instead of casamino acids, glycerol at 30 g/liter was used as a carbon source and the induction time was increased to 18 h. The purification procedure was identical to that used for the native complex.

Protein Crystallization—C. albicans Mtr2 was crystallized by vapor diffusion at 20 °C using hanging drops containing equal volumes of the protein solution and of a reservoir solution made of 0.8 M NaH2PO4/K2HPO4 at pH 7.0. Needle-shaped Mtr2 crystals appeared within 3 days and belong to the monoclinic space group P21 with cell dimensions a = 62.5 Å, b = 54.3 Å, c = 61.3 Å, and β = 90.0° and contain one Mtr2 molecule per asymmetric unit. Crystals selected for data collection were briefly soaked by successive steps into the reservoir solution supplemented with increasing amounts of glycerol up to 20–25% (v/v), flash-cooled at 100 K in the nitrogen gas stream and stored in liquid nitrogen.

A 1.75 Å resolution data set was collected on beamline ID4-EH2 at ESRF (Grenoble, France).

The C. albicans Se-Met Mtr2-Mex67 complex was crystallized at 20 °C after mixing equal volumes of the complex solution and of a reservoir solution made of 18% PEG 4000, 0.2 M imidazole-malate, pH 6.0. Thick plate-shaped crystals appeared within 1 week; they belong to the monoclinic space group P21, with cell dimensions a = 70.1 Å, b = 54.1 Å, c = 109.7 Å, and β = 96.1° and contain two Mtr2-Mex67 complexes in the asymmetric unit. A 3.5-wavelength MAD experiment was performed on beamline BW7A (peak, inflection) and X11 (remote) (DESY, Germany) using the Se-Met Mtr2-Mex67 complex crystals. All oscillation images were integrated with DENZO (24), and data were scaled and merged with SCALA (25). Data collection statistics are reported in Table I.

Structure Solution and Refinement—The SOLVE program (26) was used to identify 11 out of 18 Se atoms present in the asymmetric unit of the Mtr2-Mex67 complex crystals, giving experimental phases to 2.9 Å resolution with an overall figure of merit of 0.52. Further phase improvement techniques, including solvent flattening, non-crystallographic symmetry (NCS) averaging and phase extension with RESOLVE yielded an electron density map interpretable up to 1.95 Å resolution. The model was automatically built using ARP/wARP (27), carefully examined using TURBO-FRODO (28), and further refined using REFMAC (29). The final Mtr2 model comprises residues 3–176 with a disordered loop from residues 125–136; the Mex67 NTF2-like domain residues 305–509 could be modeled with the exception of two disordered surface loop regions (residues 346–351 and 446–457).

The structure of C. albicans Mtr2 in its apo form was solved by the molecular replacement method with AMoRe (30) using the coordinates from the Mtr2-Mex67 complex as search model. Further refinement steps using REFMAC (29), alterned with a graphic inspection of the model using TURBO-FRODO (28), yielded the final model. The structure of apo-Mtr2, which encompasses residues 3 to 176 with the exception of a disordered surface loop region (residues 127–135), is very similar to that of Mtr2 in the complex with an r.m.s.d. of 0.5 Å for 152 Ca atoms. The r.m.s.d. between the two complexes present in the asymmetric unit is 0.58 Å for 333 Ca atoms. Refinement statistics are reported in Table I. The stereochemistry of the refined structures were analyzed using PROCHECK (31). No residue was found in the disallowed region of the Ramachandran plot. The coordinates of the apo-Mtr2 and Mtr2-Mex67 complex have been deposited with the Protein Data Bank, accession numbers 1Q42 and 1Q40, respectively. Figs. 1 and 3–5 were generated with SPOCK (32) and Raster3D (33), and Fig. 2 with ESPript (prodes.toulouse.inra.fr/ESPrpt).

RESULTS AND DISCUSSION

Clustering and Structure Determination—Initial efforts to solve a Mtr2 structure by MAD phasing using Se-Met-substituted protein were hampered by the weak phase information provided by a single well ordered Se-Met residue out of three. In addition, the low solvent content of these crystals along with the presence of a single molecule in the asymmetric unit precluded the use of phase improvement techniques. Subsequent attempts to solve the Mtr2 structure using crystals soaked/co-crystallized with heavy atom derivatives or halides or using various NTF2-like structures as search model were again unsuccessful. However, success in obtaining a soluble complex by co-expression in bacteria along with the high number of Met residues (six) present in the sequence of the Mex67 NTF2-like domain prompted us to solve the structure of Mtr2 in complex with the Mex67 NTF2-like domain by MAD phasing using a Se-Met-substituted protein complex. Then the structure of apo-Mtr2 could be solved by molecular replacement using, as a search model, the coordinates of Mtr2 from the Mtr2-Mex67 complex (Table I).

Mtr2 Retains Its NTF2 Fold and Does Not Assemble as a Dimer—The sequences of yeast Mtr2 proteins are significantly longer than those of any other members of the NTF2 family (180 amino acids in Mtr2, 125 in human p15, and 140 in yeast NTF2). Yet, Mtr2 shares the overall α/β barrel made of a highly curved, central 5-stranded β-sheet that is flanked on one side by one long and two shorter α-helices and is characterized by an atypical cone-like shape reminiscent of other members of
the NTF2 fold family, as also found for *S. cerevisiae* Mtr2 bound to the Mex67 NTF2-like domain (Fig. 1) (5, 19, 34).

Structure-based sequence alignment of Mtr2 with either p15 or NTF2 shows only 18 and 15% identities (Fig. 2), respectively, resulting in r.m.s. deviations of 1.4 Å and 1.5 Å for only 104 and 102 Cα atoms. In fact, Mtr2 displays several new structural features that are unique to yeast proteins compared with other members of the NTF2 fold family, but were not visible in the crystal structure of the homologous *S. cerevisiae* Mtr2 bound to the Mex67 NTF2-like domain (19) (Fig. 1). In comparison to human p15 and *S. cerevisiae* Mtr2, and starting from the N-terminal region, the *C. albicans* Mtr2 structure reveals: a large extension in the α1-α2 loop (insert 1, 18 residues) that protrudes 20 Å away from the p15 β-hairpin; a newly formed helix 2 along with melting of p15 helix 2; a second insertion within p15 β2 that defines a new loop region (insert 2, seven residues); a third insertion that protrudes 20 Å away from the short turn (insert 3, 31 residues) in the p15 β4-β5 loop and a 5-residue elongation of β5 (Fig. 1). The conservation of most of these structural elements within yeast Mtr2 proteins suggests that they are involved in novel biological functions and that Mtr2 alone can fulfill these additional functions independently from its association with Mex67 (Fig. 2).

Gel-filtration experiments showed that Mtr2 is monomeric in solution (data not shown) as it is in the crystals. An insertion in the p15 β3-β4 loop has been reported to prevent homodimer formation of human p15 (17), but no such impairment is found in a putative Mtr2-Mtr2 homodimer modeled from the typical NTF2 homodimer (34). Instead, the interface of this Mtr2-Mtr2 dimer presents unfavorable acidic bridges between Asp residues and steric clashes involving the His91, His102, and Arg169 side chains. Therefore, in contrast to NTF2, which is functional as a homodimer for binding GDP Ran and nucleoporin FG repeats (5), yeast Mtr2 and human p15 have evolved to specifically form functional heterodimers with the Mex67 and TAP NTF2-like domains, respectively.

Mtr2 Possesses the Hydrophobic Cavity Identified in NTF2 but Not in p15—As a consequence of the unusual NTF2 conical shape, the αβ barrel opening out at the wider end creates a hydrophobic cavity between the three helices on one side and the curved β sheet on the other side (34). The crystal structure of the NTF2-Ran complex revealed that residues from the NTF2 hydrophobic cavity largely contribute to the binding interface with Ran where Phe72 from the switch II loop is inserted (35). Mtr2, unlike p15, exposes at the open end of the cone a similar hydrophobic cavity, 15 Å long, 6–7 Å wide, and 6 Å deep, that is lined by hydrophobic residues (Val54, Ile59, Phe71, Trp75, Pro79, Phe109, Tyr168, Phe144, Phe170, and Val173) and surrounded by charged/polar residues (Asn55, Glu57, Lys74, Glu77, Glu111, and Gln175) (Figs. 1 and 3). This hydrophobic patch is also present in the homologous *S. cerevisiae* Mtr2 but the bulkier Phe54 side chain in place of the *C. albicans* Mtr2 Val significantly reduces the depth of the cavity (19).

The hydrophobic residues present in the upper part of the NTF2 cavity along with the surrounding negatively charged residues significantly contribute to GDP Ran binding (35). Interestingly, two (Glu42, Asp94) out of the three negatively charged residues in NTF2 that form key salt bridges to Arg/Lys residues in the Ran switch II loop are replaced by uncharged residues (Asn55 and Glu111) in Mtr2 (Fig. 3). Structural comparison of Mtr2 and NTF2 bound to GDP Ran unambiguously reveals that the tip of insert 3 and the shifted and longer C-terminal region in Mtr2 prevent such interaction. Moreover, the side chains of Phe109 and Phe170 in Mtr2 occupy the position of Ran switch II Phe72, as does Phe135 in human p15 (17), making the formation of a complex between Mtr2 and GDP Ran unlikely. This is in agreement with previous data showing the existence of a Ran-independent pathway for nuclear export of spliced mRNA (36).

The Mtr2 hydrophobic pocket is ideally shaped to accommodate flat, slightly bent molecules containing both aromatic and polar groups, and several export factors, that were shown to

### Table I

| Data collection | Apo Mtr2 |
|----------------|----------|
| Beamline | DESY-BW7A | DESY-BW7A | DESY-X11 | ESRF-ID14-EH2 |
| Wavelength (Å) | 0.9811 | 0.9809 | 0.915 | 0.932 |
| Space group | P21 | P21 | P21 | C2 |
| a, b, c (Å), β (°) | 70.10 | 54.14 | 109.66, 96.09 | 62.49, 54.31, 61.28, 99.02 |
| Resolution range (Å) | 25-2.50 | 15-2.55 | 20-1.95 | 25-1.75 |
| Rmerge (%) | 5.4 (0.252) | 5.4 (0.337) | 5.3 (0.311) | 5.6 (0.36) |
| Rfree (%) | 4.5 (0.252) | 5.9 (0.236) | 4.0 (0.345) | — |
| No observations | 222 255 | 239 905 | 530 670 | 125 926 |
| No unique | 26 795 | 26 773 | 56 333 | 19 910 |
| Completeness (%) | 98.4 (88.3) | 98.4 (88.7) | 91.6 (67.0) | 94.8 (84) |
| Redundancy | 3.4 | 3.7 | 4.3 | 2.3 |
| Refinement | 7.1 (1.5) | 5.8 (1.9) | 9.5 (2.3) | 4.7 (1.8) |

**Data collection and refinement statistics**

### Se-Met Mtr2-Mex67 complex

| Edge (Se) | Peak (Se) | Remote (Se) |
|-----------|-----------|-------------|
| Protein atoms | 5526 | 2636 |
| Solvent/fugid atoms | 321/18 | 70— |
| Rcryst/Rfree (%) | 18.0/27.9 | 19.9/23.4 |
| r.m.s. deviations | | |
| Bond distances (Å) | 0.1 | 0.09 |
| Bond angles (°) | 1.19 | 1.22 |
| Chiral volume (Å³) | 0.072 | 0.081 |
| B-factor (Å²) | 38.7 | 28.03 |
| Side chain | 40.0 | 30.45 |
| Solvent | 42.0 | 27.87 |

* Values in parentheses refer to the last resolution shell.
* Rmerge = Σ|Fo-Fc|/ΣFo; Rfree = Σ|Fo-Fc|/ΣFo (Fo<sub>≤2L</sub>, Fo<sub>≤2L</sub>, Fo<sub>≤2L</sub>).
* Per asymmetric unit, corresponding to two Mtr2-Mex67 complexes and to a single molecule of Mtr2, respectively.
* Rcryst = Σ|Fo-Fc|/Σ|Fo|.
interact specifically with Mtr2 during mRNA translocation, would represent excellent candidates. Potential partners include Nup85 that belongs to the Nup84 hexameric assembly, a key component for nuclear mRNA export (13), and SacIII, a nuclear pore-associated protein that associates with Mtr2, Mex67 and other factors involved in mRNA export (20). Further biochemical studies are required to clarify the mode of binding of these export receptors, but the hydrophobic cavity identified at the molecular surface of Mtr2 is likely to be involved in these recognition events.

A Novel Functional Core Domain Involved in the Nuclear Export of the Large Ribosomal Subunit—A dominant and striking feature of Mtr2, compared with other members of the NTF2 family, resides in the junction of inserts 1 and 3, which are confined at the open end of the cone and define a novel functional core domain. The center of this domain is delineated by the tight packing of a /H9252-turn (residue 115–118) from insert 3 with side chains belonging to insert 1, along with the participation of few residues within helix /H9251 and /H9252-strand /H9252. The conserved Arg116 residue plays an important role in stabilizing this additional core: the Arg116 side chain, which protrudes from the /H9252-turn of insert 3, is deeply buried within the core where it establishes a network of hydrogen bonds with the side chains of Tyr38, Thr78, and Ser81 and carbonyl atoms of Leu20 and Thr78 (Fig. 1). This additional domain, that protrudes 20 Å from the open end of the cone, adopts an elongated shape and exposes two fully solvent-accessible surface regions located on different faces of the molecule. These two regions could represent recognition sites for unidentified export factors.

On one face of the additional core, a small depression is created with its center located only 16 Å away from the hydrophobic pocket. This small depression, which is separated from the larger hydrophobic pocket by a protruding segment formed by the Gln77-Thr78-Pro79 residue triplet located in the /H9252 loop, is lined by aliphatic residues (Val35, Leu76, Leu117) surrounded by charged/polar residues (Lys73, Gln77, Asn115, Glu119) as found for the NTF2-like hydrophobic pocket of Mtr2. In yeast Mtr2, the close proximity of these two pockets on the same face of the molecule at the open end of the cone, and their lining with invariant or conserved residues, form an extended surface region that may have important biological functions.

The opposite face of the additional core domain exposes a large, 18 Å long, patch of invariant residues that include the Lys and Arg residues from stretch 106KVRFD110 in /H9254, and the

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**Fig. 1. Overall folds of yeast Mtr2/Mex67 versus human p15/TAP.** Ribbon representation of the structure of Mtr2 (cyan) (A) compared with that of human p15 (yellow) (B), the Mex67 (orange) (C), and human TAP (red) (D) NTF2-like domains, viewed in a similar orientation. The three insertion loops characteristic of Mtr2, namely insert 1, 2, and 3, are displayed in magenta, orange, and green, respectively, and those that occur in the /H1-α2 and /H4-β5 loops of Mex67 in magenta and green, respectively. The secondary structure elements of Mtr2/p15 and Mex67/TAP are indicated. The middle panel in A shows a close-up view of the tight interactions between the conserved Mtr2 Arg116 (green carbon atoms) located in insert 3 and surrounding residues from insert 1 (magenta carbon atoms), the α3-β3 loop and the C-terminal region of α1 (blue carbon atoms). The right panel in A shows a close-up view of the additional core domain with mutated side chains affecting Mtr2 function (orange), the long conserved stretch (yellow), and the invariant residues (green) along with the bound glycerol molecule (magenta).
Pro and two Trp residues from stretch $^{137}$RPIWGSW$^{143}$ in the C-terminal part of insert 3 (Fig. 1). To know whether this surface region is involved in a recognition event during yeast mRNA export must await further biochemical experiments guided by our structural results, but two temperature-sensitive mutations in *S. cerevisiae* Mtr2, Leu$^{140}$ (CaIle$^{139}$)Pro and Gly$^{142}$ (CaGly$^{141}$)Asp, that are lethal when combined with the *mex67-5* mutation have been identified within this surface-exposed region (13, 20). Moreover, the recent identification of a mutant allele of *S. cerevisiae* Mtr2 (*mtr2-33*), Glu$^{106}$ (CaGln$^{111}$)Gly and Arg$^{109}$ (CaArg$^{114}$Gly), which displays a defect in ribosome but not mRNA export, indicates that this separate functional region at the molecular surface of Mtr2 is directly involved in nuclear export of the large 60S ribosomal subunit (21). A glycerol molecule arising from the cryoprotectant solution used for flash-cooling of the crystals is bound to Mtr2 conserved residues Asp$^{110}$, Arg$^{114}$, Asn$^{115}$, Gly$^{118}$, and Pro$^{138}$, and is very close (9 Å) to the conserved Trp$^{140}$ residue located in the extended surface patch region (Fig. 1), where it may mimic part of a potential ligand, as is often seen into the catalytic site of crystalline proteins. Therefore, the Mtr2 NTF2-like scaffold, compared with its human homolog, may have evolved to acquire additional functions specific for yeast nuclear ribosomal export, in addition to its conserved role in Mex67 recognition and NPC association.

**Fig. 2.** Structure-based sequence alignments of members of the NTF2 fold family. A, alignment of *C. albicans* (Ca) Mtr2 with other yeast Mtr2 proteins from *S. cerevisiae* (Sc) and *C. glabrata* (Cg), Ca NTF2, and human p15, based on a structural comparison performed with LSQMAN (41). Invariant and conserved residues between these proteins are highlighted with a black background and a boxed area, respectively. The secondary structure elements of Ca Mtr2 are shown in blue above the sequences, and those of p15 underneath. The three insertions in Ca Mtr2 (namely insert 1, 2, and 3) are indicated. B, sequence alignments of the Ca, *S. pombe* (Sp) and Sc NTF2-like domains of Mex67 are presented with that of human TAP. Invariant and conserved residues as well as the secondary structure elements between these members of the NXF family are indicated as in A.

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**Dual Role of Mtr2 for Yeast Nuclear Export**

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a noncanonical RNP-type RNA binding domain exclusively present in vertebrate NXF proteins, four leucine-rich repeats, an NTF2-like domain and an ubiquitin-associated domain (9). Whereas no obvious sequence similarity can be detected between Mtr2 and p15, the Mex67 NTF2-like domain shows significant sequence homology with the TAP homologous domain (20% identity) and other NXFs as previously described (Fig. 2) (10). This high sequence homology is supported by the high structural homology of these two NTF2-like domains, with r.m.s. deviations of 1.1 Å for 129 Ca atoms. Compared with NTF2, Mex67 possesses the macroscopic insertion, made of two short α-helices (named 2A and 2B in the β1-β2 loop) located at the open end of the cone, that prevents both a possible homodimeric association and the binding of GDP Ran, as previously identified for TAP (17) but that was removed in the construct used to solve the S. cerevisiae Mex67 crystal structure (19) (Fig. 3). In Mex67, the occlusion of the GDP Ran binding pocket is even more pronounced with the presence, in the β4-β5 loop, of a 29-residue insertion that is packed on top of helices α2A and α3 and protrudes 16 Å away into the solvent (Fig. 1). The biological significance of these insertions that are found in both Mtr2 and Mex67 and are mostly conserved in yeasts is not known, but it is intriguing that the largest loop insertions emerge from the same structural elements, i.e. between β-strands 4 and 5, and are all located at the open end of the cone characteristic of the NTF2 fold.

The Mtr2-Mex67 NTF2-like Domain Complex—The NTF2-like domains of Mex67 and Mtr2 interact to form a compact heterodimer similar to the NTF2-NTF2 and TAP-p15 homodimers (Fig. 4). The overall conformations of Mtr2 in the apo form and in complex with the Mex67 NTF2-like domain are highly similar, indicating that binding of the Mex67 NTF2-like domain does not require significant conformational changes in Mtr2. The two Mtr2 and Mex67 NTF2-like domains face each other across the convex faces of their β sheets, resulting in a near 2-fold symmetry. The major interacting β surfaces are lined mostly by hydrophilic residues buried at the heterodimer interface with the participation of few hydrophobic residues that are clustered at each extremity of the heterodimer interface. The surface area buried to a 1.6 Å probe radius is ~1700 Å², a value similar to that found in the TAP-p15 complex. Surprisingly, a few invariant residues are found in the binding interface, suggesting that the predominantly hydrophilic interface seen in the Mtr2-Mex67 complex can accommodate side chain substitutions without drastic perturbation of its binding properties; this would be consistent with the high number (~20) of solvent molecules buried at the heterodimer interface. The center of the Mtr2-Mex67 heterodimer interface is rich in polar Ser and Thr residues that belong to buried β-strands. Mutation of His409 to Tyr (mex67–5) in the S. cerevisiae Mex67 NTF2-like domain (C. albicans Mex67 His431) impairs interaction with its Mtr2 partner and association with nuclear pores, resulting in mislocalization of the protein to the cytoplasm at the nonpermissive temperature (10). Indeed, C. albicans Mex67 His431 is deeply buried at the heterodimer interface where it is surrounded by several charged residues from both Mtr2 and Mex67, thus preventing accommodation of a bulkier side chain.

Overall, the structures of the human and yeast heterodimer complexes are well conserved but significant differences are observed in the structural determinants and the nature of the residues that form the heterodimer interfaces (Fig. 4). Indeed, the 3 A shifted position of the N-terminal region of helix α1 in Mtr2, compared with its position in p15, modifies the mode of interaction with the Mex67 helix α2B. In addition, the conformational differences of the two β1-α2A and β3B-β4 surface loops in Mex67, located at each extremity of the interface, significantly contribute to the species specificity of the complex. The tight interaction between the Asp<sup>561</sup>-Trp<sup>567</sup> stretch from the extended C terminus in Mex67 (that replaces TAP helix 4 due to the presence of Pro<sup>560</sup> that impairs α-helix formation) and residues from the extended C-terminal region in Mtr2 participates to the species specificity of the heterodimer interface (Fig. 4). Among the 11 Mtr2 and 15 Mex67 side chains that establish direct hydrogen bonds at the heterodimer interface, only 3 are invariant in both p15 and TAP. This low number of conserved residues along with the structural differences between the two complex structures are consistent with previous
observations showing that the TAP-p15 complex cannot fully substitute for the Mtr2-Mex67 complex and that cross-species complexes cannot form (17, 18).

**Structural Comparison with the S. cerevisiae Mtr2-Mex67 NTF2-like Domain Complex**—The two yeast complex structures are highly similar with r.m.s. deviations of 1.2 Å for 234 Ca atoms, corresponding to 111 and 123 Ca atoms from Mtr2 and Mex67, respectively (Fig. 4). The large Mex67 macroscopic insertion along with the additional β-strand 7 identified in the C terminus of S. cerevisiae Mex67 NTF2-like domain significantly contribute to the binding interface. The high structural homology between these two yeast complexes does not include those Mtr2 side chains that establish key hydrogen bonds at the heterodimer interface. Indeed, only four out of the 11 Mtr2 residues are invariant between the two yeast Mtr2 proteins, while 11 out of 15 Mex67 residues are invariant. Large structural differences are found within Mtr2 with the largest, of up to 7 Å, being located in the α2 helical region upstream the novel core domain. This suggests that, in solution, this region adopts distinct conformations that, in turn, could affect the overall conformation of the novel core domain.

**Nucleoporin FG Repeat Binding Sites**—The crystal structures of different nuclear transports (NTF2, TAP-p15, and importin-β) bound to FG-nucleoporin repeats provide clues about the key recognition process that involves one or two Phe side chains of the FG/FxF-repeat cores and hydrophobic residues from a surface depression along with polar or even charged side chains that surround the cavity; the aliphatic portions of the later can form hydrophobic interactions with the Phe ring while their hydrophilic ends can be hydrogen-bonded to the peptide backbone of the repeat core (37). Moreover, GLFG and FXFG repeats bind to overlapping sites on importin-β, indicating that functional differences between these two repeats probably arise from differences in their spatial organization, thus highlighting the nature of the linker in conferring repeat specificity (38).

Given the high structural homology found between Mex67 and TAP, we were confident in finding, at the molecular surface of Mex67, a FG repeat binding site analogous to that identified in the crystallographic structure of the TAP-p15 complex bound to a FG-repeat nucleoporin (17). Indeed, in Mex67, a similar hydrophobic pocket where the Phe moiety of the FG core could be inserted, is made of the conserved residues Ile426 (TAP Leu491) and Val481 (Ala519) that define the floor of the cavity and are surrounded by Leu308 (Leu386), Ser305 (Leu383), Pro483 (Pro521), and Met489 (Leu527) (Fig. 5). However, the conformational change seen at the tip of the Mex67 3b–4 loop, compared with TAP, significantly affects the sequence specificity for the additional polar interactions that are required between Mex67/TAP and the peptide backbone. Indeed, the protruding Mex67 Lys419 and Asn424 side chains within this loop are well positioned to promote this interaction. In the homologous S. cerevisiae complex, this cavity is not present due to a crystalline symmetry-related molecule that modifies the conformation of the 5b–6 loop containing Pro453 (Ca Mex67 Pro463) (19).

Superimposition of Mtr2 with one subunit from the NTF2 homodimer bound to FXFG repeats shows a Mtr2 FG binding pocket similar to the NTF2 pocket, that is located across the like domain are shown in gray. D, close-up views of the significant structural differences between the heterodimer interfaces of the Mtr2-Mex67 (left) and the TAP-p15 (right) complexes. The secondary structural elements are shown under a transparent surface with each molecule color-coded as in Fig. 1. The shallow pocket is visible in the Mtr2-Mex67 (left) complex, while it is occluded in the TAP-p15 complex (right).
The coordinates of the human TAP-p15 complex (accession code 1JN5).

Through a transparent surface was fitted into the Mex67 cavity based on the Dpocket (views of C right each of the three FG repeat binding sites respectively located in Mtr2)

The crescent-shaped surface patches (white/H11011 region (symbolized with brown filled circles along with the Mtr2 hydrophobic Phe side chain of the FG core) that is shown with the Mtr2 inserts 1 and 3 color-coded as in Fig. 1 A, FG repeat binding sites). The alignment of the three FG binding sites, on one face of the Mtr2-Mex67 complex, is also consistent with the distances found between two FXFG repeat binding sites located at the molecular surface of the structurally unrelated importin-β (38). These features illustrate the important role of the spatial organization of the FG repeats at the molecular surface of nucleoporins within the NPC, as demonstrated by the simultaneous binding of Mex67 and Kap95, a member of the karyopherin transport pathway, to distinct regions of Nup116 rich in FG repeats (15).

Although further experiments using site-directed mutagenesis will definitely validate the functionality of these FG binding pockets for yeast nuclear mRNA import, earlier biochemical and structural data are consistent with our structural interpretation. Indeed, a remarkable structural feature of these FG binding sites, beyond the conserved hydrophobic character of the pocket, is the presence of a Pro residue surrounded by polar residues at the periphery of the pocket. Second, the residues that line these pockets are mostly invariant or conserved in yeast Mtr2 or Mex67 proteins. The structure reveals a crescent-shaped arrangement, on one face of the heterodimeric surface, of the three FG binding sites that are thus ideally positioned to interact with nucleoporin FG repeats during transport. In contrast, the surface region that contains the NTF2-like hydrophobic pocket in Mtr2 is located on the opposite face and this overall spatial organization might account for Mtr2-Mex67 binding both FG repeats and a yet unknown export receptor simultaneously (Fig. 5).

In summary, we have shown that yeast Mtr2 proteins have acquired novel structural determinants during evolution, a feature that supports the biological involvement of Mtr2 in both the nuclear mRNA and ribosome export pathways. The crystal structure of the Mtr2-Mex67 complex reveals novel FG repeat binding sites not found in its human homolog, highlight-
ing a discrepancy in mRNA nuclear export between yeast and higher eukaryotes. Considerable genetic, biochemical and structural data are now available on the Mtr2-Mex67 complex but less is known about the precise mechanism by which carrier-cargo complexes are translocated and released through the NPCs. Clearly, it will be important to delineate at the atomic level how Mtr2 associates with the nuclear export of the large ribosomal subunit. Finally, since C. albicans is an opportunistic fungal pathogen that often causes death of immunocompromised patients despite anti-fungal therapies, our findings contribute a highly detailed structural template for the design of new antifungal agents.

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REFERENCES

1. Ryan, K. J., and Wente, S. R. (2000) Curr. Opin. Cell Biol. 12, 361–371
2. Kuretien, S., Ohno, M., and Mattaj, I. W. (2003) Trends Cell Biol. 11, 497–503
3. Stewart, M., Baker, R. P., Bayliss, R., Clayton, L., Grant, R. P., Littlewood, T., and Matsura, Y. (2001) FEBS Lett. 498, 145–148
4. Nakielny, S., and Dreyfuss, G. (1999) Cell 99, 677–680
5. Stewart, M. (2000) Cell Struct. Funct. 25, 217–225
6. Conti, E., and Izaurralde, E. (2001) Curr. Opin. Cell Biol. 13, 310–319
7. Weis, K. (2000) Curr. Opin. Cell Biol. 14, 328–335
8. Reed, R., and Hurt, E. (2002) Cell 108, 523–531
9. Herold, A., Soyma, M., Rodrigues, J. P., Braun, I. C., Kutay, U., Carmo-Fonseca, M., Bork, P., and Izaurralde, E. (2000) Mol. Cell Biol. 20, 8996–9008
10. Segref, A., Sharma, K., Doye, V., Helwig, A., Huber, J., Luhrmann, R., and Hurt, E. (1997) EMBO J. 16, 3256–3271
11. Hieronymus, H., and Silver, P. A. (2003) Nat. Genet. 33, 155–161
12. Kadowaki, T., Hitomi, M., Chen, S., and Tartakoff, A. M. (1994) Mol. Biol. Cell 5, 1253–1263
13. Santos-Rosa, H., Moreno, H., Simos, G., Segref, A., Fahrenkrog, B., Pante, N., and Hurt, E. (1998) Mol. Cell. Biol. 18, 6826–6838
14. Strasser, K., Bassler, J., and Hurt, E. (2000) J. Cell Biol. 150, 695–706
15. Strawn, L. A., Shen, T., and Wente, S. R. (2001) J. Biol. Chem. 276, 6443–6452
16. Braun, I. C., Herold, A., Rode, M., and Izaurralde, E. (2002) Mol. Cell Biol. 22, 5405–5418
17. Fribourg, S., Braun, I. C., Izaurralde, E., and Conti, E. (2001) Mol. Cell 8, 645–656
18. Katahira, J., Strasser, K., Podtelejnikov, A., Mann, M., Jung, J. U., and Hurt, E. (1999) EMBO J. 18, 2583–2609
19. Fribourg, S., and Conti, E. (2003) EMBO Rep. 4, 699–703
20. Lei, E. P., Stern, C. A., Fahrenkrog, B., Kreibier, H., May, T. I., Aebi, U., and Silver, P. A. (2003) Mol. Biol. Cell 14, 836–847
21. Bassler, J., Grandi, P., Gadol, O., Lessmann, T., Petfalski, E., Tollervey, D., Lechner, J., and Hurt, E. (2001) Mol. Cell 8, 517–529
22. Nissan, T. A., Bassler, J., Petfalski, E., Tollervey, D., and Hurt, E. (2002) EMBO J. 21, 5539–5547
23. Riesenberg, D., Schulz, V., Knorre, W. A., Pohl, H. D., Korz, D., Sanders, E. A., Ross, A., and Deckwer, W. D. (1991) J. Biotechnol. 21, 17–27
24. Otwinowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326
25. CCP4 (1994) Acta Crystallogr. D. Biol. Crystallogr. 50, 760–763
26. Terwilliger, T. C., and Berendzen, J. (1999) Acta Crystallogr. D. Biol. Crystallogr. 55, 849–861
27. Lamzin, V. S., and Perrakis, A. (2000) Nat. Struct. Biol. 7, (suppl.) 978–981
28. Roussel, A., and Cambillau, C. (1989) TURBO-FRODO, Silicon Graphics Geometry Partners Directory (Committee, S. G., ed), Mountain View, CA
29. Mursudov, G. N., Vagin, A. A. & Dodson, E. J. (1997) Acta Crystallogr. D. Biol. Crystallogr. 53, 249–255
30. Navaza, J. (1994) Acta Crystallogr. Sect. A 50, 157–163
31. Laskowski, R., MacArthur, M., Moss, D., and Thornton, J. (1993) J. Appl. Crystallogr. 26, 283–291
32. Christoffers, J. A. (1998) SPDOCK, The Center for Macromolecular Design, Texas A&M University College Station, TX
33. Merritt, E., and Bacon, D. (1997) Methods Enzymol. 277, 505–524
34. Bullock, T. L., Clarkson, W. D., Kent, H. M., and Stewart, M. (1996) J. Mol. Biol. 260, 422–431
35. Stewart, M., Kent, H. M., and McCoy, A. J. (1998) J. Mol. Biol. 277, 635–646
36. Close, K. N., Luo, M. J., Zhou, Z., and Reed, R. (2001) Nat. Cell Biol. 3, 97–99
37. Bayliss, R., Leung, S. W., Baker, R. P., Quimby, B. B., Corbett, A. H., and Stewart, M. (2002) EMBO J. 21, 2843–2853
38. Bayliss, R., Littlewood, T., Strawn, L. A., Wente, S. R., and Stewart, M. (2002) J. Biol. Chem. 277, 50597–50606
39. Liang, J., Edelsbrunner, H., and Woodward, C. (1998) Protein Sci. 7, 1884–1897
40. Bayliss, R., Littlewood, T., and Stewart, M. (2000) Cell 102, 99–108
41. Klewegt, G. J. (1999) Acta Crystallogr. D. Biol. Crystallogr. 55, 1878–1884