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Note

The *Saccharomyces cerevisiae* Phosphatase Activator RRD1 Is Required to Modulate Gene Expression in Response to Rapamycin Exposure

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ABSTRACT

We show that mutants lacking either the phosphatase activator Rrd1 or the phosphatase Pph3 are resistant to rapamycin and that double mutants exhibit a synergistic response. This phenotype could be related to an inability of the mutants to degrade RNA polymerase II, leading to transcription of critical genes that sustain growth.

THE yPtpa1/Rrd1 protein shares 35% identity with the human phosphotyrosyl phosphatase activator, hPTPA, which has been proposed to function as a phosphatase activator (Cayla *et al.* 1994; Janssens *et al.* 1998; Ramotar *et al.* 1998). Rrd1 interacts with Sit4, a catalytic subunit belonging to the PP2A Ser/Thr phosphatase family (Douville *et al.* 2004; Zheng and Jiang 2005), and both proteins participate in the same genetic pathway to mediate resistance to 4-nitroquinoline-1-oxide and ultraviolet A (320–400 nm) light (Douville *et al.* 2004). The Rrd1–Sit4 complex may function to activate gene expression, as Sit4 is required for expression of several genes, *e.g.*, *SWI4* (Fernandez-Sarabia *et al.* 1992).

*rrd1*Δ mutants are resistant to the immunosuppressant rapamycin, suggesting that Rrd1 plays a role in the cellular response to this drug (Rempola *et al.* 2000). In yeast, rapamycin binds to the peptidyl-prolyl isomerase Fpr1 and this complex inactivates the Tor1, -2 kinases (Heitman *et al.* 1991), leading to the activation of Sit4 via dissociation from an inhibitor protein Tap42 (Di Como and Arndt 1996; Jiang and Broach 1999). The activated Sit4 dephosphorylates several targets, including the nutrient-responsive transcriptional activator Gln3 (which translocates to the nucleus to activate *GLN1* and *MEP2* expression) and the Ser/Thr kinase Npr1, which regulates the amino acid permeases (Schmidt *et al.* 1998). In general, rapamycin generates a profound modification in the transcriptional profile of yeast. While some genes, *e.g.*, the diauxic shift genes *CPA2* and *PYC1*, are upregulated by rapamycin exposure, others, such as the ribosomal protein genes including *RPS26A*, *RPL30*, and *RPL9*, are downregulated (Hardwick *et al.* 1999; Powers and Walter 1999).

Here, we investigated the cellular response mechanism of *rrd1*Δ mutants to rapamycin. We first assessed whether *rrd1*Δ mutants respond to the initial challenge of rapamycin by examining Npr1 kinase phosphorylation status and *MEP2* expression (Schmelzle *et al.* 2004). As shown in Figure 1, rapamycin quickly triggered Npr1 dephosphorylation, as well as *MEP2* expression, in both the parent and the mutant, eliminating a role for Rrd1 in these early events. We next determined the effect of rapamycin on the growth rate of the *rrd1*Δ mutant. As shown in Figure 2A, the parent and *rrd1*Δ mutant grew at nearly the same rate in the absence of rapamycin, but growth was reduced when the strains were challenged with rapamycin. Interestingly, growth of the parent ceased by 9 hr, while the mutant continued to grow (Figure 2A) and divide (data not shown) in the presence of rapamycin. Thus, the mechanism by which rapamycin induces growth arrest appears to depend on Rrd1.

A genomewide screen revealed that the Pph3 catalytic subunit of the PP2A family is involved in rapamycin resistance (Chan *et al.* 2000). We therefore tested whether Rrd1 might function via Pph3. As expected, growth of the parent ceased within 9 hr, while the *rrd1*Δ and *pph3*Δ single mutants continued growing in the presence of rapamycin (Figure 2B). Importantly, the *rrd1*Δ *pph3*Δ double mutant showed a synergistic response (Figure 2B), suggesting that Rrd1 and Pph3 act separately to mediate rapamycin-induced growth arrest. Whether Rrd1 acts via another member of the PP2A family, such as Pph21 and Pph22, is not known (Stark 1996).

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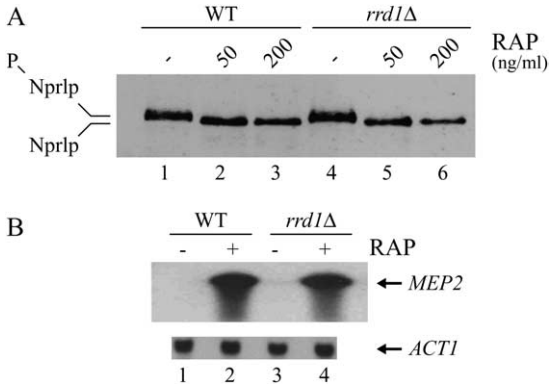

FIGURE 1.—*rrd1*Δ mutant exhibits a normal response to the early events of rapamycin exposure. (A) Rapamycin-induced dephosphorylation of the Npr1 kinase in the parent and *rrd1*Δ mutant. Exponentially growing wild-type (WT, SEY6210) (*MAT*α, *leu2-3 112, ura3-52, his3-*Δ*200, trp1-*Δ*901, lys2-801, suc2-*Δ*9, Mel*$^-$) and the *rrd1*Δ mutant bearing the plasmid pHA-NPR1 (kindly provided by Michael Hall, Basel, Switzerland) expressing a HA-Npr1 fusion protein were treated without and with rapamycin (Sigma, St. Louis) (50 and 200 ng/ml for 15 min) and the phosphorylation status of the HA–Npr1 kinase was determined by Western blot using an anti-HA monoclonal antibody (Santa Cruz). Samples without rapamycin were treated with a drug vehicle (90% ethanol, 10% Tween 20). (B) Rapamycin-induced expression of the *MEP2* gene in the parent and the *rrd1*Δ mutant. Cells were treated as in A and total RNA was probed for expression of the *MEP2* and *ACT1* genes. The data are representative of three independent experiments. RAP, rapamycin.

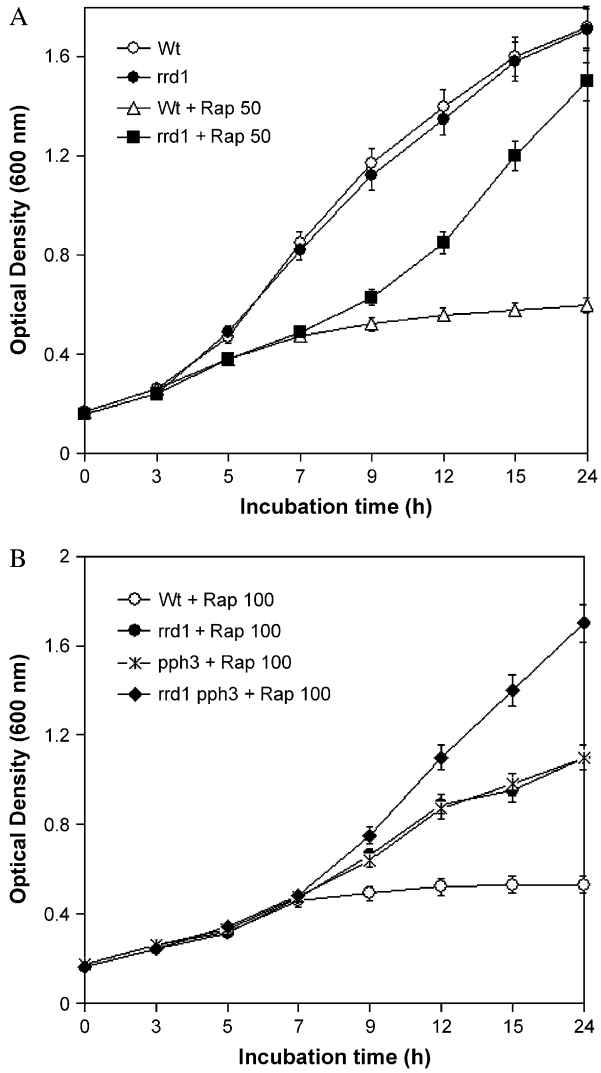

FIGURE 2.—Growth of the parent and the mutants *rrd1*Δ, *pph3*Δ, and *rrd1*Δ *pph3*Δ in the absence or presence of rapamycin. (A and B) Exponential-phase cells, wild-type strain (SEY6210), and the indicated isogenic mutants growing in YPD medium were diluted to an optical density of 600 nm (OD_{600}) of 0.2 in fresh YPD medium with the drug vehicle and with rapamycin (50 or 100 ng/ml). Growth of the cultures was monitored by measuring the OD_{600} at the indicated time. The data are the average of three independent experiments. RAP, rapamycin.

We next tested whether Rrd1 could influence expression of genes known to be regulated in response to rapamycin (HARDWICK *et al.* 1999). Expression of the *CPA2* and *PYC1* genes was induced in the parent strain, but not in the *rrd1*Δ mutant (Figure 3A), suggesting that Rrd1 is required to mediate rapamycin-induced gene expression. Since rapamycin is also known to trigger the downregulation of some genes, *e.g.*, *RPS26A* and *RPL9A*, we tested whether Rrd1 is involved in this process. The *RPS26A* gene was nearly completely downregulated in the parent strain, but remained partially expressed in the *rrd1*Δ mutant upon rapamycin exposure (Figure 3B). Since *RPL9A* is downregulated in both strains, it appears that a subset of rapamycin-responsive genes is under the control of Rrd1. In fact, Rrd1 is required to support activator-dependent *in vitro* transcription of a well-characterized reporter E4 (WU *et al.* 1996; data not shown), consistent with Rrd1 involvement in gene expression.

Since Rrd1 interacts with Sit4 and this complex has been shown to dephosphorylate the target proteins Sap185 and Gln3 (CRESPO *et al.* 2002; FELLNER *et al.* 2003), it is reasonable to assume that Rrd1 could affect gene expression by modulating the phosphorylation status of the C-terminal domain (CTD) of the Rpb1 subunit of RNA polymerase II upon rapamycin exposure. As such, the parent strain, the isogenic single mutants *rrd1*Δ and *pph3*Δ, and the double mutant *rrd1*Δ *pph3*Δ were assessed for the phosphorylation status of Rpb1. As shown in Figure 4A, the high-molecular-weight bands (~150–200 kDa), corresponding to a heterogeneous population of Rpb1 polypeptides, disappeared when the parent strain was treated with rapamycin. Interestingly, a polypeptide band of ~70 kDa was intensified following the drug treatment (Figure 4A), suggesting that Rpb1 is not dephosphorylated, but proteolytically processed. It is noteworthy that rapamycin treatment caused degradation of both the phosphorylated and the nonphosphorylated form of Rpb1 (data not shown). When a similar experiment was conducted with the *rrd1*Δ mutant, the phenomenon was significantly reduced, and

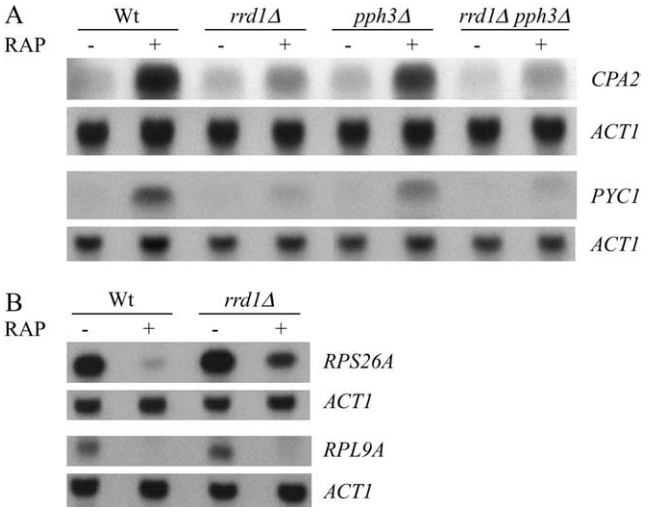

Figure 3.—Northern blot analysis of rapamycin-responsive genes in the parent and the mutants *rrd1*Δ, *pph3*Δ, and *rrd1*Δ *pph3*Δ . (A) Rapamycin-induced expression of the *CPA2* and *PYC1* genes is Rrd1 dependent. Exponentially growing cells in YPD medium were treated with a drug vehicle and with rapamycin (200 ng/ml) for 30 min before extracting total RNA. Samples of this RNA were analyzed by Northern blot for the expression of the *CPA2* and *PYC1* genes. (B) Rapamycin-induced downregulation of the ribosomal gene *RSP26A* is partially dependent on Rrd1. Cells were cultured as described in A, except that they were treated for 2 hr. Total RNA was probed for expression of the *RSP26A* and *RPL9A* genes. For A and B, the actin gene *ACT1* was used as a control for total RNA loaded on the gel. The data are representative of three independent experiments. RAP, rapamycin; Wt, wild-type strain (SEY6210).

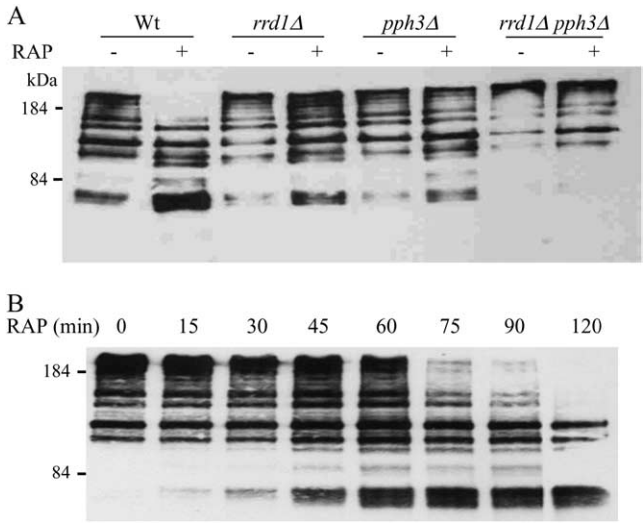

Figure 4.—Rapamycin exposure triggers degradation of RNA polymerase II large subunit Rpb1. (A) Rrd1 and Pph3 are required for rapamycin-induced degradation of Rpb1. Exponentially growing cells, wild type, and the indicated mutant strains were treated with a drug vehicle or with rapamycin (200 ng/ml) for 2 hr. Total protein extracts were derived from these samples and analyzed for the Rpb1 protein by Western blot using the H14 antibody (Covance), which recognizes phospho-Ser5 of the CTD of Rpb1. (B) Time-course analysis of Rpb1 degradation in the wild-type strain exposed to rapamycin. Exponentially growing wild-type cells were treated with a fixed concentration of rapamycin (200 ng/ml) and samples were taken at the indicated time points. Samples were processed for Western blot as in A. The data are representative of three independent experiments. RAP, rapamycin.

more dramatically in the *rrd1*Δ *pph3*Δ double mutant (Figure 4A), but not in the *gln3*Δ mutant, which also displays rapamycin resistance (data not shown) (Cardenas *et al.* 1999; Powers and Walter 1999). These data are consistent with a model whereby both Rrd1 and Pph3 contribute separately to the molecular process that acts independently of the Gln3 signaling pathway to trigger degradation of Rpb1 upon rapamycin exposure. We note that the timing of Rpb1 degradation coincided with the reported kinetics of downregulation of the ribosomal protein genes that occurred within 60 min following rapamycin treatment (Cardenas *et al.* 1999; Powers and Walter 1999). At least, >50% of Rpb1 was converted to the 70-kDa form after 75 min of rapamycin treatment (Figure 4B). Thus, the downregulation of the ribosomal protein genes triggered by rapamycin could be a consequence of increased degradation of Rpb1 in the parent strain.

The rationale underlying the potential degradation of Rpb1 during rapamycin treatment may be the necessity of the cell to drastically reduce its metabolism. Since rapamycin mimics starvation conditions, drug-treated cells will adapt to a specific energy-saving response involving the downregulation of several physiological processes, including transcription. In this context, destruction of an important fraction of Rpb1 would be an efficient mechanism to prevent unnecessary initiation of gene transcription. Although the half-life of Rpb1 (<75 min) correlates with the timing of the downregulation of the ribosomal protein genes triggered by rapamycin, the cell growth ceased only at a much later time, presumably due to the additional time required to dilute and turn over all the Rpb1 and various other molecules (Cardenas *et al.* 1999). Thus, preventing the destruction of Rpb1, as in the case of *rrd1*Δ mutants and the even more pronounced case of *rrd1*Δ *pph3*Δ double mutants, is expected to permit continuous expression of critical genes required to promote growth in the presence of rapamycin.

The only other protein documented to be degraded in response to rapamycin is the translation initiation factor eIF-4G (Berset *et al.* 1998). eIF-4G is degraded within 2–3 hr in response to rapamycin treatment, while other initiation factors, such as eIF-4E associated with eIF-4G, remain unaffected (Berset *et al.* 1998). The degradation of eIF-4G requires a functional Tor1–kinase signaling pathway (Berset *et al.* 1998). Since the time frame during which Rpb1 is degraded coincides with that of eIF-4G, it is likely that Rpb1 degradation is also via the Tor1 pathway. If so, Rrd1 might execute a function downstream of the Tor1 pathway that culminates in

the activated expression of a protease (*e.g.*, those induced by rapamycin such as Lap3, Lap 4, Pep4, and Pbr1) that could degrade key proteins involved in translation and transcription (Mewes *et al.* 1999). Clearly, additional experiments are needed to unravel the exact molecular function by which Rrd1 controls rapamycin resistance.

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LITERATURE CITED

Berset, C., H. Trachsel and M. Altmann, 1998 The TOR (target of rapamycin) signal transduction pathway regulates the stability of translation initiation factor eIF4G in the yeast Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA **95:** 4264–4269.

Cardenas, M. E., N. S. Cutler, M. C. Lorenz, C. J. Di Como and J. Heitman, 1999 The TOR signaling cascade regulates gene expression in response to nutrients. Genes Dev. **13:** 3271–3279.

Cayla, X., C. Van Hoof, M. Bosch, E. Waelkens, J. Vandekerckhove *et al.*, 1994 Molecular cloning, expression, and characterization of PTPA, a protein that activates the tyrosyl phosphatase activity of protein phosphatase 2A. J. Biol. Chem. **269:** 15668–15675.

Chan, T. F., J. Carvalho, L. Riles and X. F. Zheng, 2000 A chemical genomics approach toward understanding the global functions of the target of rapamycin protein (TOR). Proc. Natl. Acad. Sci. USA **97:** 13227–13232.

Crespo, J. L., T. Powers, B. Fowler and M. N. Hall, 2002 The TOR-controlled transcription activators GLN3, RTG1, and RTG3 are regulated in response to intracellular levels of glutamine. Proc. Natl. Acad. Sci. USA **99:** 6784–6789.

Di Como, C. J., and K. T. Arndt, 1996 Nutrients, via the Tor proteins, stimulate the association of Tap42 with type 2A phosphatases. Genes Dev. **10:** 1904–1916.

Douville, J., J. David, P. K. Fortier and D. Ramotar, 2004 The yeast phosphotyrosyl phosphatase activator protein, yPtpa1/Rrd1, interacts with Sit4 phosphatase to mediate resistance to 4-nitroquinoline-1-oxide and UVA. Curr. Genet. **46:** 72–81.

Fellner, T., D. H. Lackner, H. Hombauer, P. Piribauer, I. Mudrak *et al.*, 2003 A novel and essential mechanism determining specificity and activity of protein phosphatase 2A (PP2A) in vivo. Genes Dev. **17:** 2138–2150.

Fernandez-Sarabia, M. J., A. Sutton, T. Zhong and K. T. Arndt, 1992 SIT4 protein phosphatase is required for the normal accumulation of SWI4, CLN1, CLN2, and HCS26 RNAs during late G1. Genes Dev. **6:** 2417–2428.

Hardwick, J. S., F. G. Kuruvilla, J. K. Tong, A. F. Shamji and S. L. Schreiber, 1999 Rapamycin-modulated transcription defines the subset of nutrient-sensitive signaling pathways directly controlled by the Tor proteins. Proc. Natl. Acad. Sci. USA **96:** 14866–14870.

Heitman, J., N. R. Movva and M. N. Hall, 1991 Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. Science **253:** 905–909.

Janssens, V., C. Van Hoof, W. Merlevede and J. Goris, 1998 PTPA regulating PP2A as a dual specificity phosphatase. Methods Mol. Biol. **93:** 103–115.

Jiang, Y., and J. R. Broach, 1999 Tor proteins and protein phosphatase 2A reciprocally regulate Tap42 in controlling cell growth in yeast. EMBO J. **18:** 2782–2792.

Mewes, H. W., K. Heumann, A. Kaps, K. Mayer, F. Pfeiffer *et al.*, 1999 MIPS: a database for genomes and protein sequences. Nucleic Acids Res. **27:** 44–48.

Powers, T., and P. Walter, 1999 Regulation of ribosome biogenesis by the rapamycin-sensitive TOR-signaling pathway in Saccharomyces cerevisiae. Mol. Biol. Cell **10:** 987–1000.

Ramotar, D., E. Belanger, I. Brodeur, J. Y. Masson and E. A. Drobetsky, 1998 A yeast homologue of the human phosphotyrosyl phosphatase activator PTPA is implicated in protection against oxidative DNA damage induced by the model carcinogen 4-nitroquinoline 1-oxide. J. Biol. Chem. **273:** 21489–21496.

Rempola, B., A. Kaniak, A. Migdalski, J. Rytka, P. P. Slonimski *et al.*, 2000 Functional analysis of RRD1 (YIL153w) and RRD2 (YPL152w), which encode two putative activators of the phosphotyrosyl phosphatase activity of PP2A in Saccharomyces cerevisiae. Mol. Gen. Genet. **262:** 1081–1092.

Schmelzle, T., T. Beck, D. E. Martin and M. N. Hall, 2004 Activation of the RAS/cyclic AMP pathway suppresses a TOR deficiency in yeast. Mol. Cell. Biol. **24:** 338–351.

Schmidt, A., T. Beck, A. Koller, J. Kunz and M. N. Hall, 1998 The TOR nutrient signalling pathway phosphorylates NPR1 and inhibits turnover of the tryptophan permease. EMBO J. **17:** 6924–6931.

Stark, M. J., 1996 Yeast protein serine/threonine phosphatases: multiple roles and diverse regulation. Yeast **12:** 1647–1675.

Wu, Y., R. J. Reece and M. Ptashne, 1996 Quantitation of putative activator-target affinities predicts transcriptional activating potentials. EMBO J. **15:** 3951–3963.

Zheng, Y., and Y. Jiang, 2005 The yeast phosphotyrosyl phosphatase activator is part of the Tap42-phosphatase complexes. Mol. Biol. Cell **16:** 2119–2127.

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