Phosphorylation of Tyr-950 in the proteasome scaffolding protein RPN2 modulates its interaction with the ubiquitin receptor RPN13

Casey W. Hemmis‡, Stephanie C. Heard§, and Christopher P. Hill†

From the Departments of ‡ Biochemistry and § Medicinal Chemistry, University of Utah School of Medicine, Salt Lake City, Utah 84112

Edited by George N. DeMartino

Protein substrates are targeted to the 26S proteasome through several ubiquitin receptors. One of these receptors, RPN13, is recruited to the proteasome by binding of its N-terminal pleckstrin-like receptor of ubiquitin (PRU) domain to C-terminal residues of the scaffolding protein RPN2. The RPN13 PRU domain is followed by a flexible linker and a C-terminal deubiquitylase adaptor (DEUBAD) domain, which recruits and activates the deubiquitylase UCH37. Both RPN13 and UCH37 have been implicated in human cancers, and inhibitors of the RPN2–RPN13 interaction are being developed as potential therapeutic anticancer agents. Our current study builds on the recognition that a residue central to the RPN2–RPN13 interaction, RPN2 Tyr-950, is phosphorylated in Jurkat cells. We found that the Tyr-950 phosphorylation enhances binding to RPN13. The crystal structure of the RPN2–RPN13 pTyr-950–ubiquitin complex was determined at 1.76-Å resolution and reveals specific interactions with positively charged side chains in RPN13 that explain how phosphorylation increases binding affinity without inducing conformational change. Mutagenesis and quantitative binding assays were then used to validate the crystallographic interface. Our findings support a model in which RPN13 recruitment to the proteasome is enhanced by phosphorylation of RPN2 Tyr-950, which has important implications for efforts to develop specific inhibitors of the RPN2–RPN13 interaction, and suggest the existence of a previously unknown stress-response pathway.

The ubiquitin–proteasome system is a complex network of proteins responsible for the regulated turnover of proteins in eukaryotes (1). Ubiquitin–proteasome system pathways converge on the 26S proteasome, a ~2.5-MDa complex that selectively degrades targeted cellular proteins. The 26S proteasome comprises a central 20S core particle, which houses the proteolytic sites, and is capped on one or both ends by the 19S regulatory particle, which includes proteins that recognize, process, and translocate targeted proteins into the core particle for degradation (2). Substrates are typically targeted to the proteasome by conjugation to the small protein ubiquitin, which is recognized by the proteasome-associated ubiquitin receptors RPN1, RPN10, and RPN13 (ADRM1 in humans), or by transiently associated ubiquitin shuttles such as RAD23 (HHR23A/B in humans), DSK2 (PLIC2 in humans), and DDI1 (3–9).

The ubiquitin receptor RPN13 is one of the most commonly overexpressed genes in solid tumors (10), and increases in cellular levels of RPN13 have been found in leukemia (11), hepatocellular carcinoma (12), ovarian cancer (13–15), and gastric/colorectal cancers (16–18). RPN13 also recruits and activates the proteasome-associated deubiquitylase UCH37 (UCH-L5) (19–21), which is also found at high levels in esophageal cancer (22), ovarian cancer (23), colorectal cancer (24), multiple myeloma (25), and hepatocellular carcinoma (26). As a result of these observations, RPN13 has become the focus of recent efforts toward development of anticancer therapeutics (27–30), placing additional emphasis on understanding the mechanism and regulation of RPN13 recruitment to the proteasome.

Recent publications describe the structural and biochemical basis of RPN13 recruitment to the proteasome by the scaffolding protein RPN2 (31–34), including details of the interaction between the C-terminal residues of RPN2 (RPN2940–953) and the RPN13 PRU™ domain (residues 1–132; RPN13PROU). Three residues (Pro-945, Phe-948, and Tyr-950) make major contributions to the ~10 nM binding affinity observed for the RPN2–RPN13 interaction (31). Interestingly, this affinity does not ensure complete occupancy of RPN13 on the proteasome as endogenously purified 26S proteasomes from both yeast and humans possess substoichiometric ratios of RPN13 (33, 35).

When considering the possibility that the RPN2–RPN13 interaction might be regulated by posttranslational modification, we used the publicly accessible PhosphoSitePlus database (www.phosphosite.org)™ (36) to find two curated mass pro-

2 The abbreviations used are: PRU, pleckstrin-like receptor of ubiquitin; FP, fluorescence polarization; PDB, Protein Data Bank; r.m.s.d., room mean square deviation; GS, glutathione-Sepharose; DMF, N,N-dimethylformamide; HATU, O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate.

3 Please note that the JBC is not responsible for the long-term archiving and maintenance of this site or any other third party hosted site.
ACCELERATED COMMUNICATION: RPN2 pY950 enhances RPN13 binding

Figure 1. Binding isotherms. Fluorescence polarization binding curves of RPN13PRU binding to RPN2940–952 (black) and to RPN2940–952,pTy950 (red) are shown. Error bars represent S.E.

Results

Phosphorylation of RPN2 pTyr-950 enhances affinity for RPN13

The RPN2940–952–RPN13PRU binding interaction was quantified using fluorescence polarization (FP) assays. For these studies, RPN13PRU was recombinantly expressed and purified. Peptides corresponding to RPN2940–952 and RPN2940–952,pTy950 were chemically synthesized and C-terminally labeled with fluorescein. Consistent with previous data (31), RPN13 bound the unphosphorylated peptide with a $K_D$ of 7.15 ± 0.27 nM (Fig. 1, black). In contrast, the RPN2940–952,pTy950 peptide bound RPN13PRU with a $K_D$ of 0.90 ± 0.07 nM under identical conditions (Fig. 1, red). This indicates that the Tyr-950 phosphate causes an ~8-fold increase in binding affinity for RPN13PRU.

Crystal structure of the RPN2940–952,pTy950–RPN13PRU–ubiquitin complex

To determine the structural basis for the additional binding energy of the phosphorysine-containing peptide, we crystallized a ternary RPN2940–952,pTy950–RPN13PRU–ubiquitin complex and collected X-ray diffraction data to 1.76 Å. Crystals were grown in conditions identical to our previously reported RPN2940–953–RPN13PRU–ubiquitin complex (31), which differs only within the RPN2 peptide. Specifically, the RPN2940–952 pTy950 peptide used in the current structure lacks one residue, Asp-953, which was unresolved in the earlier structure, and possesses the phosphate on Tyr-950. Interestingly, crystals of the phosphorylated complex grew in a different space group (P31 versus P21), indicating that the two crystal structures are not isomorphous. The structure was determined by molecular replacement using the binary RPN13PRU–ubiquitin complex from the unphosphorylated structure (PDB code 5V1Y) (31) as the search model and was refined to $R_{work}/R_{free}$ values of 0.173/0.207 (Fig. 2 and Table 1). The two ternary complexes in the asymmetric unit are nearly identical, superimposing with an all-atom root mean square deviation (r.m.s.d.). of 0.45 Å. For both complexes, the main chain is clearly defined for RPN13 residues 21–88, 90–99, and 102–131; RPN2 residues 940–950; and ubiquitin residues 1–75. Residues lacking density in RPN13 are found in mobile loops (Pro-947 to Pro-950) and termini (Ser-19, Asn-20, and Gly-101) or in the C-terminal ubiquitin (Ser-19, Asn-20, and Gly-101). Importantly, density was clearly defined for the RPN2 pTy950 phosphate in both complexes in the asymmetric unit (Fig. 2B).

Comparison with our previously reported RPN2940–953–RPN13PRU–ubiquitin structure (PDB code 5V1Y) revealed that phosphorylation of RPN2 Tyr-950 does not cause substantial changes to the overall structure. Structural alignment revealed an r.m.s.d. of 0.85 Å over backbone atoms of the 184 residues present in both structures and an r.m.s.d. of 1.36 Å over all atoms, indicating that RPN2 Tyr-950 phosphorylation does not
The RPN2 pTyr-950–RPN13 interaction

Only small differences in RPN2 are observed between the phosphorylated and unphosphorylated structures. The largest differences occur near the peptide termini and include an ~1.2-Å shift of Glu-941 backbone atoms and an ~1.0-Å shift of Glu-949 and Tyr-950 backbone atoms. These differences include a slight change in the curvature of the RPN2 peptide from Pro-947 to Tyr-950 (Fig. 2C). Nevertheless, the r.m.s.d. of all RPN2 backbone atoms is only 0.53 Å, and the overall structure of the RPN13-bound RPN2 peptide is maintained upon phosphorylation. Hydrogen bonding interactions of backbone atoms of RPN2 Phe-948 with RPN13 Val-48 and of RPN2 Tyr-950 with RPN13 Thr-36 are conserved in the unphosphorylated and phosphorylated structures. Similarly, the salt bridge of RPN2 Glu-941 with RPN13 Arg-64 and hydrogen bonding of RPN2 Glu-949 with RPN13 Thr-36 and Thr-37 are also conserved. Interestingly, a salt bridge between RPN2 Glu-941 and RPN13 Arg-27, which was observed in the unphosphorylated complex, is no longer observed in the phosphorylated structure.

Coordination of the RPN2 pTyr-950 phosphate

Well-defined electron density was observed for the RPN2 pTyr-950 phosphate (Fig. 3A), which is seen to coordinate the side chains of RPN13 Lys-97 and Arg-104, as well as a molecule of ethylene glycol (cryoprotectant). The Lys-97 side chain amine lies within hydrogen-bonding distances of two phosphate oxygens (3.1 and 3.2 Å) (Fig. 3B). The ε-amine of RPN13 Arg-104 forms hydrogen bonds with the RPN2 pTyr-950 phosphate and η-oxygens (2.6 and 2.9 Å), and an RPN13 R104 η-amine forms a single hydrogen bond with a pTyr-950 phosphate oxygen (3.2 Å). Our model also suggests that additional phosphate coordination might be provided by RPN13 Lys-83, which is near RPN2 pTyr-950, but lacks observable density beyond the β-carbon (Fig. 3A).

Table 1
Crystallographic statistics

<table>
<thead>
<tr>
<th>Data collection</th>
<th>Space group</th>
<th>P31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell dimensions</td>
<td>a, b, c (Å)</td>
<td>99.4, 99.4, 141.7</td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>90.0, 90.0, 120.0</td>
<td></td>
</tr>
<tr>
<td>Rmerge (%)</td>
<td>6.9 (66.3)</td>
<td></td>
</tr>
<tr>
<td>f/diff (%)</td>
<td>12.8 (1.3)</td>
<td></td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>0.99 (0.98)</td>
<td></td>
</tr>
<tr>
<td>Redundancy</td>
<td>9.2 (7.7)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2
Mutation of coordinating residues abrogates preferential binding of RPN2 pTyr-950

<table>
<thead>
<tr>
<th>Mutation</th>
<th>KD (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K97A</td>
<td>0.1 nM</td>
</tr>
<tr>
<td>R104A</td>
<td>0.9 nM</td>
</tr>
<tr>
<td>K97D</td>
<td>11.2 ± 0.9 nM</td>
</tr>
<tr>
<td>R104D</td>
<td>18.0 ± 0.07 nM</td>
</tr>
<tr>
<td>K97E</td>
<td>16.8 ± 0.9 nM</td>
</tr>
<tr>
<td>R104E</td>
<td>27.2 ± 0.15 nM</td>
</tr>
</tbody>
</table>

ACCELERATED COMMUNICATION: RPN2 pY950 enhances RPN13 binding

To verify our structural model, additional FP assays were performed using RPN13 constructs with mutations in residues that coordinate RPN2 pTyr-950. Specifically, residues Lys-97 and Arg-104 of RPN13 were independently mutated to alanine, aspartic acid, and glutamic acid.

In agreement with the crystal structure, K97A and R104A mutations in RPN13 disrupt the ~8-fold preferential binding of phosphorylated RPN2 peptide (Table 2). Specifically, RPN13 K97A binds RPN2940–952 with a KD of 18.0 ± 0.07 nM, whereas it binds RPN2940–952:pTyr950 with a KD of 11.2 ± 0.9 nM, thereby displaying a modest ~1.6-fold preference for the phosphorylated peptide. An even stronger effect was seen for RPN13 R104A, which bound unphosphorylated peptide slightly tighter than RPN2940–952:pTyr950, with observed KD values of 16.8 ± 0.9 and 27.2 ± 0.15 nM, respectively.

To further validate the structural model, the positively charged residues that coordinate RPN2 pTyr-950 were replaced with the negatively charged residues aspartate and glutamate. Each of the K97D, K97E, R104D, and R104E mutants modestly disrupted binding of the unphosphorylated peptide (Table 2, column 3). In every case, however, binding of the phosphorylated peptide was substantially impaired (Table 2, column 5), consistent with the anticipated charge repulsion.
ACCELERATED COMMUNICATION: RPN2 pY950 enhances RPN13 binding

Table 2

<table>
<thead>
<tr>
<th>RPN13 construct</th>
<th>(K_D) (nM)</th>
<th>-Fold change</th>
<th>(K_D) (nM)</th>
<th>-Fold change</th>
<th>pTyr preference (-fold change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wildtype</td>
<td>7.2 ± 0.3</td>
<td>1</td>
<td>0.9 ± 0.09</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>K83A</td>
<td>8.9 ± 0.3</td>
<td>1.2</td>
<td>9.4 ± 0.7</td>
<td>10.4</td>
<td>0.9</td>
</tr>
<tr>
<td>K97A</td>
<td>18.0 ± 0.7</td>
<td>2.5</td>
<td>11.2 ± 0.9</td>
<td>12.4</td>
<td>1.6</td>
</tr>
<tr>
<td>K97D</td>
<td>67.0 ± 3.5</td>
<td>9.3</td>
<td>427.3 ± 72.8</td>
<td>474.8</td>
<td>0.2</td>
</tr>
<tr>
<td>K97E</td>
<td>21.4 ± 1.3</td>
<td>3.0</td>
<td>262.5 ± 31.4</td>
<td>58.2</td>
<td>0.1</td>
</tr>
<tr>
<td>R104A</td>
<td>16.8 ± 0.9</td>
<td>2.3</td>
<td>27.2 ± 1.5</td>
<td>30.2</td>
<td>0.6</td>
</tr>
<tr>
<td>R104D</td>
<td>42.6 ± 3.7</td>
<td>5.9</td>
<td>188.9 ± 26.6</td>
<td>209.9</td>
<td>0.2</td>
</tr>
<tr>
<td>R104E</td>
<td>66.2 ± 6.0</td>
<td>9.2</td>
<td>1115 ± 341</td>
<td>1223.9</td>
<td>0.1</td>
</tr>
</tbody>
</table>

The most severe binding defects occurred in RPN13 K97D \((K_D = 427.3 \pm 72.8 \text{ nm})\) and RPN13 R104E \((K_D = 1115 \pm 341 \text{ nm})\) for which binding was disrupted ~500- and ~1200-fold, respectively. In addition to greatly weakening binding, these mutations reverse the binding preference such that the unphosphorylated peptide bound ~5–10-fold more tightly than the phosphorylated peptide (Table 2, column 6).

RPN13 Lys-83 contributes to binding of pTyr-950

In addition to residues that directly coordinate RPN2 pTyr-950 in the crystal structure, we propose that Lys-83 coordinates pTyr-950 under physiological conditions that lack ethylene glycol. To test this possibility, binding studies were performed using the RPN13 K83A mutant, which revealed that the binding of unphosphorylated peptide is essentially unchanged \((K_D = 8.9 \pm 0.5 \text{ nm})\) but that the phosphorylated peptide is no longer preferentially bound \((K_D = 9.4 \pm 0.7 \text{ nm})\) (Table 2). These observations verify that Lys-83 contributes to the additional binding energy observed upon phosphorylation of Tyr-950.

Discussion

Previous work mapped the RPN13-binding epitope within RPN2 (31–34), described the molecular determinants of binding between RPN13PRU and the RPN2 C-terminal peptide (31, 34), and described how RPN13PRU is auto-ubiquitylated and restricts degradation of ubiquitylated protein substrates in impaired proteasomes (38). In this work, we advance the model of RPN13 regulation by describing a phosphorylation “switch” within RPN2 that might regulate recruitment of RPN13 to the proteasome.

Defining the precise physiological roles of RPN13 is complicated by the functional redundancy it shares with the proteasome ubiquitin receptors RPN1 and RPN10 (39–42). Although the down-regulation or loss of RPN13 does not impact the bulk turnover of ubiquitylated proteins, certain physiological substrates of RPN13 have been identified, including the NF-κB inhibitor IkBα (43). NF-κB is best known for its role in inflammation, which fosters multiple hallmarks of cancer (44); impacts most, if not all, stages of tumorigenesis (45); and has been described as the “matchmaker” that connects inflammation to cancers through expression of genes that promote survival, proliferation, and metastasis (46–49). Increased levels of RPN13 result in enhanced rates of IkBα degradation, thus releasing inhibition of the NF-κB signaling pathway. In addition, it has been established that increased cellular levels of RPN13 correlate with the onset and progression of several cancers (11–18, 28).

RPN13 is observed at substoichiometric ratios in endogenously purified proteasomes (33, 35), suggesting that under typical cell culture conditions the RPN13-binding epitope in RPN2 is unsaturated. Thus, Tyr-950 phosphorylation may serve to increase the number of RPN13-associated proteasomes. A related possibility is that phosphorylation decreases the off-rate for RPN13 binding, which may be a factor in optimizing association with substrates that are slow to engage with the proteasome ATPases or are at risk of stalling and premature release. In either case, it is likely that phosphorylation regulates RPN2–RPN13 interactions within the proteasome. Future efforts will be required to definitively validate and quantify this possibility, including quantification of the endogenous pools of free and proteasome-associated RPN2 pTyr-950 as well as the identification of the stress-response pathways, kinases, and phosphatases that modulate it.

Our findings have implications for the development of anticancer therapeutics targeting the RPN2–RPN13 interaction (27–30). In particular, the identification of a specific phosphate-binding site will inform efforts to develop inhibitors mimicking the RPN2 interaction. Moreover, recognizing that phosphorylation enhances binding imposes limits on the affinity required for an inhibitor to effectively block the interaction in vivo. In addition to the inhibitor/drug development interest, important future goals include identifying cellular conditions associated with altered phosphorylation of RPN2 Tyr-950 and determining whether this modification is associated with a cellular pathway that may function in protein quality control, a stress response, or other facet of cellular regulation.

Experimental procedures

Protein expression and purification

All expression plasmids have been submitted to the Addgene plasmid repository (plasmid numbers 73742 and 125823–125829). Recombinant RPN13 proteins were expressed and purified as described previously (31). Briefly, cell pellets were resuspended in GS-BIND buffer (20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM DTT, 1 mM EDTA) supplemented with protease inhibitors (Sigma) and incubated with 1 mg/ml lysozyme for 1 h at 4 °C prior to sonication. Lysis by sonication was followed by clarification by centrifugation. RPN13 constructs were purified by 1) GSH-Sepharose (GS) resin, 2) on-column HRV3C
protease cleavage to remove glutathione S-transferase, and 3) gel-filtration chromatography.

Specifically, clarified lysate was incubated with pre-equilibrated GS resin (GE Healthcare) for 4 h. After washing the resin twice with 10 column volumes of GS-BIND buffer, bound protein was incubated with HRV3C protease in 5 column volumes of GS-BIND buffer at 4 °C overnight before elution. Eluted protein was analyzed by SDS-PAGE, concentrated with a 3,000 molecular weight-cutoff Vivaspin centrifugal concentrator, and loaded onto an SD75 gel filtration column (GE Healthcare) equilibrated with SEC buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM DTT) for final purification. Purified protein was analyzed by SDS-PAGE, concentrated, and stored in SEC buffer with 10% glycerol at −80 °C. Recombinant ubiquitin was expressed and purified as described previously (50).

Peptide synthesis

Peptides were synthesized on a Prelude or Prelude X instrument (Gyros Protein Technologies) using Fmoc (N-(9-fluorenylethoxycarbonyl)) solid-phase peptide synthesis at 30-μmol scale. Deprotection cycles employed three treatments of 2 ml of 20% piperidine in DMF for 3 min followed by three washes for 30 s using 2 ml of DMF. Coupling cycles consisted of addition of 0.65 ml of 200 mM amino acid in 4-methylmorpholine, 0.65 ml of 195 mM HATU in DMF, and 0.5 ml of 600 mM (30 mol of resin for 15 min. C-terminally labeled peptide of 0.20 mmol/g) was utilized for C-terminal acids.

N-terminal capping was achieved through the addition of 1 ml of acetic anhydride and 1 ml of 0.6 M 4-methylmorpholine/30 μmol of resin for 15 min. C-terminally labeled peptide was synthesized through the addition of 5-(6)-carboxyfluorescein (Acros Organics) to a Lys ε-amine using standard coupling conditions. After completion of syntheses, peptide resins were thoroughly washed with methylene chloride and dried under vacuum.

Cleavage of peptide resins was achieved by 180-min agitation with 4 ml of TFA containing 2.5% water and 2.5% trisopropylsilane/30 μmol of peptide resin. The TFA solution was precipitated into 40 ml of ice-cold diethyl ether and centrifuged at 5,000 × g for 10 min. The supernatant was decanted, and pellets were triturated twice with ether before being dried under vacuum. Peptides were then HPLC-purified before lyophilization for long-term storage.

Formation of RPN2–RPN13 complex for crystallization

The RPN2–RPN13 complex was formed by addition of a slight (1.2:1) molar excess of RPN2 peptide to RPN13 in SEC buffer followed by size-exclusion chromatography. RPN2–RPN13 complex was concentrated with a 3,000 molecular weight-cutoff Vivaspin centrifugal concentrator and loaded onto an SD75 gel filtration column equilibrated with crystallization buffer (20 mM HEPES, pH 7.2, 150 mM NaCl, 1 mM tris(2-carboxyethyl)phosphine, 1 mM EDTA). Eluted fractions were analyzed by SDS-PAGE and MS to confirm the presence of the RPN2 peptide, which is not easily visible by Coomassie staining.

Crystallization and structure determination

Purified RPN2-RPN13 complex was mixed in a 1:1.5 molar ratio with ubiquitin and concentrated to 17 mg/ml (E280 = 20,935 cm−1 m−1). The crystal complex grew at 4 °C from a 0.6-μl drop in a 1:2 ratio of protein and reservoir solutions (0.1 M citric acid, pH 4.6, 20% PEG-6000). Because the pKs of tyrosine phosphate is 5.8, crystals were soaked in reservoir solution at pH 6.0 with 25% ethylene glycol prior to plunging into liquid nitrogen to promote a more physiologically relevant state.

Diffraction data were collected on a Rigaku MicroMax-007HF rotating anode generator and RAXIS IV++ detector and processed using HKL2000 (51). Phases were determined by molecular replacement using PHASER (52) using the binary RPN13–ubiquitin complex from our previous structure (PDB code 5V1Y) (31) as the search model. Model building and refinement were performed using Coot (53) and PHENIX (54).

Model geometries were analyzed by MolProbity (55) within PHENIX. 98.4% of residues have favorable backbone dihedrals, and 1.6% fall into allowed regions.

Coordinates and structure factor amplitudes have been deposited at the PDB under the accession code 6O14. Figures of molecular structures were generated using Chimera (37).

Fluorescence polarization

FP assays were performed on a Biotek Synergy Neo HTS Multi-Mode Microplate Reader using 485/528-nm excitation/emission wavelengths. Dilution series of purified RPN13 were made in FP assay buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, 1 mg/ml casein, 0.005% Tween 20) in the presence of 100 pM (unphosphorylated complex) or 50 pM (phosphorylated complex) fluorescein-labeled RPN2 peptides. Dissociation constants (KD values) were calculated using GraphPad Prism 8 (GraphPad Software) by fitting raw polarization data to the following equation.

\[
\text{FP} = \frac{(FP_{\text{max}} - FP_{\text{min}}) \times [RPN13]}{K_D + [RPN13]} + FP_{\text{min}} \quad (\text{Eq. 1})
\]


Acknowledgments—We thank Frank Whitby, Heidi Schubert, and Kath Ferrell for technical assistance and James Fulcher, Mark Peterson, and Michael Kay for peptide synthesis. Mass spectrometry validation of proteins was performed at the University of Utah Mass Spectrometry and Proteomics Core Facility, which is supported by National Institutes of Health Grant P30CA042014 from the NCI. Sequencing of constructs was performed at the DNA Sequencing Core Facility, University of Utah.
null
ACCELERATED COMMUNICATION: RPN2 pY950 enhances RPN13 binding


Downloaded from http://www.jbc.org/ at UNIV OF UTAH on June 24, 2019
Phosphorylation of Tyr-950 in the proteasome scaffolding protein RPN2 modulates its interaction with the ubiquitin receptor RPN13
Casey W. Hemmis, Stephanie C. Heard and Christopher P. Hill

doi: 10.1074/jbc.A119.008881 originally published online May 7, 2019

Access the most updated version of this article at doi: 10.1074/jbc.A119.008881

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 55 references, 7 of which can be accessed free at
http://www.jbc.org/content/294/25/9659.full.html#ref-list-1