The Cyclophilin-like Domain Mediates the Association of Ran-Binding Protein 2 with Subunits of the 19 S Regulatory Complex of the Proteasome*

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Paulo A. Ferreira‡, Cai Yunfei, Diana Schick, and Ronald Roepman
From the Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

The combination of the Ran-binding domain 4 and cyclophilin domains of Ran-binding protein 2 selectively associate with a subset of G protein-coupled receptors, red/green opsins, upon cis-trans prolyl isomerase-dependent and direct modification of opsin followed by association of the modified opsin isoform to Ran-binding domain 4. This effect enhances in vivo the production of functional receptor and generates an opsin isoform with no propensity to self-aggregate in vitro. We now show that another domain of Ran-binding protein 2, cyclophilin-like domain, specifically associates with the 112-kDa subunit, P112, and other subunits of the 19 S regulatory complex of the 26 S proteasome in the neuroretina. This association possibly mediates Ran-binding protein 2 limited proteolysis into a smaller and stable isoform. Also, the interaction of Ran-binding protein 2 with P112 regulatory subunit of the 26 S proteasome involves still another protein, a putative kinesin-like protein. Our results indicate that Ran-binding protein 2 is a key component of a macro-assembly complex selectively linking protein biogenesis with the proteasome pathway and, thus, with potential implications for the presentation of misfolded and ubiquitin-like modified proteins to this proteolytic machinery.

G protein-coupled receptors or so called seven-transmembrane receptors constitute the largest gene family known to date (1–5). It is estimated that this family consists of 5,000 members (4). They play a key role in mediating environmental cues to the intracellular signaling machinery (1–3, 5); thus, they have become prime targets for novel pharmacophores (1, 4). In addition, congenital receptor dystrophies lead to a wide variety of functional heterogeneous disorders for which the molecular pathogenesis remains unknown (6). Many of these mutations seem to affect the biogenesis of these receptors. Yet, the molecular components and mechanisms underlying the biogenesis of seven-transmembrane receptors have for the most part not been identified.

The light receptors, opsins, constitute a class of homologous receptors that are key players in the activation of the phototransduction cascade across species (7–10). Opsins are continuously produced in large amounts in photoreceptors and constitute about 90% of the membrane protein content of the outer segments of vertebrate photoreceptors (11). These cells likely express a highly efficient biogenic machinery to process and sort in a vectorial fashion the vast amounts of opsin produced in these cells. Thus, photoreceptors constitute an excellent model system to dissect the biogenic machinery of seven-transmembrane receptors. In Drosophila melanogaster, at least nine different gene products specifically affect the functional production of several opsin subclasses in photoreceptors (12–14). Among these, the ninaA gene encodes a retina-specific cyclophilin/peptidyl prolyl cis-trans isomerase (PPlase) (15, 16). The ninaA gene is expressed in all classes of photoreceptor cells (17), but mutations in this gene affect the production of only a subset of opsins, R1–6 opsin (12, 17). The molecular basis for the NinaA substrate specificity remains to be understood.

We have identified in bovine retina a cyclophilin-related protein made up of multiple and well defined structural modules (18) that is the counterpart protein of the human and murine RanBP2 reported by the Nishimoto (19), Coutavas (20), and Dabauvalle (21) groups. Expression of RanBP2 is highly tissue-restricted in the retina (18) (albeit expression has been found in the liver (19, 20)) and present at high levels in cone photoreceptors (18). We have shown that the C-terminal superdomain of RanBP2, Ran-binding domain 4 (RBD4) and cyclophilin (CY), mediate the selective association of RanBP2 with a subclass of opsins, red/green opsins (22). This effect consists in the sequential, direct, and selective PPlase-dependently-mediation of modification of opsin by CY, production of an RBD4-binding competent isoform of opsin, binding of modified opsin to the C-terminal half of RBD4 and, in concert with the N-terminal half domain of RBD4, CY-mediated chaperoning of the modified opsin isoform to the C-terminal half domain of RBD4 (22, 23). This leads to the production in vitro of a novel and immuno-reactive opsin isoform with no propensity to self-aggregate and an increase in vivo of functional opsin receptor (22, 23). Based on our results, we have proposed that prolyl-isomerization constitutes a novel molecular switch in receptor biogenesis (23). This switch is modulated by the PPlase activity of cyclophilin required for the formation of a transient opsin isoform competent to be loaded onto component(s) of the processing and/or sorting machinery (22, 23). Recent evidence suggests that this molecular switch is widespread to other biological functions such as controlling cell-cycle division (24) and gene expression activity (25), possibly through PPlase-mediated conformational changes in substrate activity.

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† To whom correspondence should be addressed: Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226. Tel.: 414-456-8877; Fax: 414-456-6545; E-mail: ferreira@post.its.mcw.edu.

‡ The abbreviations used are: RBD4, Ran-binding domain 4; CY, cyclophilin; CLD, cyclophilin-like domain; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; PVD, polynylvlendine difluoride; CHAPS, 3-[3-cholamidopropyl]dimethylammoniomio]propanesulfonic acid; PPlase, peptidyl prolyl cis-trans isomerase; ATPγS, adenosine 5'-O-(thiotriphosphate); GTPγS, guanosine 5'-O-(thiotriphosphate); GDPβS, guany1-5'-yl thiophosphate.
RanBP2 is also a component of the Ran-GTPase cycle (26, 27). Analysis of Saccharomyces cerevisiae mutants and other studies have implicated Ran-GTPase and many of its regulatory and putative effector components in a wide variety of pleiotropic functions ranging from nuclear protein import to nucleocytoplasmic export of mRNA (26–32). Yet, there is no RanBP2 orthologue in the yeast genome (27), and RanBP2 is highly expressed in terminally differentiated neuroretinal cells (18). RanBP2 has also been localized at cytoplasmic fibrils emanating from the nuclear pore complex (19–21), thus supporting the proposal that RanBP2 may play a key role in the nucleocytoplasmic transport of certain proteins and mRNAs. To better understand the RanBP2 role in cell function, it is of paramount importance to identify its molecular partners. To this end, we have extended structure-function studies to another RanBP2 domain, the cyclophilin-like domain (CLD) (18). We identified this domain as a region with low but significant homology to the C-terminal cyclophilin domain of RanBP2 (18). The results reported here link RanBP2 to the proteasome proteolytic pathway and possible proteolytic processing of RanBP2. Moreover, this process may be mediated by another protein such as a putative and novel kinesin-like motor protein. We discuss the implication of these findings in protein biogenesis.

EXPERIMENTAL PROCEDURES

Materials—Glutathione S-agarse beads, Coomassie stain, silver stain kit, and Fast Stain were purchased from Amersham Pharmacia Biotech, Acros, Bio-Rad, and Zoin, respectively. Polyvinylidene difluoride membranes were purchased from Millipore and Applied Biosystems. Bovine retinas were purchased from Pee-Freeze (Roger, AR) and a local slaughter house. All nonhydrolyzable nucleotides, thiorbitol, and apyrase (grade VI, catalog number 6410) were purchased from Sigma. Mixture of protease inhibitors were from Boehringer Mannheim. Lactacystine and MG-132 were from BIOMOL. Protein markers (regular and pre-stained) were from New England Biolabs. Protein quantitation was carried out with Bio-Rad protein assay reagent.

Preparation of Retinal Extracts—Crude retinal extracts were prepared exactly as described before (22, 23) and stored at −80 °C with the exception that tablets containing a mixture of protease inhibitors were added to the homogenization buffer as recommended by the manufacturer. When applicable, proteasome inhibitors, lactacystine and MG-132, were also added to the homogenization buffer at final concentrations of 20 μM and 20 μM, respectively.

Expression and Purification of GST-fused Protein Constructs—GST-RBD3-CLD-W1-W2, GST-CLD-W1-W2, GST-CLD, GST-RBD3-CLD, and GST-W1-W2 constructs were prepared by subcloning, respectively, Klenow-treated Sau96I-EcoRI and Klenow-treated pGEX-KG vector (33). GST-W1-W2 protein construct was prepared by subcloning the Sau96I-SalI fragment of CY15 (18) into EcoRI and Klenow-treated pGEX-KG vector (33). GST-W1-W2 protein construct was prepared by subcloning the EcoRI-BamHI fragments of CY15 (18) into EcoRI and Klenow-treated pGEX-KG vector digested with XhoI and treated with Klenow. Expression, purification, and concentration of GST-fused and thiorbitol-cleaved (unfused) constructs were carried out exactly as described before (18). Purified GST-fused and unfused proteins were resolved on SDS-PAGE followed by Coomassie and/or silver-stain analysis.

Binding Activity Assays—Incubation reactions of GST-fused constructs (2.2 μg) with bovine retinal extracts (80 μl, ~2.5 mg of extract) and pull-down assays were carried out exactly as described previously (22, 23). When applicable, nonhydrolyzable nucleotides were added at a concentration of ~1 mM and apyrase in the amount of 50 units. Unfused construct (competitor) was used at 10 fold molar excess. Coprecipitates were analyzed on 7.5% SDS-PAGE after boiling in SDS-sample buffer.

Purification of Retinal P112 and P99—P112 purification was carried out by scaling-up the incubation binding assays ~180-fold between eight 15-ml conical tubes. The washings of coprecipitates were scaled-up accordingly. Coprecipitates were boiled in SDS-sample buffer for 3–5 min and loaded on 7.5% preparative SDS-PAGE, stained with Fast Stain followed by electroelution of P112 bands into Centronics. Eluted P112 protein was concentrated, and 5% (v/v) of the total concentrated protein was resolved in SDS-PAGE in parallel with coprecipitates of analytical retinal binding reactions and analyzed by silver-stain. P99 purification procedure was carried out exactly the same way with the exception that incubation assays were scaled-up ~360-fold, and 20% (v/v) of concentrated eluted protein was loaded on SDS-PAGE for silver-stain analysis. The rest of purified proteins were loaded on SDS-PAGE, blotted onto PVDF membranes, stained for 1–2 min with 0.1% Coomassie Blue in 50% methanol and 1% acetic acid, washed several times with destaining solution (50% methanol, 1% acetic acid), followed by several washes with Millipore H₂O. The blue-stained bands were cut and subjected to Edman degradation and amino acid composition analysis.

Western Blot Analysis of CLD-interacting Components of the 19 S Regulatory Complex—Incubation reactions and pull-down assays were carried out as described before. GST-CLD coprecipitates were resolved on SDS-PAGE, blotted onto PVDF membranes, blocked, and incubated with PA700 antibody at 1:2,500 (gift from Dr. George DeMartino) by the same exact procedures previously described (22, 23) with the exception that IgY secondary antibody (Promega) and a more sensitive chemiluminescent substrate (Super Signal, Pierce) were used for the development of the blot.

Western Blot Analysis of RanBP2—Bovine retinal extracts (150 μg) were loaded and resolved on SDS-PAGE, blotted onto PVDF membranes, blocked, and incubated with Cy321 antibody (1:5,000) by the same exact procedures previously described (22, 23) with the exception of the use of a more sensitive chemiluminescent substrate (Super Signal, Pierce).

RESULTS

The CLD Domain of RanBP2 Specifically Associates with P112 and P99 Retinal Proteins—We continued the structure-function analysis of RanBP2 by the same exact methods we previously reported (22, 23). To this end, we prepared a series of GST-fused and unfused protein constructs containing the whole RBD3, CLD, W1, and W2 supradomain and several fragments thereof (Fig. 1). The GST-fused constructs were incubated with CHAPS-solubilized retinal extracts, and pull-down assays were carried out in search for coprecipitating proteins with binding activity to the bait proteins. Coprecipitating proteins were subjected to several washes followed by SDS-PAGE and silver-stain analysis. To test the specificity of the binding of retinal proteins to GST-fused constructs, free (unfused) bait was added to the incubation reactions to compete with the GST-fused construct for the target substrate (Fig. 2a, lane 4). In certain reactions, nonhydrolyzable nucleotide analogs were also added to test for nucleotide-dependent association of retinal substrate(s) with the bait moiety (Fig. 2a, lane 5).

In contrast to RBD4-CY (18, 22, 23), the large GST-fused construct containing RBD3, CLD, and W1 and W2 in the same polypeptide chain was highly unstable when expressed and purified from Escherichia coli (not shown). Thus, we expressed these domains singly to facilitate SDS-PAGE analysis of binding activity of retinal substrates to GST constructs. In this study, we searched for retinal substrates that specifically associated with the CLD domain of RanBP2. Incubation of GST-
CLD with retinal extracts under physiological conditions lead to the association to this construct of two proteins with an apparent molecular mass of 112 kDa, P112 (Fig. 2, arrow, lane 3), and 99 kDa, P99 (Fig. 2, filled arrowhead, lane 3). Binding of these proteins to the CLD moiety of GST-CLD was highly specific because these were the only two proteins whose association with the GST-construct could be disrupted by coincubation with free (unfused) CLD (Fig. 2a, lane 7). This also showed that the 112- and 99-kDa bands represented single protein species. Moreover, these proteins did not bind other unrelated GST-fusion constructs such as GST-W1-W2 (not shown) and GST-RBD3 (not shown). P112 had a much higher binding activity toward CLD than P99, suggesting that P99 is specifically copurifying with P112. Association of P112 and P99 with CLD was reduced in the presence of ATPγS (Fig. 2a, lane 5), but it was not affected by the presence of the ATP-degrading enzyme, apyrase (Fig. 2a, lane 6), GTPγS, and GDPβS (not shown). Finally, incubation reactions carried out with retinal extracts prepared in the presence of the proteasome inhibitors (34, 35), lactacystine and MG-132, did not alter significantly the binding activity of these proteins to CLD (Fig. 2a, lane 7).

To investigate if the association of P112 and P99 with CLD was selective for retinal extracts, we incubated GST-CLD exactly under the same conditions with three different extracts prepared from bovine liver, kidney, and spleen tissues. The protein concentration of these was normalized to the same scale preparation reactions were loaded on large preparative SDS-PAGE, and retinal P112 and P99 were electroeluted and concentrated. To confirm the isolation and purity of the CLD-binding proteins, 5% (v/v) of the total amount of purified protein was used in incubation reactions. The bands below GST-CLD are lower molecular weight degradation products of GST-CLD. In retina-selective association of P112 and P99 with CLD of RanBP2. P112 and P99 did not coprecipitate with GST-CLD after incubation with liver (lane 2), kidney (lane 3), and spleen (lane 4) extracts. Concentration of tissue extracts were normalized to those of retinal extracts. Lane 1, protein markers; asterisk, GST-CLD; R.E. and R.E.*, retinal extracts prepared, respectively, in the presence of mixture of protease inhibitors and, in addition, the proteasome inhibitors, lactacystine and MG-132; GST-CLD, GST-fused CLD; CLD, free (unfused) CLD.

**Purification of Retinal P112 and P99**—To determine the identity of CLD-binding P112 and P99 proteins, we performed large scale retinal incubation reactions with GST-CLD. To this end, analytical incubation reactions were scaled-up approximately 180- and 360-fold for the purification of P112 and P99, respectively. GST-CLD coprecipitating proteins from large scale preparation reactions were loaded on large preparative SDS-PAGE, and retinal P112 and P99 were electroeluted and concentrated. To confirm the isolation and purity of the CLD-binding proteins, 5% (v/v) of the total amount of purified protein was run side-by-side in SDS-PAGE with analytical incubation reactions as well as with the remaining bulk of the purified protein. As seen in Figs. 3a and 5a, respectively, purified P112 and P99 comigrated exactly with those resolved from coprecipitates of analytical incubation reactions, albeit some lower molecular weight species were observed as a result of some degradation of the purified proteins.

To ascertain the identity of P112, this purified protein was blotted onto PVDF membranes, and the 112-kDa band (Fig. 3) was subjected to Edman degradation. N-terminal sequencing of the first 19 amino acids of P112 showed 100% identity to the counterpart sequence of the bovine (36) and human (37) P112 subunits of the 19 S regulatory complex of the 26 S proteasome (Fig. 4). N-terminal sequencing of P99 (Fig. 5) lead to no signal, suggesting that the N terminus of this protein is blocked. Thus, we performed Edman degradation on the 97-kDa band (Fig. 5) just below P99 since this band resulted likely from partial proteolysis of P99 during concentration of this protein (Fig. 5a). Degradation of P99 into P97 was supported by the fact that amino acid composition analysis of P99 and P97 were similar, in particular, in those residues...
acid residue not determined by Edman degradation.

of a cDNA clone isolated from a hepatoblastoma cell line (37).

X

ical incubation reactions (lanes 3–5) as described in Fig. 2a.

Purified P112 (arrow) migrated exactly at the same position as the specific CLD-binding protein, P112, observed in analytical incubation reactions (lane 4). b, Coomassie Blue-stained PVDF blot of purified P112. P112 protein (arrow) was purified from retinal extracts, blotted on a PVDF membrane, and stained with Coo massie Blue before being subjected to Edman degradation analysis. P112, purified CLD binding protein of 112 kDa; arrowhead, P99.

with low propensity to be modified by the hydrolysis procedure (not shown). In addition, N-terminal sequence of retinal P97 revealed 100% identity to a yeast hypothetical kinesin-like protein in the Genbank translated data base (Fig. 6) and the first residue sequenced began 16 amino acids downstream of the predicted starting methionine of the putative counterpart yeast protein. The predicted molecular mass of 99 kDa of the yeast hypothetical protein also closely matched the apparent molecular weight of retinal P99 in SDS-PAGE. Like all other kinesin-related motor proteins (38, 39), the yeast protein contains an ATP-binding domain (P-loop). Thus, altogether these data suggest that P97 is a proteolytic product of P99.

CLD Forms a Specific Macro-assembly Complex with Some Subunits of the 19 S Regulatory Complex of the 26 S Proteasome—In view of CLD-specific association with P112 subunit of the proteasome, we investigated whether other components of the 19 S regulatory complex (herein also referred to as PA700 or 19 S cap) specifically associate with the CLD domain of RanBP2. It is possible also that some PA700 components are expressed at low levels in the retina and/or associate with the CLD-P112 complex with a lower affinity than P112; thus, identification of all components forming the putative CLD macro-assembly complex may not be detectable by silver-stain analysis. To this end, we used a more sensitive method by using an antibody generated against the whole purified PA700 complex (gift from George DeMartino) for Western blot analysis of GST-CLD retinal coprecipitates (Fig. 7) prepared by the same exact methods we carried out for the silver-stain analysis of retinal CLD binding proteins (Fig. 2). This would also allow us to confirm the interaction of P112 with CLD seen by silver-stain analysis. As shown in Fig. 7, PA700 antibody recognizes in retinal extracts (Fig. 7, lane 1) three PA700 subunits of the proteasome of 112 kDa (arrow), 97 kDa (arrowhead), and another of approximately 58 kDa (open arrowhead). These are commonly referred to as P112 (36, 37, 40, 41), P97 (36, 40–42), and P58 (36, 40, 41). P97 and P58 are non-ATPase subunits of the 19 S proteasome. The former is identical to type-1 tumor necrosis factor receptor-associated protein-2 (42), whereas P58 is highly homologous to P91A tumor transplantation antigen (36). Overexposure of this Western blot also detected in retinal extracts a protein of ~48 kDa, which is likely the P48 ATPase subunit of the 19 S proteasome (not shown) (36, 40, 41). P112 and P48 seem to be expressed in the retina at much lower levels than P97 and P58 (Fig. 7, lane 1). Western blots of retinal incubation reactions with GST-CLD confirmed the specific association of P112 with CLD (Fig. 7, lanes 2 and 3), which was decreased by approximately 2-fold in the presence of ATPγS (Fig. 7, lane 4). This ATP-dependent binding decrease was also observed for P58. Association of CLD with P112 occurs with very high affinity in comparison with P97 and P58, and despite P112 lower expression in the retina than P97 and P58. The same is true for the association of P48 with CLD or CLD-proteasome complex (Fig. 7, lanes 2 and 4).

Limited Proteolytic Processing of RanBP2 in the Neuroretina—In face of RanBP2 association with subunits of the 19 S cap of the 26 S proteasome and emerging evidence of the role of the proteasome in protein biogenesis (43), we investigated if RanBP2 may be proteolytically processed in the retina into a smaller and stable isoform. To this end, we performed Western blot analysis with antibodies generated against the C-terminal half domain of RBD4 (18) on retinal extracts prepared in the presence or absence of proteasome inhibitors. We have shown that this antibody stained the nuclear envelope rims as well as inner and outer segments of most cone photoreceptors (18). In Western blots, this antibody detected the presence of a 55-kDa band (Fig. 8a), which completely disappeared when this antibody was blocked by the cognate antigen (Fig. 8b). Furthermore, the 55-kDa protein was present at comparable levels in retinal extracts with and without proteasome inhibitors. In overexposed blots, we could also detect the specific presence of a very large protein of above 300 kDa, albeit this protein was

a

b
present at very low levels (not shown). This protein likely represents the minor fraction of unprocessed RanBP2 protein.

DISCUSSION

We provide further evidence that the multi-domain RanBP2 protein is part of a macro-assembly complex. The interaction of RanBP2 with different molecular components is determined through specific interactions with single or a combination of structural modules of RanBP2. To this end, we have previously shown that the RBD4 and RBD4-CY domains interact with Ran-GTPase and red/green opsin, respectively (22, 23). Here, we report that the CLD domain of RanBP2 specifically mediates the interaction with subunits of the 19 S regulatory complex of the 26 S proteasome.

The CLD domain of RanBP2 associates with the largest subunit, P112, of the 19 S regulatory complex of the 26 S proteasome (36, 37) as well as with other subunits of this complex (Figs. 2 and 7). In particular, interaction of P112 (Figs. 2 and 7) and P48 (Fig. 7) with CLD is highly specific and occurs with very high affinity. This is supported by data that association of P112 and P48 with GST-CLD is disrupted by the addition of free (unfused) CLD to the incubation reactions, formation of CLD-P112 complex can be seen on silver-stained SDS-PAGE, and P112 does not associate with other GST-fused constructs unrelated to CLD (not shown). Observation of P112 interaction with CLD on silver-stained SDS-PAGE implies that P112 is likely binding directly to CLD, and this 19 S cap subunit mediates the interaction with other proteasome components (e.g., P97, P58, P48). Also, it has been reported that the gene encoding P112 expresses at higher levels another smaller and alternative spliced isoform with a predicted molecular mass of 90 kDa (37); however, our data show that the CLD of RanBP2 associates only with the P112 isoform in retinal extracts (Figs. 2 and 7). Finally, association of P112 with CLD seems to be selective for the neuroretina, as no binding activity of P112 to CLD was observed in other tissues such as the liver, spleen, and kidney. These are intriguing observations because P112 is expressed in different tissues (37) and is commonly isolated as an integral part of a 700,000-kDa multi-subunit complex (PA700) (36).

We also observed that incubation of GST-CLD in the presence of nonhydrolyzable ATPgS leads to a uniform decrease of binding activity (~2-fold) of the 19 S cap subunits to CLD (Fig. 7). Degradation of any endogenous ATP present in retinal extracts resulted in no change of binding activity of P112 to CLD (Fig. 2). In light of these results, characterization of P112 as a non-ATPase (36, 37) and its reported tight association with ATPase subunits of the 19 S cap for the formation of an active...
creased in the presence of unfused CLD (competitor). The presence of the binding of all of these subunits to GST-CLD is dramatically decreased in the presence of unfused CLD (competitor). The presence of ATPyS leads to a 2-fold and 5-fold decrease and abolishes completely, respectively, association of P112 and P58, and P97 and P48, to GST-CLD. Prestained markers are indicated on the left. The 83-kDa prestained marker has an abnormal mobility, as it co-runs with the 97.2-kDa regular marker.

The formation of active NF-κB1 transcription factor, p50, has been shown to require the direct involvement of the 26 S proteasome (36, 37, 40, 41), it is possible that addition of ATPyS to incubation reactions leads to the recruitment of P112 from RanBP2 by 19 S cap ATPase subunits of the proteasome. These results imply that some subunits of the 19 S cap of the proteasome may exist in a latent and/or free form and associate in a tissue-specific fashion with high affinity binding proteins such as retinal RanBP2.

The targeting of NF-κB to the cytoplasm requires similar protein-tagging modifications. RanBP2 is a member of Ran GTPase-binding proteins (27). Components of the Ran-GTPase cycle have been implicated in a host of cellular functions such as nuclear protein import (30–32) and mRNA export (26–32). As it seems to be the case for other small molecular weight GTPase proteins involved in intracellular transport, the spatial and temporal separation of the coupling events with many of the different modulators of this cycle (some possibly tissue-specific) are key to impart a vectorial property to the transport of cargoes into different molecular assemblies and/or subcellular compartments. In contrast to poly-ubiquitination of protein substrates (44), emerging evidence suggests that monoubiquitin-like tagging of proteins also leads to selective activation of specific targeting events of these proteins (45–49). For example, RanGAP, the GTPase-activating protein of Ran, has been localized in the cytoplasm, but modification by a ubiquitin-like protein, SUMO-1, leads to its targeting to the cytoplasmic face of the nuclear pore complex (46–49), where RanBP2, Ran-GTPase, and other factors of the Ran-GTPase cycle seem to colocalize. Our results raise the possibility of yet another role of some components of this cycle in proteasome function. Also, it is possible that covalent modification of RanGAP with SUMO-1 (46–49) leads to its processing or degradation by the proteasome. It will be interesting to investigate if RanBP2 processing requires similar protein-tagging modifications.

Our data shows that RanBP2 constitutes a scaffold protein possibly participating in multiple biological processes as a result of its mosaic primary structure, selective association of specific domains with diverse molecular partners, and a dy-
namic subcellular localization. Its association with P112 pro-
teaseosome subunit, possibly in a latent stage, may constitute an
excellent surveillance mechanism for the proper and early
processing of opsin receptors, which upon misprocessing may
be driven to the proteasome pathway. This process may be also
mediated by other proteins closely interacting with components
of the 19 S regulatory complex such as the putative kinesin-like
motor protein, P99. In face of RanBP2-dependent production of
functional opsin receptor (22, 23), one could envision that mu-
tations in vertebrate opsins may turn these into poor sub-
states for the required PPIase-mediated conformational mod-
ification and/or binding of opsin to the RBD4-CY supradomain
of RanBP2. This could ultimately target the mutated receptor
for proteasome-dependent degradation and thus prevent the
compromise of the secretory pathway. Interestingly, dominant
mutations in vertebrate opsins, to our knowledge, do not lead to
the over-proliferation of the endoplasmic reticulum (50). This is
in contrast to what has been reported in the fruit fly, D. melano-
gaster (51, 52). Understanding the function of the molecular
assembly components of RanBP2 will provide us clues about
the mechanisms underlying the biogenesis of these receptors
and associated molecular pathogenesis of receptor dystrophies.
Physical chromosomal colocalization of P112 (37) and RanBP2
(53) at genetically mapped loci of heterogenous genetic retin-
opathies, recessive Oguchi disease (54, 55), and achromatopsia
(total color blindness) (56), respectively, make these genes ex-
cellent candidates for inherited retinal dystrophies.

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