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Yeast Functional Analysis Report

Control of 5-FOA and 5-FU resistance by *Saccharomyces cerevisiae YJL055W*

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Abstract

In a *URA3*/5-FOA-based dosage-suppressor screen, we isolated a plasmid containing the little-characterized ORF *YJL055W*. Further analysis showed that this gene did not suppress the mutation of interest. Instead, overexpression of Yjl055Wp directly suppressed the non-viability of *URA3*$^+$ cells in the presence of 5-FOA. Overexpression of Yjl055Wp also suppressed the lethality induced by 5-FU, but deletion of *YJL055W* had no detectable effect on resistance to either 5-FOA or 5-FU. Based on these observations and a previous report that a *yjl055w*Δ mutant has increased sensitivity to purine-analogue mutagens, we suggest that Yjl055Wp may function in one of several pathways for the detoxification of base analogues. However, its precise mechanism of action remains unknown. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: 5-fluoro-orotic acid; 5-fluorouracil; base analogues; detoxification pathway; dosage-suppressor screen; *URA3*

Introduction

In *Saccharomyces cerevisiae*, 5-fluoro-orotic acid (5-FOA) has long been used in various genetic screens to select against *URA3*$^+$ cells (Boeke *et al*., 1984; Rothstein, 1991; Sikorski and Boeke, 1991). *URA3* encodes orotidine-5′-phosphate decarboxylase, an enzyme in the uracil biosynthetic pathway that (in conjunction with Ura5p, orotate phosphoribosyl transferase) can convert 5-FOA into 5-fluorouracil (5-FU) in the form of 5-fluorouridylic acid (5-FUMP) (Jones and Fink, 1982). 5-FU is a pyrimidine analogue that can be misincorporated into RNA and DNA in place of uracil or thymine, leading to interference with RNA and DNA processing and function and thus to cell death. *ura3* mutants require exogenous uracil for growth and are viable in medium containing 5-FOA. The presence of a *URA3*-marked plasmid makes *ura3* cells sensitive to 5-FOA, but they can survive on medium containing 5-FOA and uracil by spontaneously losing the plasmid. Thus, dosage-suppressor screens can be conducted by transforming a genomic or cDNA library in a vector with a non-*URA3* marker into a strain that harbours both a lethal mutation and a *URA3*-marked plasmid that contains a wild-type copy of the mutated gene. The original strain is non-viable on 5-FOA medium, but the presence of a library plasmid that carries either a wild-type copy of the mutated gene or a dosage suppressor allows cells to lose the *URA3* plasmid and thus survive in the presence of 5-FOA. A potential problem with such a screen is that a gene might be isolated not because it suppresses the lethal mutation of interest but because it directly suppresses the lethality caused by 5-FOA in *URA3*$^+$ cells. We report here that the largely uncharacterized ORF *YJL055W* is such a gene.

Materials and methods

Strains, growth conditions, growth-rate measurements, and genetic methods

The *S. cerevisiae* strains used in this study were RNY757 (*MAT***a** *his3-Δ200 leu2-Δ1 lys2-801*

Table 1. Primers used in this study

Primer	Purpose	Sequence
YJL55dF	5′ primer to construct *yjl055wΔ*	5′-TAGAGTAAATCGCAGGCAATCCAG TACTAAGAAACGGCGCGGATCCCCGGGTTAATTA-3′
YJL55dR	3′ primer to construct *yjl055wΔ*	5′-TACAGATCGTTTAAACTAGATACAT GACTTACGTTTCTAGAATTCGAGCTCGTTTAAAC-3′
YJL55dK	Upstream check primer for *yjl055wΔ*	5′-TGCAAAAGAATGATTATTAGAATTATACGT-3′
YJL55dC	Downstream check primer for *yjl055wΔ*	5′-GCCCATCCGTCGATGCCAATATTTCCTTTC-3′
3′PTEF	Internal check primer of the *His3MX6* marker	5′-GTATGGGCTAAATGTACGGGCGACAGTCAC-3′
5′TTEF	Internal check primer of the *His3MX6* marker	5′-TATTTTTTTTCGCCTCGACATCATCTGCCC-3′
YEp13sp168	5′ primer to construct p757-8-2-ΔYJL055W	5′-CTTGGAGCCACTATCGACTACGCG ATCATGGCGACCACACGGATCCCCGGGTTAATTA-3′
YJL55dsr2	3′ primer to construct p757-8-2-ΔYJL055W	5′-CTGATTTAAGCGGACAGGTTCACG GTATCATTCCAAATGGAATTCGAGCTCGTTTAAAC-3′
YEp13cp168	Upstream check primer for p757-8-2-ΔYJL055W	5′-GCATAGTACCGAGAAACTAGTGCGAAGTAG-3′
YEp13cp169	Downstream check primer for p757-8-2-ΔYJL055W	5′-CTGTCCCTGATGGTCGTCATCTACCTGCCT-3′
YJL055WF	5′ primer to construct YEp13-YJL055Wsub	5′-CTTGGAGCCACTATCGACTACGCG ATCATGGCGACCACAACTCGAAATGTTGGCAGTCG-3′
YJL055WR	3′ primer to construct YEp13-YJL055Wsub	5′-ACCGCACCTGTGGCGCCGGTGATG CCGGCCACGATGCGTGCATAATACAGTACAGGCTT-3′

trp1-Δ63 ura3-52 myo1Δ::kanMX6 hof1Δ::TRP1 [pRS316-MYO1]; Ko N, Nishihama R, Pringle JR. Identification of additional proteins involved in septin-dependent, actomyosin-ring-independent cytokinesis in *Saccharomyces cerevisiae*; in preparation), DC-5 (*MAT***a** *leu2-3,112 his3 can*^{R} *URA3*$^{+}$; Broach *et al*., 1979), and KO1468 (as DC-5, except *yjl055wΔ::His3MX6*). To construct KO1468, the entire coding region of *YJL055W* in strain DC-5 was deleted using the PCR method (Baudin *et al*., 1993; Longtine *et al*., 1998) and the primers indicated in Table 1. The success of the deletion was confirmed by two PCR tests that used check primers, upstream and downstream of the deleted region, that were respectively, together with primers internal to the selectable marker (Longtine *et al*., 1998; Table 1).

Yeast were grown on YP rich medium, synthetic complete (SC) medium, or SC medium lacking specific nutrients as needed to select plasmids or transformants (Guthrie and Fink, 1991). 2% glucose was used as carbon source. Liquid cultures were grown at 23 °C, and plates were incubated at 30 °C. 5-FOA (Research Products International, Mt. Prospect, IL, USA) was used at 1 mg/ml (except where indicated) to select against *URA3*$^{+}$ cells. 5-FU (Sigma-Aldrich, St. Louis, MO, USA) was used at various concentrations, as indicated. To determine growth rates, exponential-phase cultures ($OD_{600} \approx 0.4$) were diluted two-fold with fresh medium, incubation was continued, and the times needed to return to the original OD_{600} were recorded as the doubling times. Standard procedures were used for growth of *Escherichia coli*, genetic manipulations, polymerase chain reaction (PCR), and other molecular biological procedures (Sambrook *et al*., 1989; Guthrie and Fink, 1991; Ausubel *et al*., 1995).

Plasmids

Plasmid YEp13 (high-copy, *LEU2*) was described by Broach *et al*. (1979). Plasmid pRS316–MYO1 contains the *MYO1* gene in the low-copy-number, *URA3*-marked vector pRS316 (Sikorski and Hieter, 1989; Nishihama R, Ko N, Perdue T, Pringle JR. Roles of Cyk3p and Iqg1p in septin-dependent, actomyosin-ring-independent cytokinesis in yeast: in preparation). Plasmid p757-8-2 (see Figure 1A) was isolated in a dosage-suppressor screen strain RNY757 and a genomic-DNA library (DeMarini *et al*., 1997) constructed by cloning *Sau*3A fragments into the *Bam*HI site of YEp13. Plasmid p757-8-2–ΔYJL055W (see Figure 1A) was constructed using the PCR method (Baudin *et al*., 1993). Cells of a *leu2Δ his3Δ* strain containing p757-8-2 were transformed with a PCR fragment

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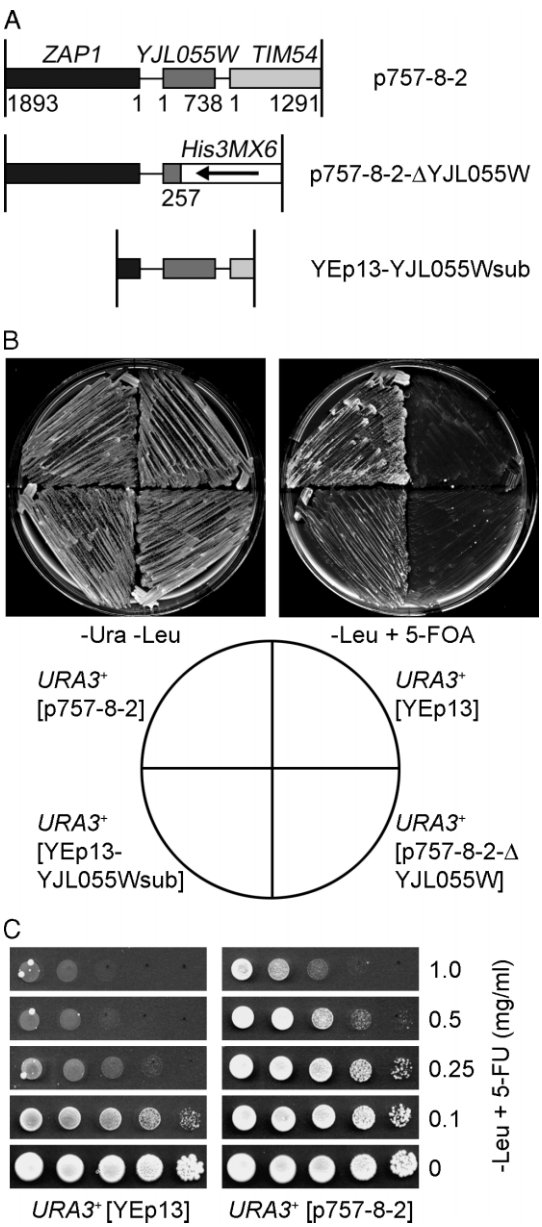
obtained using plasmid pFA6a–His3MX6 (Longtine *et al*., 1998) as template and the primers YEp13sp168 and YJL55dsr2 (Table 1). This was expected to replace the region from nucleotide +258 relative to the start site of *YJL055W* to nucleotide +10 relative to the *Bam*HI cloning site of YEp13 with the *His3MX6* marker, and the success of the construction was confirmed by two PCR tests that used the isolated plasmid as template and check primers that were upstream and downstream of the deleted region respectively, together with primers internal to the selectable marker (Table 1). Plasmid YEp13–YJL055Wsub (see Figure 1A) was constructed in two steps. First, a fragment containing nucleotides from −621 relative to the start site of *YJL055W* to +521 relative to its stop codon was PCR-amplified using p757-8-2 as template and the primers YJL055WF and YJL055WR (Table 1). YJL055WF contained nucleotides −49 to −11 relative to the *Bam*HI site of YEp13 plus nucleotides −621 to −601 relative to the start site of *YJL055W*, whereas YJL055WR contained nucleotides +49 to +11 relative to the *Bam*HI site of YEp13 plus nucleotides +521 to +501 relative to the stop codon of *YJL055W*. In the second step, the PCR product from the first step was purified and transformed together with *Bam*HI-cut YEp13 into *leu2Δ* yeast cells, and transformants (containing plasmids resulting from homologous recombination) were selected on SC-Leu plates. Several independent plasmids were isolated and gave identical results in subsequent tests.



Results and discussion

To identify additional proteins involved in cytokinesis, we used a high-copy, *LEU2*-marked genomic library to screen for dosage suppressors of the 5-FOA-induced lethality of a *myo1Δ hof1Δ* (*URA3 MYO1* plasmid) strain (Ko N, Nishihama R, Pringle JR. Identification of additional proteins

Figure 1. Suppression of 5-FOA- and 5-FU-induced lethality by overexpression of *YJL055W*. (A) The inserts of the relevant plasmids. Plasmid p757-8-2 contains an *N*-terminal fragment of the 2643-nucleotide *ZAP1* ORF, the complete *YJL055W* ORF, and an *N*-terminal fragment of the 1437-nucleotide *TIM54* ORF. Plasmid p757-8-2–ΔYJL055W contains the same *N*-terminal *ZAP1* fragment, a 257-nucleotide *N*-terminal YJL055W fragment, and a *His3MX6* marker (the arrow indicates the direction of transcription). Plasmid YEp13–YJL055Wsub contains a 336-nucleotide *N*-terminal *ZAP1* fragment, the complete *YJL055W* ORF, and a 349-nucleotide *N*-terminal *TIM54* fragment. (B, C) The *URA3*$^+$ strain DC-5 was transformed with the indicated plasmids and tested for growth in the presence of 5-FOA or 5-FU. (B) Cells were streaked on SC −Ura−Leu and SC−Leu + 5-FOA plates and grown for 2 and 6 days, respectively. Only the plasmids harbouring the complete *YJL055W* ORF could confer 5-FOA resistance. (C) Cells were grown to exponential phase [$OD_{600} \approx 0.4$] in SC-Leu medium, and 10-μl aliquots of a dilution series (undiluted and 4×, 10×, 100×, and 1000× diluted) were spotted onto SC-Leu plates containing 5-FU at the indicated concentrations and grown for 3 days

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involved in septin-dependent, actomyosin-ring-independent cytokinesis in *Saccharomyces cerevisiae*, in preparation; see Materials and Methods). Among the several plasmids isolated, p757-8-2 displayed the strongest suppression (data not shown); it proved to contain the complete *YJL055W* ORF plus truncated copies of the neighbouring genes (Figure 1A). We then asked if p757-8-2 could suppress a deletion of *IQG1* (whose product, like Myo1p and Hof1p, is involved in cytokinesis). p757-8-2 strongly suppressed the 5-FOA-induced lethality of an *iqg1Δ* [*URA3 IQG1* plasmid] strain, but it could not suppress the lethality of *iqg1Δ* spores on YP plates (data not shown). These discordant results suggested that p757-8-2 might not suppress the *myo1Δ hof1Δ* and *iqg1Δ* defects but instead interfere with the action of 5-FOA. Indeed, when we transformed plasmid p757-8-2 or YEp13-YJL055Wsub (which contains *YJL055W* plus small fragments of the neighbouring genes; Figure 1A) into a strain that was wild-type at the *URA3* chromosomal locus, the transformants were viable in the presence of 5-FOA (Figure 1B). In contrast, a plasmid in which most of *YJL055W* had been deleted could not rescue the same strain on a 5-FOA plate (Figure 1A, B).

One possible explanation for the suppression of 5-FOA-induced lethality is that overexpression of *YJL055W* downregulates *URA3* or *URA5*, or in some other way decreases Ura3p or Ura5p activity, and thus decreases the formation of 5-FU-containing nucleotides from 5-FOA. In this case, *YJL055W* overexpression should either not affect the sensitivity of cells to 5-FU itself or actually increase such sensitivity (because of a reduction in the pool of normal uridine nucleotides). However, we observed that *YJL055W* overexpression also significantly suppressed 5-FU-induced lethality (Figure 1C).

We next asked whether deletion of *YJL055W* would lead to an increased sensitivity to 5-FOA and/or 5-FU. *yjl055wΔ* cells appeared to grow slightly less well than wild-type *URA3*$^{+}$ cells in the presence of low concentrations of 5-FOA or 5-FU (Figure 2A, B; top four rows in each panel). However, this growth difference appeared to be independent of 5-FOA and 5-FU (Figure 2A, B; bottom row in each panel), an observation that was confirmed by determining the growth rates of the strains in liquid medium (Figure 2C; 22 mg/l adenine, which represents normal SC-Ura medium). Thus, deletion of *YJL055W* has little or no effect on 5-FOA or 5-FU resistance.

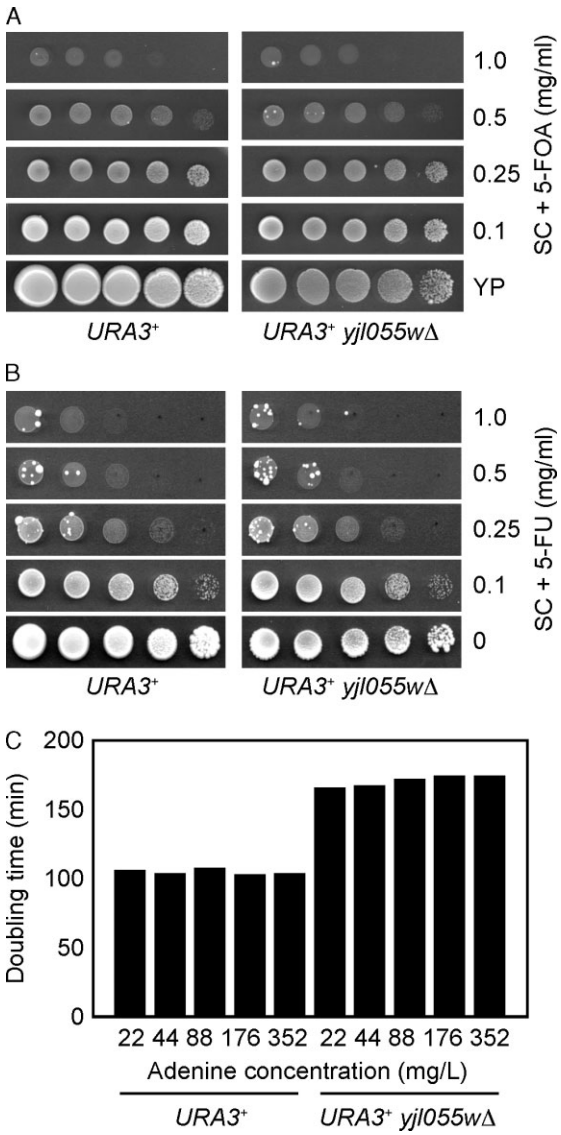

Figure 2. Phenotypic consequences of a *yjl055wΔ* mutation. (A, B) Lack of effect on sensitivity to 5-FOA or 5-FU. Strain DC-5 and its *yjl055wΔ* derivative KO1468 were grown and spotted as in Figure 1C, using YP plates (A, bottom row) or SC plates containing 5-FOA (A, top four rows) or 5-FU (B) at the indicated concentrations. Plates were then incubated for 4 (A) or 3 (B) days. (C) Slow growth that is not rescued by additional adenine in the medium. Strains DC-5 and KO1468 were grown in SC-Ura medium containing adenine at the indicated concentrations, and doubling times (averages of two measurements) were determined as described in Materials and methods. Normal SC-Ura medium (Guthrie and Fink, 1991) contains ~22 mg/l adenine

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In the one previous publication on *YJL055W*, Stepchenkova *et al*. (2005) showed that *yjl055wΔ* strains have modestly elevated sensitivities to the purine-analogue mutagens 6-hydroxylaminopurine (HAP) and 2-amino-6-hydroxylaminopurine (AHA), which function by misincorporation into DNA (Pavlov *et al*., 1991; de Serres, 1991). Because the levels of Yjl055Wp can affect responses to both pyrimidine and purine analogues, it seemed possible that the slow growth of the *yjl055wΔ* strain might reflect a link between the pyrimidine and purine pathways. In particular, if ureidosuccinic acid accumulates in the *yjl055wΔ* mutant, this could produce both slow growth and sensitivity to purine analogues by decreasing purine pools (Korch *et al*., 1974). In this case, the slow growth of the mutant should be rescued by increasing the adenine concentration in the medium (Korch *et al*., 1974). However, we observed that increasing the adenine concentration in defined medium had little or no effect on the growth rate of the *yjl055wΔ* mutant (Figure 2C) and that this mutant also grew more slowly than wild type (doubling times of 153 and 96 min, respectively) in rich (YP) liquid medium.

There are few other clues to the function of Yjl055Wp. BLASTP searches reveal homologues in Archaea, Eubacteria, fungi, and plants (but, interestingly, apparently not in animals), suggesting that at least some aspects of Yjl055Wp function are ancient in origin and have been conserved. Some of these homologues have been annotated as 'possible lysine decarboxylases', based on a PGGxGTxxE motif that is shared with Yjl055Wp but whose association with lysine decarboxylase activity does not actually seem very clear, given that several bona fide enzymes of this type lack the motif (Kikuchi *et al*., 1997). Most of the Yjl055Wp homologues also share with Yjl055Wp a Rossmann-fold motif ($Gx_{1-2}GxxG$) that is indicative of possible nucleotide-binding activity (Kleiger and Eisenberg, 2002; Kukimoto-Niino *et al*., 2004).

Because Yjl055Wp can protect cells from both pyrimidine and purine analogues and presumably from effects at the levels of both DNA and RNA, it seems unlikely that it functions by affecting the transport of analogues into cells, the levels of normal nucleotide pools (such as an increase in the pyrimidine pool through activation of Ura2p), or DNA repair. Instead, Yjl055Wp seems most likely to function through the metabolic detoxification of the base analogues, a hypothesis consistent with its possible ability to bind nucleotides (see above). Because there are probably multiple pathways for such detoxification, it is not really surprising that the effects of deleting *YJL055W* are modest (HAP and AHA) or undetectable (5-FOA and 5-FU), despite the strong effect of *YJL055W* overexpression on 5-FOA and 5-FU sensitivity. Because *yjl055wΔ* cells grow significantly more slowly than congenic wild-type cells even in the absence of added base analogues, we speculate that Yjl055Wp functions in the detoxification of base analogues that accumulate in the course of normal metabolism (see e.g. Simandan *et al*., 1998) and/or that its detoxification ability is a by-product of another metabolic activity. However, further studies will clearly be necessary to define the molecular details of Yjl055Wp function.

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