Structural Requirements for the Ubiquitin-associated Domain of the mRNA Export Factor Mex67 to Bind Its Specific Targets, the Transcription Elongation THO Complex Component Hpr1 and Nucleoporin FXFG Repeats*

Received for publication, February 19, 2009, and in revised form, April 8, 2009 Published, JBC Papers in Press, April 28, 2009, DOI 10.1074/jbc.M109.004374

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The ubiquitin-associated (UBA) domain of the principal Saccharomyces cerevisiae mRNA nuclear export factor, Mex67, can bind both nuclear pore protein (nucleoporin) FG repeats and Hpr1, a component of the TREX-THO complex that functions to link transcription and export. Using fluorescence resonance energy transfer-based assays, we show here that Hpr1 and the FG repeats interact with overlapping binding sites on the Mex67 UBA domain. We present the solution structure of the Mex67 UBA domain (UBA-Mex67) complexed with a FXFG nucleoporin peptide and define residues engaged in the interaction and those involved in the FXFG-induced conformational change. We show by NMR titration that the binding of Hpr1 produces analogous changes in chemical shifts in similar regions of the UBA domain. Together the data presented here indicate that both Hpr1 and FXFG nucleoporins may bind in a similar way to the UBA-Mex67 domain. However, whereas binding of Hpr1 allows UBA-Mex67 to interact with tetra-ubiquitin, the complex between UBA-Mex67 and FXFG is unable to bind mono- or tetra-ubiquitin, suggesting that both substrate binding and also the nature of the substrate may influence the affinity of the UBA-Mex67 domain for ubiquitin.

Transcripts generated by RNA polymerase II undergo a carefully orchestrated series of processing steps, including 5′ capping, splicing, 3′ end cleavage, and polyadenylation before being exported to the cytoplasm. The steps of mRNA biogenesis leading to export-competent ribonucleoprotein particles (mRNPs) are intimately coupled (1, 2). This coordination of mRNA biogenesis is mediated by a diverse range of RNA-binding proteins and maturation enzymes (reviewed in Refs. 2 and 3). Moreover, during processing, the transcripts are under the constant surveillance of RNA quality control mechanisms that ensure that only correctly processed mRNAs are exported to the cytoplasm for translation (4–7). From yeast to human, translocation of fully mature mRNPs to the cytoplasm through nuclear pore complex is thought to be mediated primarily by Mex67/NXF1 (also known as TAP), the primary mRNA export receptor, which forms a heterodimer with Mtr2/NXT1 (P15) that interacts directly with nuclear pore complex proteins (FG nucleoporins) that have characteristic FG sequence repeats (8–10). Previous work has shown that, like other nuclear transport factors (reviewed in Refs. 11 and 12), the Mex67/TAP UBA domain binds specifically to the two Phe rings of the FXFG nucleoporin-binding motif (13, 14). Because Mex67/NXF1 has low intrinsic affinity for mRNAs, it is recruited to the mRNP by several RNA-binding adaptors, including members of the THO-TREX complex that couples transcription elongation to mRNA export (2, 15, 16) as well as the TREX-2 complex, Yra1/ALY and Sub2/UAP56 (reviewed in Ref. 3).

Recent studies have shown that the ubiquitin pathway participates in the regulation of several key cellular functions including mRNA nuclear export (17–19). Ubiquitin is a small 76-residue protein that can be covalently linked as a monomer or as a polyubiquitin chain to a lysine residue of a specific substrate (20, 21). This process, known as ubiquitylation, targets the substrate for a range of possible fates. The diverse functions of ubiquitylation are mediated through effector proteins that contain one or several ubiquitin-binding domains. Those domains are classified into different groups according to their structural fold, the best known of which is the ubiquitin-associated (UBA) domain (22, 23). Interestingly, the essential

* This work was supported in part by a Program Grant from the Wellcome Trust (to C. D. and G. D.), and the Ligue contre le Cancer.

† Supported by the Federation of European Biochemical Societies.

‡ Supported by the Association de Recherche contre le Cancer.

§ Supported by the Association de Recherche contre le Cancer.

¶ Supported by the Thiono-Phosphate transferase and glutathione S-transferase; PBS, phosphate-buffered saline; dansyl, 5-dimethylaminonaphthalene-1-sulfonyl; FITC, fluorescein isothiocyanate; NOESY, nuclear Overhauser effect spectroscopy; HSQC, heteronuclear single quantum coherence; Bis-Tris, 2-[bis(2-hydroxyethyl)amino]-2-[hydroxymethyl]propane-1,3-diol; MES, 4-morpholineethanesulfonic acid; TOCSY, total correlation spectroscopy.

The atomic coordinates and structure factors (code 2KHH) 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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2 Supported by the Association de Recherche contre le Cancer.

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The on-line version of this article (available at http://www.jbc.org) contains links to: "The ubiquitin pathway participates in the regulation of several key cellular functions including mRNA nuclear export (17–19). Ubiquitin is a small 76-residue protein that can be covalently linked as a monomer or as a polyubiquitin chain to a lysine residue of a specific substrate (20, 21). This process, known as ubiquitylation, targets the substrate for a range of possible fates. The diverse functions of ubiquitylation are mediated through effector proteins that contain one or several ubiquitin-binding domains. Those domains are classified into different groups according to their structural fold, the best known of which is the ubiquitin-associated (UBA) domain (22, 23). Interestingly, the essential..."
mRNA export receptor Mex67/NXF1 has a UBA domain at its C terminus that has been shown to interact with nucleoporin FG repeats (13). We recently reported that the UBA domain of Mex67 (UBA-Mex67) is not only required for mRNA nuclear export but also contributes to early co-transcriptional recruitment of the receptor to the mRNP (3, 24). In addition to showing that the Mex67 UBA domain was able to bind both ubiquitin and FG nucleoporins, our studies identified Hpr1 as a specific partner of the UBA domain of Mex67. Hpr1 is a component of the THO-TREX complex that links transcription on mRNA export and that is regulated by ubiquitylation in a transcription-dependent manner (25). We proposed that the interaction with the Mex67 UBA domain transiently protects Hpr1 from ubiquitin/proteasome-mediated degradation and thereby coordinates recruitment of the mRNA export machinery with transcription and early mRNP assembly (24, 25).

With the exception of the UBA2 domain of HHR23A, most of the UBA domains studied to date have shown little or no substrate specificity (26–28). Therefore, because of its specific interactions with ubiquitinated Hpr1, the Mex67 UBA domain appears to be a promising model for understanding molecular basis of specific substrate recognition by UBA domains. UBA domains share high structural homology and are characterized by the presence of three α helices that are connected by two short loops, forming a compact fold with a hydrophobic core and surface hydrophobic patches implicated in protein-protein interactions. Structural analysis of several UBA domains in complex with ubiquitin has indicated that UBA domains interact with ubiquitin mainly via the C terminus of helix 1, loop 1, and helix 3 (29–31). The structure in solution of ubiquitin polymers varies according to the lysine residue implicated in the polyubiquitin chain formation, which leads to dif-
different modes of recognition by the UBA domains (32). In particular, UBA domains bind to each ubiquitin moiety of Lys$^{63}$-diubiquitin, whereas it binds simultaneously both ubiquitin moieties of Lys$^{48}$-diubiquitin (33–35). Unlike other UBA domains, the UBA domain of Mex67/NXF1 contains an additional C-terminal helix, helix H4, which stabilizes the core structure by closing the UBA fold (14, 36). We recently found that although helix H4 interferes with the ability of UBA-Mex67 to interact with ubiquitin, it is essential for the interaction of this UBA domain with its specific partner, Hpr1. We thus proposed that the helix H4 acts as a molecular switch that restricts the ubiquitin binding ability of the UBA domain of Mex67 to specific partners (36).

In this paper, we characterize the structural requirements for UBA-Mex67 to bind distinct and specific partners. Solution binding studies based on fluorescence titration show that Hpr1 and nucleoporin FXFG interact with UBA-Mex67 with similar affinities and compete for binding. We present the solution structure of the Mex67 UBA domain complexed with a FXFG nucleoporin peptide and characterized, by NMR titration, the residues involved in both the binding of FG repeats and Hpr1 and also those involved in the conformational changes induced by the interaction of the FXFG peptide. In addition, we present evidence indicating that, whereas Hpr1 binding allows UBA-Mex67 to interact with tetra ubiquitin, FXFG binding does not facilitate this interaction. These data indicate that both substrate binding and the nature of the substrate may influence the affinity of the UBA-Mex67 domain for ubiquitin.

**EXPERIMENTAL PROCEDURES**

**Plasmids and Cloning**—Plasmid constructs encoding His$_6$-tagged UBA-Mex67 (amino acids 543–599) and GST-Hpr1 (amino acids 548–752; Hpr1-C) were described previously (24). The pET-30a-FF18-Nsp1 construct that contains 18 FG repeats from the yeast nucleoporin Nsp1 was expressed and purified as described (37).

**Protein Purification**—Recombinant proteins were expressed in *Escherichia coli* strain BL21 (DE3). The strains transformed with pET-28a-UBA-Mex67, pGEX4T-1-Hpr1-C, pET-30a-FF18-Nsp1 were grown at 37 °C to A$_{600}$ of 0.6. Protein expression was then induced for 24 h at 23 °C with 0.5 mM of isopropyl $\beta$-D-thiogalactopyranoside after cold and chemical shock. His$_6$-tagged UBA-Mex67 and GST-Hpr1-C fusion proteins were purified as described (24). Hpr1-C was cleaved from glutathione S-transferase using thrombin. His$_6$-tagged FF18-Nsp1 (FF18-Nsp1) protein was purified on nickel-agarose beads (Qiagen) according to the manufacturer’s instructions. All of the recombinant proteins were dialyzed in PBS buffer containing 10% glycerol. For the NMR experiments UBA-Mex67 was purified as described (36).

**Fluorescence Labeling**—Recombinant proteins were labeled in PBS buffer using a 10-fold molar excess of fluorescein-5′-maleimide (for Hpr1-C, the FXFG peptide, or His-FF18-Nsp1) or dansyl-maleimide (for UBA-Mex67) (Molecular Probe). The reactions were allowed to proceed for 12 h at 4 °C. Nonincorporated label was removed by gel filtration on a NAP 5 column (GE Healthcare) equilibrated with PBS buffer.

**Fluorescence Assays**—Fluorescence titrations were carried out using a SPEX-PTI spectrofluorometer in a 0.5-cm-path length quartz cuvette, thermostatted at 25 °C in 50 mM Tris-buffer (pH 7.5) containing 150 mM NaCl, 4% glycerol. The fluorescence measurements were integrated for 2 s, repeated twice, averaged, and corrected for equipment and dilution. Binding of a FXFG peptide (DSGFSFGSK; from 100 to 4000 nM) to UBA-Mex67 (400 nM) was measured by following changes in intrinsic tryptophan fluorescence of UBA-Mex67. Trp residues were routinely excited at 290 nm, and the emission spectrum was recorded between 310 and 400 nm. Because FXFG contains no tryptophan or tyrosine residues, its intrinsic fluorescence is negligible and fluorescence emission is only due to Trp$^{575}$ of UBA-Mex67. For binding experiments using a fluorescence resonance energy transfer (FRET) assay, dansyl-labeled UBA-Mex67 (400 nM) was titrated with increasing concentrations of FITC-labeled proteins ranging from 100 to 5000 nM. Control experiments were performed with an unrelated FITC-labeled peptide (GTKALTEVIPLTEEAEC) (38). For displacement experiments using the FRET assay, complexes between dansyl-UBA-Mex67 (1 μM) and FITC-Hpr1-C-ter (1 μM) or FITC-FF18-Nsp1 (0.8 μM) were formed at concentrations higher that their dissociation constants, to start essentially with a 1:1 complex between the two species, and titrated using increasing concentrations of unlabeled Hpr1-Cter or His-FF18-Nsp1. For ubiquitin binding experiments, dansyl-UBA-Mex67 (1 μM) complexed with unlabeled Hpr1-Cter (1 μM) or FF18-Nsp1 (0.8 μM) was titrated with increasing concentrations of FITC-labeled mono- and Lys$^{48}$-linked tetra-ubiquitin. In each case, binding to dansyl-UBA-Mex67 was monitored by measuring dansyl fluorescence at 440 nm, following excitation at 350 nm.

### TABLE 1

**In vitro measurement of dissociation constants using fluorescence**

| Recombinant proteins | $K_D$ | $K_D$ |
|----------------------|--------|--------|
| His-UBA-Mex67FG      | 276 ± 80 nM | 12 ± 37 nM |
| His-UBA-Mex67FXFG    | 503 ± 125 nM | 118 ± 37 nM |
| His-FF18-Nsp1        | Not detectable | Not detectable |
| His-UBA-Mex67/Hpr1-C | Not detectable | Not detectable |
| His-UBA-Mex67/FF18-Nsp1 | 6.6 ± 14 mM | 118 ± 37 nM |

$^a$ Dissociation constant between His-UBA-Mex67 and FXFG was measured in *vivo* by monitoring the tryptophan fluorescence of His-UBA-Mex67.

$^b$ Dissociation constant between His-UBA-Mex67 and different partners were measured in *vivo* by monitoring fluorescence quenching of dansyl-labeled His-UBA-Mex67 in presence of increasing concentrations of FITC-labeled partners.

$^c$ Dissociation constant between the UBA domain of Mex67 in complex with Hpr1-C or His-FF18-Nsp1 and mono- and Lys$^{48}$-linked tetra-ubiquitin. Dansyl-labeled UBA-Mex67 (1 μM) complexed with unlabeled Hpr1-C (1 μM) or His-FF18-Nsp1 (1 μM) were titrated with increasing concentrations of FITC-labeled mono-ubiquitin or Lys$^{48}$-linked tetra-ubiquitin. Binding of ubiquitin was monitored by measuring dansyl fluorescence at 440 nm, following excitation at 350 nm.
spectra were processed using the XWINNMR software (Bruker Biospin GmbH). Backbone resonance assignment was carried out based on the triple resonance experiment CBCA(CO)NH/CBCANH pair of spectra (41, 42). Side chain resonances were identified using HBHA(CO)NH, HCCCH-TOCSY, and HCC-TOCSY (43) experiments. Interproton distance information for structure calculation was derived from a 15N-NOESY-HSQC spectrum and a pair of 13C-NOESY-HSQC (44) spectra recorded for both aliphatic and aromatic resonances. All of the NOESY spectra were acquired using a mixing time of 100 ms. Collaborative Computing Project for NMR analysis (45) was used for resonance assignment of the protein. Assignments of the FXFG peptide were obtained from two-dimensional 1H-1H NOESY and -TOCSY spectra of the free peptide. Secondary structure predictions were made using the chemical shift based dihedral angle prediction software TALOS (46) and confirmed by manual analysis of 15N- and 13C three-dimensional NOESY spectra. The distance restraints were manually refined by iterative assignment corrections/structure calculations using XPLOR-NIH (47). Final structure calculations were based on a total of 1322 NOE-derived inter-residue distance restraints and 24 distance restraints mimicking H-bonds based on characteristic short range NOE patterns (see Table 1). The final ensemble of 20 structures selected by lowest energy shows the following distribution in the Ramachandran plot: 81.7, 14.5, 3.8, and 0% for ordered regions. For analysis of the refined ensemble MOLMOL (48) and Procheck-NMR (49) were used.

**Chemical Shift Mapping—NMR titration experiments** were carried out using samples containing 100 μM UBA-Mex67 in the buffer described above and increasing amounts of either FXFG or Hpr1 in the same buffer. 15N HSQC experiments at each concentration were recorded on a Bruker DMX500 spectrometer.
Both the intrinsic tryptophan fluorescence and the FRET assays resulted from a specific binding between UBA-Mex67 and the peptide, confirming that the decrease in dansyl fluorescence in the upper panel was caused by the interaction between UBA-Mex67 and the FGF peptide (Fig. 1). As previously reported for NXF1/TAP (13), the Mex67 were first monitored by steady state fluorescence titration with increasing concentrations of the FITC-labeled Hpr1 and the FGF peptide (DSGFSFGSK) with a \( K_D \) of \( 276 \pm 80 \text{ nM} \) when measured by changes in the intrinsic UBA-Mex67 Trp fluorescence (Fig. 1A). This result was confirmed by a FRET assay in which dansyl-labeled UBA-Mex67 was incubated with increasing concentrations of the FITC-labeled FGF peptide. Because of the low quantum yield of the dansyl-fluorophore, binding to dansyl-UBA-Mex67 results in a limited increase in FITC-FGF fluorescence at 512 nm. The interaction between UBA-Mex67 and the FGF peptide (Fig. 1B and Table 1) was therefore monitored by following the quenching of dansyl fluorescence at 440 nm (when excited at 350 nm). In contrast, no quenching in dansyl fluorescence was observed using either an unlabeled FGF or an unrelated FITC-labeled peptide, confirming that the decrease in dansyl fluorescence resulted from a specific binding between UBA-Mex67 and the FGF peptide and FRET between dansyl and FITC groups. Both the intrinsic tryptophan fluorescence and the FRET assays indicated similar \( K_D \) values for the interaction between UBA-Mex67 and FGF (Table 1), thus validating the FRET approach. Because FG nucleoporins are generally present multiple FGF repeats, a fragment of the nucleoporin Nsp1 containing 18 FGF repeats (37) was also used to test the same FRET assay and found to bind UBA-Mex67 with a \( K_D \) of \( 118 \pm 37 \text{ nM} \). As one would expect as a consequence of avidity effects, the construct having multiple repeats of the FXFG motif showed an apparently increased affinity for UBA-Mex67. Use of the fluorescence-based approach to measure protein-protein interaction also confirmed that the C-terminal domain of Hpr1 (Hpr1-C) was able to interact with UBA-Mex67 and led to a \( K_D \) of \( 503 \pm 125 \text{ nM} \) (Fig. 1B and Table 1). In summary, these results indicate that both the FGF peptide and Hpr1 have similar affinities for Mex67-UBA that are in the 0.1 \( \mu \text{M} \) range.

Hpr1 and FGF Repeats May Bind Overlapping Sites at the Surface of the UBA Domain of Mex67.—To investigate whether FGF repeats and Hpr1 could bind on similar binding sites on UBA-Mex67, pulldown experiments were performed in which we tested the ability of the UBA-Mex67 domain to bind to the FGF peptide coupled to agarose beads in the absence or presence of Hpr1. As shown in Fig. 2A, incubation of UBA-Mex67 with Hpr1-C prior to the addition of the FGF coupled beads prevented binding of UBA-Mex67 to FGF (compare lanes 2 and 4). Noncoupled beads were also tested for nonspecific interactions but found not to interact with either Hpr1C or UBA-Mex67 (data not shown). These data suggest that Hpr1C and FGF peptide binding to UBA-Mex67 are mutually exclusive and can directly or indirectly compete for binding to UBA-Mex67.

To confirm this result, displacement experiments between FGF repeats and Hpr1 were performed in vitro using a FRET-based assay (Fig. 2B). Dansyl-labeled UBA-Mex67, complexed with either FITC-labeled Hpr1-C (upper panel) or FITC-labeled FF18-Nsp1 (lower panel), was challenged by increasing concentrations of unlabeled Hpr1-C or FF18-Nsp1. Competition with the unlabeled UBA-Mex67 partner led to an increase in dansyl fluorescence caused by a change of the labeled partner and recovery of fluorescence. As shown in Fig. 2B, both Hpr1-C and FF18-Nsp1 were able to compete with themselves for binding to UBA-Mex67. Similarly, unlabeled FF18-Nsp1 was also able to compete with FITC-labeled Hpr1 for binding to UBA-Mex67 and vice versa. These results support the hypothesis that the FGF motif and Hpr1 bind to similar and at least partially overlapping binding sites at the surface of the UBA domain.

Solution Structure of the UBA Domain of Mex67 Complexed with a FGF Peptide.—To further characterize how UBA-Mex67 interacts with its specific targets, the solution structure of the UBA-Mex67-FXFG complex was determined by NMR. A well defined ensemble of 20 structures (Fig. 3A) was generated from a set of 1322 structural restraints (see supplemental Table S1 and supplemental Fig. S1) and had an average root mean square deviation to the mean structure of 0.13 \( \pm 0.04 \text{ Å} \) for all backbone heavy atoms and an average root mean square deviation to the mean structure of 0.64 \( \pm 0.10 \text{ Å} \) when all non-hydrogen atoms in ordered regions were included. The Mex67 UBA domain in the complex had the same overall fold as the

**Figure 2.** Hpr1 and FGF repeats bind overlapping sites at the surface of the UBA domain of Mex67. A, FGF repeat peptides linked to CNBr-activated Sepharose beads were used for pulldown experiments. 40 nmol of UBA alone (lanes 1 and 2) or 40 nmol each of UBA-Mex67 plus Hpr1-C (lanes 3 and 4) were incubated with FXFG repeat peptide-coupled beads at 25 °C for 4 h in PBS, and the beads were then washed in PBS before being boiled in SDS-PAGE loading buffer. Odd lanes show input, and even lanes show proteins bound to beads. B, dansyl-labeled UBA-Mex67 (1 \( \mu \text{M} \)) complexed to FITC-labeled Hpr1-C (1 \( \mu \text{M} \); upper panel) or FITC-labeled FF18-Nsp1 (0.8 \( \mu \text{M} \); lower panel) was titrated with increasing concentrations of unlabeled Hpr1-C or His-FF18-Nsp1. Competition between unlabeled and FITC-labeled UBA-Mex67 partners was monitored by measuring dansyl fluorescence at 440 nm, upon excitation at 350 nm.
isolated domain (36) and was based on four α-helices (helices H1–H4) that encompassed residues 546–559, 563–572, 578–586, and 593–596, respectively. The overall structure of UBA-Mex67-FXFG complex closely resembled the structure of its metazoan homolog NXX1/TAP (14). The main interaction site for the FXFG peptide was a hydrophobic pocket located between helices 2 and 3. The aromatic rings of tryptophan residues of the peptide were buried into a hydrophobic pocket composed primarily of residues Leu561, Leu569, and Ile591 (Fig. 3B). These main contacts were preserved between NXX1/TAP and Mex67. In the UBA-Mex67-FXFG peptide complex, serines 586 and 587, located at the end of helix 3, make additional hydrophilic contacts to the peptide. Fig. 3C compares the structure of the Mex67 UBA domain before and after binding the FXFG peptide (shown in blue). There was a small conformational change that accompanied binding of the peptide, although both structures retained the same fold. Thus, the changes in NMR resonances observed on binding arose from two separate sources, namely from direct interaction between the UBA domain and the FXFG peptide and also from the conformational changes that accompanied binding.

Previous work has shown the high degree of structural plasticity of the Mex67/TAP UBA domain (20, 33) and has also shown that this potential for conformational change makes interpretation of mutagenesis data problematic. Thus, for example, although the W595A and F617A mutations eliminate the interaction with FXFG nucleoporins, neither Trp595 nor Phe617 is involved directly in the interaction, and indeed both residues are located some distance from the FXFG-binding site (33). We therefore employed chemical shift mapping to define the residues on Mex67-UBA that were interacting with the FXFG peptide and Hpr1.

Chemical Shift Mapping of UBA-Mex67 Residues upon Binding of FXFG and Hpr1—The interaction between the UBA-Mex67 domain and Hpr1 was then compared with the interaction with the FXFG peptide using NMR titrations (Fig. 4). Because the structure of the UBA-Mex67-FXFG complex was known, it was possible to distinguish between chemical shift changes directly associated with binding and changes instead associated with the conformational changes in the UBA domain generated by formation of the complex. In the case of FXFG binding, these changes can be assigned confidently to representing primarily direct interactions between the peptide and the protein. However, the titration also showed perturbations in three additional regions, which, because these residues are not in direct contact with the peptide, were most likely due to conformational changes within the UBA domain (Fig. 3C). These regions were located in loop 1 (residues 560–562), around Trp575, and in helix 4. The NMR titrations showed that the chemical shift changes upon binding of either the FXFG peptide or Hpr1C occurred in remarkably similar regions of the UBA domain (Fig. 4C). The binding of either partner to the UBA-Mex67 domain produced strong shifts in the FXFG-binding site (Fig. 4, A and B) as well as on the opposite face of the UBA domain where a hydrophobic patch is exposed to the solvent. Thus, most of the strong chemical shift perturbations observed upon adding the FXFG peptide were located between residues 566–573 and 583–591, which lie within 6 Å of the FXFG peptide (Fig. 4B). Generally, the strongest perturbations observed upon the addition of Hpr1 occurred in the same regions (Fig. 4C), with the changes at helix 4 showing an especially similar pattern.

Together, data obtained by FRET analysis, NMR, and chemical shift changes indicate that both Hpr1 and FXFG nucleoporins bind in a similar way to the UBA-Mex67 domain. As we proposed previously (36), these interactions induce conformational changes in the UBA domain mainly located in loop 1, around Trp575, and in helix 4, but also around loop 2 upon Hpr1 binding.

Binding of Hpr1 but Not Nucleoporin FXFG Repeats to the UBA Domain of Mex67 Promotes Its Interaction with Ubiquitin—We previously reported that UBA-Mex67 has a low affinity for mono-ubiquitin or Lys48-linked tetra-ubiquitin (36). To determine whether the interaction of the UBA-domain of Mex67 with FG nucleoporins was also able to facilitate ubiquitin binding by the UBA domain, the affinity of the UBA-Mex67-FXFG complex for ubiquitin was measured using a FRET-based assay (Table 1 and supplemental Fig. S2). For this purpose, dansyl-labeled UBA-Mex67 complexed to unlabeled FF18-Nsp1 or Hpr1-C was
titrated with increasing concentrations of FITC-labeled mono-
ubiquitin and Lys48-linked tetra-ubiquitin. As observed for
Hpr1 or FXFG, binding of ubiquitin to UBA-Mex67 induced a
marked quenching of dansyl fluorescence caused by fluores-
cence energy transfer to the FITC group. The purified UBA-
Mex67/H18528 complex interacted specifically with Lys 48-
linked tetra-ubiquitin with a $K_D$ of 6.6 $\pm$ 1.4 M as described
previously (36). However, neither UBA-Mex67 alone nor the
UBA-Mex67/FF18-Nsp1 complex were able to bind mono-
ubiquitin nor Lys48-linked tetra-ubiquitin.

**DISCUSSION**

UBA domains have been classified based on whether or not
they are able to bind ubiquitin itself, and only a few investiga-
tions have assessed the extent to which UBA interactions with
ubiquitin depend on the presence of specific partners. One such
example, however, is the UBA2 domain of HHR23A that inter-
acts specifically with the VIH-1 protein Vpr (26). The NMR
solution structure of the HHR23A UBA domain together with
analysis of its interaction with Vpr revealed that the amino acid
sequence of loop 1 is critical for the binding of HIV-1 Vpr (27,
28, 50). The UBA2 domain of HHR23A is also able to bind other
specific partners, including the human base excision repair pro-
tein 3-methyladenine DNA glycosylase, MPG (51), the deglyco-
sylating enzyme N-glycanase png1 (52), and the transcription
regulator p300/CBP (53). However, the precise binding inter-
face between UBA2 of HHR23A and its different partners
remains to be established.

The UBA domain of Mex67, which binds specifically the FG
repeats of the nucleoporins as well as the export factor Hpr1,
provides an interesting model for analyzing way in which a UBA
domain interacts with two partners that have different specific
functions. The in vitro FRET assays (Fig. 1) show that both Hpr1
and the FXFG peptide bind UBA-Mex67 with comparable
affinities. Moreover these different UBA-Mex67 partners com-
pete for the binding to the Mex67 UBA domain, indicating that
both of these molecules cannot bind to the Mex67 UBA domain
simultaneously (that is to say, their binding is mutually exclu-
sive). The solution NMR structure of the UBA-Mex67 domain
in complex with a FXFG peptide together with the NMR titra-
tion assays of UBA-Mex67 following FXFG or Hpr1 binding
showed that the residues that were perturbed when either part-
ter bound occurred mainly at similar sites. The residues that
Mex67 UBA Domain and Specific Target Binding

were perturbed on UBA-Mex67 were quite different to the binding interface between UBA2 of HHR23A and Vpr. Thus, a hydrophobic pocket located between helices 2 and 3 constituted the main interaction site for the FXFG peptide, whereas other examples bind primarily at different sites (22, 23). This difference in binding site location suggests that, despite high structural homology between the different UBA domains, they probably use diverse ways to recognize their cognate targets.

Based on surface plasmon resonance data, we recently proposed that initial binding of Hpr1 to UBA-Mex67 might be followed by a conformational change of the UBA domain (36). The NMR results presented here clearly showed that the binding of either Hpr1 or the FXFG peptide resulted of small but consistent conformational changes mainly located in loop 1, helix 4, and a region around Trp575 (end of helix H2 and loop2) of the UBA domain. Although deletion of helix H4 or the F596A point mutant greatly reduced interaction of UBA-Mex67 with Hpr1 (36), we found that F596A does not affect the interaction with FXFG. These results are consistent with helix H4 having a regulating role and suggest that the conformational change involving helix H4 could be either required to stabilize interaction of UBA-Mex67 with its partners (rather than for initial binding) or alternatively to maintain, through its interaction with loop 1, the overall conformation of UBA-Mex67 that is compatible with binding to its specific targets.

Although the binding sites of UBA-Mex67 for FXFG and Hpr1 appear to share many similarities, interaction with each partner has a different effect on the affinity of the Mex67 UBA domain for ubiquitin. Importantly, whereas binding to Hpr1 promotes binding of the Mex67 UBA domain to bind Lys48-linked tetra-ubiquitin, binding to nucleoporin FG repeats does not.

Further work will be required to determine the atomic structure of the tripartite complex between UBA-Mex67, Hpr1, and ubiquitin to enable the precise molecular details of this interaction to be established. However, there was a suggestion that, relative to the changes seen with the FXFG peptide bound, the perturbation of the region surrounding Trp575 may have been greater when Hpr1 was bound. Although we cannot presently distinguish between direct interactions and more distant conformational changes associated with binding, these data support the idea that the binding sites for these partners are not completely identical. The region near Trp575 corresponds to the region of other UBA domains determinant for their interaction with Lys48-linked di-ubiquitin and not with mono-ubiquitin (33, 35). Further work will be required to decide whether residues around Trp575 are directly engaged in the interaction with Hpr1, because the resonance changes reported here would be consistent with a conformational change of helix2/loop2 region induced by Hpr1 binding, which would facilitate or stabilize interaction of UBA-Mex67 with poly-ubiquitin.

The molecular bases responsible for the selectivity of ubiquitin-binding domains for their specific targets remain poorly described. However, the results presented here on the UBA domain of the mRNA export receptor open several new perspectives on the cross-talk between ubiquitin binding and substrate recognition by ubiquitin-binding domains that influence their cellular functions and may serve as a paradigm for understanding the manner in which several complex cellular processes can be modulated by ubiquitylation.

Acknowledgments—We are most grateful to our colleagues, especially Y. Mely, C. Gwizdek, and J.-C. Yang, for most helpful advice, assistance, and criticisms.

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