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Rck1 up-regulates pseudohyphal growth by activating the Ras2 and MAP kinase pathways independently in *Saccharomyces cerevisiae*

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ABSTRACT

Previously, we reported that Rck1 regulates Hog1 and Slt2 activities and affects MAP kinase activity in *Saccharomyces cerevisiae*. Recently, we found that Rck1 up-regulates phospho-Kss1 and phospho-Fus3. Kss1 has been known as a component in the pseudohyphal growth pathway, and we attempted to identify the function of Rck1 in pseudohyphal growth. Rck1 up-regulated Ras2 at the protein level, not the transcriptional level. Additionally, *FLO11* transcription was up-regulated by *RCK1* over-expression. *RCK1* expression was up-regulated during growth on SLAD + 1% butanol medium. On nitrogen starvation agar plates, *RCK1* over-expression induced pseudohyphal growth of colonies, and cells over-expressing *RCK1* showed a filamentous morphology when grown in SLAD medium. Furthermore, 1-butanol greatly induced filamentous growth when *RCK1* was over-expressed. Moreover, invasive growth was activated in haploid cells when *RCK1* was over-expressed. The growth defect of cells observed on 1-butanol medium was recovered when *RCK1* was over-expressed. Interestingly, Ras2 and phospho-Kss1 were up-regulated by Rck1 independently. Together, these results suggest that Rck1 promotes pseudohyphal growth by activating Ras2 and Kss1 *via* independent pathways in *S. cerevisiae*.

1. Introduction

Microorganisms adapt to nutritional conditions, and dimorphic growth of yeast cell is an adaptation pattern of growth against glucose and nitrogen starvation [1–4]. *Candida albicans*, a human pathogen, exhibits dimorphic growth as single cells and filaments and shows strong pathogenicity when in the filamentous form [5–8]. *Saccharomyces cerevisiae* also exhibits dimorphic growth during glucose and nitrogen starvation [1,9,10]. In *S. cerevisiae*, pseudohyphal growth is activated when nitrogen is depleted, and its nutritional signal is regulated by a well-understood cellular signaling pathway [11].

In *S. cerevisiae*, pseudohyphal growth is regulated by two signaling pathways, the MAP kinase (mitogen-activated protein kinase) and cAMP-dependent PKA (protein kinase A) pathways. These two pathways converge at Flo11, which is localized at a downstream region [9,11–16]. The MAP kinase pathway is activated by Ras2, which is an upstream element of the pathway. Ras2 activates Cdc24 and then Cdc42 [17–20]. Activated Cdc42 activates a core MAP kinase, which is composed of Ste20, Ste11, Ste7, Kss1, and Ste12. Ste12 forms a complex with Tec1, binds to the filamentous growth response elements (FREs) of *FLO11*, and then activates filamentous growth [21–24]. Deletion of Kss1 results in the failure of invasive or filamentous growth [9,12,25].

Another pathway is the cAMP-PKA pathway. This adenylate cyclase receives signals from the upstream activators Ras2 and Gpa2 [11,15,16,26–28]. Mep2, which is a high-affinity ammonium permease, acts as an ammonium sensor and regulator of Gpa2 and regulates pseudohyphal growth [29,30]. Activated adenylate cyclase synthesize cAMP, and cAMP activates the PKA complex [18]. The PKA complex is composed of two regulatory subunits (Bcy1) and two catalytic subunits (Tpks) [18,31,32]. The binding of cAMP to the regulatory subunits of the PKA complex induces the release of the catalytic subunits from the PKA complex, and activated Tpk2 up-regulates Flo8 expression, which is a transcriptional activator of filamentous target genes [11,24,33]. Furthermore, Tpk1 also activates filamentous growth by activating Phd1 and up-regulates Flo11 [34,35].

Rck1 and Rck2 are identified as radiation sensitivity complementation kinases from *S. cerevisiae* [36]. Rck1 has been reported to be involved in the cell wall stress response pathway by zymolyase stress and is regulated by the transcriptional activator Rlm1 [37]. Furthermore, Kdx1, a pseudokinase with high homology to Slt2, is involved in pseudohyphal growth in *S. cerevisiae* [38,39]. Previously, we reported that Kdx1 up-regulates Rck1, implying

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that Rck1 may activate pseudohyphal growth. We found that over-expression of Rck1 up-regulated the phosphorylated form of Kss1, a MAP kinase involved in pseudohyphal growth. Furthermore, we found that over-expression of Rck1 up-regulated Ras2 at the protein level, and here, we report the role of Rck1 in pseudohyphal growth in *S. cerevisiae*.

2. Materials and methods

2.1. Strains, media, and growth conditions

The yeast strains used were the *S. cerevisiae* Σ1278b background and its derivatives. The Δ*rck1::G418* mutant strain was created by the PCR-mediated gene disruption metho using the G418 resistance cassette from the plasmid pFA6a-kanMX6. Independently derived haploid strains (created in strains MLY40α and MLY41a) were mated to produce the homozygous diploid strain. Yeast strains were grown in 1% yeast extract, 2% peptone, 2% glucose (YPD), or synthetic defined (SD) medium (6.7 g/L yeast nitrogen base) supplemented with the required auxotrophic supplements. Limiting nitrogen medium contains 0.17% yeast nitrogen base (YNB) without amino acids or ammonium sulfate, 2% glucose, and 50 μM ammonium sulfate (SLAD). Haploid filamentous growth was induced in standard growth medium supplemented with 1% butanol or SLAD medium plus 1% butanol. Diploid filamentous growth was induced in low-nitrogen medium. Invasive growth was assayed on synthetic complete (SC)-Ura.

2.2. Plasmids

The plasmid pRS426-*RCK1* was constructed as follows. A 4.1-kb (*Xho*I/*BamH*I) DNA fragment containing *RCK1* was prepared by PCR using 5′-CTCGAGGAACTATAGCTAAATAACCCA-3′ and 5′-GGATCCC GAGCCACAATATGCGGCAACG-3′ as the forward and reverse primers, respectively. The nucleotide sequences were confirmed by DNA sequencing. The amplified fragments were subcloned into pRS426.

2.3. Photomicroscopy

Whole colony photographs were taken directly on agar plates with a microscope (AXIO Imager A1/M1, Carl Zeiss, Germany). Whole colonies were photographed at 10× magnification.

2.4. Live cell microscopy

Cells were grown overnight, diluted to an optical density at 600 nm (OD_{600}) of 0.2, and grown at 30 °C for 3 h 30 min. The cells were grown in standard medium or under inducing conditions. Filamentous growth was induced as follows: overnight cultured cells were centrifuged, washed, and inoculated at an OD_{600} of 0.2 into low-nitrogen medium or growth mediums plus 1% butanol at 30 °C for 18 h before observation.

2.5. Haploid invasion assays

Strains were patched to synthetic complete (SC)-Ura and incubated for 5 days at 30 °C. Surface cells were gently washed off, and the remaining invaded cells were imaged.

2.6. Cell viability assays

The yeast cells were grown overnight at 30 °C in SD-Ura medium. The cells were pelleted and washed with distilled water. Then, the cells (1×10^6 cells/ml) were plated on a fresh SD-Ura, SLAD-Ura, and SLAD-Ura plus 1% butanol plates and incubated overnight at 30 °C for 7 days.

2.7. Northern blot analysis

Yeast cells were incubated in SD-Ura liquid medium overnight, transferred to fresh SD-Ura medium, and incubated to an OD_{600} of 1.0. Cells were washed with water, transferred to SLAD liquid medium, and incubated for 3 h at 30 °C. The cells were then collected and washed with ice-cold water. Total RNA was extracted using the TRIzol reagent (Life Technologies). Equal amounts (6 μg) of total RNA were separated by 1% formaldehyde agarose gel electrophoresis, transferred to nylon membranes, and hybridized with a ^{32}P-labeled probe at 65 °C in rapid-hybridization buffer (Amersham). Hybridized membranes were washed twice with 2× SSC and 0.1% SDS at RT and twice with 0.2× SSC and 0.1% SDS at 65 °C and exposed to X-ray film. The total RNA and expression of specific genes were probed using radiolabeled PCR fragments containing the ORFs of *FLO11*, *RCK1*, *RAS2*, and *ACT1*. The following forward and reverse primers, respectively, were used: *FLO11*, 5′- ATGCAAAGACCATTTCTACT-3′ and 5′-TGGTATGTGTTGTCTTG AAC -3′; *RCK1*, 5′- GTGAACAGAGCTGCTTGGAAA-3′ and 5′-TACCT GATAATGACATGACG-3′; *RAS2*, 5′- ATGCCTTTGAACAAGTCGAAC-3′ and 5′-AGTCGGTATCTTTGAGTCTC-3′; *ACT1*, 5′-ACACGGTATTGTCA CCAACTGGG-3′ and 5′-AGGACAAAACGGCTTGGAGG-3′.

2.8. Immunoblot analysis

The cells were grown in SD medium until an OD_{600} of approximately 0.5 was attained. Haploid filamentous growth was induced in standard growth medium supplemented with 1% butanol. Cells were harvested by centrifugation. The cell pellet was broken by vortexing with glass beads in 250 μl of cold lysis buffer (50 mM Tris–HCl (pH 7.5), 10% glycerol, 1% Triton X-100, 0.1% SDS, 150 mM NaCl, 50 mM NaF, 1 mM sodium orthovanadate, 50 mM β-glycerol phosphate, 5 mM sodium pyrophosphate, 5 mM EDTA, 1 mM phenylmethylsulfonylfluoride, and protease inhibitors) [40]. Fifty micrograms of protein was separated on SDS-polyacrylamide gels and transferred to nitrocellulose membranes. Immunoblotting was performed with the anti-phospho-p44/42 MAPK (Thr^{202}/Tyr^{204}) antibody at 1:2000, the anti-phospho-p38 antibody at 1:2000 (Cell Signaling), the anti-Ras2 at 1:3000 (y-130 Santa Cruz Biotechnology), and the anti-Pgk1 at 1:50,000 (Invitrogen).

3. Results

3.1. Rck1 up-regulated the phosphorylated form of Kss1

Previously, we reported that Rck1 up-regulates MAP kinase in response to cell wall stress [37]. Interestingly, we found that over-expression of *RCK1* up-regulated phospho-Kss1, and it was detected simultaneously with Slt2. Kss1 functions in the pseudohyphal growth pathway in *S. cerevisiae*. To investigate the function of Rck1 in pseudohyphal growth, we investigated the effect of Rck1 on MAP kinase activity in pseudohyphal growth. As shown in Fig. 1A, we tested the effect of Rck1 on Kss1 phosphorylation and found that phospho-Kss1 was up-regulated by Rck1. Wild type cells and a *RCK1* deletion strain showed similar levels of phospho-Kss1, and 1% 1-butanol up-regulated phospho-Kss1. However, when *RCK1* was over-expressed by a multi-copy plasmid, phospho-Kss1 increased severalfold, suggesting the involvement of Rck1 on pseudohyphal growth in *S. cerevisiae*. To further investigate the function of Rck1 in pseudohyphal growth, we performed Northern blot analysis with *FLO11*, a key protein where the signals for pseudohyphal growth converge and affect *FLO11* expression. As

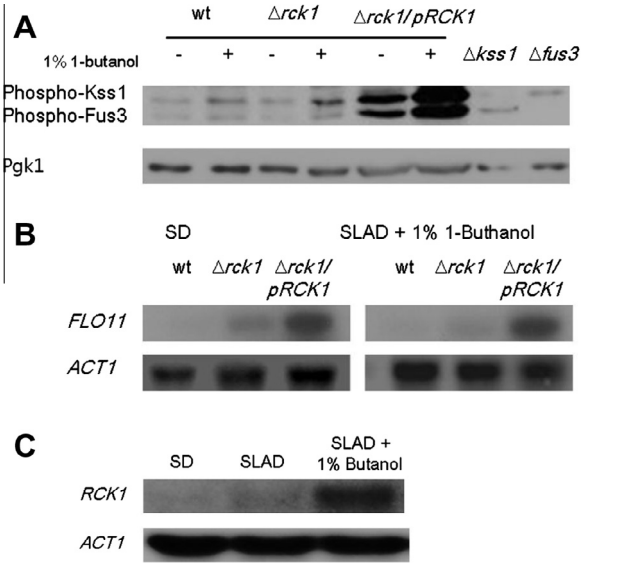

Fig. 1. Rck1 up-regulated phospho-Kss1. (A) The indicated strains were cultured on SLAD or SLAD + 1% butanol media, and total proteins were extracted. Western blotting was performed and probed with an anti-phospho-Kss1 or phosphor-Fus3 antibody. Pgk1 was used as a loading control. Δ*kss1* and Δ*fus3* were used as negative controls. (B) Gene expression of *FLO11* was investigated. The cells were cultured in the indicated media until mid-log phase, and total RNA was then extracted. Northern blots were performed to investigate the expression of *FLO11* and *RCK1*. (C) *ACT1* was used as a loading control.

shown in Fig. 1B, the indicated strains were cultured in SD or SLAD plus 1% 1-butanol media, and total RNAs were extracted. The wild type and *RCK1* deletion strains showed the same level of *FLO11* expression. However, over-expression of *RCK1* caused *FLO11* expression to be up-regulated. Moreover, the up-regulation of *FLO11* by Rck1 was detected in the absence of 1-butanol treatment. Furthermore, as shown in Fig. 1C, the expression of *RCK1* itself was up-regulated by SLAD + 1% butane medium. These results indicate that *RCK1* is involved in pseudohyphal growth by up-regulating *FLO11* expression *via* the MAP kinase pathway.

3.2. Rck1 up-regulated Ras2 at the protein level

Ras2 is a protein localized in an upstream region of the *S. cerevisiae* pseudohyphal growth signaling pathway [41,42]. Nitrogen starvation is recognized on the plasma membrane by Mep2, and the signal is transferred to Cdc25 and Ras2, which in turn affect *FLO11* gene expression [10,29,43]. Ras2 activates Kss1, which is localized in the MAP kinase pathway, and Ras2 and MAP kinase communicate to activate pseudohyphal growth. Conversely, activated Kss1 up-regulates Ras2 again by feedback regulation [44,45]. We investigated the effect of Rck1 on Ras2 expression. As shown in Fig. 2A, we performed a Northern blot analysis of *RAS2* with cells grown in the pseudohyphal growth-inducing medium. Cells were cultured in SD medium or nitrogen starvation SLAD medium, and total RNA was extracted. From the Northern blot analysis, no difference in the transcription of *RAS2* was observed in different media. Neither the *RCK1* wild type, deletion, or over-expression strains exhibited activated transcription of *RAS2*. No effect was observed with 1-butanol treatment. Additionally, we performed Western blot analysis to identify the translational levels of Ras2. As shown in Fig. 2B, the protein level of Ras2 was up-regulated by *RCK1* over-expression. Up-regulation of Ras2 by Rck1 did not depend on 1-butanol. The *RCK1* wild type and deletion strains did not show any difference in Ras2 protein levels. These results indicate that Rck1 up-regulates Ras2 protein level and *FLO11* expression.

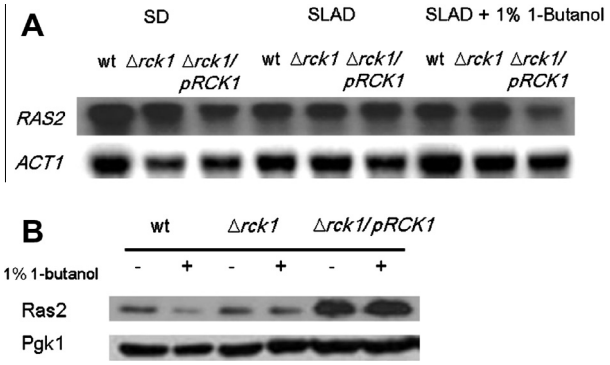

Fig. 2. Rck1 up-regulated the Ras2 protein level. (A) The indicated strains were cultured in SD, SLAD, and SLAD + 1% butanol media. Total RNA was extracted, and Northern blotting was performed to investigate the expression of *RAS2*. ACT1 was used as a loading control. (B) The Ras2 protein level was measured. Total cell lysates were prepared from the cells cultured in SLAD or SLAD + 1% butanol media, and Western blotting was performed. Pgk1 was used as a loading control.

3.3. Rck1 promoted pseudohyphal growth

To investigate pseudohyphal growth further, we constructed diploid cells that originated from the Σ1278b strain, and *RCK1* was then deleted from these diploid cells. Then, the cells were inoculated on SD or SLAD plates. After 14 days, we investigated the colony morphologies and found that *RCK1* over-expression promoted filamentous colony growth on SLAD plates. As shown in Fig. 3A, colonies of wild type cells showed filamentous growth, and the *RCK1* deletion strain failed to grow as filaments. Furthermore, *RCK1* over-expression accelerated pseudohyphal growth compared with the wild type cells. On SD plates, no pseudohyphal growth was found from any of the three strains. Next, we investigated pseudohyphal growth from individual cells. As shown in Fig. 3B, no pseudohyphal growth was observed on SD medium, even when *RCK1* was over-expressed. However, a higher level of pseudohyphal growth was found when *RCK1* was over-expressed. These results indicate that Rck1 is involved in pseudohyphal growth in *S. cerevisiae*. Furthermore, we tested whether Rck1 is involved in invasive growth in haploid cells. As shown in Fig. 3C, the cells were grown on SD or SLAD plates for 5 days, washed thoroughly with tap water, and then imaged. Colonies of cells containing the *RCK1* deletion washed out when washed with tap water. However, most cells remained when *RCK1* was over-expressed. This result indicates that Rck1 is involved in the invasive growth of haploid cells. Generally, cells undergoing invasive growth are resistant to 1-butanol. We investigated the resistance of *RCK1* over-expressing cells to 1-butanol. As shown in Fig. 3D, the cells were inoculated on SD, SLAD, and SLAD plus 1% 1-butanol plates and incubated for 7 days. The wild type cells and *RCK1* deletion strains were sensitive to 1-butanol. However, the *RCK1* over-expression cells grew better than the other strains. This result indicates that Rck1 is involved in pseudohyphal and invasive growth *via* the MAP kinase pathway.

3.4. Rck1 up-regulated Ras2 and Kss1 independently

As shown in Figs. 1 and 2, Rck1 up-regulated Kss1 and Ras2 protein levels, and in turn, Kss1 and Ras2 both up-regulated the gene expression of *FLO11*. Previous studies have shown that Ras2 and Kss1 are components of different pseudohyphal growth pathways [16,44]. However, Ras2 has also been suggested to affect activity upstream of Kss1 [44]. To investigate whether Rck1 regulates Ras2 and Kss1 independently, we performed western blot analysis against Kss1 with the *RAS2* deletion mutant. As shown in Fig. 4A,

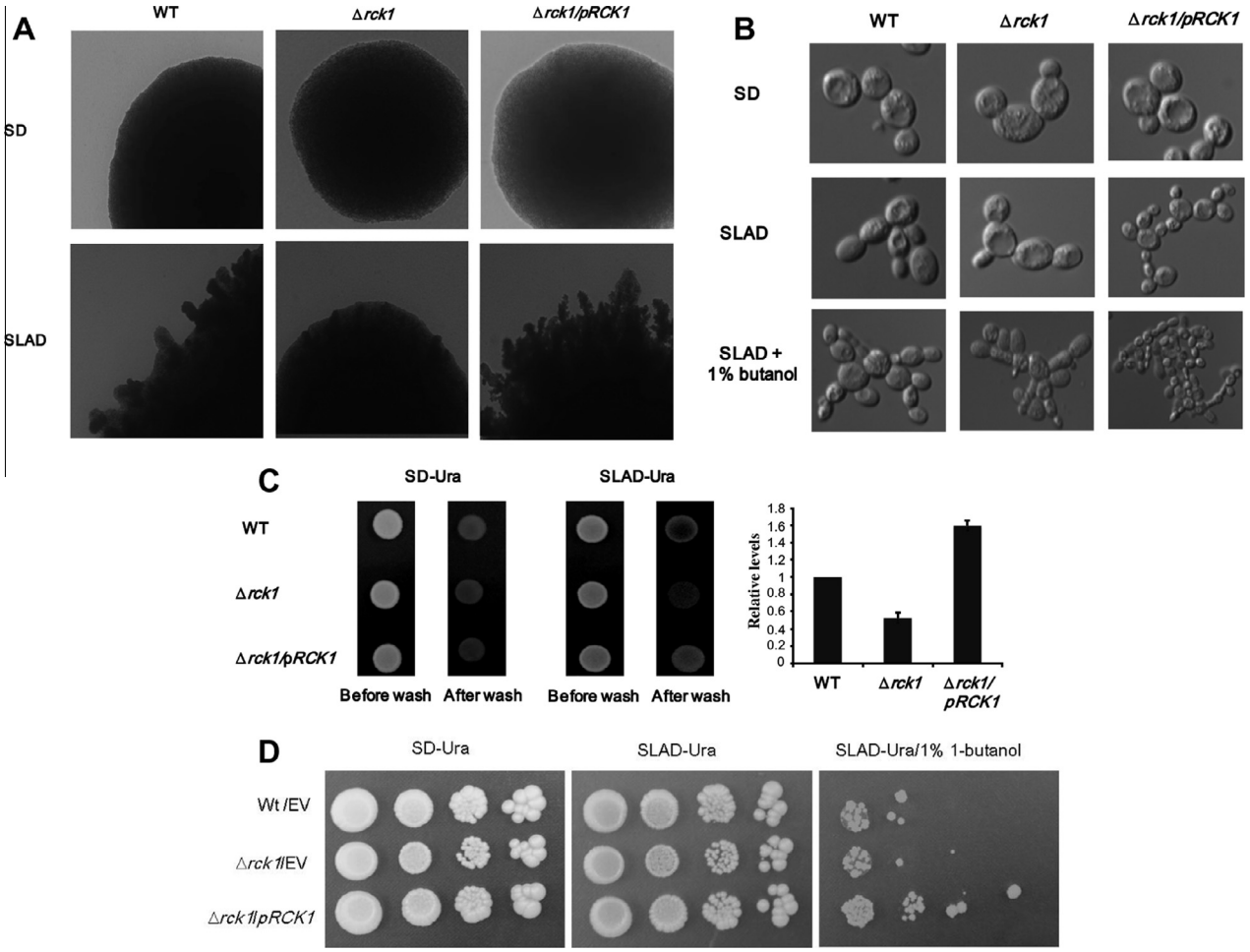

Fig. 3. Rck1 promoted pseudohyphal growth. (A) The effect of Rck1 on pseudohyphal growth was investigated. Indicated strains were inoculated on SD or SLAD plate and incubated for 14 days and then imaged. (B) Filamentous growth of the indicated cells was investigated. The indicated strains were cultured in SD, SLAD or SLAD plus 1-butanol broth for 18 h and then imaged. (C) The effect of Rck1 on the invasive growth of haploid cells was investigated. The indicated haploid strains were incubated on SD or SLAD plate for 7 days, washed with tap water, and then imaged. Relative amounts are normalized to WT. (D) The effect of Rck1 on 1-butanol resistance was investigated. The indicated strains were inoculated on SLAD or SLAD + 1% 1-butanol plates for 5 days and then imaged.

RAS2 deletion had no effect on Kss1 expression. Phospho-Kss1 levels increased, even when *RAS2* was deleted. Phospho-Fus3 levels also increased when Rck1 was over-expressed. Furthermore, we constructed a *KSS1* deletion strain and assayed the Ras2 levels using a western blot. As shown in Fig. 4B, no effect was found on Ras2 expression by Rck1 in the *KSS1* deletion mutant. Based on these results, we suggest that Rck1 regulates Ras2 and Kss1 independently and that the detailed mechanism should be identified.

4. Discussion

In *S. cerevisiae*, pseudohyphal growth is regulated by two signaling pathways, the MAP kinase and cAMP-dependent PKA (protein kinase A) pathways, and these two pathways function independently [9,11–16]. However, these pathways converge downstream at *FLO11* [11,16,33]. Furthermore, previous studies have shown that Ras2 regulates Kss1 activity and that the Ras2 and Kss1-dependent pathways are linked closely to activate pseudohyphal growth [17,44].

Rck1 has been identified as a component of the MAP kinase pathway that responds to cell wall stress [37,46]. Previously, we reported that Rck1 inhibits the Slt2 MAP kinase pathway activity and then Ptp2, which subsequently activates Hog1. Based on these results, we investigated the detailed involvement of Rck1 in the MAP kinase pathway and then pseudohyphal growth, which is regulated by the MAP kinase pathway in response to nutrient deprivation. Kss1 and Fus3 were detected simultaneously when phospho-Slt2 was detected. When we tried to detect phospho-Slt2, we found that the expression of Kss1 and Fus3 was up-regulated by *RCK1* over-expression. It has been reported that Kss1 is involved in pseudohyphal growth, and the phosphorylated form of Kss1 activates pseudohyphal growth [21–24]. From these results, we attempted to identify the role of Rck1 on pseudohyphal growth.

As shown in Fig. 1, we found that *RCK1* over-expression up-regulated phosphorylated Kss1 and *FLO11*. *RCK1* over-expression also resulted in colonies that exhibited filamentous and invasive growth. When *RCK1* was over-expressed, cells showed a more filamentous morphology than the wild type. These results indicate that Rck1 is involved in pseudohyphal growth. Next, we investigated how Rck1 activates pseudohyphal growth. As shown in Fig. 1, Rck1 up-regulated Kss1 and *FLO11*. Interestingly, *RCK1* over-expression also up-regulated Ras2 protein levels, which activates pseudohyphal growth in response to nutrient signals. The Ras2 and MAP kinase pathways are two independent pathways shown to regulate pseudohyphal growth [9,11–16]. However, Ras2 also regulates and communicates with the MAP kinase pathway [17–20].

The Hog1 MAPK pathway is essential for survival in high osmolarity environments. Furthermore, the Hog1 MAPK pathway is known to inhibit pseudohyphal growth under conditions of

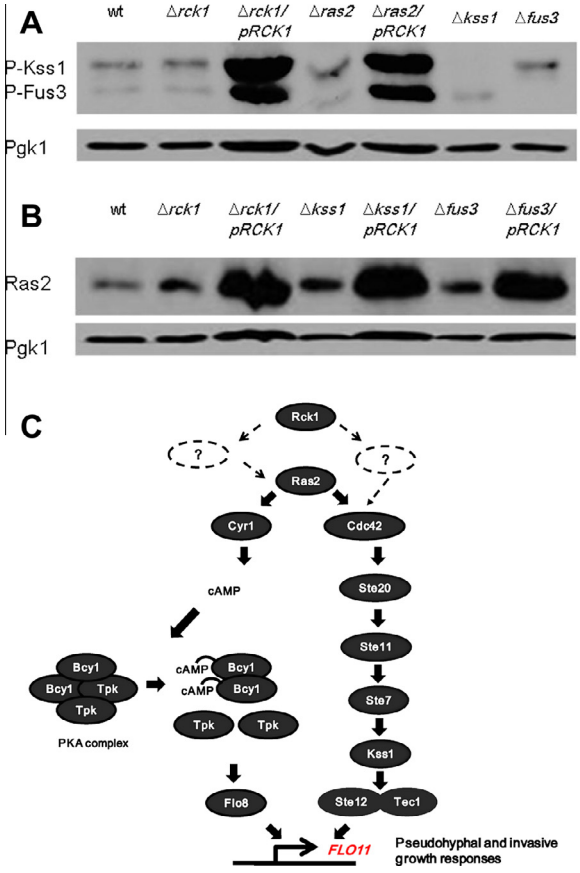

Fig. 4. Rck1 up-regulated Ras2 and Kss1 independently. (A) The effect of Ras2 on the expression of phospho-Kss1 was investigated. The indicated cells were cultured in SD media. The total cell lysates were extracted, and then, Western blotting was performed. Δ*kss1* and Δ*fus3* were used as negative controls. (B) The indicated cells were cultured in SD medium. Total cell lysates were extracted, and then, Western blotting was performed. (C) Rck1 up-regulates yeast pseudohyphal differentiation *via* Ras2 and MAP kinase pathway independently. In this model, Rck1 stimulates phosphorylation of the Kss1 and expression of Ras2. The up-regulation of these genes activate *FLO11* gene expression, which is required for pseudohyphal differentiation.

nitrogen sufficiency. Notably, *PTP2* involved in the nuclear export of Hog1 was stimulated pseudohyphal growth upon overexpression, and our data show the similar patterns of expression by Western blotting (data not shown). Under conditions of nitrogen sufficiency, deletion of Rck1 strain showed slightly up-regulated phosphorylated Hog1 and over-expressed Ptp2 [39]. As shown in Fig. 1B, deletion of Rck1 strain resulted in increased expression of the Ptp2 and thus may enable expression of *FLO11*. We do not understand the exact molecular mechanism by which deletion of Rck1 is transduced into signals affecting filamentous growth. As shown in Fig. 4, we tested whether Rck1 regulates both pathways independently. A *RAS2* deletion mutant did not up-regulate Kss1 by Rck1 and *vice-versa*. These results indicate that Rck1 up-regulates pseudohyphal growth *via* the Ras2 and MAP kinase pathways independently and suggest the possibility of other factors in the upstream region of the Ras2 and MAP kinase pathways; efforts should be made to uncover these factors (Fig. 4C).

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References

[1] C.J. Gimeno, P.O. Ljungdahl, C.A. Styles, G.R. Fink, Unipolar cell divisions in the yeast *S. cerevisiae* lead to filamentous growth: regulation by starvation and RAS, Cell 68 (1992) 1077–1090.
[2] J.R. Broach, R.J. Deschenes, The function of ras genes in *Saccharomyces cerevisiae*, Adv. Cancer Res. 54 (1990) 79–139.
[3] M.I. Borges-Walmsley, A.R. Walmsley, CAMP signalling in pathogenic fungi: control of dimorphic switching and pathogenicity, Trends Microbiol. 8 (2000) 133–141.
[4] R. Dechant, M. Peter, Nutrient signals driving cell growth, Curr. Opin. Cell Biol. 20 (2008) 678–687.
[5] S.C. Cheng, L.A. Joosten, B.J. Kullberg, M.G. Netea, Interplay between *Candida albicans* and the mammalian innate host defense, Infect. Immun. 80 (2012) 1304–1313.
[6] N.A. Gow, F.L. van de Veerdonk, A.J. Brown, M.G. Netea, *Candida albicans* morphogenesis and host defence: discriminating invasion from colonization, Nat. Rev. Microbiol. 10 (2012) 112–122.
[7] I.D. Jacobsen, D. Wilson, B. Wachtler, S. Brunke, J.R. Naglik, B. Hube, *Candida albicans* dimorphism as a therapeutic target, Expert Rev. Anti Infect. Ther. 10 (2012) 85–93.
[8] C. Antachopoulos, T.J. Walsh, E. Roilides, Fungal infections in primary immunodeficiencies, Eur. J. Pediatr. 166 (2007) 1099–1117.
[9] R.L. Roberts, G.R. Fink, Elements of a single MAP kinase cascade in *Saccharomyces cerevisiae* mediate two developmental programs in the same cell type: mating and invasive growth, Genes Dev. 8 (1994) 2974–2985.
[10] P.J. Cullen, G.F. Sprague Jr., The regulation of filamentous growth in yeast, Genetics 190 (2012) 23–49.
[11] X. Pan, J. Heitman, Cyclic AMP-dependent protein kinase regulates pseudohyphal differentiation in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 19 (1999) 4874–4887.
[12] J.G. Cook, L. Bardwell, J. Thorner, Inhibitory and activating functions for MAPK Kss1 in the *S. cerevisiae* filamentous-growth signalling pathway, Nature 390 (1997) 85–88.
[13] H. Liu, C.A. Styles, G.R. Fink, Elements of the yeast pheromone response pathway required for filamentous growth of diploids, Science 262 (1993) 1741–1744.
[14] H.D. Madhani, C.A. Styles, G.R. Fink, MAP kinases with distinct inhibitory functions impart signaling specificity during yeast differentiation, Cell 91 (1997) 673–684.
[15] M.C. Lorenz, J. Heitman, Yeast pseudohyphal growth is regulated by GPA2, a G protein alpha homolog, EMBO J. 16 (1997) 7008–7018.
[16] S. Rupp, E. Summers, H.J. Lo, H. Madhani, G. Fink, MAP kinase and cAMP filamentation signaling pathways converge on the unusually large promoter of the yeast *FLO11* gene, EMBO J. 18 (1999) 1257–1269.
[17] H.U. Mosch, R.L. Roberts, G.R. Fink, Ras2 signals via the Cdc42/Ste20/mitogen-activated protein kinase module to induce filamentous growth in *Saccharomyces cerevisiae*, Proc. Natl. Acad. Sci. USA 93 (1996) 5352–5356.
[18] M. Gagiano, F.F. Bauer, I.S. Pretorius, The sensing of nutritional status and the relationship to filamentous growth in *Saccharomyces cerevisiae*, FEMS Yeast Res. 2 (2002) 433–470.
[19] H.U. Mosch, G.R. Fink, Dissection of filamentous growth by transposon mutagenesis in *Saccharomyces cerevisiae*, Genetics 145 (1997) 671–684.
[20] R.L. Roberts, H.U. Mosch, G.R. Fink, 14-3-3 proteins are essential for RAS/MAPK cascade signaling during pseudohyphal development in *S. cerevisiae*, Cell 89 (1997) 1055–1065.
[21] L. Bardwell, J.G. Cook, J.X. Zhu-Shimoni, D. Voora, J. Thorner, Differential regulation of transcription: repression by unactivated mitogen-activated protein kinase Kss1 requires the Dig1 and Dig2 proteins, Proc. Natl. Acad. Sci. USA 95 (1998) 15400–15405.
[22] J.G. Cook, L. Bardwell, S.J. Kron, J. Thorner, Two novel targets of the MAP kinase Kss1 are negative regulators of invasive growth in the yeast *Saccharomyces cerevisiae*, Genes Dev. 10 (1996) 2831–2848.
[23] V. Gavrias, A. Andrianopoulos, C.J. Gimeno, W.E. Timberlake, *Saccharomyces cerevisiae* TEC1 is required for pseudohyphal growth, Mol. Microbiol. 19 (1996) 1255–1263.
[24] W.S. Lo, A.M. Dranginis, The cell surface flocculin Flo11 is required for pseudohyphae formation and invasion by *Saccharomyces cerevisiae*, Mol. Biol. Cell 9 (1998) 161–171.
[25] N. Hao, N. Yildirim, M.J. Nagiec, S.C. Parnell, B. Errede, H.G. Dohlman, T.C. Elston, Combined computational and experimental analysis reveals mitogen-activated protein kinase-mediated feedback phosphorylation as a mechanism for signaling specificity, Mol. Biol. Cell 23 (2012) 3899–3910.
[26] S. Colombo, P. Ma, L. Cauwenberg, J. Winderickx, M. Crauwels, A. Teunissen, D. Nauwelaers, J.H. de Winde, M.F. Gorwa, D. Colavizza, J.M. Thevelein, Involvement of distinct G-proteins, Gpa2 and Ras, in glucose- and intracellular acidification-induced cAMP signalling in the yeast *Saccharomyces cerevisiae*, EMBO J. 17 (1998) 3326–3341.
[27] L. Kraakman, K. Lemaire, P. Ma, A.W. Teunissen, M.C. Donaton, P. Van Dijck, J. Winderickx, J.H. de Winde, J.M. Thevelein, A *Saccharomyces cerevisiae* G-protein coupled receptor, Gpr1, is specifically required for glucose activation of the cAMP pathway during the transition to growth on glucose, Mol. Microbiol. 32 (1999) 1002–1012.

[28] Y. Xue, M. Batlle, J.P. Hirsch, GPR1 encodes a putative G protein-coupled receptor that associates with the Gpa2p Galpha subunit and functions in a Ras-independent pathway, EMBO J. 17 (1998) 1996–2007.
[29] M.C. Lorenz, J. Heitman, The MEP2 ammonium permease regulates pseudohyphal differentiation in *Saccharomyces cerevisiae*, EMBO J. 17 (1998) 1236–1247.
[30] M.C. Lorenz, J. Heitman, Regulators of pseudohyphal differentiation in *Saccharomyces cerevisiae* identified through multicopy suppressor analysis in ammonium permease mutant strains, Genetics 150 (1998) 1443–1457.
[31] J.F. Cannon, K. Tatchell, Characterization of *Saccharomyces cerevisiae* genes encoding subunits of cyclic AMP-dependent protein kinase, Mol. Cell. Biol. 7 (1987) 2653–2663.
[32] T. Toda, S. Cameron, P. Sass, M. Zoller, J.D. Scott, B. McMullen, M. Hurwitz, E.G. Krebs, M. Wigler, Cloning and characterization of BCY1, a locus encoding a regulatory subunit of the cyclic AMP-dependent protein kinase in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 7 (1987) 1371–1377.
[33] L.S. Robertson, G.R. Fink, The three yeast A kinases have specific signaling functions in pseudohyphal growth, Proc. Natl. Acad. Sci. USA 95 (1998) 13783–13787.
[34] X. Pan, J. Heitman, Sok2 regulates yeast pseudohyphal differentiation via a transcription factor cascade that regulates cell-cell adhesion, Mol. Cell. Biol. 20 (2000) 8364–8372.
[35] C.J. Gimeno, G.R. Fink, Induction of pseudohyphal growth by overexpression of PHD1, a *Saccharomyces cerevisiae* gene related to transcriptional regulators of fungal development, Mol. Cell. Biol. 14 (1994) 2100–2112.
[36] A. Dahlkvist, G. Kanter-Smoler, P. Sunnerhagen, The RCK1 and RCK2 protein kinase genes from *Saccharomyces cerevisiae* suppress cell cycle checkpoint mutations in *Schizosaccharomyces pombe*, Mol. Gen. Genet. 246 (3) (1995) 316–326.
[37] M. Chang, H.J. Kang, I.J. Baek, C.M. Kang, Y.S. Park, C.W. Yun, Kdx1 regulates RCK1 gene expression by interacting with Rlm1 in *Saccharomyces cerevisiae*, Biochem. Biophys. Res. Commun. 435 (2013) 350–355.
[38] R. Garcia, C. Bermejo, C. Grau, R. Perez, J.M. Rodriguez-Pena, J. Francois, C. Nombela, J. Arroyo, The global transcriptional response to transient cell wall damage in *Saccharomyces cerevisiae* and its regulation by the cell integrity signaling pathway, J. Biol. Chem. 279 (2004) 15183–15195.
[39] C.A. Shively, M.J. Eckwahl, C.J. Dobry, D. Mellacheruvu, A. Nesvizhskii, A. Kumar, Genetic networks inducing invasive growth in *Saccharomyces cerevisiae* identified through systematic genome-wide overexpression, Genetics 193 (2013) 1297–1310.
[40] H. Martin, J.M. Rodriguez-Pachon, C. Ruiz, C. Nombela, M. Molina, Regulatory mechanisms for modulation of signaling through the cell integrity Slt2-mediated pathway in *Saccharomyces cerevisiae*, J. Biol. Chem. 275 (2000) 1511–1519.
[41] M. Barbacid, Ras genes, Annu. Rev. Biochem. 56 (1987) 779–827.
[42] J.M. Gancedo, Control of pseudohyphae formation in *Saccharomyces cerevisiae*, FEMS Microbiol. Rev. 25 (2001) 107–123.
[43] J.R. Broach, Nutritional control of growth and development in yeast, Genetics 192 (2012) 73–105.
[44] N. Sengupta, P.K. Vinod, K.V. Venkatesh, Crosstalk between cAMP-PKA and MAP kinase pathways is a key regulatory design necessary to regulate *FLO11* expression, Biophys. Chem. 125 (2007) 59–71.
[45] V.A. Cherkasova, R. McCully, Y. Wang, A. Hinnebusch, E.A. Elion, A novel functional link between MAP kinase cascades and the Ras/cAMP pathway that regulates survival, Curr. Biol. 13 (2003) 1220–1226.
[46] E. Bilsland, S. Molin, S. Swaminathan, A. Ramne, P. Sunnerhagen, Rck1 and Rck2 MAPKAP kinases and the HOG pathway are required for oxidative stress resistance, Mol. Microbiol. 53 (6) (2004) 1743–1756.