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Loss of *RPS41* but not its paralog *RPS42* results in altered growth, filamentation and transcriptome changes in *Candida albicans*

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ABSTRACT

Although ribosomal proteins (RPs) are components of the ribosome, and function centrally in protein synthesis, several lines of evidence suggest that S4 ribosomal proteins (Rps4ps) can function in other cellular roles. In *Candida albicans*, ribosomal protein S4 (Rps4p) is encoded by two distinct but highly similar genes, *RPS41* (*C2_10620W_A*) and *RPS42* (*C1_01640W_A*). Previous studies indicated that in *Saccharomyces cerevisiae* loss of one isoform generated distinct phenotypes. To probe this relationship in *C. albicans*, *rps41*Δ and *rps42*Δ homozygous null mutants were generated. The transcript levels of the *RPS41* and *RPS42* genes are asymmetric in *C. albicans*, *RPS41* mRNA levels were similar in wild-type strains and *rps42*Δ null mutants, while *RPS42* gene transcript levels were induced 20 fold relative to wild type in *rps41*Δ null mutants. We found that the *rps41*Δ homozygous null mutant showed a reduced growth rate, and had defects in filament formation in liquid media and on solid media, while these phenotypes were not observed in the *rps42*Δ mutant strain. Neither the *rps41*Δ nor *rps42*Δ mutant strains displayed differential sensitivity to azoles, although intriguingly ectopic expression of either *RPS41* or *RPS42* in a wild-type strain leads to decreased sensitivity to fluconazole (FLC). *C. albicans* cDNA microarray analysis experiments found that carbohydrate and nitrogen metabolic processes were repressed but transport-process-related genes were up-regulated in the *rps41*Δ mutant. Overall, our present study suggests that loss of the *RPS41* gene but not its paralog the *RPS42* gene can generate distinct phenotypes including effects on growth rate, morphological transitions, and susceptibility to osmotic stress due to the fact that mRNA levels of *RPS41* is much higher than *RPS42* in *C. albicans*.

1. Introduction

The ribosome, which is the site of mRNA translation and protein synthesis, is a complex molecular machine (Sonenberg and Hinnebusch, 2009). For example, in *Saccharomyces cerevisiae*, the active 80S ribosome consists of a small 40S subunit made up of an 18S rRNA and 32 proteins and a large 60S subunit including 25S, 5.8S, and 5S rRNAs and 46 proteins (Green and Noller, 1997; Spahn et al., 2001). The eukaryotic ribosome is responsible for translating mRNA into protein, and it is generally perceived as a key component of the cell, with a non-selective role in polypeptide synthesis (Byrne, 2009). However, in fungi, plants and animals, mutations in genes encoding ribosomal proteins (RPs) and ribosome assembly factors can lead to specific physiological defects (Dignard et al., 2008; Strittmatter et al., 2006; Szakonyi and Byrne, 2011). Although some of the consequences of reduced ribosomal protein (RP) function probably result from a global reduction in protein synthesis, the existence of specific physiological defects suggests that the ribosome may have a regulatory role in physiological functions.

The eukaryotic ribosomal protein S4 (Rps4p), which has no homolog in bacteria, is one of the proteins of the small ribosomal subunit. In *S. cerevisiae*, Rps4p is encoded by the duplicated genes *RPS4A* and *RPS4B*. Mutations in these two paralogous ribosomal protein genes generate completely distinct phenotypes. Loss of *RPS4A* gives phenotypes showing differences in cell size (Jorgensen et al., 2002) and CG4-theopalaumide sensitivity

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(Parsons et al., 2006), while deletion of *RPS4B* can lead to abnormal telomere length (Askree et al., 2004), modified neomycin sulfate sensitivity and hydrogen peroxide sensitivity (Parsons et al., 2006). The Rps4p of wheat has been suggested to act as a general cysteine protease, and can hydrolyze a variety of cysteine protease substrates (Sudhamalla et al., 2012). In mammals, Rps4p is encoded by three genes, *RPS4X*, *RPS4Y1* and *RPSY2*, which are located on the X and the Y chromosomes (Lopes et al., 2010), and might be involved in Turner syndrome (Fisher et al., 1990; Zinn et al., 1994). In human males, *RPS4X* and *RPS4Y1* are expressed ubiquitously, but *RPSY2* expression is restricted to the testis and prostate, suggesting a male-specific role for this RP and the possibility of testis-specific ribosomes (Lopes et al., 2010). Although the sequences of the three Rps4ps are very similar, distinct amino acids in the carboxyl terminus of *RPS4Y2* possibly facilitate unique interactions with distinct, potentially testis-specific, RPs or extra-ribosomal factors (Lopes et al., 2010). In *Candida albicans*, Rps4p is encoded by two isogenes, *RPS41* (*C2_10620W_A*) and *RPS42* (*C1_01640W_A*). Recent studies showed that the mRNA level of Rps4p is repressed in Spider-medium-induced biofilms (Nobile et al., 2012). A *rps41::Tn5/RPS41* mutant strain, with one copy of *RPS41* disrupted by *Tn5*, showed defects in invasion (Oh et al., 2010). However, are the duplicated genes *RPS41* and *RPS42* coding Rps4p equally important to *C. albicans*? Currently, these questions remain to be answered.

Meanwhile, the possible complexity of function of Rps4ps in *S. cerevisiae*, wheat and mammals provided an interesting direction to answer the above questions in *C. albicans*, and in the present study we have focused on the phenotypes of *rps4* mutants. We constructed *rps41*Δ and *rps42*Δ null mutant strains in *C. albicans,* and showed that expression of either *RPS41* or *RPS42* is sufficient for growth of yeast form cells, but expression of *RPS42* alone does not support filamentous growth and reduces overall growth rate. Although loss of *RPS41* and *RPS42* does not affect the sensitivity to azoles of *C. albicans*, ectopic expression of *RPS41* or *RPS42* in a wild-type strain increased tolerance to fluconazole (FLC). The *RPS41* isogene produces more transcript than *RPS42*. To investigate the mechanisms of the phenotypic changes in the *rps41*Δ mutant at the molecular level, *C. albicans* cDNA microarray analysis experiments were used to identify gene expression profiles of the wild-type strain and the *rps41*Δ mutant. Overall, our observations suggested that loss of the *RPS41* gene but not the *RPS42* gene can generate distinct phenotypes including changes in growth rate, and morphological transitions, and susceptibility to osmotic stress.

2. Materials and methods

2.1. Strains, media and growth conditions

The strains of *C. albicans* used in this study are listed in Table 1. For *C. albicans,* cells were routinely grown in either YPD medium (1% yeast extract, 2% bacto peptone, 2% glucose, and 2% agar for solid medium) (Guthrie and Fink, 1991) or synthetic complete (SC) medium (0.67% yeast nitrogen base without amino acids, 2% glucose, 0.15% amino acid mix with uridine at 50 μg/ml, and 2% agar for solid medium) at 30 °C at 200 rpm in an orbital shaker overnight, diluted to an OD_{600} of 0.1–0.2, grown to logarithmic phase (6–8 h) and used for subsequent experiments. For morphogenesis analysis, mid-log phase *C. albicans* cells were adjusted to 1×10^3 cells/ml. Cellular filamentation was induced in either liquid and solid Spider medium (1% nutrient broth, 1% mannitol, 0.2% K_2HPO_4, and 2% agar for solid medium, PH 7.2) or in YPD + 10%FBS (with fetal bovine serum at 10% concentrations) at 37 °C. These filament-inducing media were pre-warmed to 37 °C. YPD medium was supplemented with 50 μg ml^{-1} uridine for growth of *URA3*$^-$ strains. YPD/-Uri medium (YPD medium lacking uridine) and SC/-Uri medium (SC medium lacking uridine) were used for growth of *URA3*$^+$ strains. For selection of Ura$^-$ clones, SC/-Uri medium was supplemented with 50 μg ml^{-1} uridine and 1 mg ml^{-1} 5-fluoroorotic acid (5-FOA) (Staab and Sundstrom, 2003).

Table 1
Strains used in this study.

Strain	Parent	Description	Source or reference
CAI4	CAF2-1	*ura3Δ::imm434/ura3Δ::imm434*	Fonzi and Irwin (1993)
CaLH031	CAI4	*RPS41/RPS41/rps41Δ::hisG-URA3-hisG*	This study
CaLH033	CaLH031	*RPS41/RPS41/rps41Δ::hisG*	This study
CaLH035	CaLH033	*RPS41/rps41Δ::hisG/rps41Δ::hisG-URA3-hisG*	This study
CaLH037	CaLH035	*RPS41/rps41Δ::hisG/rps41Δ::hisG*	This study
CaLH039	CaLH037	*rps41Δ::hisG/rps41Δ::hisG/ rps41Δ::hisG-URA3-hisG*	This study
CaLH041	CaLH039	*rps41Δ::hisG/rps41Δ::hisG/ rps41Δ::hisG*	This study
CaLH032	CAI4	*RPS42/RPS42/rps42Δ::hisG-URA3-hisG*	This study
CaLH034	CaLH032	*RPS42/RPS42/rps42Δ::hisG*	This study
CaLH036	CaLH034	*RPS42/rps42Δ::hisG/rps42Δ::hisG-URA3-hisG*	This study
CaLH038	CaLH036	*RPS42/rps42Δ::hisG/rps42Δ::hisG*	This study
CaLH040	CaLH038	*rps42Δ::hisG/rps42Δ::hisG/ rps42Δ::hisG-URA3-hisG*	This study
CaLH042	CaLH040	*rps42Δ::hisG/rps42Δ::hisG/ rps42Δ::hisG*	This study
CaLH051	CAI4	*ura3Δ::imm434/ura3Δ::imm434/ RPS1::URA3*	This study
CaLH052	CaLH041	*rps41Δ::hisG/rps41Δ::hisG/ rps41Δ::hisG/RPS1::URA3*	This study
CaLH053	CaLH042	*rps42Δ::hisG/rps42Δ::hisG/ rps42Δ::hisG/RPS1::URA3*	This study
CaLH054	CAI4	*ura3Δ::imm434/ura3Δ::imm434/ RPS1:: RPS41-URA3*	This study
CaLH055	CAI4	*ura3Δ::imm434/ura3Δ::imm434/ RPS1:: RPS42-URA*	This study
CaLH056	CaLH041	*rps41Δ::hisG/rps41Δ::hisG/ rps41Δ::hisG/RPS1::RPS41-URA3*	This study
CaLH057	CaLH042	*rps42Δ::hisG/rps42Δ::hisG/ rps42Δ::hisG/RPS1::RPS42-URA3*	This study

2.2. Disruption of the RPS41 gene and RPS42 gene

To investigate the function of Rps4ps in *C. albicans*, we constructed *C. albicans rps41*Δ *and rps42*Δ null mutants in wild-type strain CAI4 by the ura-blaster method as described previously (Fonzi and Irwin, 1993). The *RPS41* gene is on chromosome II, while the *RPS42* gene is on the chromosome I of *C. albicans* (http://www.candidagenome.org/). Both the chromosome I and II of *C. albicans* in the CAI4 background are trisomic(Selmecki et al., 2005, 2010); there are three copies of the *RPS41* gene and three copies of the *RPS42* gene in the CAI4 strain. For the disruption of the *RPS41* gene, a 300 bp 3′ flanking region of the *RPS41* gene was amplified by PCR with oligonucleotides RPS41dn-F plus RPS41dn-R and cloned into the *Bam*HI and *Hind*III sites at one side of the *hisG–URA3–hisG* cassette in p5921, yielding new plasmid pLH001. Similarly, a 400 bp DNA fragment which is promoter region of *RPS41* was amplified by PCR with oligonucleotides RPS41up-F plus RPS41up-R and cloned into the *Kpn*I and *Bgl*II sites at another side of the *hisG–URA3–hisG* cassette in pLH001, yielding new plasmid pLH003, which was used to disrupt the three alleles of *RPS41* (see Table 3). The pLH003 was linearized with KpnI for transformation *C. albicans* strain CAI4 by the lithium acetate–polyethylene glycol (LiAc–PEG) method as described previously