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Deletion of the *DEF1* gene does not confer UV-immutability but frequently leads to self-diploidization in yeast *Saccharomyces cerevisiae*

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ABSTRACT

In yeast *Saccharomyces cerevisiae,* the *DEF1* gene is responsible for regulation of many cellular processes including ubiquitin-dependent degradation of DNA metabolism proteins. Recently it has been proposed that Def1 promotes degradation of the catalytic subunit of DNA polymerase δ at sites of DNA damage and regulates a switch to specialized polymerases and, as a consequence, DNA-damage induced mutagenesis. The idea was based substantially on the severe defects in induced mutagenesis observed in the *def1* mutants. We describe that UV mutability of *def1*Δ strains is actually only moderately affected, while the virtual absence of UV mutagenesis in many *def1*Δ clones is caused by a novel phenotype of the *def1* mutants, proneness to self-diploidization. Diploids are extremely frequent (90%) after transformation of wild-type haploids with *def1::kanMX* disruption cassette and are frequent (2.3%) in vegetative haploid *def1* cultures. Such diploids look "UV immutable" when assayed for recessive forward mutations but have normal UV mutability when assayed for dominant reverse mutations. The propensity for frequent self-diploidization in *def1*Δ mutants should be taken into account in studies of the *def1*Δ effect on mutagenesis. The true haploids with *def1*Δ mutation are moderately UV sensitive but retain substantial UV mutagenesis for forward mutations: they are fully proficient at lower doses and only partially defective at higher doses of UV. We conclude that Def1 does not play a critical role in damage-induced mutagenesis.

1. Introduction

The *DEF1* gene in *S. cerevisiae* encodes for a protein with yet-to-be-found biochemical activity and multiple biological functions. The *DEF1* gene has been shown to promote ubiquitination and proteolysis of RNAPII [1]. Null *def1* mutants exhibit diverse phenotypes, including slow growth [2], defective cytokinesis and meiosis [3], abnormal cell size [4] and increased sensitivity to mutagens [3]. *DEF1* is involved in genome stability control: *def1*Δ is synthetically sick with mutations in the *PIF1* gene encoding for DNA helicase that participates in DNA maintenance and replication associated with DNA breaks [5,6]; Def1 is found at sites of double strand breaks [7]; Def1 assists repair of abasic sites on the transcribed strands [8].

It has been proposed that Def1 participates in DNA damage induced mutagenesis in yeast, by promoting degradation of the catalytic subunit of DNA polymerase (pol) δ (encoded by the *POL3* gene) at stalled forks, which subsequently leads to a switch to translesion (TLS) DNA polymerases, ultimately responsible for mutagenesis [9]. Biochemical evidence in favor of the hypothesis was Def1-dependent Pol3 ubiquitylation leading to subsequent degradation of Pol3 in proteasomes [9]. Genetic support was a complete absence of UV-induced mutagenesis in *def1* strains [9]. Here we re-investigated the effect of *def1* mutation on mutagenesis. Our initial experiments revealed amazing heterogeneity of mutability of newly generated *def1*Δ*::kanMX* strains. We show that, in fact, the deletion of *DEF1* leads to only a relatively small reduction of UV-mutagenesis. The apparent complete loss of induced mutagenesis seen in many strains with the deletion of the *DEF1* (including the strain present in the collection of deletions of all yeast reading frames in BY4742 background created in Genome Deletion Project, "The Yeast Knockout Collection" (YKO)) can be explained by a novel finding of

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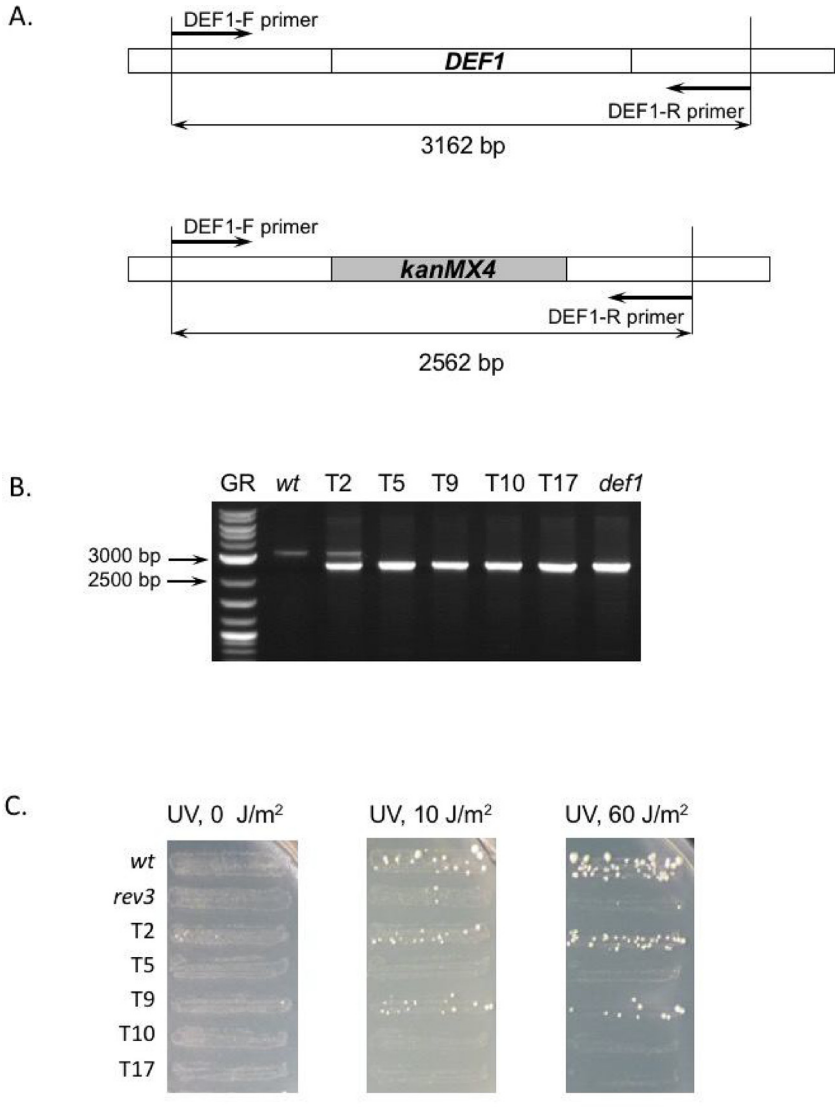

Fig. 1. PCR-based generation, verification and mutability of the *DEF1* knockouts. (A). Expected length of amplicon in PCR with genomic DNA of *DEF1* and *def1::kanMX4* strains. (B). Agarose gel electrophoresis of PCR for presence of the *def1::kanMX* disruption in different strains. GR – GeneRuler 1 kb DNA Ladder; wt – LAN201-ura3Δ strain; T2 – T17 – transformants by *def1::kanMX* cassette; def1 – BY4742-def1Δ strain from YKO. (C). Variable UV-mutability of def1 mutants in qualitative test. Single colonies of wild-type (wt), *rev3* and *def1*Δ strains were streaked on YPDAU plates as wide patches. After 24 h of incubation at 30 °C, yeast patches were replica plated on selective media with L-canavanine (3 plates). Two of Can-plates were irradiated by different doses of UV. One (left) plate of three is a negative control without irradiation. Can-plates were then incubated at 30 °C for 5 days. Relative mutability was scored.

frequent self-diploidization of *def1* haploids. Diploids have sharply reduced recovery of forward recessive mutations in reporters commonly used for mutagenesis assays [10–13]. Our study extends the knowledge on pleiotropic functions of the *DEF1*. The results suggest that the propensity for ploidy changes in strains with *def1*Δ should be taken into account when interpreting the *DEF1* role in the control of genome stability.

2. Materials and methods

We used *S. cerevisiae* strains LAN201-ura3Δ (*MAT*a *ade5-1 ura3*Δ *lys2-Tn5-13 trp1-289 his7-2 leu2-3,112*) [14], its *def1::kanMX* and corresponding diploid derivatives. Deletion of the *DEF1* gene in haploid strain was made by one-step gene replacement using PCR product amplified from genomic DNA of the BY4742-*def1*Δ*::kanMX* strain from yeast YKO collection (Dharmacon, U.S.A.). To amplify *def1*Δ*::kanMX* allele, we used primers DEF1-F (5′ GCAGCTCTCGTCAAACAAGG) and DEF1-R (5′ AGTGGCACCTGTTACTATCGC), Fig. 1A. Diploid derivative of the LAN201-ura3Δ strain was obtained by HO-endonuclease expression in transformants by HO-LEU plasmid, followed by selection for diploids. Diploid *def1* strain was a result of spontaneous self-diploidization in haploid *def1* mutant. Yeast strains were cultivated in standard conditions and media (YPDAU, standard YPD supplemented with extra adenine and uracil, YPDAU with G418 (200 μg/mL) and various selective synthetic media, SD) [15]. Qualitative and quantitative mutagenesis tests on induction of canavanine-resistant (Can^r) mutants and His^+ and Trp^+ reversions were done as described before [14,16,17]. Ploidy of yeast strains was determined by flow cytometry as described in [18], but we used SYBR GREEN I instead of SYTOX dye. Data were analyzed using FlowJo software. To estimate the frequency of self-diploidization during vegetative growth, 3–6 independent cultures of LAN201-ura3Δ or its *def1::kanMX* derivative where cultivated in YPDAU broth overnight, then 4×10^4-fold dilutions where plated on YPDAU agar. Individual colonies where picked up (500–1400 for each culture) and streaked as small patches on new YPDAU plates (72 patches per plate). After 2 days of growth, the patches were replica-plated on minimal complete medium with canavanine and UV irradiated (20 J/m^2). UV mutability was evaluated after 5–7 days of incubation. To find how integrative transformation affects the recovery of UV-immutable clones, we used integrative plasmid pRS306-TRP1, with *URA3* and *TRP1* genes (*TRP1* gene was amplified by PCR using Trp1-BamHI (5′-AAGCCCAAGGATCCGATTGTACTGAGAGTGCACC) and Trp1- XhoI (5′- TTCGGGAACTCGAGTTTACAATTTCCTGATGCGG) and cloned using BamH1 and XhoI sites into pRS306 vector). Wild-type and *def1* strains were transformed by the plasmid cut inside the *TRP1* gene by HindIII. Transformants were selected on SC-ura media. Individual transformants were re-cloned on selective medium and then the proportion of UV-immutable clones was then determined as described