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Genetics

A genetic system based on split-ubiquitin for the analysis of interactions between membrane proteins *in vivo*

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ABSTRACT **A detection system for interactions between membrane proteins *in vivo* is described. The system is based on split-ubiquitin [Johnsson, N. & Varshavsky, A. (1994) *Proc. Natl. Acad. Sci. USA* 91, 10340–10344]. Interaction between two membrane proteins is detected by proteolytic cleavage of a protein fusion. The cleavage releases a transcription factor, which activates reporter genes in the nucleus. As a result, interaction between membrane proteins can be analyzed by the means of a colorimetric assay. We use membrane proteins of the endoplasmic reticulum as a model system. Wbp1p and Ost1p are both subunits of the oligosaccharyl transferase membrane protein complex. The Alg5 protein also localizes to the membrane of the endoplasmic reticulum, but does not interact with the oligosaccharyltransferase. Specific interactions are detected between Wbp1p and Ost1p, but not between Wbp1p and Alg5p. The new system might be useful as a genetic and biochemical tool for the analysis of interactions between membrane proteins *in vivo*.**

The analysis of interactions between proteins *in vivo* is essential for understanding their functions in the cellular context. The yeast two-hybrid system is a powerful method for the *in vivo* analysis of protein-protein interactions (1), but is limited to the analysis of soluble proteins or soluble domains of membrane proteins, i.e., interactions between integral membrane proteins cannot be studied. The split-ubiquitin system, which has recently been introduced for the analysis of interactions between soluble proteins, provides an alternative for the *in vivo* analysis of protein interactions (2). Ubiquitin is a conserved protein of 76 amino acids, which is usually attached to the N terminus of proteins as a signal for their degradation (3). The ubiquitin moiety is recognized by ubiquitin-specific protease(s) (UBP), resulting in the cleavage of the attached protein. The cleavage can be visualized with a stable reporter protein attached to the C terminus of ubiquitin (Fig. 1*A*). The ubiquitin-fusion can be divided and expressed in two parts, a N-terminal part (Nub*I*, amino acids 1–34, with *I* being isoleucine at position 13) and a C-terminal part of ubiquitin (Cub) (amino acids 35–76 of ubiquitin) fused to a reporter protein (2). Nub*I* and Cub-reporter assemble in the cell and form split-ubiquitin ([Nub*I*:Cub]-reporter). The split-ubiquitin is recognized by UBP, resulting in the cleavage of the reporter protein attached to Cub (Fig. 1*B*). Wild-type Nub*I* has a high affinity for Cub and assembles spontaneously to form a split-ubiquitin heterodimer. Replacement of Ile-13 of wild-type Nub*I* by alanine (Nub*A*) or glycine (Nub*G*) decreases the affinity between Nub and Cub (Fig. 1*C*). The association of Nub*G* with Cub-reporter is now dependent on additional protein contacts (Fig. 1*D*). The protein contacts can be provided by two test proteins, protein1 fused to Nub and protein2 to Cub-reporter. The detection of the cleaved reporter protein indicates interactions between protein1 and protein2.

The split-ubiquitin system was able to detect specific homotypic interactions between the zipper region of Gcn4p *in vivo* (2). The interaction between the two zippers was measured by immunoprecipitation and Western blot analysis of the cleaved reporter. We reasoned that the split-ubiquitin system would also be applicable to membrane proteins, provided that Nub and Cub are attached to parts of the protein, which localize to the cytosol. This is a prerequisite, because the necessary UBP is present in the cytosol and not in the lumen of the endoplasmic reticulum (ER) (4). Wbp1p is an essential component of the yeast oligosaccharyltransferase complex (5, 6) and in close proximity to Ost1p, another essential protein of the same complex (7–10). Both Ost1p and Wbp1p are type I transmembrane proteins with cytoplasmic C termini (9, 11). Alg5p is a type II transmembrane protein with both N and C termini in the cytoplasm, which synthesizes dolichol-phosphoglucose from dolicholphosphate and UDP-glucose (12). Alg5p is not known to interact with the oligosaccharyltransferase complex, but localizes to the membrane of the ER and is therefore suited as a control for the system. Using the oligosaccharyltransferase complex as a model, we established a detection system for interactions between integral membrane proteins. A transcription factor, protein A-LexA-VP16 (PLV) was used as the reporter molecule. PLV is liberated upon cleavage from Cub, hence able to activate *LacZ* and *HIS3* reporter genes, therefore providing a potentially useful tool for the screening of interaction between membrane proteins.

MATERIALS AND METHODS

Strains, Media. All constructs were expressed in the *Saccharomyces cerevisiae* strain L40 (*MATa trp1 leu2 his3 LYS2*::*lexA-HIS3 URA3*::*lexA-lacZ*) (13). pRS305(Δwbp1-Cub-PLV) was integrated at the single *Spe*I site into the *WBP1* gene resulting in strain YG0673 (*MATa trp1 leu2 his3 LYS2*::*lexA-HIS3 URA3*::*lexA-lacZ wbp1*::*pRS305(Δwbp1-Cub-PLV)*). YG0673

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This paper was submitted directly (Track II) to the *Proceedings* office. Abbreviations: Nub, amino acids 1–37 of ubiquitin, either Nub*I* or Nub*A* or Nub*G*; Nub*I*, wild-type Nub, isoleucine at position 13; Nub*A*, Ile-13 replaced by alanine; Nub*G*, Nub Ile-13 replaced by glycine; Cub, C-terminal part of ubiquitin (amino acids 35–76). UBP, ubiquitin-specific protease(s); PLV, protein A-LexA-VP16 fusion; β-gal, β-galactosidase; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside; ER, endoplasmic reticulum; ARS, autonomous replicating sequence.

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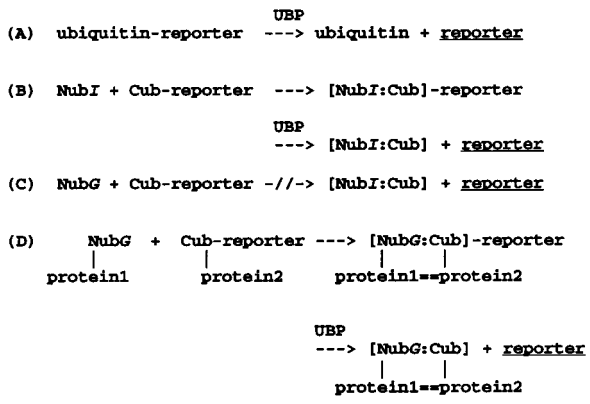

FIG. 1. Principle of the split-ubiquitin system. Split-ubiquitin is drawn as [Nub*I*:Cub] or [Nub*G*:Cub]. The liberated reporter protein is underlined. ":" indicates ubiquitin interaction; "=" indicates interaction between protein1 and protein2; for more information see the text.

was the recipient for different Nub-constructs. The *Escherichia coli* strain DH5α (*F*$^{-}$, *endA1 hsdR17 (rk-mk*$^{+}$*) supE44 thi recA1 gyrA96 relA1 f80d lacZ*Δ*M15*) (14) was used as the bacterial host for all plasmid constructions. Standard yeast media and techniques (15) were used. For selection of plasmids, dropout media containing all except the specified amino acids were used. All plasmids were transformed into yeast by lithium acetate transformation (16).

Construction of Plasmids. Sequences of the plasmid constructs and the detailed construction schemes are available upon request. All plasmids were verified by sequencing. The expression of all constructs was checked by Western blot analysis using suitable antibodies.

pRS305(Δwbp1-Cub-PLV). The *Apa*I-*Hin*dIII insert within pRS305 (17) encodes amino acids 251–430 of Wbp1p (Δ*wbp1*) (accession number P33767), fused to LEGSTMSG and the amino acids 35–76 of the yeast Ubi4p (Cub) (P04838), which in turn is linked by the sequence MHRSACGRMAG to the amino acids 151–275 of *Staphylococcus aureus* protein A (X96612) (18). The sequence ASGR links the protein A sequence to the amino acids 1–202 of LexA (J01643), which in turn is fused to EFPGIW and the amino acids 402–479 of VP16 (P04486). The sources of the DNAs were as follows: *WBP1*, p45–11 (11); Cub, pRS306(Δswp-Cub-dha) (N.J., unpublished data); protein A, p28NZZtrc (19); LexA-VP16, pLexA202+VP16 (gift from Alcide Barberis, University of Zürich).

pRS314(Nub*I*-ALG5). The region encompassing the *CUP1* promoter and Nub*G*GSTM was amplified from construct X (2) by PCR and fused to the ORF of the *ALG5* gene. The fusion protein consists of amino acids 1–37 of Ubi4p (Nub), followed by GGST and the 334 amino acids of Alg5p (P40350) (12). The fusion gene was inserted between the *Not*I-*Pst*I of pRS314. pRS314(Nub*A*-ALG5) and pRS314(Nub*G*-ALG5) were constructed by exchanging the small *Bam*HI fragment carrying *CUP1* and the mutated Nub-sequence from construct XII and construct XIII (2). The 2-μm plasmids pNub*I*-ALG5, pNub*A*-ALG5, and pNub*G*-ALG5 were constructed by exchanging the small *Pst*I-*Nru*I fragment of pAS2 (20) with the *Pst*I-*Sac*II fragment of the corresponding pRS314(Nub*I*-ALG5), pRS314(Nub*A*-ALG5), or pRS314(Nub*G*-ALG5).

pRS304(Δost1-Nub). A truncated *ost1* gene (codons 102–476) (P41534) (9) was fused to Nub*I*, Nub*A*, and Nub*G* in the vector pRS306 resulting in pRS306(Δost1-Nub*I*), pRS306(Δost1-Nub*A*), and pRS306(Δost1-Nub*G*). The encoded sequence after the C terminus of Ost1p is LEGGST followed by the amino acids 1–37 of Ubi4p (Nub), which in turn is fused to Thr-Leu-Glu and a stop codon. The Δost1-Nub*I*, Δost1-Nub*A*, and Δost1-Nub*G* gene fragments were transferred to the pRS304 vector to give pRS304 (Δost1-Nub*I*), pRS304(Δost1-Nub*A*), and pRS304 (Δost1-Nub*G*). For site-directed integration into the *OST1* locus, plasmids were linearized at the single *Sph*I site.

pOST1-Nub. The 5′-end of the *OST1* gene, including its promoter, was fused to Δost1-Nub*I*, Δost1-Nub*A*, and Δost1-Nub*G*, using the single *Sph*I site. The complete *OST1*-Nub*I,* *OST1*-Nub*A,* *OST1*-Nub*G* genes were used to replace the small *Eco*RI/*Sma*I fragment of the 2-μm plasmid pAS2 (20), resulting in the 2-μm plasmids pOST1-Nub*I*, pOST1-Nub*A*, and pOST1-Nub*G*.

β-Galactosidase (β-Gal) Activity Tests. Cells were grown for two days at 30°C on sterile Whatman filters on dropout agar plates lacking tryptophan and leucine and supplemented with 0.2 mM $CuSO_4$. The cells were permeabilized by dipping the filters into liquid nitrogen for 1 min. After thawing, the filters were overlaid with 1.5% agarose in 0.1 M $NaPO_4$-buffer (pH 7.0) containing 0.4 mg/ml 5-bromo-4-chloro-β-D-galactopyranoside (X-Gal), and incubated at 30°C for 1–23 h. For the quantitative assay, cells were grown in liquid dropout medium lacking tryptophan and leucine, supplemented with 0.2 mM $CuSO_4$ to an OD_{546} of 0.5–0.7. Cells (1.5 ml) were pelleted, washed once in Z buffer (113 mM Na_2HPO_4/40 mM NaH_2PO_4/10 mM KCl/1 mM $MgCl_2$, pH 7.0), suspended in 300 μl Z buffer. One hundred microliters were taken, lysed by 3 freeze/thaw cycles. Z buffer (700 μl) containing 0.27% (vol/vol) 2-mercaptoethanol and 160 μl 2-nitrophenyl-β-D-galactopyranoside (4 mg/ml in Z buffer) were added and incubated for 1–20 h at 30°C. Four hundred microliters of 0.1 M $NaCO_3$ was added, the sample centrifuged and the OD_{420} measured. β-Gal units were calculated as $1{,}000 \times OD_{420}/(OD_{546} \times \text{min})$.

Protein Extraction and Western Blot Analysis. Cells were grown at 30°C to OD_{546} of 0.3–1.2 in liquid dropout medium lacking tryptophan and leucine supplemented with 0.2 mM $CuSO_4$. Proteins were extracted essentially according to Horvath and Riezman (21). Cells were pelleted and suspended in 50 μl 1.85 M NaOH per 3 OD units of cells and incubated on ice for 10 min. The same volume of 50% trichloroacetic acid was added and proteins were precipitated by centrifugation for 5 min. The pellet was suspended in 50 μl of SDS/sample buffer containing 8 M urea. The proteins were dissolved after addition of 20 μl of 1 M Tris·base for 1.5 h at 37°C. Samples were centrifuged for 2 min and 10 μl extract was used for SDS/PAGE/Western blot analysis. Membranes were probed with peroxidase-IgG at 1:5,000 dilution. Protein A-fusion proteins were detected by enhanced chemiluminescence (Amersham). The amount of protein loaded was verified by Coomassie blue staining of the SDS gels.

RESULTS

Experimental Design. Wbp1p is a transmembrane protein of the ER and a subunit of the oligosaccharyltransferase complex (5). To analyze interactions between Wbp1p and other proteins, we elongated the C terminus of Wbp1p by Cub as the interaction module, followed by a modified transcription factor (PLV). The final fusion protein was termed Wbp1-Cub-PLV (Fig. 2*A*). The rationale was to anchor the transcription factor to the membrane of the ER, thereby preventing it from activating genes in the nucleus (Fig. 2*B*). Coexpression of Wbp1-Cub-PLV protein with a noninteracting Nub-fusion-protein, like Nub*G*-Alg5p, does not lead to activation (Fig. 2*C*). Coexpression of an interacting protein fused to Nub*G* results in the assembly and the recognition of the split-ubiquitin heterodimer by UBP(s). The protease liberates PLV, which probably enters the nucleus by diffusion and activates then *lacZ* and *HIS3* reporter genes (Fig. 2*D*).

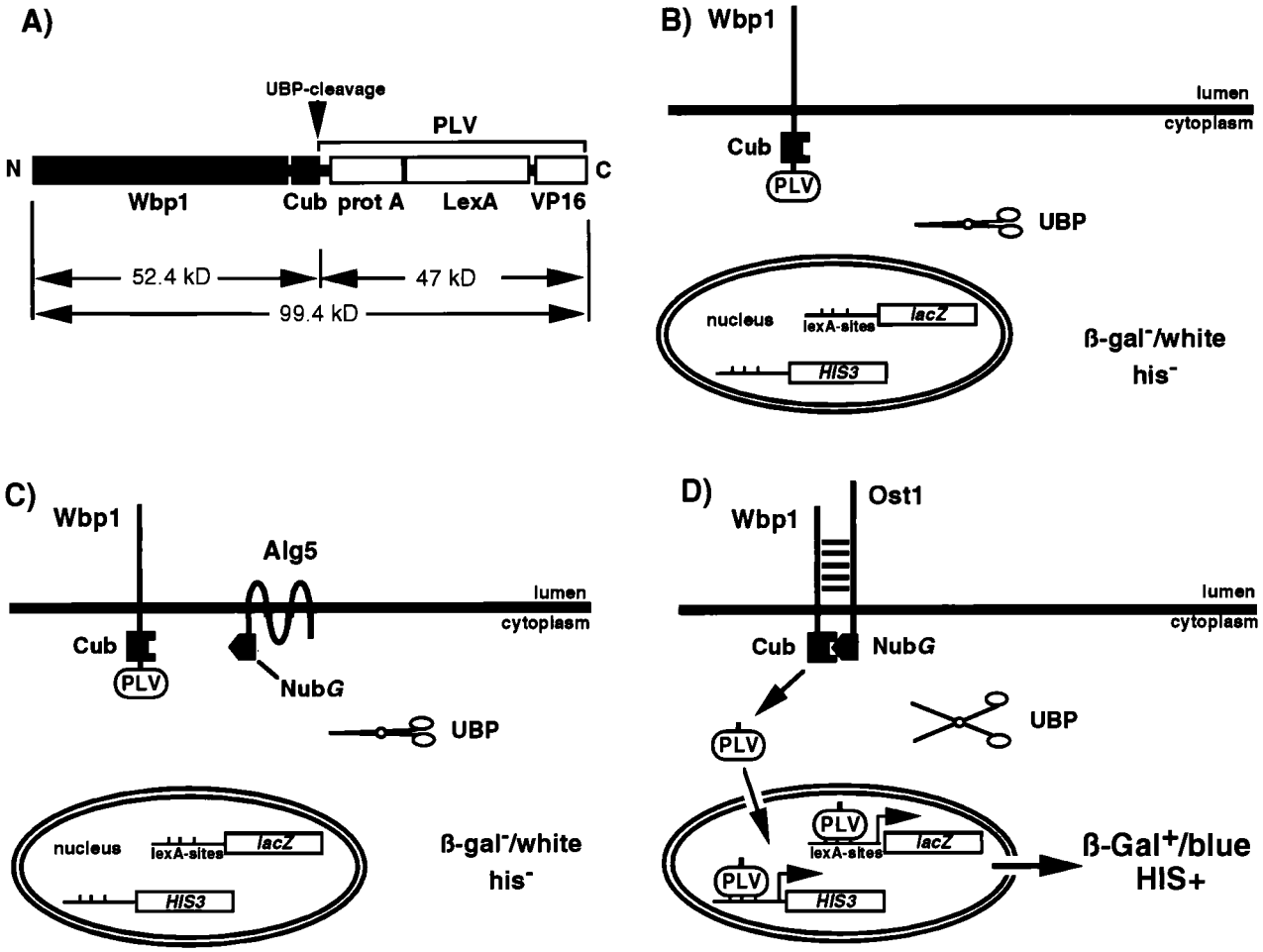

FIG. 2. Design of Wbp1-Cub-PLV fusion protein and the principle of detection of the interaction. (*A*) The structure of the mature Wbp1-Cub-PLV fusion protein. Cleavage by the UBP(s) occurs at the C terminus of Cub, cleaving the Wbp1-Cub-PLV fusion protein of ≈100 kDa into Wbp1-Cub (52 kDa) and PLV (47 kDa). (*B*) Expression of PLV as a fusion to the ER membrane protein Wbp1p prevents the transcription factor from gene activation in the nucleus. Cleavage of Wbp1-Cub-PLV by UBP does not occur (solid scissors) in the absence of Nub, the cells are white in the presence of X-Gal and are His auxotrophs. (*C*) Coexpression of Wbp1-Cub-PLV with the noninteracting Nub*G*-Alg5p does not lead to formation of the split-ubiquitin heterodimer, nor cleavage by UBP (solid scissors) and gene activation. (*D*) Interaction between Wbp1 and Ost1 results in formation of the split-ubiquitin heterodimer. The heterodimer is recognized and cleaved by the UBP (open scissors), liberating PLV. PLV can enter the nucleus by diffusion and bind to the LexA-binding sites leading to activation of transcription of the *lacZ* and *HIS3* reporter genes. This results in blue cells in the presence of X-Gal and growth of the cells on agar plates lacking histidine.

The protein A sequence from *Staphylococcus aureus* contains two IgG-binding domains, which allow easy and sensitive detection of the fusion protein as well as of the cleaved product. The LexA-VP16 cassette consists of the entire DNA-binding protein LexA followed by the transcriptional activation domain of VP16 (22). LexA-VP16 can activate reporter genes with LexA binding sites in the promoter region. The *WBP1-Cub-PLV* fusion gene was generated by site-directed integration of a PLV cassette containing a 5′-truncated Δ*wbp1* gene (Δ*wbp1-Cub-PLV*) into the genomic *WBP1* locus. Thus, only the modified Wbp1-Cub-PLV, but no wild-type Wbp1p, was present in the cell. The insertion of Cub-PLV at the C terminus of Wbp1p did not inactivate the essential Wbp1p function in the oligosaccharyltransferase complex (11). Nub*I*, Nub*A*, and Nub*G* were fused to the 3′-end of the ORF of a 5′-truncated Δ*ost1* gene. The resulting fusion genes were integrated into the *OST1* locus to give only one active Ost1 copy, expressing Ost1-Nub*I*, Ost1-Nub*A*, or Ost1-Nub*G*. The generated cells were viable. Hence, the addition of Nub to the C terminus did not inactivate the essential Ost1 protein (9). To analyze the competition between the wild-type Ost1p and Ost1-Nub, we placed the complete fusion genes OST1-Nub*I*, OST1-Nub*A*, or OST1-Nub*G* on a 2-μm plasmid and expressed each of them together with the wild-type chromosomal *OST1* gene. As a control, Nub*I*, Nub*A*, or Nub*G* were fused to the 5′-end of the ORF of the *ALG5* gene. The fusion of Nub to the N terminus of Alg5p did not inactivate the protein. All constructions using 2-μm vectors resulted in 10- to 20-fold overexpression of the respective Nub-fusion protein (data not shown).

Interaction of Wbp1-Cub-PLV with Ost1-Nub and Nub-Alg5p. Ost1p is a member of the oligosaccharyltransferase complex and expected to interact with Wbp1p (7–10). In contrast, Alg5p, dolicholphosphoglucose synthetase (12), is not expected to interact with Wbp1p. To test for interactions, Ost1-Nub or Nub-Alg5 were coexpressed with Wbp1-Cub-PLV in a *S. cerevisiae* strain carrying *lacZ* and *HIS3* reporter genes under the control of LexA-binding sites (13). Cells were tested for β-gal activity after permeabilization on Whatman filters with X-Gal.

Expression of Wbp1-Cub-PLV with Nub*I*-Alg5 or Nub*A*-Alg5 resulted in blue cells in the presence of X-Gal. In contrast, Nub*G*-Alg5 showed very little β-gal activity (Fig. 3, lanes 1 and 2). It has been shown with soluble proteins that Nub*I*-fusions associate with Cub independent of additional protein-protein contacts, whereas interactions of Cub with Nub*A* or Nub*G* are dependent on additional contacts (4). The absence of β-gal activity in the case of Nub*G*-Alg5 suggests that there are no specific interactions between Wbp1p and Alg5p.

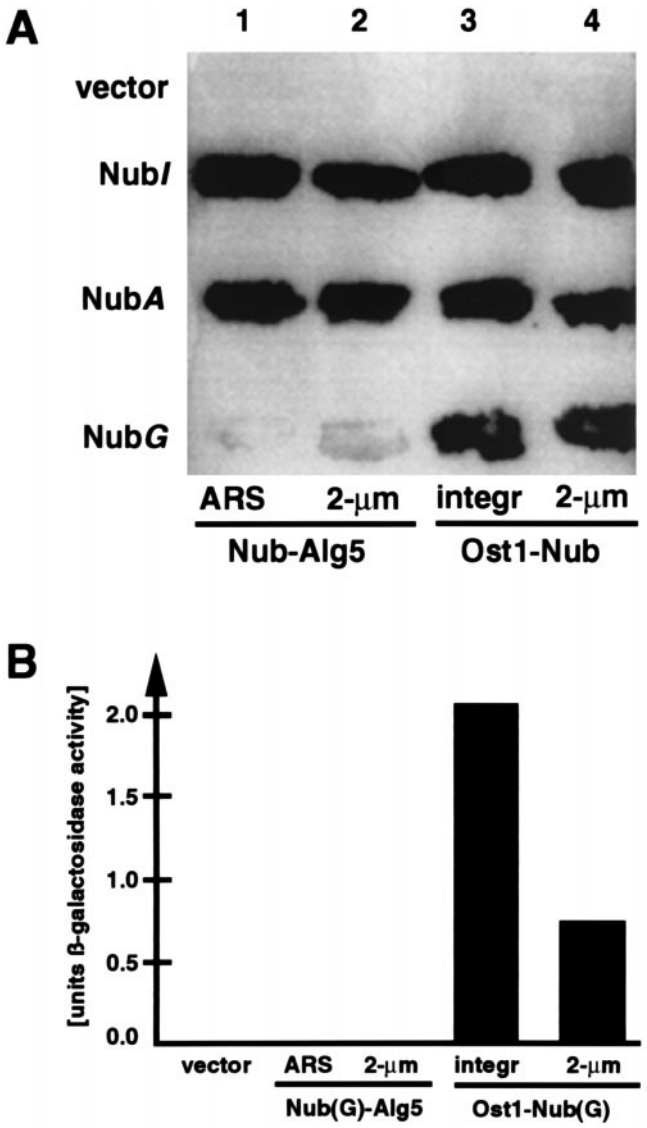

FIG. 3. β-Gal activity of cells expressing Wbp1-Cub-PLV together with Nub-fusion proteins. (*A*) YG0673 cells expressing Wbp1-Cub-PLV and (*i*) Nub*I*-Alg5, Nub*A*-Alg5, or Nub*G*-Alg5 from a CEN/ARS plasmid; (*ii*) Nub*I*-Alg5, Nub*A*-Alg5, or Nub*G*-Alg5 from a 2-μm plasmid; (*iii*) Ost1-Nub*I*, Ost1-Nub*A*, or Ost1-Nub*G* from an integrated fusion gene (no wild-type Ost1p present in the cell); (*iv*) Ost1-Nub*I*, Ost1-Nub*A*, or Ost1-Nub*G* from a 2-μm plasmid in presence of the wild-type Ost1p. As negative control, YG0673 was transformed with the vector pRS314. Cells were grown on Whatman filters, permeabilized, and incubated in the presence of X-Gal. Expression of β-gal resulted in blue cells. (*B*) Quantitative β-gal assay of YG0673 cells expressing Wbp1-Cub-PLV together with the vector, low copy number pRS314(Nub*G*-ALG5) (ARS), Nub*G*-ALG5 (2 μm), OST1-Nub*G* (integrated fusion gene, no wild-type OST1 present), and OST1-Nub*G* (2 μm, wild-type OST1 present). Shown are the results of one out of three independent experiments.

Expression of Ost1-Nub fusions together with Wbp-Cub-PLV resulted in strongly blue cells with Ost1-Nub*I*, Ost1-Nub*A*, and, in contrast to Nub*G*-Alg5, also with Ost1-Nub*G* (Fig. 3*A*, lanes 3 and 4). We were interested to see, whether the modified proteins were able to compete with the wild-type protein for interactions. In the case of the integrated Ost1-Nub fusion, cells have to utilize the modified Ost1-Nub, because the Ost1 protein is essential. The situation is different, if Ost1-Nub is expressed in the presence of the wild-type Ost1p. Preliminary experiments had indicated that a high level of expression of Ost1-Nub is needed for successful competition with the unmodified Ost1p (data not shown). Therefore, high-copy number 2-μm yeast plasmids were used for the expression of Ost1-Nub in presence of the wild-type Ost1p (Fig. 3, lane 4). The blue color indicates that the modified Ost1-Nub proteins were able to compete with the wild-type Ost1p. As a control, Nub-Alg5 was expressed by the same vector system, resulting in blue color in the case of Nub*I*- and Nub*A*-, but not Nub*G*-Alg5 (Fig. 3, lane 2). As a further control, expression of Wbp-Cub-PLV with the empty vector plasmid did not give any β-gal activity. In summary, the β-gal activity in the case of Ost1-Nub*G* demonstrated the expected specific interactions between Wbp1 and Ost1. The absence of β-gal activity in the case of Nub*G*-Alg5 served as a stringent control for the system. This absence of activity is unlikely due to a general inaccessibility of Nub*G*-Alg5 to Wbp1-Cub-PLV, because expression of Nub*I*-Alg5 and Nub*A*-Alg5 results in β-gal activity.

We confirmed the results by the use of a quantitative assay with 2-nitrophenyl-β-D-galactopyranoside as a substrate for β-gal (Fig. 3*B*). Ost1-Nub as the sole Ost1 protein in the case of the integrated fusion gene in the cell led to high β-gal activity. This was reduced to about one-third if Ost1-NubG was expressed from a 2-μm plasmid in the presence of the wild-type Ost1p. No β-gal activity was detectable after 1 h of incubation with the vector or Nub*G*-Alg5p expressed from low copy number [autonomous replicating sequence (ARS)] or high copy number plasmid (2 μm). We also checked the growth behavior of cells expressing Wbp1-Cub-PLV together with Ost1-Nub or Alg5-Nub on agar plates lacking histidine (Fig. 4). Expression of Wbp1-Cub-PLV with the empty vector resulted in His− cells. Coexpression of Wbp1-Cub-PLV with Ost1-Nub*I*, -Nub*A*, and -Nub*G* results in His+ cells. Growth of cells expressing Nub*G*-Alg5p on agar plates lacking histidine was reduced compared with Ost1-Nub*G*.

Interaction-Mediated Cleavage of Wbp1-Cub-PLV *in Vivo*. We were interested to see whether the β-gal activity and the HIS+ phenotype correlated with the actual cleavage of the Wbp1-Cub-PLV reporter protein. Therefore, protein extracts were prepared from cells expressing Wbp1-Cub-PLV with either Ost1-Nub or Nub-Alg5 and determined by Western blot analysis and probing with peroxidase-IgG (Fig. 5). The Wbp1-Cub-PLV fusion protein was easily detected at a relative molecular mass of 95 kDa (lane 1), which is in good agreement with the calculated molecular mass of 99.4 kDa. The cleaved PLV reporter was visible upon coexpression with Nub-constructs as a band migrating at ≈50 kDa (calculated 47 kDa) (Fig. 5, lanes 2–6 and 8–13). The band migrating at a position

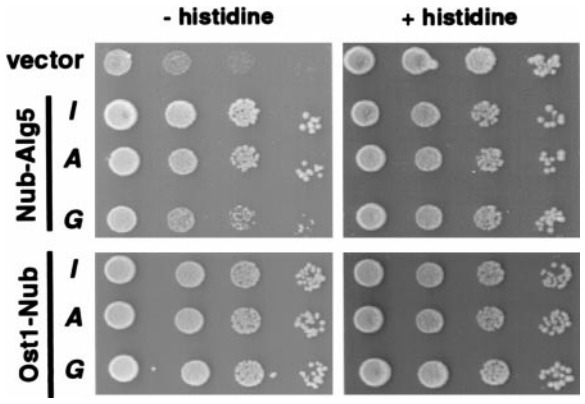

FIG. 4. Growth of cells expressing Wbp1-Cub-PLV with various Nub-fusions on agar plates lacking histidine. YG0673 cells expressing Wbp1-Cub-PLV and either Nub*I*-Alg5, Nub*A*-Alg5, or Nub*G*-Alg5 from a 2-μm plasmid or expressing Ost1-Nub*I*, Ost1-Nub*A*, or Ost1-Nub*G* from a 2-μm plasmid were grown to logarithmic phase, spotted in serial 10-fold dilutions on selective agar plates containing 0.2 mM $CuSO_4$ +/− histidine and were incubated for 5 days at 30°C. YG0673 transformed with the vector pRS314 served as negative control.

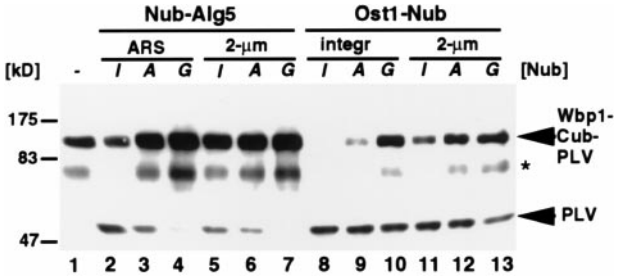

FIG. 5. Western blot analysis of cells expressing Wbp1-Cub-PLV together with Nub-fusions. YG0673 cells expressing Wbp1-Cub-PLV with the vector (lane 1); with either Nub*I*-Alg5, Nub*A*-Alg5, or Nub*G*-Alg5 from a CEN/ARS plasmid (lanes 2–4); or from a 2-μm plasmid (lanes 5–7). YG0673 expressing Ost1-Nub*I*, Ost1-Nub*A*, or Ost1-Nub*G* as the sole source of Ost1 in the cell (lanes 8–10, integr) or from a 2-μm plasmid in presence of wild-type Ost1p (lanes 11–13). Cells were grown to logarithmic phase in selective medium; proteins were extracted and determined by Western blot analysis and probing with peroxidase-IgG as described in *Materials and Methods*. *, Unspecific degradation product.

of ≈75 kDa may arise by an unspecific degradation of the fusion protein. Cleavage of Wbp1-Cub-PLV upon coexpression of Nub-Alg5 constructs occurred in the case of Nub*I*-Alg5 (Fig. 5, lanes 2 and 5), which was reduced with Nub*A*-Alg5 (Fig. 5, lanes 3 and 6) and almost completely abolished with Nub*G*-Alg5 (Fig. 5, lanes 4 and 7). These findings are in good correlation with the strong reduction of β-gal activity in the case of Nub*G*-Alg5 (Fig. 3). Integration of OST1-Nub*I* into the OST1 gene resulted in complete cleavage of the Wbp1-Cub-PLV (lane 8). Cleavage was reduced, but still occurring using Ost1-Nub*A* and Ost1-Nub*G* (lanes 9 and 10). In agreement with the β-gal activity observed in Fig. 3, expression of Ost-Nub in the presence of the wild-type Ost1p also produced significant amounts free PLV from Wbp1-Cub-PLV (lanes 11–13). Importantly, free PLV is also found with Ost1-Nub*G* (lane 13). Therefore, although the Ost1-Nub fusion proteins had to compete with the endogenous wild-type Ost1p, they were still able to interact with Wbp1-Cub-PLV protein (compare lanes 8–10 with 11–13). The use of the dhfr-ha (dihydrofolate reductase) reporter cassette described in ref. 2 as a reporter results in the same cleavage pattern as with the Wbp1-Cub-PLV in Fig. 5 (data not shown).

DISCUSSION

We were interested in generating a genetic system allowing the analysis of membrane protein interactions *in vivo*. The term "interaction" is hereby used for describing either physical contact between two proteins or close spatial arrangement, for example the presence of two proteins in the same protein complex. The split-ubiquitin system has been used to detect specific interactions between known soluble proteins (2). Some of the advantages of the split-ubiquitin system are: (*i*) *in vivo* and *in situ* detection of protein–protein interaction, there is no need for nuclear localization; (*ii*) small modules (Nub and Cub) attached to linker sequences are used to detect interactions thereby minimizing potential steric hindrance; (*iii*) the possible detection of transient interactions; and (*iv*) the detection of interactions is mediated via cleavage by UBP and not by transcription. Therefore, proteins could be tested, which carry by themselves transcriptional activating or repressing sequences. However, in contrast to the two-hybrid technique, no system suitable for the screening of interactions was available for the split-ubiquitin.

Using well characterized membrane proteins Wbp1p and Ost1p of the *S. cerevisiae* oligosaccharyltransferase, we demonstrate that the split-ubiquitin also works with membrane proteins. In addition, the introduction of the PLV-cassette as the reporter molecule provides a potentially useful tool for the screening of interactions. PLV is not known to contain a nuclear localization signal and does not lead to gene activation, when fused to the ER membrane protein Wbp1p (Fig. 3 *A* and *B*). Although strict evidence is lacking, it is unlikely that the PLV transcription factor is simply inactivated by the fusion to Wbp1p. First, the transcriptionally active module LexA-VP16 is already extended by the protein A sequence. Second, overexpression of WBP1-Cub-PLV or the expression of a soluble Cub-PLV results in gene activation in the absence of Nub (data not shown). Therefore, we believe that the inactivation of PLV is because of its compartmentalization to the ER membrane, as shown in Fig. 2. As a consequence, the Cub-PLV system might also work for testing for interactions of soluble proteins, provided they are fused to membrane anchors.

It has been shown that Nub*A*-fusions to soluble proteins can assemble with Cub only in the presence of additional protein-protein contracts (4). In our experiments, Nub*A*-Alg5 assembles unspecifically with Wbp1-Cub-PLV. This is understandable in the light that the membrane protein interactions are limited to two dimensions of the lipid bilayer, thereby increasing the probability of unspecific reactions. However, Nub*G*-Alg5 cannot assemble with Wbp1-Cub-PLV.

The modified split-ubiquitin system could be used to screen a library of cDNAs or yeast genomic DNA linked to Nub*G* as 5′ or 3′ fusions for interaction with a protein of interest. Two other systems have been published recently, which potentially might allow also the testing of interactions between membrane proteins, but which have not been examined with membrane proteins so far. One system uses activation of plasma membrane anchored yeast Ras bound to GDP by human SOS protein (23), and the second system uses fusions to β-gal for α-complementation of β-gal (24). To our knowledge, our study represents the first report of a system capable of the *in situ* analysis of interactions between membrane proteins.

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