Biochemical and Biophysical Research Communications 464 (2015) 1248–1253

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The yeast chromatin remodeler Rsc1-RSC complex is required for transcriptional activation of autophagy-related genes and inhibition of the TORC1 pathway in response to nitrogen starvation

Feifei Yu [a, 1], Yuko Imamura [a, 1], Masaru Ueno [a], Sho W. Suzuki [b], Yoshinori Ohsumi [b], Masashi Yukawa [a, *], Eiko Tsuchiya [a]

[a] Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8530, Japan
[b] Frontier Research Center, Tokyo Institute of Technology, Yokohama 226-8503, Japan

ARTICLE INFO

Article history:
Received 11 July 2015
Accepted 22 July 2015
Available online 26 July 2015

Keywords:
RSC
Chromatin remodeling
Autophagy induction
TORC1
Saccharomyces cerevisiae

ABSTRACT

The yeast RSC, an ATP-dependent chromatin-remodeling complex, is essential for mitotic and meiotic growth. There are two distinct isoforms of this complex defined by the presence of either Rsc1 or Rsc2; however, the functional differences between these complexes are unclear. Here we show that the RSC complex containing Rsc1, but not Rsc2, functions in autophagy induction. Rsc1 was required not only for full expression of *ATG8* mRNA but also for maintenance of Atg8 protein stability. Interestingly, decreased autophagic activity and Atg8 protein stability in *rsc1Δ* cells, but not the defect in *ATG8* mRNA expression, were partially suppressed by deletion of *TOR1*. In addition, we found that *rsc1Δ* impaired the binding between the Rho GTPase Rho1 and the TORC1-specific component Kog1, which is required for down-regulation of TORC1 activity. These results suggest that the Rsc1-containing RSC complex plays dual roles in the proper induction of autophagy: 1) the transcriptional activation of autophagy-related genes independent of the TORC1 pathway and 2) the inactivation of TORC1, possibly through enhancement of Rho1-Kog1 binding.

1. Introduction

Chromatin structure plays significant and dynamic roles in the regulation of DNA metabolism including DNA replication, transcription, recombination, and repair. The access of regulatory proteins involved in these processes to chromatin is regulated by covalent post-translational modifications of histones and by the action of ATP-dependent chromatin-remodeling complexes [1]. *Saccharomyces cerevisiae* RSC (Remodels the Structure of Chromatin) is an essential chromatin-remodeling enzyme consisting of 17 subunits [2]. RSC exists as at least two isoforms, containing either the Rsc1 or Rsc2 subunit [3]. Strains lacking either *RSC1* or *RSC2* are viable, but deletion of both genes results in inviability. By genome-wide localization analysis, these two RSCs were shown to occupy the same region of the genome [4], suggesting that they share overlapping functions. However, *rsc1Δ* and *rsc2Δ* strains do not exhibit identical phenotypes [3,5–9]. Moreover, the intracellular levels of Rsc1 and Rsc2 are different; Rsc2 is estimated to be 10-fold more abundant than Rsc1 [3]. Therefore, functional differences between these two RSCs are still unknown. Recently, we identified two autophagy-related genes, *ATG17* and *VPS34*, through a synthetic lethal screen using a temperature-sensitive mutant allele of *NPS1/STH1*, encoding an ATPase subunit of RSC [10]. The deletion of *ATG17* was reported to be synthetically lethal in combination with the deletion of *RSC7*, which encodes another non-essential component of RSC [11]. These results suggest a functional relationship between autophagy and RSC.

Autophagy is a highly conserved catabolic pathway for recycling cytoplasmic materials and maintaining cellular homeostasis [12]. Upon starvation of nutrients, such as carbon, nitrogen, sulfur, and various amino acids, or upon environmental or intracellular stresses, cells initiate the formation of a double-membrane vesicle, termed the autophagosome, that mediates this process. Approximately 30 autophagy-related (Atg) proteins have been identified in

* Corresponding author.
E-mail address: myukawa@hiroshima-u.ac.jp (M. Yukawa).
[1] These authors equally contributed to this work.

http://dx.doi.org/10.1016/j.bbrc.2015.07.114
0006-291X/

S. cerevisiae, including highly conserved core components such as Atg1 (ULK1/2 in mammals), Atg13, and Atg8 (LC3 in mammals). Multiple signaling pathways regulate autophagy among which target of rapamycin kinase complex 1 (TORC1) plays a crucial role in the induction of autophagy [13]. In both yeast and mammalian cells, nutrient starvation inhibits the ability of TOR kinase to induce autophagy. Autophagy is also regulated via the transcriptional up-regulation or induction of autophagy genes. However, little is known about the mechanisms regulating the transcriptional activation of these genes and the accumulation/turnover of these gene products. In addition, the role of ATP-dependent chromatin remodelers in this process has not yet been evaluated.

In this report, we show that Rsc1-containing RSC complex (Rsc1-RSC) is required for transcriptional induction of autophagic genes and inactivation of the TORC1 pathway.

2. Materials and methods

2.1. Strains, plasmids and media

All yeast strains used in this study were S288C derivatives, and are listed in Table S1 of Supplementary Material. Standard procedures were used for mating, sporulation, transformation, and tetrad dissection. All media were prepared as described previously [14]. Alleles of *nps1-105* and *nps1-13* were described previously [15,16]. C-terminal 6×HA tagging of *ATG13*, *KOG1*, and *ATG8* was carried out by homologous recombination using a simple PCR-based strategy as described previously [10]. Correct insertion was verified by sequencing. All PCR primers are listed in Table S2 of Supplementary Material. Plasmids used were pRS316*GFP-ATG8* [17], pTN3 (including *PHO8Δ60*) [18], YEp13*RSC1*-3Myc, and YEp24*RSC2*-2HA [15]. Yeast strains were grown or incubated in YPD (1% yeast extract, 2% peptone, 2% glucose), synthetic complete medium with glucose (SDC; 0.67% yeast nitrogen base without amino acids, 2% glucose, amino acids) or nitrogen-starvation (N-starvation) medium (SD-N; 0.17% yeast nitrogen base without amino acids and ammonium sulfate, 2% glucose). SDC media lacking appropriate amino acids for selection were prepared as previously described [14]. Cultures were incubated at 30 °C with shaking, unless otherwise indicated.

2.2. Autophagy assay

For monitoring bulk autophagy, the alkaline phosphatase (ALP) activity of Pho8Δ60 was measured, as described previously [18]. In this study, the plasmid pTN3 was used for Pho8Δ60 overexpression. Detection of the processing of GFP-Atg8 expressed under the *ATG8* promoter was carried out as previously described [17].

2.3. Immunoblotting

Yeast cells were grown to log phase on YPD and then shifted to SD-N, or incubated with 400 ng/ml of rapamycin (Focus Biomolecules), and cell lysates were thereafter prepared at the appropriate times. Proteins in each cell lysate were resolved by SDS-PAGE followed by immunoblotting or processed for immunoprecipitation as described previously [15]. The intensities of protein bands obtained by immunoblotting were measured using the image analyzing software, Image-J (NIH, USA). The following antibodies were used: anti-GFP (Roche Diagnostics), anti-Cdc28 (Santa Cruz Biotechnology, Inc), anti-HA (Covance), and anti-Rho1 (y-260, Santa Cruz Biotechnology, Inc).

2.4. Quantitative RT-PCR analysis of mRNA and ChIP analysis

Total RNA was isolated from yeast cells grown in YPD or incubated in SD-N by using an RNeasy Protect Mini kit (Qiagen) according to the manufacturer's instructions. Quantitative real-time RT-PCR was performed with a One Step SYBER PrimeScript RT-PCR kit II (Takara Bio Inc.) by using a LightCycler (Roche Diagnostics). Primers used are listed in Table S2 of Supplementary Material. Primers for the specified genes were validated before use with standard curves. Transcript abundance was normalized to *ACT1* transcripts. The chromatin immunoprecipitation (ChIP) procedure was performed as described previously [19].

3. Results

3.1. The Rsc1-RSC complex is required for autophagy induction

To investigate the role of RSC in autophagy, we first measured the level of autophagy induction by using the Pho8Δ60 assay [18], which measures autophagy-dependent activation of an altered alkaline phosphatase (ALP) marker. Wild-type (WT) and *rsc* mutant cells were grown in YPD medium and then shifted to nitrogen (N-) starvation medium (SD-N) for 3 h to induce autophagy. As shown in Fig. 1A, the level of activity in *rsc1Δ* cells was significantly lower than that in the WT cells and comparable to that in the *atg1Δ* cells,

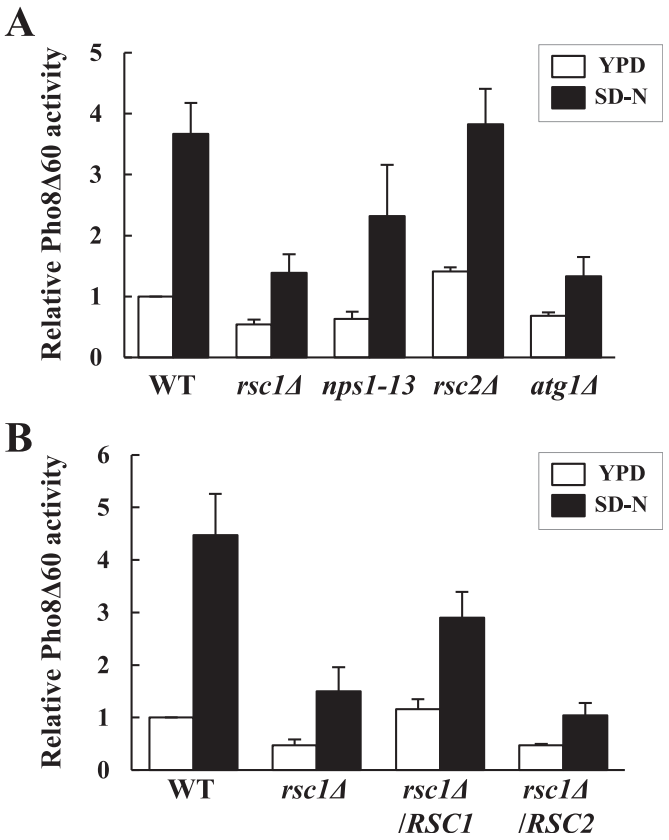

Fig. 1. The Rsc1-RSC complex is required for the proper induction of autophagy. (A) Pho8Δ60-derived alkaline phosphatase (ALP) activities in wild type (BY4743), *rsc1Δ* (BYI-1), *rsc2Δ* (BYI-2), *nps1-13* (BYI-3), and *atg1Δ* (BYI-4) cells expressing Pho8Δ60. The activities are indicated as values relative to the value for WT cells grown in YPD, which was set at 1. Data are presented as means ± SEM ($n = 3$). (B) The ALP activities in wild type (BY4743) cells carrying YEp13 and *rsc1Δ* (BYI-1) cells carrying YEp13, YEp13*RSC1*-3Myc, or YEp13*RSC2*-2HA (all expressing Pho8Δ60). The activities are indicated as values relative to the value for WT cells grown in YPD, which was set at 1. Data are presented as means ± SEM ($n = 3$).

which fail to undergo autophagy. The level of activity in *nps1-13*, a temperature-sensitive mutant allele of the RSC ATPase subunit Sth1/Nps1 [15], was also slightly decreased compared with that of WT (Fig. 1A). In contrast, deletion of *RSC2* did not affect its activity, despite the fact that Rsc2 is about 10 times more abundant than Rsc1 [3]. To further examine the functional distinction between Rsc1 and Rsc2 in autophagy, we tested whether the overexpression of Rsc2 could rescue the defect of autophagy in *rsc1Δ* cells. As shown in Fig. 1B, the defects in the ALP activity of *rsc1Δ* cells were suppressed by the overexpression of Rsc1, but not by that of Rsc2. Therefore, these data indicate that the Rsc1-RSC has a unique function in autophagy induction.

3.2. Rsc1-RSC is required for full expression of Atg8 protein

To confirm the above results, we next examined the processing of green fluorescent protein (GFP)-Atg8 in various *rsc* mutants. Atg8 is an ubiquitin-like protein that is important for autophagy. When autophagy is induced, GFP-labeled Atg8 is transported into the vacuole inside the autophagic body. Atg8 is degraded after lysis of the autophagic body, whereas the GFP moiety is relatively resistant to proteolysis. Accordingly, monitoring free GFP processed from GFP-Atg8 corresponds to the level of autophagy (autophagic flux) [17]. As shown in Fig. 2A and Fig. S1A, total GFP signals (GFP-Atg8 and free GFP) in *rsc1Δ* and *nps1-13* cells were significantly lower than that in WT cells, although these mutations did not affect the ratio of free GFP to GFP-Atg8. These data indicate that autophagy induction was significantly delayed, but still occurred, in these mutants because of the reduction of Atg8 protein expression. In contrast, the total GFP signal in *rsc2Δ* cells was substantially higher than that in WT cells. In accordance with this, the level of Atg8-HA expression in *rsc1Δ* cells under N-starvation was also lower than that in WT cells (Fig. S1B). However there was no difference in the levels between *rsc2Δ* and WT cells. These results suggest that Rsc1-RSC was required for *ATG8* transcription.

To test this possibility, we measured the level of *ATG8* mRNA after the shift to N-starvation. After a 2 h incubation, the level of *ATG8* mRNA in WT cells increased to about 18 times higher than that in the YPD medium (Fig. 2B). In contrast, the increase in the level of *ATG8* mRNA in *rsc1Δ* cells was approximately 70% of that in WT cells. In addition, transcriptional levels of *ATG7* and *ATG17* in *rsc1Δ* under N-starvation were also lower than those in WT cells (Fig. S2A, B), indicating that Rsc1-RSC is required for the transcriptional activation of a subset of autophagic genes. To further address whether Rsc1-RSC directly regulates the transcriptional activation of *ATG8*, we examined the binding of RSC to the *ATG8* promoter following the shift to N-starvation by using a strain expressing Nps1-TAP [19]. Chromatin immunoprecipitation (ChIP) analysis was performed using an anti-TAP antibody. This analysis showed that Nps1 binding to the *ATG8* promoter following the shift to N-starvation increased by 3.5-fold (Fig. 2C).

Interestingly, we noticed that in *rsc1Δ* cells, the decrease in both the GFP-Atg8 protein level and autophagic activity was much more pronounced (approximately 70%, Fig. 2A and approximately 65% reductions, Fig. 1A, respectively) compared with the rather modest decrease in the *ATG8* mRNA level under N-starvation conditions (approximately 30% decrease, Fig. 2B). These results suggested that Rsc1-RSC was also required for the stability of the Atg8 protein independent of its mRNA expression. To explore this possibility, WT and *rsc1Δ* cells were treated with a protein synthesis inhibitor cycloheximide (CHX) following the shift to N-starvation for 1 h, and GFP-Atg8 levels were monitored thereafter. As shown in Fig. 2D, the levels of GFP-Atg8 and its cleaved product free GFP did not change during a 2 h treatment with CHX in WT cells, consistent with a report by Bicknell et al. [20]. In contrast, in *rsc1Δ* cells, the levels of

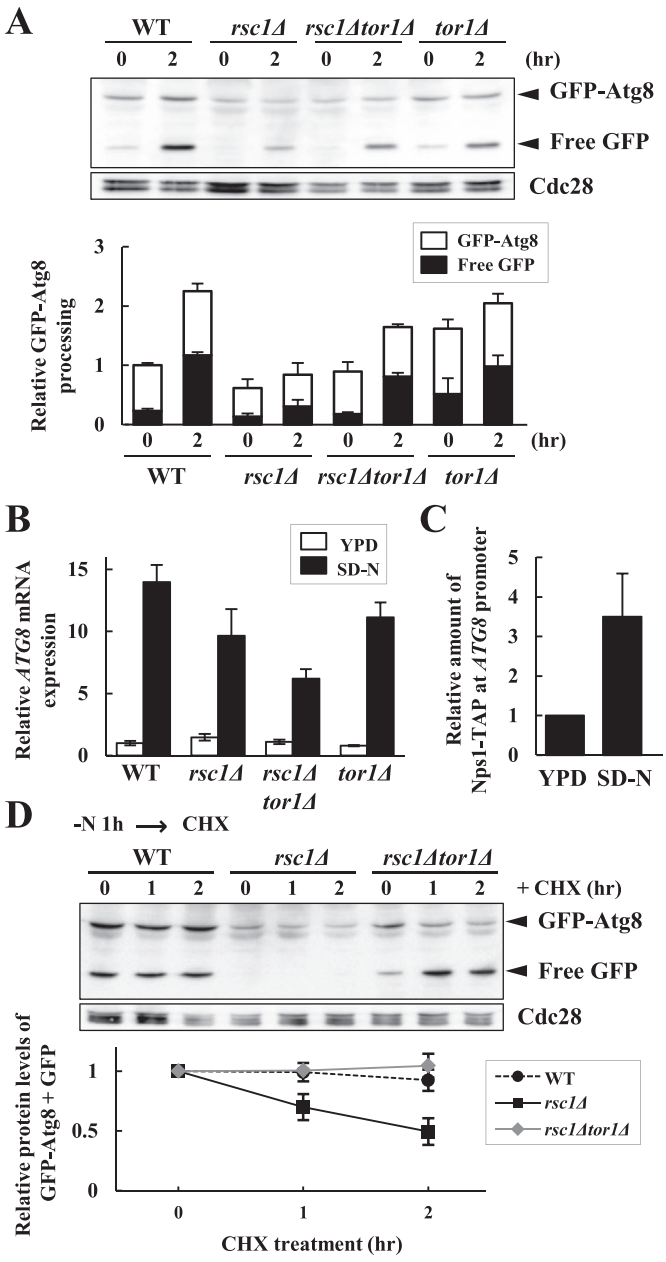

Fig. 2. The Rsc1-RSC complex is required for full expression of Atg8 protein under N-starvation. (A) Expression and processing of GFP-Atg8 in wild type (BY4743), *rsc1Δ* (BYI-1), *rsc1ΔtorΔ* (BYI-9), and *tor1Δ* (BYI-10) cells carrying pRS316*GFP-ATG8*. Proteins in each cell lysate were separated by SDS-PAGE, followed by immunoblotting with anti-GFP (upper panel) and anti-Cdc28 (lower panel) antibodies. Each protein signal was quantified, and GFP-Atg8 and free GFP signals were normalized by the Cdc28 signal. The total GFP signals (GFP-Atg8 and free GFP) in each strain are indicated as values relative to the value for WT cells grown in YPD, which was set at 1. Data are presented as means ± SEM ($n = 3$). (B) Expression of *ATG8* mRNA in wild type (BY4743) and *rsc1Δ* (BYI-1) cells. *ATG8* mRNA levels were normalized to levels of *ACT1* mRNA and indicated as values relative to the value for WT cells grown in YPD, which was set at 1. Data are presented as means ± SEM ($n = 3$). (C) Nps1-TAP binds the *ATG8* promoter. *NPS1-TAP* cells (BYI-7) were grown to log phase in YPD and shifted to SD-N for 2 h. ChIP analysis was conducted on the *ATG8* promoter −54 to −186 bp upstream of the *ATG8* start codon. As a control, a region of *HTA1-E* where Nps1 could not bind was analyzed. The ChIP results of *ATG8* promoter were normalized to that of the control and indicated as values relative to the value of YPD, which was set at 1. Data are presented as means ± SEM ($n = 3$). (D) Turnover of GFP-Atg8. Wild type (BY4743), *rsc1Δ* (BYI-1) and *rsc1Δtor1Δ*(BYI-9) cells were grown to log phase in YPD and shifted to SD-N for 1 h, after which 100 mg/ml of CHX was added. GFP-Atg8 processing was monitored as described in Fig. 2A. The total GFP signals (GFP-Atg8 and free GFP) at each time point are indicated as values relative to the value of 0 h in each strain, which was set at 1. Data are presented as means ± SEM ($n = 3$).

both proteins were decreased to approximately 50% after 2 h incubation. Because of lower mRNA expression (see Fig. 2B), the levels of both proteins prior to CHX addition were also reduced (approximately 70%) in *rsc1Δ* cells. Taken together, these results indicate that Rsc1-RSC ensures the transcriptional activation of autophagic genes and also facilitates the stabilization of Atg8 under N-starvation conditions.

3.3. The TORC1 signaling pathway is not properly down-regulated in Rsc1-RSC deficient cells following the shift to N-starvation

TOR is one of the major nutrient-signaling regulators across eukaryotes. The TOR-signaling pathway plays a pivotal role in controlling the induction of autophagy. We aimed to determine whether Rsc1-RSC was under the control of the TOR-signaling pathway or modulated it to activate autophagy. First, we investigated if deletion of *TOR1* could suppress the reduction of autophagic activity in *rsc1Δ* cells following the shift to N-starvation. Remarkably, deletion of *TOR1* restored the ALP activity to near WT levels in *rsc1Δ* cells (Fig. 3A).

It is known that TORC1 inhibits autophagy induction through direct phosphorylation of Atg13, thereby preventing the binding of this protein to Atg1 [21]. Inactivation of TORC1 results in Atg13 dephosphorylation, leading to the formation of the Atg1–Atg13–Atg17 complex, which functions in the induction of autophagy [22]. Given this finding, we subsequently examined whether dephosphorylation of Atg13 occurred in *rsc1Δ* cells. As shown in Fig. 3B, the amount of the slow-migrating form of Atg13-HA was rapidly decreased by a 15 min incubation in N-starvation medium in both WT and *tor1Δ* cells [21]. In the *rsc1Δ* cells, the amount of slow-migrating Atg13-HA remained unchanged under the same condition. Importantly, deletion of *TOR1* in the *rsc1Δ* cells resulted in a substantial reduction of slow-migrating Atg13-HA. These results indicate that Rsc1-RSC is involved in down-regulation of TORC1 activity under N-starvation conditions, thereby inducing autophagy.

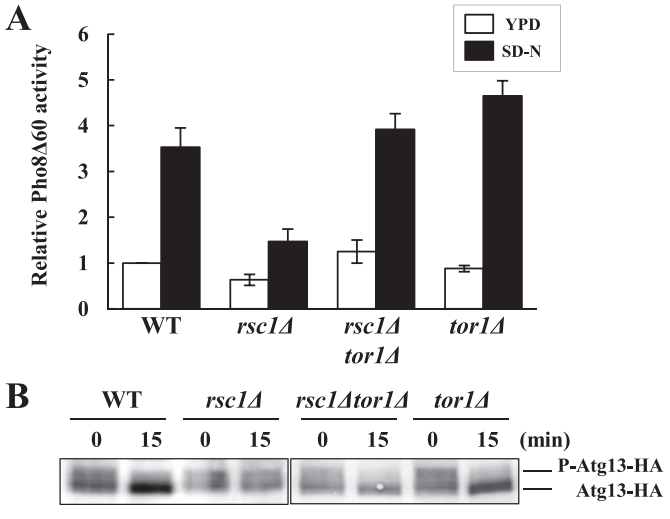

Fig. 3. The Rsc1-RSC complex is required for inactivation of TORC1 signaling pathway under N-starvation. (A) The ALP activities in wild type (BY4743), *rsc1Δ* (BYI-1), *rsc1Δtor1Δ* (BYI-9), and *tor1Δ* (BYI-10) cells expressing Pho8Δ60. The ALP assay was performed as determined in Fig. 1A. The results are indicated as values relative to the value for WT cells grown in YPD, which was set at 1. Data are presented as means ± SEM ($n = 3$). (B) Immunoblot of Atg13 in wild-type (BY1-11), *rsc1Δ* (BYI-12), *rsc1Δtor1Δ*(BY1-13), and *tor1Δ* (BYI-14) cells expressing Atg13-HA. The phosphorylation state of Atg13 (P-Atg13-HA, phospho-Atg13-HA) was assessed by immunoblotting.

3.4. The TORC1 signaling pathway is involved in maintenance of Atg8 protein stability

Next, we examined whether TORC1 is required for full expression of Atg8 protein by synergizing with Rsc1-RSC. To this end, the level of GFP-Atg8 and free GFP were measured in *tor1Δ* and *rsc1Δtor1Δ* cells. As shown in Fig. 2A, deletion of *TOR1* partially restored the amount of GFP-Atg8 and free GFP in *rsc1Δ* cells, consistent with the ALP activity (see Fig. 3A). Interestingly, *ATG8* mRNA levels remained lower in the *rsc1Δtor1Δ* double mutant than in each single mutant (Fig. 2B). In contrast, the GFP-Atg8 protein was substantially stabilized in *rsc1Δ* cells by the *TOR1* deletion (Fig. 2D). Hence, the inactivation of TORC1 in *rsc1Δ* led to stabilization of Atg8 protein without restoring its mRNA expression.

3.5. Rsc1-RSC promotes Rho1-Kog1 binding to down-regulate the TORC1 pathway

Previous results indicated that Rsc1-RSC inhibited TORC1 signaling under N-starvation. How did this complex participate in TORC1 inactivation? Upon activation by stresses, the Rho GTPase Rho1 binds to the TORC1-specific subunit Kog1, which results in down-regulation of TORC1 activity [23]. Rho1 is the core sensor of the cell wall integrity (CWI) pathway, a major stress response pathway in yeast that controls actin polarization and cell wall expansion in response to various stress conditions [24]. Our group and others identified the factors involved in this CWI pathway as high-copy suppressors of temperature-sensitive *nps1-105* and *sth1-3* mutants, suggesting a functional relationship between RSC and this signaling pathway [25,26].

Accordingly, we assessed whether Rsc1-RSC leads to TORC1 inactivation through the Rho1-Kog1 binding. In WT cells, the level of Kog1-HA co-immunoprecipitated with anti-Rho1 was increased approximately 3-fold 40 min after treatment with rapamycin (Fig. 4A). In contrast, in *rsc1Δ* cells, rapamycin treatment failed to enhance the Rho1-Kog1 interaction. Yan et al., previously showed that the Rho1-Kog1 binding was transiently induced after rapamycin treatment [23]. However, we repeatedly observed this binding even after 40 min of rapamycin treatment. Although dissociation of Rho1 from TORC1 is not sufficient for the immediate restoration of the TORC1 activity [23], this difference may be dependent on the strain background or antibody used in this assay. Nevertheless, this result suggested that Rsc1-RSC is required for the proper down-regulation of TORC1 activity through stimulation of the Rho1-Kog1 interaction under stress conditions, including N-starvation.

4. Discussion

Our data have uncovered a pivotal role for the Rsc1-RSC complex in the proper induction of autophagy. To the best of our knowledge, this is the first report showing the function of an ATP-dependent chromatin remodeler in autophagy induction. It should also be noted that we show that the Rsc1-RSC complex, but not Rsc2-RSC, is required for autophagy. ATP-dependent chromatin remodeling complexes are conserved from yeast to humans. Mutations in components of human chromatin-remodeling complexes have been identified at high frequencies in human cancers; however, the molecular mechanisms underlying the carcinogenic effects of these mutations are largely unknown [27,28]. Autophagy is thought to play dual roles in cancer; it can prevent tumor initiation through its quality control function for cells, and it can sustain tumor metabolism through nutrient recycling [29]. Our findings suggest that the impaired function of mammalian chromatin remodelers impacts autophagy and provides insight into the role of

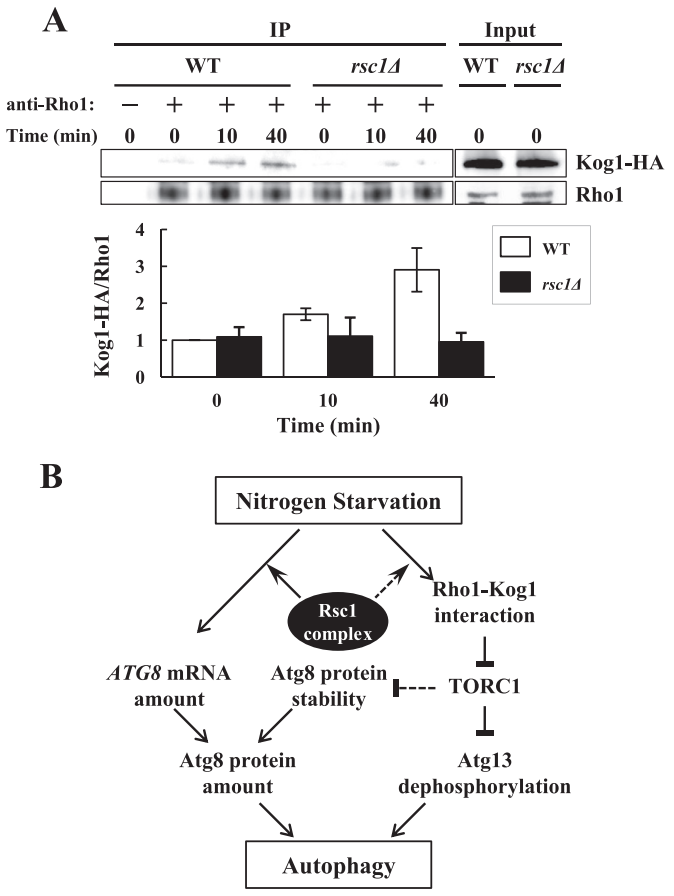

Fig. 4. The Rsc1-RSC complex promotes Rho1-Kog1 binding to down-regulate the TORC1 pathway. (A) Interaction between Rho1 and Kog1-HA is impaired in *rsc1Δ*. Wild type and *rsc1Δ* cells expressing Kog1-HA (BYI-15 and BYI-16, respectively) were grown to log phase in YPD at 23 °C, after which 400 ng/ml rapamycin was added. The densities of immunoblot bands labeled with anti-HA were normalized to those labeled with anti-Rho1 and indicated as values relative to the value for WT cells grown in YPD for 0 min, which was set at 1. Data are presented as means ± SEM ($n = 3$). (B) Schematic diagram showing the signaling pathways leading to autophagy regulated by Rsc1-RSC. On one branch, the Rsc1-RSC complex directly activates the transcription of a subset of *ATG* genes under N-starvation. On the other branch, this complex promotes the Rho1-Kog1 interaction, thereby down-regulating the TORC1 signaling pathway. Both pathways result in autophagy induction. TORC1 might be involved in the maintenance of Atg8 protein stability.

ATP-dependent chromatin remodelers in carcinogenesis.

Our results identified Rsc1-RSC as a transcriptional activator of *ATG7*, *ATG8*, and *ATG17*. Atg17 is a regulatory subunit of an autophagy-specific complex that includes Atg1 and Atg13, and it stimulates Atg1 kinase activity. Atg7 is a dual-specificity member of the E1 family, which mediates the conjugation of Atg12 to Atg5 and that of Atg8 to phosphatidylethanolamine. Both steps are essential for autophagosome formation [30,31]. Therefore, decreased expression of these *ATG* genes would account for the defective autophagy in *rsc1Δ* (Fig. 4B). The mechanism by which Rsc1-RSC activates these *ATG* genes upon N-starvation is an important question that remains to be answered.

In addition to transcriptional activation of these *ATG* genes, Rsc1-RSC is also required for proper inactivation of the TORC1 pathway through enhancement of Rho1-Kog1 binding (Fig. 4B), although we cannot rule out the possibility that Rsc1-RSC might possibly regulate the expression of other genes to inhibit the TORC1 pathway. Interestingly, Rsc1-RSC is required for the maintenance of Atg8 protein stability, which depends on TORC1 (Fig. 4B). Atg8 is rapidly induced after the shift of the cells to N-starvation conditions, which is required for autophagosome expansion and cargo binding [32]. However, little is known about how Atg8 protein levels are regulated during autophagy. Hog1 MAP kinase participates in autophagy by enhancing the stability of the Atg8 protein during the late stage of persistent ER stress, although the kinase was dispensable for autophagy induced by N-starvation [20]. Given the functional role of autophagy in both normal cellular homeostasis and response to various stress conditions, it is unsurprising that Atg8 protein levels are tightly controlled at multiple levels. Further studies on the regulation of TORC1 and Rsc1-RSC should provide greater insight into how cells respond to starvation conditions to maintain cellular homeostasis through autophagy.

Conflict of interests

No potential conflicts of interest were disclosed.

Acknowledgments

This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan to ET (22580086), and MY (19780076).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.07.114.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.07.114.

References

[1] C.R. Clapier, B.R. Cairns, The biology of chromatin remodeling complexes, Annu. Rev. Biochem. 78 (2009) 273–304.
[2] B.R. Cairns, Y. Lorch, Y. Li, et al., RSC, an essential, abundant chromatin-remodeling complex, Cell 87 (1996) 1249–1260.
[3] B.R. Cairns, A. Schlichter, H. Erdjument-Bromage, et al., Two functionally distinct forms of the RSC nucleosome-remodeling complex, containing essential AT hook, BAH, and bromodomains, Mol. Cell 4 (1999) 715–723.
[4] H.H. Ng, F. Robert, R.A. Young, et al., Genome-wide location and regulated recruitment of the RSC nucleosome-remodeling complex, Genes Dev. 16 (2002) 806–819.
[5] M. Yukawa, H. Koyama, K. Miyahara, et al., Functional differences between *RSC1* and *RSC2*, components of a for growth essential chromatin-remodeling complex of *Saccharomyces cerevisiae*, during the sporulation process, FEMS Yeast Res. 2 (2002) 87–91.
[6] K.K. Baetz, N.J. Krogan, A. Emili, et al., The *ctf13-30/CTF13* genomic haploin sufficiency modifier screen identifies the yeast chromatin remodeling complex RSC, which is required for the establishment of sister chromatid cohesion, Mol. Cell. Biol. 24 (2004) 1232–1244.
[7] D. Bungard, M. Reed, E. Winter, *RSC1* and *RSC2* are required for expression of mid-late sporulation-specific genes in *Saccharomyces cerevisiae*, Eukaryot. Cell 3 (2004) 910–918.
[8] V. Rossio, E. Galati, M. Ferrari, et al., The RSC chromatin-remodeling complex influences mitotic exit and adaptation to the spindle assembly checkpoint by controlling the Cdc14 phosphatase, J. Cell Biol. 191 (2010) 981–997.
[9] A.L. Chambers, P.M. Brownlee, S.C. Durley, et al., The two different isoforms of the RSC chromatin remodeling complex play distinct roles in DNA damage responses, PLoS One 7 (2012) e32016.
[10] Y. Imamura, F. Yu, M. Nakamura, et al., RSC chromatin-remodeling complex is important for mitochondrial function in *Saccharomyces cerevisiae*, PLoS One 10 (2015) e0130397.
[11] B. Wilson, H. Erdjument-Bromage, P. Tempst, et al., The RSC chromatin remodeling complex bears an essential fungal-specific protein module with broad functional roles, Genetics 172 (2006) 795–809.
[12] N. Mizushima, T. Yoshimori, Y. Ohsumi, The role of Atg proteins in autophagosome formation, Annu. Rev. Cell Dev. Biol. 27 (2011) 107–132.
[13] T. Noda, Y. Ohsumi, Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast, J. Biol. Chem. 273 (1998) 3963–3966.
[14] M. Rose, F. Winston, P. Hieter, Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, New York, 1990.

[15] H. Koyama, M. Itoh, K. Miyahara, et al., Abundance of the RSC nucleosome-remodeling complex is important for the cells to tolerate DNA damage in *Saccharomyces cerevisiae*, FEBS Lett. 531 (2002) 215–221.
[16] E. Tsuchiya, T. Hosotani, T. Miyakawa, A mutation in *NPS1*/*STH1*, an essential gene encoding a component of a novel chromatin-remodeling complex RSC, alters the chromatin structure of *Saccharomyces cerevisiae* centromeres, Nucleic Acids Res. 26 (1998) 3286–3292.
[17] T. Shintani, D.J. Klionsky, Cargo proteins facilitate the formation of transport vesicles in the cytoplasm to vacuole targeting pathway, J. Biol. Chem. 279 (2004) 29889–29894.
[18] T. Noda, A. Matsuura, Y. Wada, et al., Novel system for monitoring autophagy in the yeast *Saccharomyces cerevisiae*, Biochem. Biophys. Res. Commun. 210 (1995) 126–132.
[19] T. Inai, M. Yukawa, E. Tsuchiya, Interplay between chromatin and trans-acting factors on the *IME2* promoter upon induction of the gene at the onset of meiosis, Mol. Cell. Biol. 27 (2007) 1254–1263.
[20] A.A. Bicknell, J. Tourtellotte, M. Niwa, Late phase of the endoplasmic reticulum stress response pathway is regulated by Hog1 MAP kinase, J. Biol. Chem. 285 (2010) 17545–17555.
[21] Y. Kamada, K. Yoshino, C. Kondo, et al., Tor directly controls the Atg1 kinase complex to regulate autophagy, Mol. Cell. Biol. 30 (2010) 1049–1058.
[22] Y. Kabeya, Y. Kamada, M. Baba, et al., Atg17 functions in cooperation with Atg1 and Atg13 in yeast autophagy, Mol. Biol. Cell 16 (2005) 2544–2553.
[23] G. Yan, Y. Lai, Y. Jiang, The TOR complex 1 is a direct target of Rho1 GTPase, Mol. Cell 45 (2012) 743–753.
[24] B.B. Fuchs, E. Mylonakis, Our paths might cross: the role of the fungal cell wall integrity pathway in stress response and cross talk with other stress response pathways, Eukaryot. Cell 8 (2009) 1616–1625.
[25] T. Hosotani, H. Koyama, M. Uchino, et al., *PKC1*, a protein kinase C homologue of *Saccharomyces cerevisiae*, participates in microtubule function through the yeast EB1 homologue, *BIM1*, Genes Cells 6 (2001) 775–788.
[26] B. Chai, J.M. Hsu, J. Du, et al., Yeast RSC function is required for organization of the cellular cytoskeleton via an alternative PKC1 pathway, Genetics 161 (2002) 575–584.
[27] L.A. Garraway, E.S. Lander, Lessons from the cancer genome, Cell 153 (2013) 17–37.
[28] C. Kadoch, D.C. Hargreaves, C. Hodges, et al., Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy, Nat. Genet. 45 (2013) 592–601.
[29] E. White, Deconvoluting the context-dependent role for autophagy in cancer, Nat. Rev. Cancer 12 (2012) 401–410.
[30] K. Suzuki, Y. Ohsumi, Molecular machinery of autophagosome formation in yeast, *Saccharomyces cerevisiae*, FEBS Lett. 581 (2007) 2156–2161.
[31] F. Reggiori, D.J. Klionsky, Autophagic processes in yeast: mechanism, machinery and regulation, Genetics 194 (2013) 341–361.
[32] Z. Xie, U. Nair, D.J. Klionsky, Atg8 controls phagophore expansion during autophagosome formation, Mol. Biol. Cell 19 (2008) 3290–3298.