Tsc1+ and *tsc2*+ Regulate Arginine Uptake in *S. pombe*

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Mutations in either *TSC1* or *TSC2* cause tuberous sclerosis. Homologs for *TSC1* and *TSC2* genes have been identified in mouse, rat, *Drosophila*, and in the yeast *Schizosacharomyces pombe.* Data shows that S. pombe lacking tsc1+ or tsc2+ exhibit decreased arginine uptake and low intracellular levels of four members of the arginine biosynthesis pathway. Intracellular levels of amino acids were measured using a Biochrom 30 Amino Acid Analyzer.

Tuberous sclerosis complex (TSC) is a tumor suppressor syndrome characterized by seizures, mental retardation, and benign tumors of the skin, brain, heart, and kidneys. TSC is caused by mutations in either *TSC1* or *TSC2*. Hamartin, the *TSC1* gene product, and tuberin the *TSC2* gene product, are known to interact and function in a complex. Tuberin has a highly conserved GTPase-activating protein (GAP) domain with activity with activity for Rheb1.

Schizosacharomyces pombe contains genes with significant similarity to *TSC1* and *TSC2*, which are named *tsc1*+ and *tsc2*+, and a Rheb1 homolog *rhb1*+. Here data shows that S. pombe lacking *tsc1*+ or *tsc2*+ have similar phenotypes, including a defect in arginine uptake and low intracellular levels of four members of the arginine biosynthesis pathway. The transcriptional profile and intracellular amino acid levels associated with Δ*tsc1* and Δ*tsc2* overlap extensively, suggesting similar roles for *tsc1*+ and *tsc2*+ in *S. pombe*. Results suggest that *S. pombe* can be utilized as a model for studying TSC and indicate that *S. pombe* Tsc1 and Tsc2 proteins play a central role in amino acid biosynthesis and sensing.

Method and Results.

972Δ*tsc1* and 972Δ*tsc2* Have a Defect in Argnine Uptake

Δ*tsc1* and Δ*tsc2* were crossed into the 972 background, a strain that does not require amino acid supplements. Results showed that 972Δ*tsc1* and 972Δ*tsc2* were resistant to 60μ/ml canavanine, a toxic analog of arginine. Canavanine is toxic to wild-type 972. To determine whether canavanine resistance was the result of decreased uptake, the uptake of [^{3}H] arginine was measured. After 10 mins., arginine uptake was ~3.5 fold less in the 972Δ*tsc1* and 972Δ*tsc2* compared with wildtype 972, indicating that the canavanine resistance is the result of decreased uptake.

Dominant negative Rhb1 Can Rescue the Arginine Uptake Defect in uraΔtsc2.

Rhb1 has been previously shown to regulate arginine uptake in S. pombe. Rhb1-D60K is a dominant negative form of Rhb1 that is unable to bind GTP or GDP. Results obtained showed that decreased arginine uptake observed in Δtsc2 was restored by expression of Rhb1-D60K, but not by wild-type Rhb1 suggesting that arginine uptake is regulated through Tsc1, Tsc2, and Rhb1 in S. pombe.

Intracellular amino acid concentrations are decreased in 972Δtsc1 and 972Δtsc2

Protein extracts were prepared from 972Δ*tsc1* and 972Δ*tsc2* and 972. The sample (100 μl) was injected into the Biochrom 30 Amino Acid analyzer for analysis.

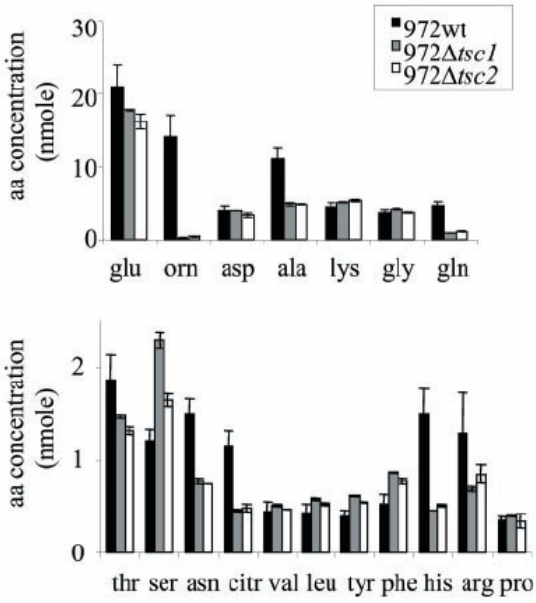

Figure 1. *Intracellular amino acid levels in 972Δ*tsc1 *and 972Δ*tsc2 *were compared with wild-type yeast. A decrease in at least 40% was detected for alanine, asparagine, histidine, glutamine, ornithine, citrulline, and arginine in 972Δ*tsc1 *and 972Δ*tsc2*. Two biological replicates were run for each sample, and similar results were seen in two independent experiments.*

A standard amino acid mixture (10 nM) was also included. Results showed that the intracellular levels of multiple amino acids were low in 972Δ*tsc1* and 972Δ*tsc2* compared to the 972 wild-type yeast (Figure 1). Ornithine, which is a product of both glutamate and arginine metabolism, showed the largest relative decrease from ~15 nM in wild-type 972 to nearly undetectable levels in 972Δ*tsc1* and 972Δ*tsc2*, whereas lysine was not changed (Figure 2). A decrease in at least 40% was detected for alanine, asparagine, histidine, glutamine, ornithine, citrulline, and arginine. The latter four are linked to arginine biosynthesis. Low intracellular amino acid levels and decreased arginine uptake suggests that yeast lacking *tsc1*+ and *tsc2*+ have an intrinsic defect in amino acid sensing.

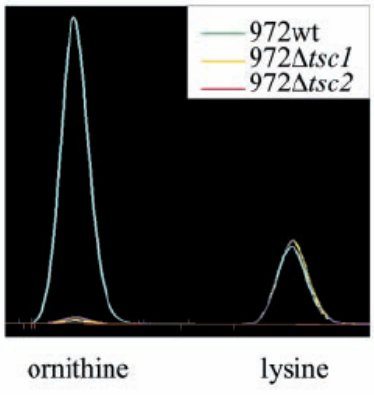

***Figure 2**. Intracellular amino acid levels in 972Δtsc1 and 972Δtsc2 were compared with wild-type yeast. Ornithine and lysine amino acid profile in wild-type 972, 972Δtsc1 and 972Δtsc2. Ornithine levels were decreased in 972Δtsc1 and 972Δtsc2, whereas lysine levels were similar for wildtype, 972Δtsc1 and 972Δtsc2.*

Conclusions

• S. pombe lacking tsc1+ and tsc2+ have defects in arginine uptake and low intracellular amino acid levels, suggesting defects in amino acid sensing.

• Rhb1 regulates arginine uptake in S. pombe. The arginine uptake defect in Δtsc2 was rescued by the expression of a dominant negative form of S. pombe Rhb1-D60K. The rescue suggests that Rhb1 is downstream of Tsc2 in S. pombe as well as other species and further strengthens the relevance of S. pombe as a model system to study human TSC.

• Low intracellular amino acids, including alanine, asparagine, histidine, glutamine, ornithine, citrulline, and arginine were observed. The inability to respond appropriately to low amino acid levels suggests that Tsc1 and Tsc2 play a role in amino acid sensing. In Drosphila S2 cells subjected to RNA interference using siRNA against TSC2, amino acid levels of valine, leucine, phenylalanine and lysine were measured and no difference in intracellular levels was observed. These four amino acids were not changed in S. pombe lacking tsc1+ and tsc2+.

• Findings support S. pombe as a model for studies of human TSC.

Data excerpted from **M van Slegtenhorst, E Carr, R Stoyanova, W D. Kruger, and E Petri Henske *Tsc1*+ and *tsc2*+ Regulate Arginine Uptake and Metabolism in *Schizosaccharomyces pombe* J. Biol. Chem., Mar 2004; 279: 12706 - 12713**