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Phosducin-like protein (PhLP), a regulator of G$\beta\gamma$ function, interacts with the proteasomal protein SUG1

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Abstract

Phosducin-like protein (PhLP) and phosducin are highly homologous proteins that interact with the $\beta\gamma$ subunits of guanine nucleotide binding proteins. While phosducin has a well-characterized role in retinal signal transduction, PhLP function remains unclear. To further understand the function of PhLP, we have examined other potential protein:protein interactions with PhLP using the yeast two-hybrid system. PhLP was found to interact with a mouse homologue of the yeast SUG1, a subunit of the 26S proteasome which may also indirectly modulate transcription. This interaction was further confirmed by an in vitro binding assay and co-immunoprecipitation of the two proteins in overexpression studies. Inhibition of proteasome function by lactacystin led to accumulation of high molecular weight, ubiquitin-immunoreactive protein precipitated by PhLP antiserum. We suggest that PhLP/SUG1 interaction may target PhLP for proteasomal degradation.

Keywords: Phosducin-like protein; G protein; Proteasome; Two-hybrid

1. Introduction

Phosducin-like protein (PhLP) is the product of a widely expressed gene first isolated by subtractive cloning of ethanol-responsive genes [1]. Different splice variants of the gene have been isolated which potentially generate two forms of the protein: PhLP long (PhLP) and PhLP short ($PhLP_S$), a truncated form of PhLP missing the first 83 N-terminal amino acids [1]. PhLP has a high degree of protein sequence homology to phosducin, a cytosolic phosphoprotein primarily expressed in the retina and pineal gland that interacts with the $\beta\gamma$ subunits of guanine nucleotide binding proteins (G proteins) [2–5]. In the retina, phosducin scavenges the $\beta\gamma$ subunits of the G protein transducin ($G_t\,\beta\gamma$), thus, preventing their reassociation with the α subunit of G_t ($G_t\,\alpha$) [4,6]. Since phosducin has a higher affinity for $G_t\,\beta\gamma$ than does $G_t\,\alpha$, it has been suggested that the formation of the phosducin/$G_t\,\beta\gamma$ complex is a major factor regulating photoreceptor responsiveness [7]. Phosducin also inhibits the GTPase activity of several other G pro-

Abbreviations: GA, Gal4 activation domain; GB, Gal4 DNA-binding domain; G protein, heterotrimeric GTP-binding protein; GST, glutathione S-transferase; HA, hemagglutinin; mSUG1, mouse homologue of the yeast SUG1 protein; PhLP, phosducin-like protein; $PhLP_S$, phosducin-like protein, short form; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate

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teins [8], and inhibits G$\beta\gamma$ activation of effectors such as adenylyl cyclase or phospholipase C [9–11]. We and others have recently suggested that PhLP may also act as an endogenous regulator of G proteins [12,13]. PhLP interacts in vitro and in vivo with G$\beta\gamma$ subunits [13]. In addition, Schröder and Lohse have reported that PhLP inhibits several functions of G$\beta\gamma$ subunits in vitro [12].

To further characterize PhLP function, we chose to identify other proteins which might interact with PhLP using the yeast two-hybrid system [14]. $PhLP_S$ was used to screen gene products from a mouse liver cDNA library for PhLP-binding activity. One candidate, a mouse homologue (mSUG1) of the yeast SUG1 protein [15–20], was found to be the primary PhLP-binding protein in this assay. This interaction was then confirmed using an in vitro binding assay and by co-immunoprecipitation of the two proteins in overexpression studies. Finally, we provide data suggesting that PhLP interaction with mSUG1 may have functional implications by targeting PhLP for proteasomal degradation.

2. Materials and methods

2.1. DNA constructs

The PhLP–GAL4 and $PhLP_S$–GAL4 DNA binding domain fusions (PhLP–GB and $PhLP_S$–GB) were constructed by first amplifying the PhLP coding region from a full length rat PhLP cDNA clone [1] using polymerase chain reaction (PCR), introducing *Eco*RI and *Bam*HI restriction sites in the 5′ and 3′ PCR primers, respectively (PhLP 5′ primer: 5′GAATTCATGACAACCCTGGAT3′; $PhLP_S$ 5′ primer: 5′GAATTCATGGAGCGGCTGATC3′; PhLP/ $PhLP_S$ 3′ primer: 5′GGATCCATTATCAGTTCAATC3′). The fragments were then ligated into vector pGBT9 (Clontech) between *Eco*RI and *Bam*HI sites and the constructs were transformed into XL1-blue *E. coli* cells (Stratagene).

A glutathione S-transferase (GST) + mSUG1 fusion construct was obtained by inserting the full length mSUG1 coding region in-frame at the carboxyl-terminus of GST. The mSUG1 coding sequence, which contains an internal *Eco*RI site, was isolated by partial *Eco*RI digestion of a mSUG1 cDNA clone obtained in the two-hybrid library screen. This fragment contains the full length mSUG1 coding region and 15 bases of the 5′-untranslated region which codes in-frame for an additional five amino acids (RERGK) upstream of the initiator methionine. The mSUG1 fragment was ligated into *Eco*RI-digested pGEX-4T-1 (Pharmacia Biotech), and the ligation product was transformed into BL21 *E. coli* cells, a protease-deficient *E. coli* strain (Pharmacia Biotech).

A hemagglutinin (HA) epitope-tagged PhLP expression vector was generated as described previously [13]. Similarly, this HA epitope (YDVPDYAS) was also fused to the carboxyl-terminus of mSUG1. The mSUG1 coding region was amplified by PCR using primers containing *Bam*HI and *Eco*RI restriction sites at the 5′ and 3′ ends, respectively (5′ primer: 5′GGATCCATGGCGCTTGATGGG3′; 3′ primer: 5′GAATTCCTTCCATAGCTTCTT3′). This fragment was then fused in-frame, using a two-step ligation, with the HA epitope previously inserted into the polylinker region of pcDNA3, between *Bam*HI and *Eco*RI sites of the modified vector (In Vitrogen) [13].

All constructs were verified by DNA sequence analysis (Sequenase version 2.0, United States Biochemical).

2.2. Yeast two-hybrid system

The two hybrid assay was performed using the Matchmaker two-hybrid system essentially as described by the manufacturer (Clontech). A cDNA library (Clontech #ML4000AB) containing mouse liver cDNA's fused to the transcriptional activation domain of GAL4 (GA) in the pGAD10 vector was co-transformed with the $PhLP_S$–GB fusion into yeast strain HF7c (MATa, ura3–52, his3–200, lys2–801, ade2–101, trp1–901, leu2–3, 112, gal4–542, gal80–538, LYS2::GAL1-HIS3, URA3::$(GAL4\ 17mers)_3$-CYC1-*lacZ*). Approximately 7.5×10^5 transformants were plated on −His plates and His^+ colonies were isolated and tested for β-galactosidase activity using a filter assay. The $His^+/\beta\text{–}gal^+$ cells were then grown in +Trp media for 48 h to allow the loss of the $PhLP_S$–GB fusion plasmid. Cells that could not grow in −Trp media were then used to isolate mouse library plasmid DNA. These plasmids were re-intro-

duced into HF7c cells and SFY526 yeast cells (MATa, ura3–52, his3–200, ade2–101, lys2–801, trp1–901, leu2–3, 112, can^r, gal4–542, gal80–538, URA3::GAL1-*lacZ*), in which *lacZ* is under the control of a different Gal4-responsive promoter than the one in HF7c. As suggested by the manufacturer, additional controls included testing the interaction of library fusions with the GAL4 DNA-binding domain alone (GB) and a human lamin C–GB fusion (Clontech). Clones satisfying all genetic screening tests were subsequently sequenced and compared to DNA sequence databases to determine their identity or similarity to known proteins [21].

2.3. GST-fusion binding to [S^{35}]-PhLP in vitro

[^{35}S]-PhLP was generated as described previously [1] by T3 RNA polymerase (Promega) in vitro transcription from a *Xho*I cleaved, full length rat PhLP cDNA template plasmid. PhLP–RNA was then translated in vitro with rabbit reticulocyte lysate (Promega) in the presence of [^{35}S]-methionine (DuPont). The GST–mSUG1 fusion protein was produced and purified on glutathione Sepharose-4B resin (Pharmacia) exactly as described previously [13,22]. Following immobilization on the beads, the GST–mSUG1 protein was washed four times in PBS and twice in binding buffer (50 mM $KHPO_4$, pH 6.0, 100 mM KCl, 10 mM $MgCl_2$, 10% glycerol, 1% Tween 20) [20]. The fusion protein was then incubated overnight in the same buffer containing [^{35}S]-PhLP along with blocking proteins from naive BL21 cell lysate. Following three washes with binding buffer, the proteins were eluted with SDS sample buffer and resolved on a 10% SDS–polyacrylamide gel. Bound radiolabeled PhLP was detected by autoradiography or phosphoimager analysis (Bio-Rad). As controls, [^{35}S]-PhLP binding to GST itself or endogenous [^{35}S]-reticulocyte lysate proteins (no added PhLP RNA) binding to GST–mSUG1 were also tested.

2.4. Transient transfection of COS-7 cells and immunoprecipitation

COS-7 cells (3×10^5 cells well^{-1}) were seeded, 48 h before transfection, in 6-well plates in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS). Cells were transfected for 5 h in serum-free DMEM with plasmid DNA pre-mixed with lipofectAMINE Reagent (BRL). The total amount of DNA in all transfections was 1.0 μg well^{-1} and yielded a 10% transfection efficiency as determined by immunocytochemistry using the 12CA5 monoclonal antibody directed against the HA epitope (Boehringer Mannheim Biochemicals) (C. Thibault, unpublished data). When required, the empty pcDNA3 vector was used to maintain a constant amount of DNA. The transfected cells were incubated overnight at 37°C in DMEM containing 10% FCS. Cells were then washed twice with ice-cold PBS and lysed in 500 μl of hypotonic buffer (10 mM HEPES pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 μM dithiothreitol, 2 μg/ml leupeptin, 2 μg/ml aprotinin, 20 μg/ml soybean trypsin inhibitor) well^{-1}. Since PhLP/mSUG1 interaction was found to be unstable in the presence of detergents, the immunoprecipitation was conducted on the cytosolic fraction of transfected cells. A soluble fraction was obtained by centrifugation of a post-nuclear supernatant at $150\,000 \times g$ for 1 h at 4°C and was precleared for 30 min in the presence of protein A-agarose (Santa Cruz Biotechnology). A polyclonal anti-PhLP antibody was produced against a maltose binding protein-$PhLP_S$ fusion protein and affinity purified as described previously [13]. The pre-cleared lysate was incubated overnight at 4°C with the anti-$PhLP_S$ antibody or pre-immune serum (2 μg/1.5 ml of lysate), followed by protein A- agarose. Immunoprecipitates were washed four times with hypotonic buffer. Protein complexes were eluted in SDS sample buffer and analyzed by Western blot using the 12CA5 anti-HA antibody.

2.5. Immunodetection of PhLP and ubiquitinated PhLP in NG 108-15 cells

NG108-15 neuroblastoma × glioma cells were grown as described [1] in DMEM containing 10% Serum$^+$ (JRH Biosciences). For Western blot analysis, cells were rinsed twice in ice-cold PBS and homogenized in 10 mM Tris–HCl, 1 mM EDTA, pH 7.6. Total cell lysates were analyzed by Western blot using affinity-purified, polyclonal anti-$PhLP_S$ antiserum [13]. For in vivo ubiquitination studies, cells at 70% confluence in 10 cm petri dishes were treated for 6 h in culture media containing 10 μM lacta-

cystin (Calbiochem) or the drug vehicle alone (DMSO). At the end of this incubation, cells were rinsed twice in ice-cold PBS and suspended in Nonidet P-40 lysis buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% (v/v) Nonidet P-40, 2 μg/ml aprotinin and leupeptin, 1 mM phenylmethylsulfonyl fluoride). After 15 min on ice, the insoluble material was removed by centrifugation at $10\,000 \times g$ for 10 min at 4°C. Immunoprecipitations were then done as above with anti-$PhLP_S$ antiserum or pre-immune serum. Immunoprecipitates were analyzed by Western blot using an anti-ubiquitin monoclonal antibody (Immunotech).

3. Results

Using $PhLP_S$–GB as bait, a yeast two-hybrid screen was used to detect PhLP-interacting proteins in a library of mouse cDNA–Gal4 activation domain fusion clones. Screening of 7.5×10^5 transformants on −His media yielded 75 His^+ colonies and four of these proved to be *lacZ*$^+$ (Fig. 1). These four clones were sequenced and three of them were identified as mSUG1, the mouse homologue of the yeast SUG1 gene [15,23]. These three clones contained the full length coding region of mSUG1 fused in-frame with the Gal4 DNA binding domain and their DNA sequence was identical to that previously reported for mSUG1 (accession #Z54219) [15]. The fourth clone was the mouse alcohol dehydrogenase (accession #M11307), the significance of which is currently being studied. In addition, full length PhLP protein (PhLP–GB) also interacted with mSUG1 in the yeast two-hybrid system (S. Barhite and M.F. Miles, unpublished data).

The specificity of the PhLP/mSUG1 interaction was confirmed by in vitro binding studies using a GST pull down assay. GST–mSUG1 fusion protein, or GST alone, was incubated with [^{35}S]-PhLP produced by in vitro transcription/translation of a rat PhLP cDNA clone. Fig. 2 shows that the GST–mSUG1 fusion protein bound a radiolabeled protein migrating at the expected molecular weight for PhLP

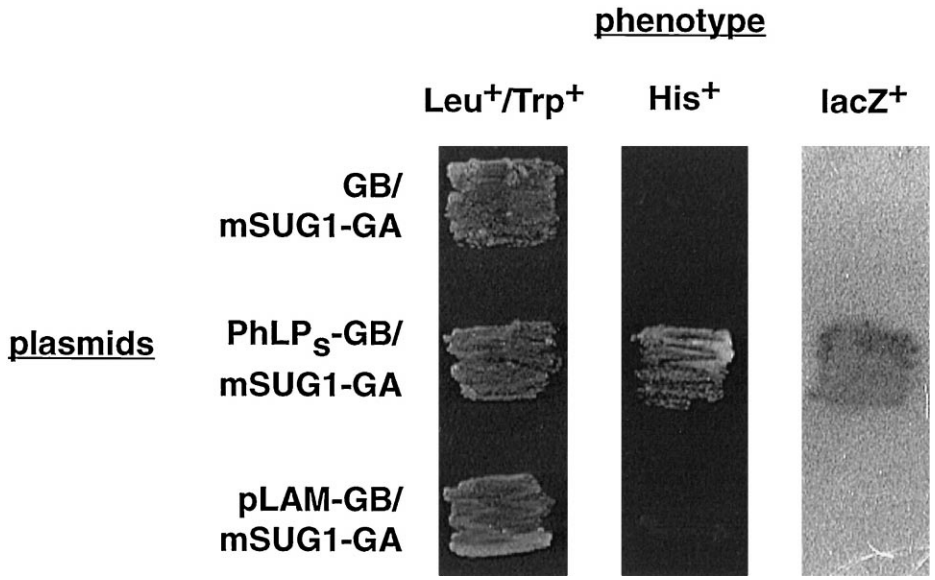

Fig. 1. Identification of a PhLP-interacting protein by yeast two-hybrid screening. Mouse cDNA clones fused to the transcriptional activation domain of Gal4 (GA) were screened using a yeast two-hybrid approach with $PhLP_S$ as the bait protein coupled to the Gal4 DNA binding domain (GB). Following screening of 7.5×10^5 clones, 4 putative $PhLP_S$-interacting clones were identified. Positives were confirmed by clonal isolation of the interacting cDNA and repeated genetic screening. Results obtained with mSUG1–GA are depicted. Yeast cells were transformed with mSUG1–GA + GB alone, $PhLP_S$–GB or pLAM–GB. The latter is human lamin C fused to GB as an additional control for nonspecific binding. Growth on + Leu/ + Trp confirmed the presence of both plasmids in all transfections. Only cells transformed with $PhLP_S$–GB + mSUG1–GA showed growth on −His media (His^+) and β-galactosidase activity (*lacZ*$^+$).

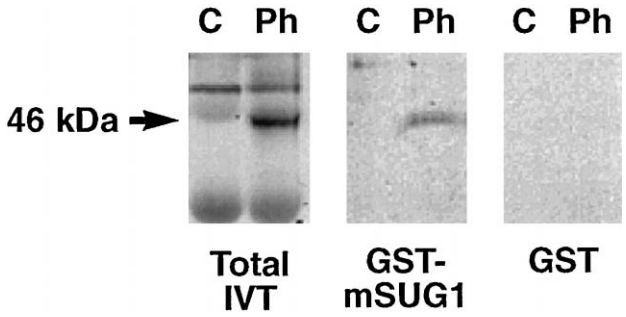

Fig. 2. GST–mSUG1 binds [^{35}S]-PhLP. In vitro translation reactions were programmed with no added template (C) or with PhLP RNA transcribed from a full length PhLP cDNA construct using T3 RNA polymerase (Ph). [^{35}S]-labeled proteins were incubated in vitro with GST alone or GST–mSUG1 immobilized on glutathione-agarose beads. Following washing, proteins remaining on the beads were analyzed by SDS–PAGE and autoradiography. Panels depict total in vitro translation (IVT) products vs. those binding to GST–mSUG1 or GST alone. Results are representative of experiments repeated twice.

on SDS–polyacrylamide gel analysis, while no detectable PhLP binding was seen with GST alone.

The PhLP/mSUG1 interaction was further substantiated by co-immunoprecipitation of the complex with an affinity purified, polyclonal anti-PhLP$_S$ antibody, following overexpression of the proteins in COS-7 cells (Fig. 3). In order to facilitate the detection of the precipitated proteins, we added an HA epitope tag onto the carboxyl-terminal end of PhLP and mSUG1. COS-7 cells were transfected with DNA plasmids encoding PhLP–HA, mSUG1–HA, or a combination of both plasmids. Expression of the proteins in COS-7 cells was monitored by Western blot analysis using monoclonal anti-HA antibody (Fig. 3, lower panel). PhLP–HA was specifically precipitated by the anti-PhLP$_S$ antibody but not by the pre-immune serum (Fig. 3, upper panel). In addition, this antibody co-precipitated mSUG1–HA only when co-expressed with PhLP–HA (Fig. 3, upper panel).

Other SUG1-interacting proteins have been localized to the proteasome, suggesting a role of SUG1 in targeting proteins for degradation [23]. Since the

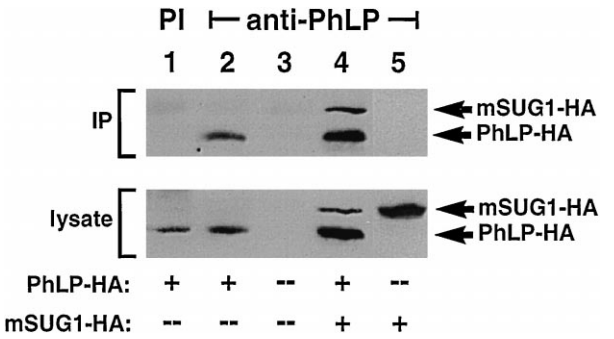

Fig. 3. Co-immunoprecipitation of mSUG1–HA and PhLP–HA from COS-7 cells. COS-7 cells were transfected with expression plasmids containing mSUG1 or PhLP coupled to a HA epitope. Cells were lysed in hypotonic media 24 h after transfection and subjected to immunoprecipitation with pre-immune serum (PI) or anti-PhLP$_S$ polyclonal antiserum. Precipitated proteins were identified by Western blot analysis using a monoclonal antibody recognizing the HA epitope (upper panel). Expression of proteins was confirmed by Western blot analysis of cell lysates prior to immunoprecipitation (lower panel). The position of mSUG1–HA and PhLP–HA are indicated. Results are representative of experiments repeated twice.

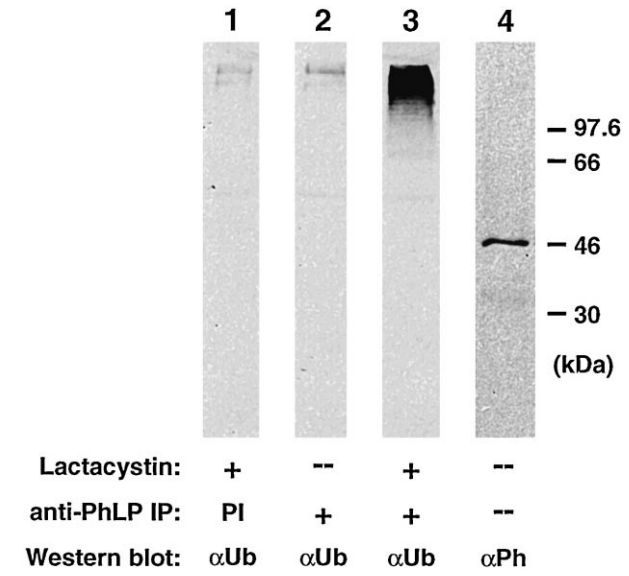

Fig. 4. PhLP antiserum immunoprecipitates polyubiquitinated protein in lactacystin-treated NG108-15 cells. NG108-15 cells were grown in the presence or absence of lactacystin, a proteasome inhibitor, for 6 h prior to preparation of whole cell cleared lysates for immunoprecipitation with affinity-purified, polyclonal PhLP$_S$ antiserum (αPh, lanes 2 and 3). Mock immunoprecipitation with pre-immune serum (PI) was also done on lactacystin-treated cells (lane 1). Immunoprecipitates were analyzed by SDS–polyacrylamide gel electrophoresis and Western blotting using a monoclonal antibody against ubiquitin (αUb). NG108-15 total cell lysates probed by Western blot analysis with the PhLP$_S$ antiserum showed a single major band migrating at the expected molecular weight (46 kDa) for full length PhLP protein (lane 4).

covalent addition of polyubiquitin to proteins is a common means used by eucaryotic cells to target proteins for proteasomal degradation [24], we examined whether PhLP was modified by polyubiquitination. For these studies, we used NG108-15 neuroblastoma × glioma cells which express predominantly the full length PhLP protein as determined by Western blot analysis (Fig. 4, lane 4). Following treatment of NG108-15 cells with lactacystin, a specific inhibitor of proteasome function [25,26], PhLP protein was immunoprecipitated from whole cell lysates. Immunoprecipitates were analyzed by SDS–polyacrylamide gel electrophoresis followed by Western blotting with antiserum against ubiquitin. PhLP immunoprecipitates from lactacystin-treated cells showed a dramatic increase in ubiquitin-immunoreactive material migrating diffusely at molecular weights extending well above the 46 kDa expected for unmodified PhLP (Fig. 4, lane 3). A small amount of ubiquitin immunoreactive material was seen in cells without lactacystin treatment or following immunoprecipitation with pre-immune serum (Fig. 4, lanes 1 and 2). This data suggests that PhLP is extensively modified by ubiquitination.

4. Discussion

In the present study, we identified interactions between the mouse SUG1 protein and PhLP using three independent assays. Repeated isolation of mSUG1 in the yeast two-hybrid system, in vitro binding of PhLP to GST–mSUG1 and co-immunoprecipitation of PhLP and SUG1 from COS-7 cells, together, provide strong evidence that these two proteins may have a biologically relevant interaction.

Since PhLP is known to interact with the β-subunits of heterotrimeric G proteins [13], it might seem somewhat surprising that the yeast two-hybrid analysis did not isolate Gβ. However, Gβ is generally not thought capable of forming a stable protein configuration without the obligate partner, Gγ. Thus, $PhLP_S$–GB interaction with G$\beta\gamma$ would have required a trimeric protein complex, an extremely unlikely event under the transfection conditions used for the two-hybrid screening unless endogenous yeast Gγ were to substitute for the mouse homologue. It is likely that formation of such a cross-species complex would be much less stable, particularly given the fusion protein configurations for PhLP and Gβ.

The function of SUG1 is not completely understood but it has been implicated in two different cellular processes. A role as a mediator in transcriptional regulation has been suggested since SUG1 has been found in the yeast RNA polymerase II complex [27] and been shown to interact with several known yeast enhancer binding proteins [20,28]. Mammalian homologues of SUG1, termed TRIP, mSUG1 and FZA-B, have been shown to interact with nuclear hormone receptors [15,19] and c-*fos* [23], respectively. Wang et al. also reported that c-*fos* co-localizes with mSUG1 in proteasomes [23]. Similarly, TRIP was later co-purified with the proteasome (see correction to Ref. [20]). It was thus suggested that SUG1 may regulate transcription either directly as a transcriptional mediator, or indirectly, via targeted degradation of transcriptional proteins [15,23]. In both yeast and mammalian cells, SUG1 has also been shown to be a component of the 26S proteasome [29–31]. SUG1 is homologous to several other conserved ATPase domain proteins also present in the 26S proteasome [32]. These ATPases have been suggested to play a regulatory role in proteasome function, or possibly serve to target specific proteins for proteasome degradation [32].

Thus, PhLP/SUG1 interaction might serve to direct PhLP for proteasomal degradation. Our initial studies support this idea, showing that polyubiquitinated protein precipitated by PhLP antiserum accumulates when proteasome function is inhibited by lactacystin. Further studies will establish whether SUG1 indeed targets PhLP for proteasomal degradation and whether PhLP/SUG1 interactions might modulate G$\beta\gamma$ signaling.

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References

[1] M.F. Miles, S. Barhite, M. Sganga, M. Elliott, Proc. Natl. Acad. Sci. USA 90 (1993) 10831–10835.
[2] R.H. Lee, A. Fowler, J.F. McGinnis, R.N. Lolley, C.M. Craft, J. Biol. Chem. 265 (1990) 15867–15873.
[3] Y. Watanabe, K. Kawasaki, N. Miki, C.-H. Kuo, Biochem. Biophys. Res. Commun. 170 (1990) 951–956.
[4] R.H. Lee, T.D. Ting, B.S. Lieberman, D.E. Tobias, R.N. Lolley, Y.-K. Ho, J. Biol. Chem. 267 (1992) 25104–25112.
[5] R.N. Lolley, C.M. Craft, R.H. Lee, Neurochem. Res. 17 (1992) 81–89.
[6] R.H. Lee, B.S. Lieberman, R.N. Lolley, Biochemistry 26 (1987) 3983–3990.
[7] T. Yoshida, B.M. Willardson, J.F. Wilkins, G.J. Jensen, B.D. Thornton, M.W. Bitensky, J. Biol. Chem. 269 (1994) 24050–24057.
[8] P.H. Bauer, S. Muller, M. Puzicha, S. Pippig, B. Obermaier, E.J.M. Helmrich, M.J. Lohse, Nature 358 (1992) 73–76.
[9] M. Hekman, P.H. Bauer, P. Söhlemann, M.J. Lohse, FEBS Lett. 343 (1994) 120–124.
[10] B.E. Hawes, K. Touhara, H. Kurose, R.J. Lefkowitz, J. Inglese, J. Biol. Chem. 269 (1994) 29825–29830.
[11] J. Xu, D. Wu, V.Z. Slepak, M.I. Simon, Proc. Natl. Acad. Sci. USA 92 (1995) 2086–2090.
[12] S. Schröder, M.J. Lohse, Proc. Natl. Acad. Sci. USA 93 (1996) 2100–2104.
[13] C. Thibault, M.W. Sganga, M.F. Miles, J. Biol. Chem. 272 (1997) 12253–12256.
[14] S. Fields, R. Sternglanz, Trends Genet. 10 (1994) 286–292.
[15] E. vom Bauer, C. Zechel, D. Heery, M.J.S. Heine, J.M. Garnier, V. Vivat, B. Le Douarin, H. Gronemeyer, P. Chambon, EMBO J. 15 (1996) 110–124.
[16] S. Ugai, T. Tamura, N. Tanahashi, S. Takai, N. Komi, C.H. Chung, K. Tanaka, A. Ichihara, J. Biochem. 113 (1993) 754–768.
[17] J.C. Swaffield, J.F. Bromberg, S.A. Johnston, Nature 357 (1992) 698–700.
[18] H. Michael, H. Schmidt, O. Fleck, H. Gutz, C. Liedtke, A. Lorentz, K. Ostermann, Gene 145 (1994) 205–210.
[19] J.W. Lee, F. Ryan, J.C. Swaffield, S.A. Johnston, D.D. Moore, Nature 374 (1995) 91–94.
[20] J.C. Swaffield, K. Melcher, S.A. Johnston, Nature 374 (1995) 88–91.
[21] S.F. Altschul, W. Gish, W. Miller, E.W. Myers, D.J. Lipman, J. Mol. Biol. 215 (1990) 403–410.
[22] J.V. Frangioni, B.G. Neel, Anal. Biochem. 210 (1993) 179–187.
[23] W. Wang, P.M. Chevray, D. Nathans, Proc. Natl. Acad. Sci. USA 93 (1996) 8236–8240.
[24] S. Jentsch, S. Schlenker, Cell 82 (1995) 881–884.
[25] G. Fenteany, R.F. Standaert, W.S. Lane, S. Choi, E.J. Corey, S.L. Schreiber, Science 268 (1995) 726–731.
[26] C.L. Ward, S. Omura, R.R. Kopito, Cell 83 (1995) 121–127.
[27] Y.-J. Kim, S. Björklund, Y. Li, M.H. Sayre, R.D. Kornberg, Cell 77 (1994) 599–608.
[28] Q. Xu, R.A. Singer, G.C. Johnston, Mol. Cell. Biol. 15 (1995) 6025–6035.
[29] D.M. Rubin, O. Coux, I. Wefes, C. Hengartner, R.A. Young, A.L. Goldberg, D. Finley, Nature 379 (1996) 655–657.
[30] M. Ghislain, A. Udvardy, C. Mann, Nature 366 (1993) 358–362.
[31] K. Akiyama, K. Yokota, S. Kagawa, N. Shimbura, G.N. DeMartino, C.A. Slaughter, C. Noda, K. Tanaka, FEBS Lett. 363 (1995) 151–156.
[32] Y. Makino, S. Yogosawa, M. Kanemaki, T. Yoshida, K. Yamano, T. Kishimoto, V. Moncollin, J.-M. Egly, M. Muramatsu, T. Tamura, Biochem. Biophys. Res. Commun. 220 (1996) 1049–1054.