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Deletion of RTS1, Encoding a Regulatory Subunit of Protein Phosphatase 2A, Results in Constitutive Amino Acid Signaling via Increased Stp1p Processing

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Received 21 September 2005/Accepted 4 November 2005

In Saccharomyces cerevisiae, extracellular amino acids are sensed at the plasma membrane by the SPS sensor, consisting of the transporter homologue Ssy1p, Ptr3p, and the endoprotease Ssy5p. Amino acid sensing results in proteolytic truncation of the transcription factors Stp1p and Stp2p, followed by their relocation from the cytoplasm to the nucleus, where they activate transcription of amino acid permease genes. We screened a transposon mutant library for constitutively signaling mutants, with the aim of identifying down-regulating components of the SPS-mediated pathway. Three isolated mutants were carrying a transposon in the RTS1 gene, which encodes a regulatory subunit of protein phosphatase 2A. We investigated the basal activity of the AGP1 and BAP2 promoters in rts1Δ cells and found increased transcription from these promoters, as well as increased Stp1p processing, even in the absence of amino acids. Based on our findings we propose that the phosphatase complex containing Rts1p keeps the SPS-mediated pathway down-regulated in the absence of extracellular amino acids by dephosphorylating a component of the pathway.

The yeast Saccharomyces cerevisiae has developed a complex regulatory network enabling it to control the production of nutrient transporters depending on substrate availability in the environment. Hexose transporters and amino acid transporters are examples of nutrient transporters that are transcriptionally induced by their substrates (14, 24, 44). Amino acids are imported into the cells through amino acid permeases (AAPs) (20). The yeast AAPs belong to the amino acid/polyamine/organocation (APC) superfamily of transporters (28). They exhibit a range of affinities and specificities for all 20 common amino acids and a number of other compounds (42). The presence of extracellular amino acids results in increased transcription of about a third of the AAP genes (14). Amino acids are sensed in proteolytic truncation of the transcription factors Stp1p and Stp2p, followed by their relocation from the cytoplasm to the nucleus, where they activate transcription of amino acid permease genes. We screened a transposon mutant library for constitutively signaling mutants, with the aim of identifying down-regulating components of the SPS-mediated pathway. Three isolated mutants were carrying a transposon in the RTS1 gene, which encodes a regulatory subunit of protein phosphatase 2A. We investigated the basal activity of the AGP1 and BAP2 promoters in rts1Δ cells and found increased transcription from these promoters, as well as increased Stp1p processing, even in the absence of amino acids. Based on our findings we propose that the phosphatase complex containing Rts1p keeps the SPS-mediated pathway down-regulated in the absence of extracellular amino acids by dephosphorylating a component of the pathway.

Transcriptional induction is mediated by the homologous transcription factors Stp1p and Stp2p, which bind to the promoters of the AAP genes at the UASaa (upstream activating sequence) first identified in the BAP3 promoter (6) and then in the BAP2 promoter (37), but later also found in the promoter sequences of the AAP genes GNPI, AGPI, MUPI, TAT1, TAT2, and DIP5 (10). In the absence of amino acids, Stp1p and Stp2p are present mainly in the cytoplasm (3, 10). When amino acids are detected in the environment, 10 kDa of the N terminus are endoproteolytically cleaved off, resulting in relocation of the transcription factors to the nucleus (3). Ssy5p is the endoprotease responsible for processing of Stp1p (1, 2). Signaling and processing are moreover dependent on the F-box protein Grr1p (1, 5, 11, 26), which is part of the E3 ubiquitin ligase SCF (Skp1-Cullin-F-box) complex SCFGrr1 (38). Though SCFCinh1 normally targets proteins for degradation by the 26S proteasome, activation of Stp1p is not dependent on the 26S proteasome (1). Other factors involved in amino induction include casein kinase I (1). Furthermore, Ptr3p is found to interact with Yfr021wp in a two-hybrid screen (18). The same work reports that yfr021wpΔ and ypl100hΔ strains exhibit ptr3Δ-like phenotypes, i.e., they are unable to grow at a high concentration of histidine.

Mutants that exhibit constitutive expression of the target AAP genes independently of the components of the SPS sensor have been identified (15). Here we report a screen likewise aimed at identifying factors down-regulating SPS-mediated signaling, but allowing for factors that, when mutated, cause (constitutive) signaling only if the sensor is present. Using a strain deficient in histidine synthesis, we selected for growth on minimal ammonium medium supplemented with the dipeptide Gly-His. Dipeptidases, including Gly-His, are taken up by the permease Ptr2p (39), which is under transcriptional control of
the amino acid-sensing pathway (4, 8). Subsequent cleavage of internalized Gly-His satisfies the growth requirement for histidine (39). Thus, growth on Gly-His reflects activation of the signaling pathway, so that growth in the absence of an inducing amino acid reveals that the amino acid-sensing pathway is turned on constitutively. The constitutive mutants thus found were tested for constitutive induction of the BAP2 gene, encoding a branched-chain amino acid permease, using the β-galactosidase reporter assay. After this secondary screen the mutants included some with transposon insertions in the RTS1 gene. RTS1 encodes a regulatory subunit of protein phosphatase 2A (PP2A). We subsequently studied the role of this gene in SP5-mediated amino acid signaling.

MATERIALS AND METHODS

Strains and media. The S. cerevisiae strains used in this study are listed in Table 1 and are all derived from strain M4054 (MATα ura3 gal1Δ) (19), which in turn is derived from S288C. The HIS3 and LEU2 genes were disrupted in M4054; the resulting strain was named M4605 and was used as the host for the transposon mutagenesis library, as described below. The histidine-independent strain M5397 (MATα ura3 his3 leu2 gal1Δ rts1Δ) was constructed by crossing M4999 (MATα ura3 his3 gal1Δ rts1Δ) with the isogenic X2180-1b (MATα). Spores with the desired genotype (ura3 HIS3) were tested for the gal1Δ phenotype on minimal citrulline medium (20, 43).

The RTS1/rtsl genotype was tested on SD plates supplemented with uracil (1% [wt/vol] succinic acid, 0.6% [wt/vol] NaOH, 0.17% [wt/vol] Bacto yeast nitrogen base without amino acids, 2% Bacto agar, 2% [wt/vol] glucose, 20 mg/liter uracil), at the center of which a filter was placed with 4 mg of the toxic dipeptide L-leucyl-L-ethionine, as described previously (8). Equal amounts of cells were tested for constitutive induction of the gal1Δ gene, encoding a branched-chain amino acid permease, using the β-galactosidase reporter assay. After this secondary screen the mutants included some with transposon insertions in the RTS1 gene. RTS1 encodes a regulatory subunit of protein phosphatase 2A (PP2A). We subsequently studied the role of this gene in SP5-mediated amino acid signaling.

<table>
<thead>
<tr>
<th>Strain</th>
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<th>Reference</th>
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<td>MATα ura3 gal2 CUP1 ura3 gal1Δ grr1Δ STP1-ZZ</td>
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</table>

Western blot analysis. Cells carrying a fusion (Stp1-2Z) of an immunoglobulin G-binding domain of the Staphylococcus aureus protein A to the C terminus of Stp1p (40) were cultivated in SD medium supplemented with uracil and 0.5 g/liter Gly-His dipeptide; their ability to grow on SD plus Ura plus 30 mg/liter Ile plus 20 mg/liter His plus 60 mg/liter Val plus 100 mg/liter mersulfuron methyl; and finally by their constitutively active BAP2 promoter.

β-Galactosidase reporter assay. Strains of interest were transformed with plasmid pRB108 or pTID1 (7, 42), centromere-based plasmids in which 1.0 kb of the amino acid-inducible AGP1 promoter and 683 bp of the BAP2 promoter, respectively, are fused to Escherichia coli lacZ. Transformants were grown overnight in SD medium to an optical density of 0.60 nm of 0.1 to 0.3. The cultures were then divided in aliquots to which different compounds were added: L-citrulline (Sigma) to a final concentration of 5 mM or an equal volume of water, and L-leucine to a final concentration of 100 μM. Each treatment was performed in duplicate on aliquots originating from the same culture. β-Galactosidase assays were performed as described previously (7). Miller unit values were calculated as follows: (optical density at 420 nm × 1.000) / (cell volume [ml] × reaction time [min] × optical density at 600 nm at harvest).

Staplypsopeptidase analysis. Cells carrying a fusion (Stp1-2Z) of an immunoglobulin G-binding domain of the Staphylococcus aureus protein A to the C terminus of Stp1p (40) were cultivated in SD supplemented with 20 mg/liter uracil to the early exponential phase. Cells were harvested and proteins were extracted under denaturing conditions with NaOH and β-mercaptoethanol before being enriched by precipitation with trichloroacetic acid. Protein concentrations were determined using the Bio-Rad protein assay. Proteins were separated on NuPAGE 10% Bis-Tris gels (Invitrogen) and transferred to polyvinylidene difluoride membranes. Chemiluminescent immunodetection was performed as described previously (41). The extent of binding of the antibody against horseradish peroxidase was quantified using the Image Quant software 5.0 (Molecular Dynamics) with a local median-based background correction.
RESULTS

Isolation of mutants constitutive in amino acid signaling. In order to select mutants with increased activity of the amino acid-sensing pathway, a screen was designed in which a his3 leu2 derivative of the reference strain M4054 was subjected to transposon mutagenesis. The host strain was transformed with a mixture of yeast genomic fragments representing a mutagenized LEU2-based plasmid library (46). Mutants of interest were selected using three different screening criteria. First, we took advantage of the fact that dipeptide uptake (39), as well as branched-chain amino acid uptake (7), is very low in cells grown on minimal ammonium medium in the absence of leucine. Dipeptides, including Gly-His, are taken up by the peptide transporter Ptr2p (39), which is under the transcriptional control of the amino acid-sensing pathway (4). Mutants with constitutive signaling were thus selected on SD medium supplemented with the Gly-His dipeptide, which, upon intracellular cleavage, provides the histidine required for growth (39). A subsequent selection was carried out on SD medium supplemented with the amino acids Ile, His, and Val, and the sulfonamide herbicide metsulfuron methyl. This type of compound inhibits synthesis of branched-chain amino acids and therefore prevents growth of cells unable to take up branched-chain amino acids from the medium (53), such as mutants deficient in amino acid signaling (29, 30). Finally, colonies of interest were tested for constitutive activity of the BAP2 promoter.

Among the 11 mutants thus isolated, three had transposon insertions in the PP2A subunit-encoding gene RTS1 (34). The remaining insertions were localized in VPS15, VPS34, and VPS16, coding for proteins involved in vacuolar protein sorting, and STH1, coding for the ATPase subunit in the chromatin remodeling complex RSC. Two others had mutations in ORFs with unknown function, YFR044C and YFR045W. Two mutants were not mapped. In the present work, the relationship between the phenotype and the locus affected by transposon insertion was analyzed in more detail for the RTS1 gene.

Deletion of RTS1 results in constitutive activation of BAP2 and AGP1 transcription. Since transposon insertion very often completely inactivates a gene, we wished to analyze the behavior of a strain deleted for RTS1. One of the selection criteria used in the original screen was the ability to take up Gly-His on SD medium, thereby promoting growth of histidine-requiring cells in the absence of leucine. Dipeptides, including Gly-His, are taken up by the peptide transporter Ptr2p (39) is thus under transcriptional control of the amino acid-sensing pathway (4). Insensitivity to L-leucyl-L-ethionine therefore reflects that the pathway is not activated, while growth inhibition in the absence of an inducing amino acid indicates constitutive activation of the amino acid-sensing pathway. The rts1Δ strain M5397 was unable to grow on minimal medium in the presence of L-leucyl-L-ethionine, suggesting constitutive activity of the signaling pathway (not shown).

We also investigated activation of the promoter of the broad-specificity AAP gene AGP1 using a fusion of the promoter to the E. coli lacZ gene. Cells were grown in SD medium and incubated for 40 min with or without 100 μM L-leucine. The experiment was repeated with 5 mM L-citrulline or an equal volume of water. These concentrations have been previously found to fully or almost fully induce the AGP1 promoter (16, 40). The results show that AGP1 transcription in rts1Δ cells is constitutive, i.e., unaffected by leucine or citrulline addition. In the wild-type control experiment, AGP1 promoter activation was low in the absence of amino acids and increased strongly in response to either leucine or citrulline (Table 2).

Transcriptional induction of AAP genes was previously shown to involve Stp1p endoproteolysis (3). We used the Stp1p-ZZ construct previously described (40), in which the C terminus of Stp1p was fused to a doublet of the IgG-binding Z domain of the S. aureus protein A. This insert was integrated in the wild-type strain and the rts1Δ strain, resulting in strains M5447 and M5474, respectively. The fraction of processed Stp1p in rts1Δ cells in the absence of leucine was greater than in wild-type cells (30% versus 13%), whereas addition of leucine lead to almost complete (above 90%) Stp1p processing in wild-type cells (30% versus 13%). We used the wild-type strain and the rts1Δ strain M5397 because of intracellular cleavage of the dipeptide. To find out if an rts1Δ strain exhibits the same phenotype, we deleted the RTS1 ORF in a his3 derivative of the reference strain M4054. Indeed, the lack of RTS1 enabled growth on the dipeptide as a histidine source (not shown).

In order to be able to analyze the rts1Δ mutation in the absence of extracellular amino acids or dipeptides, we also constructed a HIS3 rts1Δ strain (M5397). First we wished to confirm the activation of target promoters for the amino acid signaling by testing for growth on YPD supplemented with metsulfuron methyl; the strain grew, as expected (not shown). Then we tested whether this activation was constitutive by yet another criterion, using the dipeptide L-leucyl-L-ethionine. If this dipeptide is taken up, it is cleaved to yield the toxic methionine analogue L-ethionine. Also, uptake of the dipeptide L-leucyl-L-ethionine is mediated up by the peptide transporter Ptr2p (39) and is thus under transcriptional control of the amino acid-sensing pathway (4).
Remarkably, Stp1p processing to the extent of 30% is sufficient to generate an activity of the \textit{AGP1} promoter comparable to that observed in wild-type cells after amino acid addition (compare Table 2 and Table 3). In fact, this is expected from previous comparisons of dose-response relationships using quantification of Stp1p processing versus \textit{AGP1} promoter activity as the read-out (40, 41). The sensing of l-leucine exhibits a 50% effective concentration of 12 \muM under the growth conditions studied when Stp1p processing is monitored, but only 1 \muM when \textit{AGP1} promoter-driven \textit{lacZ} expression is measured (40). The most obvious interpretation is that saturation of the promoter activity occurs already at modest levels of activated Stp1p.

\textbf{Epistasis relationships.} In order to further investigate the role of \textit{RTS1}, we constructed strains M4600 (\textit{sys1A rts1A}), M5437 (\textit{ptr3A rts1A}), M5439 (\textit{sys5A rts1A}), M5470 (\textit{grr1A rts1A}), and M4608 (\textit{sys1A \textit{sys2A} rts3A rts1A}). \textit{STP3} is homologous to \textit{STP1} and \textit{STP2}, and Helge A. Andersen (personal communication) has found that deletion of \textit{STP3} further reduces the low, remaining activity of the amino acid signaling pathway in an \textit{sys1A \textit{sys2A}} mutant. All of the resulting strains were sensitive to metsulfuron methyl on YPD, and all were insensitive to l-leucyl-l-ethionine (not shown), suggesting that the amino acid-sensing pathway was inactive.

\textit{β}-Galactosidase assays were performed on extracts from cells in which the \textit{E. coli lacZ} gene was placed under the control of the \textit{AGP1} promoter. \textit{β}-Galactosidase measurements confirmed that the multiple mutants behaved similarly to the single mutants deficient in signaling, i.e., \textit{AGP1} transcription was very low even in the presence of extracellular amino acids (Table 4). Using the Stp1-ZZ construct, we also measured relative amounts of processed and full-length Stp1p in each of the strains, in the absence and presence of amino acids. Stp1p was exclusively present as the full-length protein in all single and multiple mutants (Fig. 2). In other words, \textit{sys1}, \textit{ptr3}, \textit{sys5}, and \textit{grr1} are epistatic over \textit{rts1}.

\textbf{DISCUSSION}

In this work we have attempted to identify potential negative regulators of the amino acid-sensing pathway. For this purpose we have designed a screen allowing selection of mutants with constitutive activity of the pathway. The screen described in this report was performed in the absence of inducer in cells lacking \textit{GAP1}, and we investigated transcription levels of a \textit{lacZ} reporter gene placed under the control of the amino acid-inducible promoters of the \textit{AGP1} and \textit{BAP2} genes, which are known targets of the SPS-mediated pathway. The genes that were disrupted by transposon insertion encode proteins that could be involved at any level of the signaling.

The isolated mutants include some in which the transposon insertion disrupted the \textit{RTSI} gene. This gene encodes a regulatory subunit of PP2A, which is a major serine/threonine phosphatase involved in several nutrient-induced signaling pathways, in cell growth control and cell division control (9, 35, 45, 56). Protein phosphatase 2A exists in several isoforms and is mostly present in cells as a heterotrimeric complex, consisting of a catalytic (C) subunit, encoded by \textit{PPH21}, \textit{PPH22}, or \textit{PPH3} (45, 49), a scaffolding subunit (A), encoded by \textit{TPD3} (55), and a regulatory subunit (B or B’), encoded by \textit{CDC55} (22) and \textit{RTSI} (47, 48), respectively. The B and B’ subunits are believed to regulate the activity of PP2A by determining its cellular location and modifying the substrate specificity of the C subunit (31, 54). \textit{CDC55} is required for correct cell cycle checkpoint control (35), and \textit{cdc55Δ} cells display a cold-sensi-

\begin{table}[h]
\caption{\textit{AGP1} promoter activity in cells deleted of genes encoding positive factors in amino acid signaling and/or \textit{RTSI} in the absence and presence of inducing amino acids}
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
\textbf{Inducer} & \multicolumn{6}{c|}{\textbf{Mean β-galactosidase activity ± SEM}} \\
& \textbf{M4054 (wild type)} & \textbf{M5397 (\textit{rtsiA})} & \textbf{M4600 (\textit{sys1A rtsiA})} & \textbf{M5437 (ptr3A rtsiA)} & \textbf{M5439 (sys5A rtsiA)} & \textbf{M5471 (grr1A rtsiA)} & \textbf{M4608 (\textit{rtsiA sys1A sys2A rts3A})} \\
\hline
l-Leucine (100 \muM) & & & & & & & \\
\hline
\textbf{−} & 1.7 ± 0.1 & 10.1 ± 0.2 & 1.1 ± 0.1 & −0.1 ± 0.0 & −0.8 ± 0.0 & 0.1 ± 0.0 & \\
& 7.2 ± 0.6 & 9.6 ± 0.2 & 1.1 ± 0.0 & −0.1 ± 0.0 & −0.6 ± 0.0 & −0.1 ± 0.0 & \\
\hline
\textbf{+} & 0.1 ± 0.0 & 0.95 ± 0.1 & 0.0 ± 0.0 & 0.0 ± 0.0 & 0.0 ± 0.0 & 0.0 ± 0.0 & \\
\hline
l-Citulline (5 mM) & & & & & & & \\
\hline
\textbf{−} & & & & & & & \\
\hline
& 0.1 ± 0.0 & 0.95 ± 0.1 & 0.0 ± 0.0 & 0.0 ± 0.0 & 0.0 ± 0.0 & 0.0 ± 0.0 & \\
\hline
& 11.3 ± 1.2 & 0.83 ± 0.1 & 0.0 ± 0.0 & 0.0 ± 0.0 & 0.0 ± 0.0 & 0.0 ± 0.0 & \\
\hline
\end{tabular}
\end{table}

\textit{a} Activity in Miller units in the absence (−) and 40 minutes after the addition (+) of inducer.
tive phenotype but no phenotype at elevated temperatures (22). RTS1 was found in two independent genetic screens, as a multicopy suppressor of hsp60(Ts) mutant alleles (48), and later as a Rox Three Suppressor (12); rts1Δ cells are thermosensitive and exhibit a typical cdc mutant phenotype (47).

We identified RTS1 as a negative component of the SPS-mediated amino acid-sensing pathway. Deletion of RTS1 results in constitutive transcription of AGPI and BAP2. Moreover, overs, syi1, ptr3Δ, syi3Δ, and grr1Δ were found to be epistatic over rts1. These results indicate that the PP2A is involved in the SPS-mediated pathway and suggest that a dephosphorylation step is required to down-regulate signaling in the absence of extracellular amino acids. Rts1Δr associated with Tpd3p and the C subunit might thus dephosphorylate one of the SPS proteins. Alternatively, it could dephosphorylate the Stp transcription factors, resulting in a conformational change that perhaps limits its accessibility of the cleavage site to the protease, thereby impairing Stp1Δr and Stp2Δr proteolysis and activation in the absence of amino acids.

While PP2A appears to down-regulate amino acid signaling, a corresponding kinase should be involved in the activation of the pathway. Casein kinase I is a candidate for this activity, since the amino acid-sensing pathway is inactive in temperature-sensitive mutants affected in the casein kinase I genes YCK1 and YCK2, and these strains exhibit loss of Stp1Δr processing (1). Casein kinase I and PP2A are known to act on the same substrate in Xenopus embryos (17) and we propose that they do so in yeast as well. This hypothesis is substantiated by the finding that the yeast Cdc55p subunit forms a complex with casein kinase I (23). Moreover, casein kinase I has also been found to interact with casein kinase I like Cdc55p does. This is the case for Mth1p (50) and for the G1 cyclin Cln2p (25). The finding that the yeast Cdc55p subunit forms a complex with casein kinase I (23) Moreover, casein kinase I has also been found to interact with casein kinase I like Cdc55p does. This is the case for Mth1p (50) and for the G1 cyclin Cln2p (25).

Mth1Δ and Std1Δ interact with the transcriptional repressor Rgt1Δr in the absence of glucose: the resulting complex binds promoter DNA, thereby repressing transcription from the HXT genes (33, 52). Likewise, Stp1Δr is phosphorylated in a casein kinase I-dependent way prior to its endoproteolytic activation (1). SFCGR1 recognizes phosphorylated substrates; this is the case for Mth1Δr (50) and for the G1 cyclin Cln2p (25). Although Stp1Δr is phosphorylated by casein kinase I (1), there is no reason to invoke it as a target for SFCGR1. A possible target for SFCGR1 is Ptrl3Δp, which in fact has been found to be subject to posttranslational modification, exhibiting a slower-migrating band (13) that might be due to ubiquitination. Alternatively, the target for SFCGR1 could be a protein not yet known to be part of the pathway. Further work is needed to propose which protein is a target for PP2A associated with Rts1Δp. There may, however, be cross talk between the amino acid-sensing and the target of rapamycin (TOR) pathways, since N. Eckert-Boulet (unpublished data) has observed that treatment of rts1Δ cells with rapamycin enhanced the constitutive signal, also at the level of Stpl-ZZ processing.

Interestingly, it has recently been found that PP2A is involved in regulating the induction of HXT1 by glucose and that Cdc55p is the regulatory subunit involved. The 14-3-3 proteins Bmh1Δr and Bmh2Δp also appear to be involved (51). These observations add to existing similarities between the glucose induction pathway and the amino acid induction pathway. It will be interesting to see whether Bmh1Δp and Bmh2Δp are involved in the SPS-mediated pathway and whether Rts1Δr interacts with casein kinase I like Cdc55p does.

In summary, we have shown that Rts1Δp, one of the two known regulatory subunits of PP2A, is a down-regulator of the SPS-mediated amino acid-sensing pathway. This finding illustrates a new role in nutrient-induced signaling for PP2A and further highlights the reuse of components in different signaling pathways.

ACKNOWLEDGMENTS

We acknowledge Helge A. Andersen for very fruitful discussions. Part of this work was financed by the Danish BioloInstrument Centre (DABIC). We are grateful for a fellowship from the Knut and Alice Wallenberg Foundation to Katrin Larsson.

REFERENCES


