The Conserved CPH Domains of Cul7 and PARC Are Protein-Protein Interaction Modules That Bind the Tetramerization Domain of p53*

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Cul7 is a member of the Cullin Ring Ligase (CRL) family and is required for normal mouse development and cellular proliferation. Recently, a region of Cul7 that is highly conserved in the p53-associated, Parkin-like cytoplasmic protein PARC, was shown to bind p53 directly. Here we identify the CPH domains (conserved domain within Cul7, PARC, and HERC2 proteins) of both Cul7 and PARC as p53 interaction domains using size exclusion chromatography and NMR spectroscopy. We present the first structure of the evolutionarily conserved CPH domain and provide novel insight into the Cul7-p53 interaction. The NMR structure of the Cul7-CPH domain reveals a fold similar to peptide interaction modules such as the SH3, Tudor, and KOW domains. The p53 interaction surface of both Cul7 and PARC CPH domains was mapped to a conserved surface distinct from the analogous peptide-binding regions of SH3, KOW, and Tudor domains, suggesting a novel mode of interaction. The CPH domain interaction surface of p53 resides in the tetramerization domain and is formed by residues contributed by at least two subunits.

The ubiquitin-proteasome system plays an important role in controlling diverse biological processes, ranging from signal transduction to cell cycle control (1, 2). These complex processes are controlled via specific degradation of individual or groups of proteins. Protein degradation via the ubiquitin pathway involves two successive steps: tagging of the substrate by covalent attachment of multiple ubiquitin molecules (ubiquitylation) and degradation of the tagged protein by 26S proteasome complex with release of free and reusable ubiquitin (3).

Ubiquitylation is the ultimate result of coordinated activity of an enzymatic cascade, which includes a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and ubiquitin-ligating (E3) enzymes. The E3 ligases are the “brain” of this process and determine substrate specificity (4, 5).

Cul7, a recently identified member of the Cullin family of ubiquitin E3 ligases, localizes predominantly in the cytoplasm (6), and forms a unique Skp1-Cul7-Fbx29-like complex with FBXW8, a WD40 containing F-box protein (7–9). Although a target substrate for FBXW8 has not been yet identified, Cul7 recruits RBX1 to form a Skp1-Cul7-Fbx29-like E3 ubiquitin ligase complex (7, 8). The biological function of Cul7 is unclear. However, Cul7 appears to play an important role in development (8, 9), and overexpression of Cul7 accelerates the rate of cell proliferation (6).

Cul7 has significant sequence similarity (see Fig. 1) with the p53-associated, Parkin-like cytoplasmic protein, PARC (10). Both proteins contain CPH (domain that is conserved in Cul7, PARC, and HERC2 proteins) (11), DOC (DOC1/APC10), and Cullin homology domains (see Fig. 1A) that are linked with E3 ligase function, suggesting that PARC and Cul7 may both function as E3 ubiquitin ligases. PARC has been shown to sequester p53 in the cytoplasm via interaction between the N terminus of PARC and the C terminus of p53 (10). Cul7 may perform functions similar to those of PARC given their degree of sequence similarity and have also recently been shown to interact directly with p53 via its N terminus (6, 11). Recently published data identified a domain within Cul7 that is necessary and sufficient for p53 binding (6, 11). This domain (the CPH domain) also contributes to the cytoplasmic localization of Cul7 (6, 11). This, taken together with its similarity to PARC, another p53 interacting protein and putative E3 ligase, argues for more detailed investigation of these two proteins with respect to their interactions with p53. Understanding the structure, function, and interactions of Cul7/PARC domains with p53 will help to elucidate their involvement in ubiquitylation pathways and the circumstances through which they impinge on the p53 pathway.

Inactivation of p53 is considered an important step in the development of many human cancers. It is therefore important...
to determine how p53 levels are regulated and how this regulation is altered in cancer. Transcriptionally active p53 protein is tetrameric, and in this conformation it binds with high affinity to DNA or interacts more efficiently with various other proteins (12, 13). The tetramerization domain is therefore important for p53 function because it ensures that the protein is endowed with its correct conformation. The oligomerization state of p53 is also thought to contribute to its subcellular localization by virtue of a cryptic nuclear export sequence that is only exposed in nontetrameric forms of the protein (14). Here we show that the conserved CPH domain of Cul7 interacts with the tetramerization domain (TD) of p53. To gain insight into Cul7 and its interaction with p53, we have solved the three-dimensional structure of the CPH domain of human Cul7 by NMR spectroscopy. The structure reveals a small domain with a fold similar to SH3, KOW, and Tudor domains, suggesting that it may function as a peptide-binding module. Chemical shift perturbation studies map the p53-TD interaction to regions distinct from the analogous peptide-binding surface of Tudor, KOW, and SH3 domains. Our NMR data also suggest that the Cul7-p53 interaction depends on the p53 oligomerization status. The structures and interactions were compared with the homologous region of PARC, confirming a similar interaction with the CPH domain of PARC.

**EXPERIMENTAL PROCEDURES**

**Cloning, Expression, and Purification**—The coding regions for Cul7 360–460 and PARC 366–465 (CPH-containing domains) and the p53 TD (residues 310–360) were PCR-amplified from human Cul7 cdNA and human p53 cdNA, respectively, and subcloned into the pET15b expression vector (Novagen) at the 5′-Ndel site and 3′-BamHI site. The p53 fragment was expressed in *Escherichia coli* BL21 (DE3)-pLysS cells (Stratagene), whereas Cul7 and PARC constructs were expressed in *E. coli* BL21 (DE3) Rosetta cells (Novagen). For large scale production, the cells were grown at 37 °C until A600nm of ~1.0, and then the cultures were induced with 1 mM isopropyl-β-D-thiogalactopyranoside for 5 h at room temperature before harvesting. The bacteria were grown in LB medium for nonlabeled proteins and in M9-defined medium supplemented with [15N]ammonium chloride (0.8 g/liter) and D-glucose. For the 15N/13C-labeled samples, D-glucose was replaced by 13C6-D-glucose (4 g/liter). These highly expressed proteins were purified by Talon (BD) affinity chromatography under native conditions and eluted with buffer containing 500 mM imidazole. The proteins were treated with thrombin and further purified by size exclusion chromatography using a HiLoad 26/60 Superdex-75 column (GE Healthcare).

**Gel Filtration Studies**—A calibrated Superdex 75 column was equilibrated with 25 mM Tris, pH 7.5, 250 mM NaCl, 2 mM benzamidine, 0.5 mM TCEP, 0.5 mM phenylmethylsulfonyl fluoride. Cul7-CPH and p53-TD were combined in 1:1 and 1:3 molar ratios and subjected to gel filtration chromatography (see Fig. 4A, shown in blue and black, respectively). Peak fractions were pooled and run on SDS-PAGE.

**NMR Spectroscopy**—All of the spectra were recorded at 25 °C on Varian INOVA 600 MHz and Bruker Avance 500 MHz spectrometers equipped with triple resonance 1H/13C/15N cold and cryoprobes, respectively. All of the NMR samples were prepared at pH 7.5 with 25 mM Tris, 250 mM NaCl, 2 mM benzamidine hydrochloride, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM TCEP. The final NMR samples contained 90% H2O, 10% D2O with a protein concentration ranging between 0.5 and 0.7 mM. Two-dimensional, gradient-enhanced 1H/15N HMQC root mean square deviation spectra were acquired on uniformly 15N- or 13C/15N-labeled Cul7- and PARC-CPH domains and 15N-labeled p53-TD. The spectra were processed with NMRPipe software (15) and analyzed with the SPARKY program (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco). Linear prediction in the 13C and 15N dimensions was used to improve the digital resolution. The assignment of 94.8% of 1H, 15N, and 13C resonances of Cul7-CPH domain was obtained with the automated ABACUS approach (16) combined with manual analysis using data from HNCO, CBCA(CO)NH, HBHA(CO)NH, CC(CO)NH-TOCSY, HC(CO)NH-TOCSY HC(CH)-TOCSY, and H(C)CH-TOCSY experiments. The assignment of ~80% of 1H, 15N, and 13C resonances of the PARC-CPH domain were based on the following experiments: HNCA,CB, CBCA(CO)NH, HNCO, HNCA, and (H)CCH-TOCSY. The backbone resonances (1Hα, 15N) of Cul7- and PARC-CPH domains obtained upon the addition of p53-TD were assigned assuming minimal change in chemical shifts relative to the unbound proteins.

**Structure Calculations**—Distance restraints for structure calculations were obtained from 13C-edited NOESY-HSQC (r90 = 120 ms) and 15N-edited NOESY-HSQC (r90 = 150 ms) experiments. NOESY spectra were analyzed with the SPARKY program. The NOE-derived distance restraints were classified into four categories: 1.8–2.8, 1.8–3.5, 1.8–5.0, and 1.8–6.0 Å, corresponding to strong, medium, weak, and very weak NOEs, respectively. The restraints for backbone ϕ and ψ torsion angles were derived from chemical shifts of backbone atoms using TALOS (17). No hydrogen bond constraints were used. Structure calculations were carried out using CNS version 1.1 (18) with its standard annealing protocol. Five iterative cycles of automated structure calculation and NOE assignment were applied. Automated NOE assignment was performed using the BACUS procedure (19). 15N-1H residual dipolar coupling constraints were incorporated in structure calculations on cycles 2–5 using floating alignment tensor (SANI (20) energy term with force constant of 2.0 kcal/mol·Hz2). On each cycle, the best 20 of 100 generated structures were selected for 1) filtering prior NOE assignments followed by a BACUS search for new identities and 2) estimating the magnitude of the axial and rhombic components of the alignment tensor using the PALES program (21). The 20 lowest energy structures were refined using CNS by performing a short constrained molecular dynamics simulation in explicit solvent (22). The resulting structures were analyzed using MOLMOL (23), PROCHECK-NMR (24), and PSVS validation software (25). A homology model of PARC-CPH was generated from the NMR structure of the Cul7-CPH using YASARA (www.yasara.com).

**NMR Titration Experiments**—Aliquots of 15N-labeled p53-TD and Cul7-CPH proteins were titrated into the unlabeled Cul7-CPH and p53-TD in molar ratios of 1:1, 1:2, 1:3, 1:4,
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and 1:5, respectively, until no further changes in chemical shifts were detected in the 1H-15N HSQC spectrum. HSQC spectra were acquired and analyzed after each addition of ligand. 15N-p53-TD chemi-

cal shifts were those previously reported (20). The weighted chemical shift displacements were calculated using the following formula: Δppm = [(δppm)2 + (δppm/5)2]1/2.

RESULTS

Given that Cul7 and PARC have high sequence identity in their N-terminal domains and both interact with p53 via their N-terminal domains, we sought to identify a stable domain common to both proteins that would interact with p53. In screening for stable N-terminal domain constructs of Cul7, a protease-resistant fragment (residues 360–460) was identified from limited trypsin digestion (data not shown). Cul7(360–

460) contains a CPH domain, named after a homologous

sequence found within the Cul7, PARC, and HERC2 proteins

common. PARC is distinct from Cul7 with a spacer domain (573–957 amino acids) and RING-IBR-RING domain

and/or a nucleic acid-binding protein, because the KOW

domain of NusG (27) and the Tudor domain of 53BP1 (28) have

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been implicated in both these activities.

The arrangement of β sheets in the CPH domain is almost identical to that found in SH3, Tudor, and KOW domains with backbone root mean square deviations on the order of 1.5 Å (Fig. 3). Nevertheless, there is significant variability in the length and arrangement of the loops that contribute to peptide binding in each domain. For example, the RT-Src loop of SH3 domain (29) consists of 17 residues, whereas there are only 11 residues in the corresponding loop of the Cul7-CPH domain. Furthermore, despite the similar fold, all three domains display variety in the location of functional binding sites. Peptide binding by SH3 domains is mediated by a hydrophobic surface that is rich in aromatic residues and by various polypeptide loops located in the RT-Src and N-Src loops (Fig. 3C) (30). The Tudor domain of SMN uses loops 1 and 3 to bind to symmetrically dimethylated arginines of RG-rich sequences (Fig. 3D) (31). The NusG KOW domain can bind proteins and nucleic acids at

FIGURE 1. Cul7 and PARC sequence homology. A, a schematic representation of the Cul7 and PARC polypeptides. PARC shares 60% sequence identity to Cul7 with CPH, DOC, and Cullin homology (CH) domains in common. PARC is distinct from Cul7 with a spacer domain (573–957 amino acids) and RING-IBR-RING domain

its C terminus (1953–2517 amino acids). B, sequence alignment of Human Cul7(360–460), PARC (366–465), and HERC2 (2554–2630) CPH domains. Cul7 and PARC share homology to HERC2 in this region (40% sequence identity, 65% similarity). An asterisk indicates the key surface p53 TD-interacting residues.

FIGURE 2. Solution structure of the Cul7-CPH domain. A, Cul7 and PARC are connected by a long turn,

and form a hydrophobic cluster that may be involved in ligand interac-

tions (Fig. 2C). Other conserved, charged residues Glu391 (B2), Glu399 (B2), Asn404 (loop3), Gln411 (B2), Tyr389 (loop2), Gly406 (loop3), Phe413 (loop3), Thr420 (B4), and Trp422 (B4) could form an additional platform for protein-ligand interaction.

A DALI search (www.ebi.ac.uk/dali/) for structurally similar domains yielded a list of ∼90 proteins with Z score of ≥2.0. The NusG KOW domain (Protein Data Bank code 1M1G) and the SH3 domain of the kinase p56lck (LCK; Protein Data Bank code 1H10) are the two closest matches (Z = 6.7 and 6.6, respectively), although they lack any significant sequence similarity. The next 17 hits are all SH3 domains; the Tudor domains of SMN (Protein Data Bank code 1g5v) and 53BP1 are found in the 19th and 28th positions (Z = 5.4 and 4.3, respectively). This suggests that the CPH domain may be a protein-protein interaction module, and/or a nucleic acid-binding protein, because the KOW domain of NusG (27) and the Tudor domain of 53BP1 (28) have

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the same time via different surfaces (Fig. 3B) (27). Thus, each module uses the same fold in a unique manner to interact with partners, making it difficult to predict a substrate-binding surface for Cul7 from domain comparisons.

The CPH Domain Interacts with p53-TD—The N-terminal region of Cul7 has been observed by us and others to be a major p53 binding determinant (6, 11). Recently Kasper et al. (11) reported that residues 268–438 of Cul7 were sufficient for association with p53. Residues 331–437 of Cul7 were shown to co-precipitate with p53 (6, 11). Because both of these fragments encompass the CPH domain, we further examined whether the latter was responsible for the p53 interaction using size exclusion chromatography and NMR titration experiments. As shown in Fig. 4A, when Cul7-CPH is preincubated with an excess of p53-TD at high protein concentrations (2 mg/ml), Cul7-CPH co-eluted with p53-TD as a Cul7-CPH/p53-TD complex (shown in black).

TABLE 1
Structure calculation statistics of the CUL7-CPH domain

| NMR restraints violations | Distance restraints (>0.5 Å) | 0.005 ± 0.001 |
|--------------------------|----------------------------|----------------|
| Bond angles (>5 degrees)  | 0.50 ± 0.67                | 0.32 ± 0.23    |
| Root mean square deviation from NMR restraints | 0.015 ± 0.001 | 0.32 ± 0.23 |
| Bond angles (deg.)       | 0.82 ± 0.32                | 0.54 ± 0.001   |
| Q factor for residual dipolar coupling restraints | 0.154 ± 0.005 | 0.32 ± 0.23 |
| Deviation from ideal covalent geometry | 0.007 ± 0.0001 | 0.32 ± 0.23 |
| Bond lengths (Å)         | 0.68 ± 0.02                | 0.54 ± 0.001   |
| Root mean square deviation from mean structure | 0.75 ± 0.17 | 0.32 ± 0.23 |
| Backbone atoms            | 1.23 ± 0.12                | 0.32 ± 0.23    |
| All heavy atoms           | 0.75 ± 0.17                | 0.32 ± 0.23    |
| Energies (kcal/mol)       | 380 ± 22                   | 0.54 ± 0.001   |
| Total                     | 380 ± 22                   | 0.32 ± 0.23    |
| Van der Waals’            | 380 ± 22                   | 0.32 ± 0.23    |
| Electrostatic             | −3616 ± 101                | 0.32 ± 0.23    |
| Ramachandran plot (%)     | 90.6                       | 0.32 ± 0.23    |
| Most favored regions      | 9.4                        | 0.32 ± 0.23    |
| Additionally allowed regions | 0.0                      | 0.32 ± 0.23    |
| Generously allowed regions | 0.0                      | 0.32 ± 0.23    |
| Disallowed regions        | 0.0                        | 0.32 ± 0.23    |

* Ensemble of 20 lowest energy structures out of 100 calculated.
* The values were calculated for residues 10–78.
* Energy is calculated using the PARALLHDG5.3 parameters set (1).

15N HSQC NMR experiments in which the 15N-Cul7-CPH was titrated with p53-TD revealed chemical shift perturbations for several residues (Fig. 4B). The free and bound forms of the protein are in fast exchange on the NMR time scale, suggesting that the affinity between CPH and p53-TD is likely in the micromolar range. An approximate equilibrium dissociation constant (Kd) of the Cul7-CPH/p53-TD complex was determined by least square fitting of the chemical shift changes accompanying ligand binding as a function of the total ligand concentration as described (32). Using chemical shift changes of Tyr389, Gln411, and Gln398 upon the addition of p53-TD, a Kd of ~530 μM was estimated. This result is in agreement with the gel filtration results where the co-elution of two proteins could only be observed when Cul7-CPH is saturated by p53-TD and proteins are preincubated at high concentrations (0.6 and 1.6 mM for Cul7-CPH and p53-TD, respectively). No interaction was observed when proteins were preincubated at 1:1 molar ratio (Fig. 4A, shown in blue). The central p53-TD peptide-binding region (defined as those residues with weighted chemical shift displacements greater than 0.05 ppm) includes Tyr389, Gln391, Asn404, Gly406, Val407, Gln411, Gln415, Ser416, Arg419, and Thr420 and forms a contiguous surface on the protein (Fig. 4B). In addition, the resonance peak for Trp422 completely disappeared and that of Phe413 was significantly broadened. Other residues surrounding this surface (Gly398, Gln391, Val410, and Val412) also showed chemical shift perturbations (between 0.03
and 0.05 ppm; Fig. 4C). These residues form a cluster between loops 1–3 and strands β2-β4 forming an interface for interaction with p53 (Fig. 4C). Significantly, these residues also encompass the conserved residues Tyr389, Glu391, Gly406, Val410, Gln411, Thr420, and Trp422 (Fig. 1B), suggesting that p53 binds to Cul7-CPH through a conserved surface formed by a combination of hydrophobic negatively charged residues.

Oligomeric Forms of p53-TD Residues Are Required for Binding Cul7-CPH—To assess the surface of p53-TD that interacts with Cul7-CPH, 1H-15N HSQC titration experiments were performed on uniformly 15N-labeled samples of wild type p53-TD with increasing amounts of unlabeled Cul7-CPH (Fig. 5A). The observed spectral changes that occurred after the addition of Cul7-CPH are characterized by reductions in resonance intensity of the residues Phe328, Arg335, Arg342, and Asn345. Resonances for residues Gly325, Leu330, Ile332, Arg333, and Gly334 became so weak they can no longer be detected (Fig. 5A). Residues Phe328, Leu330, Ile332, and Arg333 belong to the β-strand and are exposed to the surface of p53. In addition, these residues are hydrophobic (Fig. 5C and D) and have been proposed to be a potential site for interaction with other proteins (33, 34). Residues Gly334 and Arg335 are also surface-exposed, and they belong to the turn that links the α-helices to the β-sheets. An additional, six residues in our p53-TD spectrum had reduced intensities but could not be assigned because of spectral overlap (35). Because of the small size of p53-TD (~30 residues/subunit) and as a result of possible conformational changes in p53-TD, it is likely that residues distinct from the binding surface may also be affected by interaction with Cul7-CPH protein. Furthermore, a 1:1 complex between the symmetric p53 tetramer and the asymmetric Cul7-CPH domain would destroy the symmetry of the p53-TD, further complicating the NMR spectrum of the latter. This may explain why distinct chemical shift changes are observed for the CPH domain, but only intensity changes are observed for the peaks in p53-TD.

The β-sheet interaction surface on p53-TD is formed by at least two subunits, suggesting that oligomerization may be required for the interaction. To probe the role that oligomerization plays in p53 binding to Cul7-CPH, we performed NMR titrations using two previously designed and characterized p53 mutants, M340Q/L344R (MQLR) and L344P, which are dimeric and monomeric forms of the p53 protein, respectively (36). As shown in Fig. 5B, under the condition where the wild type p53-TD/15N-Cul7-CPH complex was readily formed, no 15N-Cul7-CPH interaction with L344P mutant was detected, whereas the MQLR mutant shows evidence of interaction based on chemical shift perturbations. These results can be explained by the lack of proper conformation and/or three-dimensional binding surface in the monomeric, unfolded L344P mutant (37), whereas MQLR is a “half-tetramer” that retains the wild type p53-TD N- and C-terminal orientations in addition to a native-like β-sheet surface (36). Taken together our results imply that p53-Cul7 complex formation requires either dimerization or tetramerization of p53.

The PARC-CPH Domain Also Interacts with p53-TD—A CPH domain with 60% sequence identity to Cul7-CPH is also present within the N-terminal region of PARC (Fig. 1). To understand whether these two proteins interact in the same way with p53, we performed an NMR titration experiment and identified the residues in PARC-CPH whose chemical shifts change upon the addition of p53-TD. These changes were mapped onto a homology model of the PARC-CPH domain. Fig. 6 shows the surface representation for both proteins with assigned p53 interacting residues for the PARC-CPH domain. The combined results of the molecular modeling and the spec-
troscopic analysis showed that the structure and interaction of both domains with p53 are conserved.

DISCUSSION

The tumor suppressor gene p53 is extensively studied because of its importance in cancer. The TD of p53 has been implicated in multiple aspects of p53 function including sequence-specific DNA binding, subcellular localization, and protein-protein interactions. Disruption of the oligomeric state of p53 can lead to cancer as evidenced by tetramerization-deficient mutations associated with Li-Fraumeni and Li-Fraumeni-like syndromes (37) and adrenocortical carcinoma in children (38). Tetramerization domain mediated protein-protein interactions can occur either by direct binding to residues in the TD or indirectly because they necessitate an oligomeric p53 molecule. Thus, the TD has been implicated in interactions with casein kinase 2 (39), the Ca$^{2+}$/H$_{11001}$2-dependent protein kinase C (40), and the gene product of adenovirus E4orf6 (41); however, a direct interaction between the TD and these proteins has not yet been demonstrated. The direct (or indirect) involvement of the TD in several protein-protein interactions shows that this domain functions not only to facilitate p53 DNA binding but also to favor the interactions with other proteins, most likely because of an increase in the apparent binding constant caused by an increase in local concentration of binding surfaces in distal regions of the tetrameric molecule. The presence of the TD is important for p53 regulation by other proteins, and a tetrameric structure may permit the simultaneous interaction of p53 tetramers with multiple different proteins. This would allow for the integration of the various signals that ultimately converge on the p53 pathway. Several post-translational modifications are dependent on the quaternary structure of p53. The ubiquitylation of p53 requires it to be oligomerized (42), and its degradation is abolished when tetramerization is inhibited (43).

In this report, we have shown that residues 360–460 (CPH domain) of Cul7 that are highly conserved within both PARC and Cul7 proteins (Fig. 1) are able to interact directly with the p53-TD. Our results are consistent with the previously reported association of the Cul7-CPH domain with p53 (6, 11). We have solved the solution structure of the CPH domain of Cul7, a putative HECT E3 ligase by heteronuclear multidimensional NMR spectroscopy. The three-dimensional structure of the CPH domain is very similar to other small protein-protein interaction domains such as the KOW motif (27), the SH3 (29) domain, and the Tudor domain (31). Unlike the SH3 and Tudor domains, which bind to small peptides, the CPH domain appears to bind to an extended surface on a tetrameric (or dimeric) p53 protein.

Our results provide novel structural insights into the interaction between Cul7 and p53 proteins. Given the similarity within Cul7 and PARC, we investigated the potential interaction between PARC-CPH domain with p53-TD by NMR. Our NMR and homology modeling data showed that the CPH domains of Cul7 and PARC appear to be very similar in structure, and they share residues that mediate the interaction with p53-TD. This raises the possibility that other CPH domain-containing proteins may also interact with p53. In fact, the conserved p53-interacting residues of Cul7 and PARC CPH domains are also conserved in the prototype protein, HERC2, suggesting that this domain in HERC2 may also bind directly to p53 (Fig. 1B).

The N-terminal region of Cul7 has been observed by our lab and others (6, 11) to be a major p53-binding determinant. In agreement with previous reports (6, 11), our results showed
that Cul7 can bind to p53 directly, and the binding between the CPH domain and p53 probably involves a number of weak interactions that rely mostly on hydrophobic residues (see NMR titration results). Although the affinity of the CPH domain for p53 is quite weak (~530 μM), it is likely to be relevant for several reasons. First, the p53 interaction surface is highly conserved, suggesting an important function. Second, the affinity of full-length Cul7 has been shown to be greater than the CPH domain alone (6), suggesting that additional domains of Cul7 (and possibly PARC) may also be involved in the interaction. In such a model, a weak $K_d$ of the CPH domain in the mid-micromolar range would be physiologically relevant in the context of additional interactions even if the latter were also weak. Thus, the CPH domain may provide specificity to the interaction, and additional domains together may add to the total binding affinity. Given that p53 oligomerization is concentration-dependent and the interaction with Cul7-CPH is weak, a possible role for Cul7 may be detection of high levels of p53 in the cytoplasm.

The tetramerization of p53 has also been found to be relevant in its activation (44). The mechanisms that control p53 activity such as stability, cellular localization, and tetramerization are tightly regulated and in some cases interdependent. The p53 nuclear export signal comprises residues 340–351 within the TD. In the case of tetrameric p53, the key hydrophobic residues recognized by the nuclear export machinery are buried in the core of TD, and it is believed that the dissociation into monomers or dimers is required for nuclear export (14, 44). Similarly, tetrameric p53 is less efficient at entering the nucleus than monomeric p53 (45). Both cases suggest that the subcellular localization of p53 is controlled, in part, by quaternary structure (14). Our results showed that Cul7-CPH domain binds preferentially to the tetrameric and dimeric forms of p53.

**FIGURE 5.** Chemical shift perturbation of the p53 oligomers upon binding to Cul7-CPH domain. A, $^1$H-$^{15}$N HSQC spectra of wild type p53-TD in the absence (black) and in the presence (red) of Cul7-CPH protein. B, chemical shift change in Cul7-CPH caused by binding of wild type p53-TD (red), p53MQLR mutant (green), and L344P mutant (blue). An asterisk is used to indicate resonances that disappear. The average shift value was calculated using the equation $\Delta \delta = (\Delta \delta_{HN}^N + (\Delta \delta_{HN}/5)^{1/2})$ when the molar ratio of Cul7-CPH to p53 peptides is 1:3. C and D, surface (C) and ribbon (D) representations of a p53 tetramer. The residues involved in interaction with Cul7-CPH domain are indicated.

**FIGURE 6.** Surface representation of PARC-CPH (A) and Cul7-CPH (B) domains with p53-interacting residues shown in white and yellow, respectively.
Cul7-CPH Domain and Its Interaction with p53

(at high protein concentration). These results suggest that Cul7 protein might play a role in the control of p53 function by sensing its oligomerization state.

It has been shown that mutations targeting the p53 nuclear export signal blocked p53 nuclear export and decreased Cul7-p53 association, whereas mutation targeting the nuclear localization signal in p53 blocked p53 nuclear import and increased p53-Cul7 association (6). In contrast to PARC, which has been reported to bind to p53 and sequester it in the cytoplasm (10), Cul7 has not shown any cytoplasmic sequestration activity toward p53, nor does it appear to be the cytoplasmic ubiquitin ligase responsible for p53 degradation (6). Thus, the exact biochemical role of Cul7, in particular its interplay with p53, remains to be determined.

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