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A High-Copy Suppressor Screen Reveals a Broad Role of Prefoldin-like Bud27 in the TOR Signaling Pathway in *Saccharomyces cerevisiae*

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Abstract: Bud27 is a prefoldin-like, a member of the family of ATP-independent molecular chaperones that associates with RNA polymerases I, II, and III in *Saccharomyces cerevisiae*. Bud27 and its human ortholog URI perform several functions in the cytoplasm and the nucleus. Both proteins participate in the TOR signaling cascade by coordinating nutrient availability with gene expression, and lack of Bud27 partially mimics TOR pathway inactivation. Bud27 regulates the transcription of the three RNA polymerases to mediate the synthesis of ribosomal components for ribosome biogenesis through the TOR cascade. This work presents a high-copy suppression screening of the temperature sensitivity of the *bud27*Δ mutant. It shows that Bud27 influences different TOR-dependent processes. Our data also suggest that Bud27 can impact some of these TOR-dependent processes: cell wall integrity and autophagy induction.

Keywords: prefoldin-like; Bud27; TOR signaling pathway; *Saccharomyces cerevisiae*

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1. Introduction

The TOR (Target of Rapamycin) signaling pathway is one of the most important mechanisms to control and coordinate cell growth and nutrient availability, and is conserved in all eukaryotes [1–5]. TOR acts as a stress sensor by responding to different stimuli, such as rapamycin or nutrient starvation [6–12]. The TOR pathway is constitutively active under favorable growth conditions to maintain ribosome production and cell growth. However, it is inhibited under stress conditions and ribosome biogenesis ceases [4,5,13,14].

It can be considered that the final function of TOR, in combination with PKA signaling, is the repression of the stress response. Some of the major regulators of the stress response in *S. cerevisiae*, which are controlled by the TOR and PKA signaling pathways, are Rho1 GTPase, Rim15, as well as transcription factors Msn2/4 and Gis1, which regulate about 150 genes by their association with the stress response element (STRE) located in the promoters of these genes [8,10]. Interestingly, either rapamycin treatment of cells or nitrogen starvation mediates responses that involve Msn2 [10,15].

TOR signaling represses autophagy, and TOR signaling inactivation by situations that induce stress, such as rapamycin or nitrogen starvation, induces autophagy [10,16]. Autophagy is essential for cells to survive under stressful conditions and is one of the most common responses of eukaryotic cells to bypass nutritional limitations [16–18].

In *S. cerevisiae*, two different TOR complexes exist, TORC1 and TORC2, which share some components [19]. TORC1 regulates ribosome biogenesis, nutrient transport, autophagy, and cell cycle progression, and is sensitive to the drug rapamycin. TORC2, which

is unaffected by rapamycin treatment, participates in the cell cycle-dependent polarization of the actin cytoskeleton, endocytosis, and cell wall integrity (CWI) [20–22].

Bud27 in *S. cerevisiae*, and its human ortholog URI, have been described as components of the TOR signaling pathway by coordinating nutrient availability with gene expression [23–26]. However, whether Bud27 acts upstream or downstream the TOR kinase is still unknown. Bud27/URI are members of the prefoldin family of ATP-independent molecular chaperones [24], interact with the Rpb5 subunit of eukaryotic RNA polymerases, and participate in nuclear and cytoplasmic functions, such as the biogenesis of RNA pols, transcription, and translation, among others [23,25–31]. Notably, Bud27 inactivation induces a transcriptional response that partially mimics the transcriptional response due to TOR inactivation by rapamycin [25]. Bud27 has been recently shown to mediate ribosome biogenesis by regulating the activity of the three RNA pols and the synthesis of ribosomal components, likely involving the activity of TORC1 kinase [25].

Yet, whether lack of Bud27 only impacts TOR activity to regulate ribosome biogenesis or other TOR-dependent processes as well is unclear. To unravel the participation of Bud27 in the mechanisms governed by the TOR signaling pathway, we performed a high-copy suppressor screen on the *bud27*Δ mutant strain to rescue the temperature-sensitive phenotype of this mutant. Our results show that Bud27 may modulate the different processes regulated by the TOR signaling cascade and impact CWI, and likely autophagy.

2. Materials and Methods

2.1. Yeast Strains, Genetic Manipulations, Media, and Genetic Analyses

Common yeast media, growth conditions, and genetic techniques were used as described elsewhere [32]. For nitrogen starvation, the SD (-N) medium was used and contained 0.17% yeast nitrogen base without amino acids or ammonium sulfate, and 2% glucose.

Calcofluor white (Fluorescent Brightener 28; Sigma-Aldrich, Darmstadt, Germany) was used at the indicated concentrations.

The employed yeast strain was BY4741 and its derivative isogenic with *bud27*Δ mutation.

The *pRS315-GFP-ATG8* (*CEN; LEU2*) plasmid was employed for the autophagy analysis (see below) [33].

Screening of a WT yeast genomic multicopy library. *bud27*Δ cells were transformed with a 2 μm-based multicopy yeast genomic DNA library constructed in plasmid pFL44L [34]. Colonies were selected in SD medium with appropriate requirements at 37 °C. In an effort to determine if suppression was plasmid-linked, cells were cured of plasmids by being grown on medium containing 5-FOA and screened for growth at 37 °C. Finally, plasmids were rescued from transformants, amplified in *Escherichia coli* and transformed into *bud27*Δ cells. The plasmids that allowed growth at 37 °C and/or 36 °C upon retransformation were selected and sequenced.

2.2. Chitin Staining with Calcofluor White and Fluorescence Microscopy

Chitin staining was performed in cells grown exponentially (OD~0.6–0.7) in SD minimal medium with the appropriate requirements. Cells were collected by centrifugation and resuspended in calcofluor white (Fluorescent Brightener 28; Sigma-Aldrich, Darmstadt, Germany) at 0.1 mg/mL concentration and incubated for 10 min at room temperature. Then cells were washed three times in distilled water. Slides were covered with Vectashield mounting solution (Vector Laboratories, San Francisco, CA, USA). Fluorescence intensity was scored with a fluorescence microscope (Olympus BX51).

2.3. Autophagy Detection

Autophagy progression was monitored by the immunological detection of the Gfp accumulation processed from Gfp–Atg8, which is delivered to the vacuole to be degraded upon autophagy induction [35]. Gfp moiety is very resistant to proteolysis compared to Atg8. For these assays, the WT and *bud27*Δ cells were transformed with the *pRS315-*

GFP-ATG8 (*CEN; LEU2*) plasmid to allow the expression of Gfp-Atg8. Cells were grown at 30 °C in SD minimal medium to the mid-log phase (OD_{600}~0.6), which corresponded to experiment time 0. Then, cells were washed three times, diluted, and shifted to SD (-nitrogen) during a time course (up to 2 h). Proteins were precipitated with TCA from 1 mL of culture cells and analyzed by western blot using an anti-Gfp antibody (GFP (D5.1), 2956; Cell Signaling). Anti-phosphoglycerate kinase, Pgk1 (22C5D8; Invitrogen, Waltham, MA, USA) was used to detect Pgk1 as the internal control.

The intensities of the immunoreactive bands on western blots were quantified by densitometry using the IMAGE STUDIO LITE software from the images acquired with an office scanner.

3. Results and Discussion

3.1. Genetic Screening for Multicopy Suppressors of the Temperature-Sensitive Growth Defect of the bud27Δ Mutant

In order to search for genes that overcome the temperature sensitivity phenotype of the *bud27*Δ mutant, we performed high-copy suppressor screening. Cells were transformed with a 2 µm-based (pFL44L) multicopy genomic DNA library [34] and selected for growth at 37 °C. Suppressors were then identified and characterized as described in the Materials and Methods. Of the 100,000 independent transformants, 71 were able to grow at 37 °C, but at different growth rates depending on the suppressor (weak growth was observed for some). Accordingly, suppressors were defined as those able to overcome the temperature sensitivity of the *bud27*Δ mutant at 36 °C. The plasmids responsible for the suppression of the *bud27*Δ mutant phenotype were extracted and the presence of the wild-type (WT) *BUD27* gene was analyzed by PCR. As expected, six independent plasmids contained the *BUD27* gene, either alone or in combination with the *FRS2* gene. A search was done by a PCR analysis for genes *RPB5* and *RPB6*, whose overexpression overcomes the temperature sensitivity of the *bud27*Δ mutant [29]. Nine plasmids containing the *RPB5* gene and twenty-six containing the *RPB6* gene were identified (Figure 1).

Finally, we sequenced the fragments contained in 19 plasmids responsible for the suppression of the *bud27*Δ temperature-sensitivity phenotype. BLAST searches against the *S. cerevisiae* genome revealed that the *RPC17* gene, which codes for the Rpc17 subunit of RNA pol III, was one of the independent suppressors (Figure 1). This finding falls in line with the role of Bud27 in not only regulating the activity of the three RNA pols in coordination with TOR signaling [25], but also in mediating their cytoplasmic assembly [29]. The gene *SUN4*, which codes for Sun4, was also identified as a high-copy suppressor. Interestingly, this protein physically interacts with the member of the glycogen synthase kinase 3 (GSK3) family, Mck1 (https://www.yeastgenome.org, accessed on 1 April 2022), which mediates the phosphorylation of RNA pol III subunit Rpc53, which is important for enzyme activity regulation [36].

Another plasmid bore an insert containing the *RPL40A* gene, which codes for the large ribosomal protein Rpl40a. Notably, three plasmids contained inserts that harbored genes *RPL8A*, *RPL34A*, or *RPL33B*, which also code for large ribosomal proteins, among other genes. Subcloning genes *RPL8A*, *RPL34A*, or *RPL33B* corroborated their function as suppressors of the *bud27*Δ mutant growth phenotype at 36 °C. These genes also overcame the sensitivity of the *bud27*Δ cells to rapamycin, a drug that inhibits the TOR signaling pathway [29] (Figure 1). These data might be related to the roles of Bud27 in ribosome biogenesis [25] and/or translation initiation [31]. In agreement with the role of Bud27 in ribosome biogenesis, *SNR69,* which codes for the C/D box small nucleolar RNA (snoRNA) Snr69 [37], was also identified as a multicopy suppressor.

SMY2 overexpression acted as a high-copy suppressor. It codes for a GYF domain protein involved in COPII vesicle formation, which suppresses ribosome biogenesis defects [38] and influences the translation initiation of some mRNAs [39]. Two other multicopy suppressors are genes *FRS2* and *MSD1,* which, respectively, code for the α subunit of cytoplasmic phenylalanyl-tRNA synthetase Frs2 [40] and for mitochondrial aspartyl-tRNA synthetase

Msd1 [41]. These results could well coincide with Bud27 participating in translation initiation [31], mediating ribosome biogenesis [25], or with a more general role of Bud27 and its human ortholog URI in coordinating nutrient availability with gene expression via the TOR signaling cascade [26].

A

Suppressor	Protein		Rapamycin resistance
-			-
BUD27	Bud27	Prefoldin-like	+++
RPB5	Rpb5	Subunit common to the eukaryotic RNA polymerases	++
RPB6	Rpb6	Subunit common to the eukaryotic RNA polymerases	++
RPC17	Rpc17	Subunit of the RNA polymerase III	++
SUN4	Sun4	Cell wall protein related to glucanases localized in birth scars	-
RPL40A	Rpl40A	Ribosomal protein (Large subunit)	+
RPL8A	Rpl8A	Ribosomal protein (Large subunit)	+
RPL34A	Rpl34a	Ribosomal protein (Large subunit)	+
RPL33B	Rpl33b	Ribosomal protein (Large subunit)	++
SNR69		C/D box small nucleolar RNA (snoRNA)	-
SMY2	Smy2	GYF domain protein involved in COPII vesicle formation	+
FRS2	Frs2	Cytoplasmic phenylalanyl-tRNA synthetase	+++
MSD1	Msd1	Mitochondrial aspartyl-tRNA synthetase Msd1	-
PAB1	Pab1	Poly(A) binding protein	-
FLC3	Flc3	Flavin adenine dinucleotide transporter	-
SPO20	Spo20	Meiosis-specific subunit of t-SNARE complex	+
SSP2	Ssp2	Sporulation specific protein required for sporulation after meiosis II	-
RMR1	Rmr1	Protein required for meiotic recombination and gene conversion	-
ZIP2	Zip2	Meiosis-specific protein involved synaptonemal complex formation	-
RME3		Antisense transcript that repress *ZIP2* gene expression	-
GCR1	Gcr1	Transcription factor that regulates glycolysis and ribosomal protein genes, the RNA pol II transcription and cell cycle	-
ATG39	Atg39	Receptor for selective autophagy of the ER and the nucleus	-
ATG36	Atg36	Pex3p interacting protein, required for pexophagy	-
PEX8	Pex8	Intraperoxisomal organizer of the peroxisomal import machinery	++

B

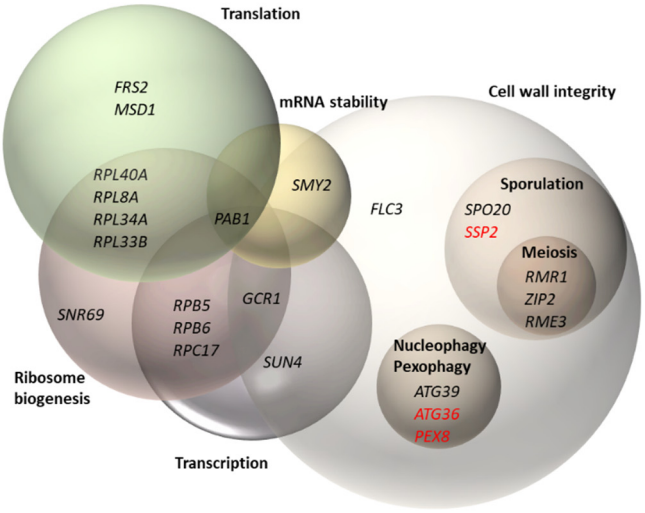

Figure 1. Suppressors of the temperature sensitivity phenotype of the *bud27*Δ mutant. (**A**) List of suppressors. Rapamycin sensitivity was strongly (+++), moderately (++), weakly (+), or not (–) overcome by suppressors. (**B**) Possible functional interactions between suppressors. Genes are grouped by their GO-Terms, and related cellular processes are enclosed together. Intersections indicate a functional or genetic relation. Red denotes genes that are putative suppressors.

PAB1 has been identified as a multicopy suppressor. This gene codes for poly(A) binding protein Pab1, which performs many cellular functions associated with the 3-poly(A)-tail of messenger RNAs, including transport, translation, and mRNA decay [42–45]. These data agree with the role of Bud27 in translation and suggest a putative connection with mRNA degradation, as observed by the general decrease in mRNA stability in the *bud27*Δ mutant (Cuevas-Bermúdez et al., in preparation). Interestingly, Smy2 (see above) has been associated with the Ccr4-NOT deadenylase complex, which participates in mRNA degradation [46].

Another identified multicopy suppressor was *FLC3,* which codes for Flc3, a flavin adenine dinucleotide transporter [47] localized in the endoplasmic reticulum (ER) [48]. This protein interacts physically with Sac7 to render a non-active CWI pathway in the ER [47]. In addition, the deletion of *FLC* genes results in poor cell wall assembly [47]. Interestingly, Sun4 (see above) contributes to the regulation of cell wall morphogenesis and septation [49]. Furthermore, Smy2 (see above) has been described to be involved in the ER-dependent secretory pathway [46] as a multicopy suppressor of the *ptc1* mutant by showing defects for the CWI pathway [50]. Notably, TORC1 signaling acts in parallel with the unfolded protein response (UPR) to regulate ER stress and to modulate the CWI pathway [51]. Taken together, these results suggest a relation between Bud27 and cell wall assembly. Thus Bud27 might participate in CWI as a co-chaperone [24] member of the TOR signaling pathway [25,26].

In addition, sporulation has been shown to be related to CWI via the CWI MAP Kinase cascade [52]. In line with this, we identified *SPO20* as a multicopy suppressor in our

screen, a gene that codes for the meiosis-specific subunit of the t-SNARE complex that is required for prospore formation during sporulation [53]. The *SSP2* gene was harbored in a plasmid capable of suppressing the phenotype of *bud27*Δ. *SSP2* codes for a sporulation-specific protein, Ssp2, which is localized in the spore wall and is required for sporulation after meiosis II [54]. Accordingly, we speculate that *SSP2* could be a suppressor of the *bud27*Δ phenotype.

Sporulation in *S. cerevisiae* is a highly regulated process that is divided into three phases, with meiosis I and II taking place in the early and middle phases [55]. In line with the identified suppressors related to sporulation, a plasmid containing a fragment with genes *RMR1*, *ZIP2*, and *RME3*, which are all involved in meiosis, suppressed the temperature sensitivity phenotype of the *bud27*Δ mutant. Rmr1 is a protein required for meiotic recombination and gene conversion [56], Zip2 is a meiosis-specific protein involved in synaptonemal complex formation [57], while *RME3* codes an antisense transcript that represses *ZIP2* gene expression [58].

GCR1 appeared as one of the strongest multicopy suppressors and was able to overcome the temperature sensitivity of the *bud27*Δ mutant, but also the sensitivity to drugs that affects translation (cycloheximide) and transcription elongation in *S. cerevisiae* (6-Azauracil and micophenolic acid [25,59]) (not shown). Gcr1 codes for a transcription factor that regulates the expression of glycolysis and ribosomal protein genes, RNA pol II transcription and the cell cycle [60,61]. Gcr1 has been reported to act in conjunction with transcription factor Rap1, which regulates the TOR signaling pathway [61,62] and could be involved in rRNA synthesis via Hmo1/Rap1 [63]. In agreement, *GCR1* overexpression overcame the sensitivity of the *bud27*Δ cells to rapamycin (Figure 1). Interestingly, the *gcr1*Δ mutant presents a defective vacuolar structure and affects autophagy in *S. cerevisiae* [61].

The *ATG39* gene has been identified as a multicopy suppressor. It codes for *Atg39*, a protein localized in the perinuclear ER that acts as a receptor for the selective autophagy of the ER and the nucleus in *S. cerevisiae*, a process known as nucleophagy [64]. Autophagy is associated with the TOR signaling cascade [10,16–18] and is related to ER stress [65], which compromises CWI [51]. Furthermore, the transcription of some autophagy-related genes has been shown to be altered after Congo Red or Zymolyase treatment in *S. cerevisiae*, two agents that induce cell wall damage [66]. Notably, a crosstalk between autophagy and sporulation has been described, two processes that correlate with meiosis [67]. Based on the above results, and on Bud27 participating in TOR signaling by coordinating nutrient availability with gene expression [25,26], we speculate that autophagy could be altered by lack of Bud27. Thus, it is tempting to speculate that genes *ATG36* and *PEX8*, which are involved in pexophagy [68–71] and are contained in two of the identified plasmids, could also act as suppressors of the *bud27*Δ growth phenotype. In line with the notion that autophagy was altered in *bud27*Δ cells, the Mlt1 cell wall receptor, which belongs to the CWI pathway and allows autophagy activation as a response to nutrient deprivation [72], was down-regulated in the *bud27*Δ mutant [25].

3.2. Transcriptomic Analyses Reinforce the Role of Bud27 in the TOR Signaling Pathway and in Cell Wall Integrity

The cells lacking Bud27 trigger a transcriptional response that partially mimics the repression of the TOR signaling pathway [25] and resembles previous data from rapamycin-treated cells [7,19,73]. In agreement, some of the identified suppressors overcame rapamycin sensitivity of the *bud27*Δ mutant (Figure 1).

By using the previously described data [25], we identified 719 genes that were differentially affected by Bud27 inactivation in relation to rapamycin treatment (288 up-regulated and 431 down-regulated). The STRING analysis [74] of the GO categories for the differentially up-regulated genes revealed biological processes that corresponded mainly to biosynthetic process (Supplemental Table S1). Contrarily and interestingly, the GO categories for the differentially down-regulated genes were mainly related to cell wall organization and cell wall biogenesis (Supplemental Table S2).

Taken together, these results reinforce the role of Bud27 in the TOR pathway and the possibility that lack of Bud27 may affect CWI.

These data led us to wonder whether lack of Bud27 could affect CWI. Then, we analyzed the growth of the *bud27*Δ mutant and its isogenic wild-type (WT) strain in YPD-rich medium and the SD minimal medium containing calcofluor white, a chitin-binding agent that can inhibit the growth of cells with an abnormally large amount of chitin [75]. As shown in Figure 2A for growth in SD minimal medium, *bud27*Δ cells were sensitive to calcofluor white. In contrast, the WT cells did not display such sensitivity (similar results were obtained in the YPD-rich medium). Then, we examined cells by fluorescence microscopy after calcofluor white staining. We found that the *bud27*Δ mutant cells showed brighter fluorescence (Figure 2B), which indicates excess chitin. This finding falls in line with data previously observed for other mutants with cell wall defects [75–79].

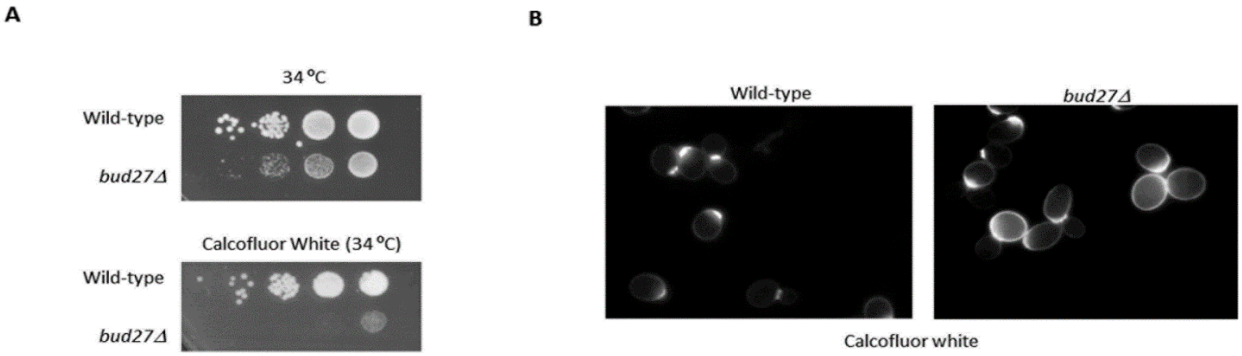

Figure 2. The *bud27*Δ mutant seems to affect cell wall integrity. (**A**) Growth of the wild-type and *bud27*Δ mutant at 34 °C in SD minimal medium w/o or with calcofluor white (10 μg/mL). (**B**) The yeast cells grown in minimal medium were stained with calcofluor white (0.1 mg/mL) and analyzed by fluorescence microscopy.

Altogether, these data indicate that lack of Bud27 leads to stress, which affects CWI. In line with this, some of the suppressors (*SPO20*, *SMY2*, the fragment containing *RMR1*, *ZIP2* and *RME3*, *SUN4*, and the putative suppressor *SSP2*) slightly overcame the sensitivity of the *bud27*Δ mutant cells to calcofluor (Supplemental Figure S1).

3.3. Lack of Bud27 May Cause Slower Autophagy Induction

As the above results suggest a relation between Bud27 and autophagy, we investigated whether this process could be altered in the *bud27*Δ mutant cells. For this purpose, we transformed the *bud27*Δ mutant and its WT isogenic strain with a plasmid harboring a *GFP-ATG8* construction coding for Gfp-Atg8 [33]. Atg8 is a member of the ubiquitin-like family proteins, and has been identified as one of the core elements in autophagy that bind to autophagic receptors and recruit cargo proteins for degradation [33,80]. During autophagy induction, Gfp is processed from Gfp-Atg8 and fragmentation can be analyzed by western blot [33,72]. Our results showed higher levels of free (processed) Gfp with respect to the unprocessed form (Gfp-Atg8) at time 0, in the *bud27*Δ mutant cells. Moreover, processing of Gfp-Atg8 to free Gfp seemed to occur more slowly in the *bud27*Δ mutant cells with respect to the WT cells during the time course under nitrogen starvation, reaching lower levels of free Gfp vs. Gfp-Atg8 after 120 min (Figure 3).

These results suggest that autophagy is active in the absence of Bud27, probably because of TOR signaling pathway inactivation [25]. In addition, autophagy induction may occur more slowly, likely by dysregulation of TOR pathway. The role of Bud27, likely downstream of TORC1 and TORC2, could impact TOR regulation affecting different processes, such as autophagy, CWI, transcription, or ribosome biogenesis, among others. The influence of Bud27 in TOR regulation could complement and not be contradictory

with the negative effects that Tor1 hyperactivation has on autophagy induction and in cell wall [51].

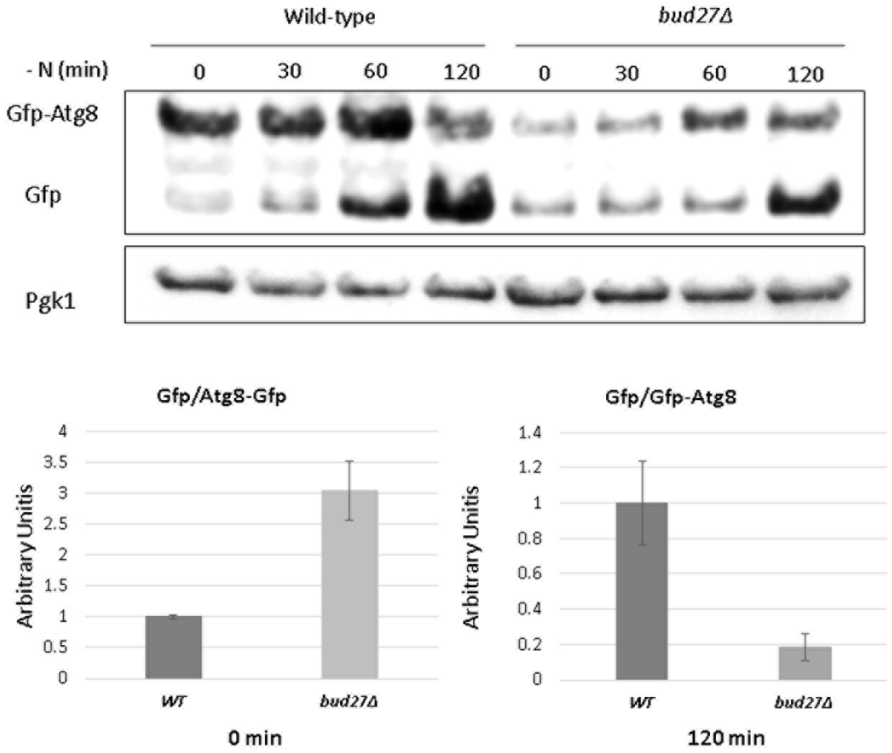

Figure 3. Gfp levels from Gfp-Atg8 degradation in the *bud27*Δ mutant seems to reflect lower autophagy induction. Upper panel: The wild-type and *bud27*Δ cultures transformed with the *pRS315-GFP-ATG8* plasmid were grown to the log phase (OD_{600}~0.6) in SD minimal medium at 30 °C. Cells were then shifted to SD (–N) medium for the indicated time course. Aliquots were collected for protein extraction at the indicated times, and western blots with the anti-Gfp antibody was performed. The anti-Pgk1 antibody was used to detect Pgk1 as the internal control. Lower panel: Quantification of western blots signals shown in upper panel, corresponding to Gfp/Gfp-Atg8 obtained from wild-type and *bud27*Δ cultures. Graphs represent median and standard deviation of two independent biological replicates.

4. Conclusions

Our work reinforces the role of Bud27 in the TOR signaling cascade by showing that it may broadly impact different TOR-dependent processes. The results herein reported indicate a new role for Bud27 in influencing CWI. Finally, our data suggest that lack of Bud27 affects autophagy induction, which probably results from TOR pathway alteration. Taken together, our data suggest a functional relation of Bud27 with TORC1 and TORC2, according to the main actions of these complexes in autophagy and CWI regulation [35,51,72].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/genes13050748/s1, Figure S1: Some suppressors overcome the sensitivity of the *bud27*Δ mutant to calcofluor; Table S1: Functional categories (Biological Process) for the up-regulated genes in bud27Δ mutant vs. wild-type strain (≥2), and differentially expressed than in a wild-type strain under rapamycin addition [74,81]; Table S2: Functional categories (Biological Process) for the down-regulated genes in bud27Δ mutant vs. wild-type strain (≥2), and differentially expressed than in a wild-type strain under rapamycin addition [74,81].

Author Contributions: Conceptualization: F.N.G. and J.P.-F.; experimental procedures and analyses: F.G.-S., M.C.-G., M.M.-E., M.d.C.M.-T. and C.C.-H.; writing: F.N.G. and J.P.-F. All authors have read and agreed to the published version of the manuscript.

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References

1. Loewith, R.; Hall, M.N. Target of rapamycin (TOR) in nutrient signaling and growth control. *Genetics* **2011**, *189*, 1177–1201. [CrossRef]
2. Loewith, R. A brief history of TOR. *Biochem. Soc. Trans.* **2011**, *39*, 437–442. [CrossRef]
3. Zaman, S.; Lippman, S.I.; Zhao, X.; Broach, J.R. How Saccharomyces responds to nutrients. *Annu. Rev. Genet.* **2008**, *42*, 27–81. [CrossRef]
4. Gentilella, A.; Kozma, S.C.; Thomas, G. A liaison between mTOR signaling, ribosome biogenesis and cancer. *Biochim. Biophys. Acta* **2015**, *1849*, 812–820. [CrossRef]
5. González, A.; Hall, M.N. Nutrient sensing and TOR signaling in yeast and mammals. *EMBO J.* **2017**, *36*, 397–408. [CrossRef]
6. Worley, J.; Sullivan, A.; Luo, X.; Kaplan, M.E.; Capaldi, A.P. Genome-Wide Analysis of the TORC1 and Osmotic Stress Signaling Network in Saccharomyces cerevisiae. *G3 (Bethesda)* **2015**, *6*, 463–474. [CrossRef]
7. Kumar, P.; Awasthi, A.; Nain, V.; Issac, B.; Puria, R. Novel insights into TOR signalling in *Saccharomyces cerevisiae* through Torin2. *Gene* **2018**, *669*, 15–27. [CrossRef]
8. Yan, G.; Lai, Y.; Jiang, Y. TOR under stress: Targeting TORC1 by Rho1 GTPase. *Cell Cycle* **2012**, *11*, 3384–3388. [CrossRef]
9. So, Y.S.; Lee, D.G.; Idnurm, A.; Ianiri, G.; Bahn, Y.S. The TOR Pathway Plays Pleiotropic Roles in Growth and Stress Responses of the Fungal Pathogen Cryptococcus neoformans. *Genetics* **2019**, *212*, 1241–1258. [CrossRef]
10. Plank, M. Interaction of TOR and PKA Signaling in S. cerevisiae. *Biomolecules* **2022**, *12*, 210. [CrossRef]
11. Sengupta, S.; Peterson, T.R.; Sabatini, D.M. Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Mol. Cell* **2010**, *40*, 310–322. [CrossRef]
12. Shen, C.; Lancaster, C.S.; Shi, B.; Guo, H.; Thimmaiah, P.; Bjornsti, M.A. TOR signaling is a determinant of cell survival in response to DNA damage. *Mol. Cell Biol.* **2007**, *27*, 7007–7017. [CrossRef]
13. Xiao, L.; Grove, A. Coordination of ribosomal protein and ribosomal RNA gene expression in response to TOR signaling. *Curr. Genom.* **2009**, *10*, 198–205. [CrossRef]
14. Vizoso-Vázquez, A.; Barreiro-Alonso, A.; González-Siso, M.I.; Rodríguez-Belmonte, E.; Lamas-Maceiras, M.; Cerdán, M.E. HMGB proteins involved in TOR signaling as general regulators of cell growth by controlling ribosome biogenesis. *Curr. Genet.* **2018**, *64*, 1205–1213. [CrossRef]
15. Duvel, K.; Santhanam, A.; Garrett, S.; Schneper, L.; Broach, J.R. Multiple roles of Tap42 in mediating rapamycin-induced transcriptional changes in yeast. *Mol. Cell* **2003**, *11*, 1467–1478. [CrossRef]
16. Farre, J.C.; Subramani, S. Mechanistic insights into selective autophagy pathways: Lessons from yeast. *Nat. Rev. Mol. Cell Biol.* **2016**, *17*, 537–552. [CrossRef]
17. Diaz-Troya, S.; Perez-Perez, M.E.; Florencio, F.J.; Crespo, J.L. The role of TOR in autophagy regulation from yeast to plants and mammals. *Autophagy* **2008**, *4*, 851–865. [CrossRef]
18. Cebollero, E.; Reggiori, F. Regulation of autophagy in yeast Saccharomyces cerevisiae. *Biochim. Biophys. Acta* **2009**, *1793*, 1413–1421. [CrossRef]
19. Crespo, J.L.; Hall, M.N. Elucidating TOR signaling and rapamycin action: Lessons from *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* **2002**, *66*, 579–591. [CrossRef]
20. Loewith, R.; Jacinto, E.; Wullschleger, S.; Lorberg, A.; Crespo, J.L.; Bonenfant, D.; Oppliger, W.; Jenoe, P.; Hall, M.N. Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. *Mol. Cell* **2002**, *10*, 457–468. [CrossRef]
21. Inoki, K.; Ouyang, H.; Li, Y.; Guan, K.L. Signaling by target of rapamycin proteins in cell growth control. *Microbiol. Mol. Biol. Rev.* **2005**, *69*, 79–100. [CrossRef]
22. Martin, D.E.; Hall, M.N. The expanding TOR signaling network. *Curr. Opin. Cell Biol.* **2005**, *17*, 158–166. [CrossRef]

23. Martínez-Fernández, V.; Garrido-Godino, A.I.; Cuevas-Bermúdez, A.; Navarro, F. Cytoplasmic and nuclear functions for the prefoldin-like URI/Bud27. In *New Research On Molecular Chaperones*; Chaperones, N.R.O.M., Ed.; Nova Science Publishers, Inc.: New York, NY, USA, 2015; pp. 57–73.
24. Martínez-Fernández, V.; Garrido-Godino, A.I.; Cuevas-Bermúdez, A.; Navarro, F. The yeast prefoldin Bud27. *Adv. Exp. Med. Biol.* **2018**, *1106*, 109–118.
25. Martinez-Fernandez, V.; Cuevas-Bermudez, A.; Gutierrez-Santiago, F.; Garrido-Godino, A.I.; Rodriguez-Galan, O.; Jordan-Pla, A.; Lois, S.; Trivino, J.C.; de la Cruz, J.; Navarro, F. Prefoldin-like Bud27 influences the transcription of ribosomal components and ribosome biogenesis in Saccharomyces cerevisiae. *RNA* **2020**, *26*, 1360–1379. [CrossRef]
26. Gstaiger, M.; Luke, B.; Hess, D.; Oakeley, E.J.; Wirbelauer, C.; Blondel, M.; Vigneron, M.; Peter, M.; Krek, W. Control of nutrient-sensitive transcription programs by the unconventional prefoldin URI. *Science* **2003**, *302*, 1208–1212. [CrossRef]
27. Mirón-García, M.C.; Garrido-Godino, A.I.; Martínez-Fernández, V.; Fernández-Pevida, A.; Cuevas-Bermúdez, A.; Martín-Expósito, M.; Chávez, S.; de la Cruz, J.; Navarro, F. The yeast prefoldin-like URI-orthologue Bud27 associates with the RSC nucleosome remodeler and modulates transcription. *Nucleic Acids Res.* **2014**, *42*, 9666–9676. [CrossRef]
28. Vernekar, D.V.; Bhargava, P. Yeast Bud27 modulates the biogenesis of Rpc128 and Rpc160 subunits and the assembly of RNA polymerase III. *Biochim. Biophys. Acta* **2015**, *1849*, 1340–1353. [CrossRef]
29. Mirón-García, M.C.; Garrido-Godino, A.I.; García-Molinero, V.; Hernández-Torres, F.; Rodríguez-Navarro, S.; Navarro, F. The prefoldin Bud27 mediates the assembly of the eukaryotic RNA polymerases in an Rpb5-dependent manner. *PLoS Genet.* **2013**, *9*, e1003297. [CrossRef]
30. Mita, P.; Savas, J.N.; Ha, S.; Djouder, N.; Yates, J.R., 3rd; Logan, S.K. Analysis of URI nuclear interaction with RPB5 and components of the R2TP/Prefoldin-Like complex. *PLoS ONE* **2013**, *8*, e63879. [CrossRef]
31. Deplazes, A.; Mockli, N.; Luke, B.; Auerbach, D.; Peter, M. Yeast Uri1p promotes translation initiation and may provide a link to cotranslational quality control. *EMBO J.* **2009**, *28*, 1429–1441. [CrossRef]
32. García-López, M.C.; Mirón-García, M.C.; Garrido-Godino, A.I.; Mingorance, C.; Navarro, F. Overexpression of *SNG1* causes 6-azauracil resistance in *Saccharomyces cerevisiae*. *Curr. Genet.* **2010**, *56*, 251–263. [CrossRef] [PubMed]
33. Krick, R.; Bremer, S.; Welter, E.; Schlotterhose, P.; Muehe, Y.; Eskelinen, E.L.; Thumm, M. Cdc48/p97 and Shp1/p47 regulate autophagosome biogenesis in concert with ubiquitin-like Atg8. *J. Cell Biol.* **2010**, *190*, 965–973. [CrossRef] [PubMed]
34. Stettler, S.; Chiannilkulchai, N.; Hermann-Le Denmat, S.; Lalo, D.; Lacroute, F.; Sentenac, A.; Thuriaux, P. A general suppressor of RNA polymerase I, II and III mutations in Saccharomyces cerevisiae. *Mol. Gen. Genet.* **1993**, *239*, 169–176. [CrossRef] [PubMed]
35. Montella-Manuel, S.; Pujol-Carrion, N.; Mechoud, M.A.; de la Torre-Ruiz, M.A. Bulk autophagy induction and life extension is achieved when iron is the only limited nutrient in Saccharomyces cerevisiae. *Biochem. J.* **2021**, *478*, 811–837. [CrossRef] [PubMed]
36. Lee, J.; Moir, R.D.; McIntosh, K.B.; Willis, I.M. TOR Signaling Regulates Ribosome and tRNA Synthesis via LAMMER/Clk and GSK-3 Family Kinases. *Mol. Cell* **2012**, *45*, 836–843. [CrossRef]
37. Aquino, G.R.R.; Krogh, N.; Hackert, P.; Martin, R.; Gallesio, J.D.; van Nues, R.W.; Schneider, C.; Watkins, N.J.; Nielsen, H.; Bohnsack, K.E.; et al. RNA helicase-mediated regulation of snoRNP dynamics on pre-ribosomes and rRNA 2′-O-methylation. *Nucleic Acids Res.* **2021**, *49*, 4066–4084. [CrossRef]
38. Okano, A.; Wan, K.; Kanda, K.; Yabuki, Y.; Funato, K.; Mizuta, K. SMY2 and SYH1 suppress defects in ribosome biogenesis caused by ebp2 mutations. *Biosci. Biotechnol. Biochem.* **2015**, *79*, 1481–1483. [CrossRef]
39. Sezen, B. Reduction of Saccharomyces cerevisiae Pom34 protein level by SESA network is related to membrane lipid composition. *FEMS Yeast Res.* **2015**, *15*, fov089. [CrossRef]
40. Sanni, A.; Mirande, M.; Ebel, J.P.; Boulanger, Y.; Waller, J.P.; Fasiolo, F. Structure and expression of the genes encoding the alpha and beta subunits of yeast phenylalanyl-tRNA synthetase. *J. Biol. Chem.* **1988**, *263*, 15407–15415. [CrossRef]
41. Gampel, A.; Tzagoloff, A. Homology of aspartyl- and lysyl-tRNA synthetases. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 6023–6027. [CrossRef]
42. Brune, C.; Munchel, S.E.; Fischer, N.; Podtelejnikov, A.V.; Weis, K. Yeast poly(A)-binding protein Pab1 shuttles between the nucleus and the cytoplasm and functions in mRNA export. *RNA* **2005**, *11*, 517–531. [CrossRef] [PubMed]
43. Brengues, M.; Parker, R. Accumulation of polyadenylated mRNA, Pab1p, eIF4E, and eIF4G with P-bodies in Saccharomyces cerevisiae. *Mol. Biol. Cell* **2007**, *18*, 2592–2602. [CrossRef] [PubMed]
44. Moqtaderi, Z.; Geisberg, J.V.; Struhl, K. Extensive structural differences of closely related 3′ mRNA isoforms: Links to Pab1 binding and mRNA stability. *Mol. Cell* **2018**, *72*, 849–861.e6. [CrossRef] [PubMed]
45. Brambilla, M.; Martani, F.; Bertacchi, S.; Vitangeli, I.; Branduardi, P. The Saccharomyces cerevisiae poly (A) binding protein (Pab1): Master regulator of mRNA metabolism and cell physiology. *Yeast* **2019**, *36*, 23–34. [CrossRef] [PubMed]
46. Ash, M.R.; Faelber, K.; Kosslick, D.; Albert, G.I.; Roske, Y.; Kofler, M.; Schuemann, M.; Krause, E.; Freund, C. Conserved beta-hairpin recognition by the GYF domains of Smy2 and GIGYF2 in mRNA surveillance and vesicular transport complexes. *Structure* **2010**, *18*, 944–954. [CrossRef] [PubMed]
47. Protchenko, O.; Rodriguez-Suarez, R.; Androphy, R.; Bussey, H.; Philpott, C.C. A screen for genes of heme uptake identifies the FLC family required for import of FAD into the endoplasmic reticulum. *J. Biol. Chem.* **2006**, *281*, 21445–21457. [CrossRef]
48. Sickmann, A.; Reinders, J.; Wagner, Y.; Joppich, C.; Zahedi, R.; Meyer, H.E.; Schonfisch, B.; Perschil, I.; Chacinska, A.; Guiard, B.; et al. The proteome of Saccharomyces cerevisiae mitochondria. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 13207–13212. [CrossRef]

49. Mouassite, M.; Camougrand, N.; Schwob, E.; Demaison, G.; Laclau, M.; Guerin, M. The 'SUN' family: Yeast SUN4/SCW3 is involved in cell septation. *Yeast* **2000**, *16*, 905–919. [CrossRef]
50. Tatjer, L.; Gonzalez, A.; Serra-Cardona, A.; Barcelo, A.; Casamayor, A.; Arino, J. The Saccharomyces cerevisiae Ptc1 protein phosphatase attenuates G2-M cell cycle blockage caused by activation of the cell wall integrity pathway. *Mol. Microbiol.* **2016**, *101*, 671–687. [CrossRef]
51. Ahmed, K.; Carter, D.E.; Lajoie, P. Hyperactive TORC1 sensitizes yeast cells to endoplasmic reticulum stress by compromising cell wall integrity. *FEBS Lett.* **2019**, *593*, 1957–1973. [CrossRef]
52. Levin, D.E. Cell wall integrity signaling in Saccharomyces cerevisiae. *Microbiol. Mol. Biol. Rev.* **2005**, *69*, 262–291. [CrossRef] [PubMed]
53. Nakanishi, H.; Morishita, M.; Schwartz, C.L.; Coluccio, A.; Engebrecht, J.; Neiman, A.M. Phospholipase D and the SNARE Sso1p are necessary for vesicle fusion during sporulation in yeast. *J. Cell Sci.* **2006**, *119 Pt 7*, 1406–1415. [CrossRef] [PubMed]
54. Li, J.; Agarwal, S.; Roeder, G.S. SSP2 and OSW1, Two Sporulation-specific Genes Involved in Spore Morphogenesis in Saccharomyces cerevisiae. *Genetics* **2006**, *175*, 143–154. [CrossRef] [PubMed]
55. Neiman, A.M. Sporulation in the budding yeast Saccharomyces cerevisiae. *Genetics* **2011**, *189*, 737–765. [CrossRef] [PubMed]
56. Jordan, P.W.; Klein, F.; Leach, D.R. Novel roles for selected genes in meiotic DNA processing. *PLoS Genet.* **2007**, *3*, e222. [CrossRef] [PubMed]
57. Chua, P.R.; Roeder, G.S. Zip2, a meiosis-specific protein required for the initiation of chromosome synapsis. *Cell* **1998**, *93*, 349–359. [CrossRef]
58. Gelfand, B.; Mead, J.; Bruning, A.; Apostolopoulos, N.; Tadigotla, V.; Nagaraj, V.; Sengupta, A.M.; Vershon, A.K. Regulated antisense transcription controls expression of cell-type-specific genes in yeast. *Mol. Cell Biol.* **2011**, *31*, 1701–1709. [CrossRef]
59. Shaw, R.J.; Wilson, J.L.; Smith, K.T.; Reines, D. Regulation of an IMP dehydrogenase gene and its overexpression in drug-sensitive transcription elongation mutants of yeast. *J. Biol. Chem.* **2001**, *276*, 32905–32916. [CrossRef]
60. Deminoff, S.J.; Santangelo, G.M. Rap1p requires Gcr1p and Gcr2p homodimers to activate ribosomal protein and glycolytic genes, respectively. *Genetics* **2001**, *158*, 133–143. [CrossRef]
61. Ravi, C.; Gowsalya, R.; Nachiappan, V. Impaired GCR1 transcription resulted in defective inositol levels, vacuolar structure and autophagy in Saccharomyces cerevisiae. *Curr. Genet.* **2019**, *65*, 995–1014. [CrossRef]
62. Tornow, J.; Zeng, X.; Gao, W.; Santangelo, G.M. GCR1, a transcriptional activator in Saccharomyces cerevisiae, complexes with RAP1 and can function without its DNA binding domain. *EMBO J.* **1993**, *12*, 2431–2437. [CrossRef] [PubMed]
63. Hall, D.B.; Wade, J.T.; Struhl, K. An HMG protein, Hmo1, associates with promoters of many ribosomal protein genes and throughout the rRNA gene locus in Saccharomyces cerevisiae. *Mol. Cell Biol.* **2006**, *26*, 3672–3679. [CrossRef] [PubMed]
64. Mochida, K.; Oikawa, Y.; Kimura, Y.; Kirisako, H.; Hirano, H.; Ohsumi, Y.; Nakatogawa, H. Receptor-mediated selective autophagy degrades the endoplasmic reticulum and the nucleus. *Nature* **2015**, *522*, 359–362. [CrossRef] [PubMed]
65. Yorimitsu, T.; Nair, U.; Yang, Z.; Klionsky, D.J. Endoplasmic reticulum stress triggers autophagy. *J. Biol. Chem.* **2006**, *281*, 30299–30304. [CrossRef] [PubMed]
66. Garcia, R.; Bermejo, C.; Grau, C.; Perez, R.; Rodriguez-Pena, J.M.; Francois, J.; Nombela, C.; Arroyo, J. The global transcriptional response to transient cell wall damage in Saccharomyces cerevisiae and its regulation by the cell integrity signaling pathway. *J. Biol. Chem.* **2004**, *279*, 15183–15195. [CrossRef]
67. Barve, G.; Manjithaya, R. Cross-talk between autophagy and sporulation in Saccharomyces cerevisiae. *Yeast* **2021**, *38*, 401–413. [CrossRef]
68. Meguro, S.; Zhuang, X.; Kirisako, H.; Nakatogawa, H. Pex3 confines pexophagy receptor activity of Atg36 to peroxisomes by regulating Hrr25-mediated phosphorylation and proteasomal degradation. *J. Biol. Chem.* **2020**, *295*, 16292–16298. [CrossRef]
69. Motley, A.M.; Nuttall, J.M.; Hettema, E.H. Pex3-anchored Atg36 tags peroxisomes for degradation in Saccharomyces cerevisiae. *EMBO J.* **2012**, *31*, 2852–2868. [CrossRef]
70. Motley, A.M.; Nuttall, J.M.; Hettema, E.H. Atg36: The Saccharomyces cerevisiae receptor for pexophagy. *Autophagy* **2012**, *8*, 1680–1681. [CrossRef]
71. Wroblewska, J.P.; Cruz-Zaragoza, L.D.; Yuan, W.; Schummer, A.; Chuartzman, S.G.; de Boer, R.; Oeljeklaus, S.; Schuldiner, M.; Zalckvar, E.; Warscheid, B.; et al. Saccharomyces cerevisiae cells lacking Pex3 contain membrane vesicles that harbor a subset of peroxisomal membrane proteins. *Biochim. Biophys. Acta Mol. Cell Res.* **2017**, *1864*, 1656–1667. [CrossRef]
72. Montella-Manuel, S.; Pujol-Carrion, N.; de la Torre-Ruiz, M.A. The Cell Wall Integrity Receptor Mtl1 Contributes to Articulate Autophagic Responses When Glucose Availability Is Compromised. *J. Fungi* **2021**, *7*, 903. [CrossRef] [PubMed]
73. Bandhakavi, S.; Xie, H.; O'Callaghan, B.; Sakurai, H.; Kim, D.H.; Griffin, T.J. Hsf1 activation inhibits rapamycin resistance and TOR signaling in yeast revealed by combined proteomic and genetic analysis. *PLoS ONE* **2008**, *3*, e1598. [CrossRef] [PubMed]
74. Szklarczyk, D.; Morris, J.H.; Cook, H.; Kuhn, M.; Wyder, S.; Simonovic, M.; Santos, A.; Doncheva, N.T.; Roth, A.; Bork, P.; et al. The STRING database in 2017: Quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Res.* **2017**, *45*, D362–D368. [CrossRef] [PubMed]
75. Roncero, C.; Valdivieso, M.H.; Ribas, J.C.; Duran, A. Isolation and characterization of Saccharomyces cerevisiae mutants resistant to Calcofluor white. *J. Bacteriol.* **1988**, *170*, 1950–1954. [CrossRef] [PubMed]

76. Kapteyn, J.C.; Ram, A.F.; Groos, E.M.; Kollar, R.; Montijn, R.C.; Van Den Ende, H.; Llobell, A.; Cabib, E.; Klis, F.M. Altered extent of cross-linking of beta1,6-glucosylated mannoproteins to chitin in Saccharomyces cerevisiae mutants with reduced cell wall beta1,3-glucan content. *J. Bacteriol.* **1997**, *179*, 6279–6284. [CrossRef] [PubMed]
77. Lesage, G.; Shapiro, J.; Specht, C.A.; Sdicu, A.M.; Menard, P.; Hussein, S.; Tong, A.H.; Boone, C.; Bussey, H. An interactional network of genes involved in chitin synthesis in Saccharomyces cerevisiae. *BMC Genet.* **2005**, *6*, 8. [CrossRef]
78. Rockwell, N.C.; Wolfger, H.; Kuchler, K.; Thorner, J. ABC transporter Pdr10 regulates the membrane microenvironment of Pdr12 in Saccharomyces cerevisiae. *J. Membr. Biol.* **2009**, *229*, 27–52. [CrossRef]
79. Queiroz, M.G.; Elsztein, C.; de Morais, M.A., Jr. The effects of the Ncw2 protein of Saccharomyces cerevisiae on the positioning of chitin in response to cell wall damage. *Antonie Van Leeuwenhoek* **2020**, *113*, 265–277. [CrossRef]
80. Ishii, A.; Kurokawa, K.; Hotta, M.; Yoshizaki, S.; Kurita, M.; Koyama, A.; Nakano, A.; Kimura, Y. Role of Atg8 in the regulation of vacuolar membrane invagination. *Sci. Rep.* **2019**, *9*, 14828. [CrossRef]
81. Cuevas-Bermúdez, A.; Garrido-Godino, A.; Navarro, F. A novel yeast chromatin-enriched fractions purification approach, yChEFs, for the chromatin-associated protein analysis used for chromatin-associated and RNA-dependent chromatin-associated proteome studies from Saccharomyces cerevisiae. *Gene Rep.* **2019**, *16*, 100450. [CrossRef]