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SBF transcription factor complex positively regulates UV mutagenesis in *Saccharomyces cerevisiae*

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Abstract

The collection of gene deletion mutants of *Saccharomyces cerevisiae* was used to screen for novel genes required for UV-induced mutagenesis. We found the SBF transcription factor (Swi4/Swi6 protein complex) to be required for wild-type levels of UV mutability in forward and reverse mutation assay. Expression of translesion polymerase ζ component Rev7 was identified as a target of SBF-dependent regulation.

Keywords

Yeast; UV Radiation; Mutagenesis; Translesion synthesis; Transcriptional regulation

Genetic instability resulting from enhanced mutagenesis is a severe consequence of exposing pro- and eukaryotic cells to DNA-damaging agents [1]. Especially for bulky adducts, such as UV-C induced pyrimidine dimers, point mutations opposite DNA lesions of reduced coding capacity mainly arise through active processes that ensure the completion of replication of a damaged template. In recent years, it became clear that high-fidelity replicative DNA polymerases are temporarily replaced at the lesion site by one of several translesion polymerases whose active sites can accommodate modified bases while lacking proofreading activity [2,3]. A concerted action of one polymerase inserting a base opposite a lesion together with another polymerase extending from the imperfectly matched primer/template junction may be required [4]. The ensuing damage bypass may be error-free or error-prone, depending on type of lesion and polymerases involved. Most of the enhanced mutability following UV radiation is generated by such error-prone translesion synthesis. The majority of UV-induced and spontaneous mutations are dependent on polymerase ζ , a complex of Rev3 and Rev7 [5-7].

Our present understanding of the events leading to mutagenic translesion synthesis also assigns a major role to proliferating cell nuclear antigen (PCNA). PCNA is subject to ubiquitination by the Rad6/Rad18 complex [8]. Monoubiquitinated PCNA appears to facilitate the switch from replicative to bypass polymerase [9,10] and thus, ubiquitin binding domains of bypass polymerases were found to be important for function [11-14]. Mutation of PCNA that prevents ubiquitination abolishes most of UV-induced mutability [15,16].

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In pro- and eukaryotic model organisms, the isolation of mutants that reduce DNA-damage induced mutability (anti-mutator mutants) was critical for defining the elements and mechanisms required for mutagenic bypass [17,18]. Some of these mutants, such as *rad6*, were initially found among the collection of radiation-sensitive mutants. Additional screens were performed to specifically isolate mutants of the mutational process in budding yeast, irrespective of their radiation sensitivity [19-21]. The current list of genes required for UV mutagenesis in *S. cerevisiae* includes *RAD6*, *RAD18*, *REV1*, *REV3* and *REV7*. Also, the inactivation of *POL32* subunit of polymerase δ results in defective UV mutagenesis [22]. *REV6* was recently shown to encode PCNA whose monoubiquitinated version appears to be important for the recruitment of translesion polymerases to sites of damage [15]. Additional proteins involved include the kinase Cdc7/Dbf4 [23,24], thymidilate kinase Cdc8 [25], ribonucleotide reductase subunit Rnr4 [26], and defects were also found for genes required for checkpoint arrest [27].

The now commercially available collection of deletion mutants of non-essential yeast genes has previously been exploited to screen for mutants with enhanced spontaneous mutability and gross chromosomal rearrangements [28,29]. Here, we used this collection to identify novel genes required for UV-induced mutagenesis that may have been missed previously. The identical approach has recently been reported in an independent study that lead to the isolation of many non-overlapping genes [30]. Here, we identify the SBF transcription factor as required for wild-type levels of UV mutability. We found the level of Rev7 to be one of the relevant targets of SBF-dependent regulation.

Material and methods

Yeast strains and plasmids

The wild type strains BY4741 (*MATa his3 Δ leu2 Δ met15 Δ ura3 Δ*) and Y300 (*MATa ade2-1 ura3-1 trp1-1 his3-11,15 leu2-3,112 can1-100*, originally from S. Elledge) were used throughout the study. Various *kanMX4*-marked gene deletions were transferred into Y300 from existing deletion strains (purchased from OpenBiosystems), following propagation by PCR and transplacement by homologous recombination. Yeast transformation was performed as described [31]. Plasmid pELGH6yhRev7 (a gift from Dr. Zhigang Wang, University of Kentucky) was used for overexpression of Rev7 from a galactose-inducible promoter.

Screen for yeast deletion mutants with altered mutability

Clones of the systematic gene deletion collection (OpenBiosystems) were grown on YPD plates, transferred by replica-plating to synthetic medium plates containing 60 mg/l canavanine sulfate (US Biological) and exposed to UV-C radiation (254 nm, 40 J/m²) or 0.2 mg/l 4-nitroquinoline-N-oxide (4-NQO). Yeast media were as described elsewhere [32]. For the patches of each deletion clone, the number of arising canavanine-resistant mutants was compared between treated plates and untreated control plate. This assay was repeated for candidate deletion clones initially showing a low number of mutants on treated plates, or no mutants whatsoever.

Measurement of UV mutation frequencies

For determining UV-induced reversion frequencies, cells were grown to stationary phase on YPD plates. Appropriate numbers of cells were spread on synthetic tryptophane-free omission or canavanine-containing plates. Dilutions were plated on the identical medium supplemented with tryptophane or without canavanine, respectively, to determine survival of colony-forming cells. Cells were irradiated directly on solid media. In the case of Rev7 overexpression, cells were precultured and plated on media containing 1.5% galactose and 0.5% dextrose.

RNA detection

Total RNA was isolated using zirconia beads and hot phenol, separated on a denaturing 1% agarose gel using formaldehyde/MOPS buffer and transferred to a nylon membrane as described [14]. Hybridization was performed in Hybrisol^R 1 (Serologicals Corp.) using DNA probes generated by PCR and labeled by random priming with HighPrime kit (Roche) and ³²P-dCTP. Signal levels were quantified using ImageJ software. For RT-PCR, 50pg DNase I-treated RNA samples served as templates for SuperScriptTM one-step RT-PCR with Platinum Taq (Invitrogen), following manufacturer's instructions. Primer pairs used were TGTATACCCACCTCAGTCATTCGA and CACGTCAGAACCGACTAAAGA for *REV7* and AGATATGGTCATCATCAGAAGA and TCTAGTTCTGTAGGTAGTACCG for *PDA1*, which served as a control. If UV treated-samples were to be analyzed, logarithmic-phase cells were exposed to 60 J/m² UV-C in suspension under constant stirring in a petridish (15 ml, 2.5 × 10⁷ cells/ml), resuspended in YPD and incubated at 30°C for various periods.

Protein detection

Chromosomal *REV7* was tagged in strain BY4741 at the C-terminus with 3 copies of HA peptide by using microhomology-mediated recombination of a PCR product derived from plasmid-borne modules [33]. Protein samples were prepared using TCA and zirconia beads [34]. Western blots were developed with a commercial HA antibody (Covance, 1:1,000 dilution), or a 3-phosphoglycerate kinase (Pgk1) antibody (Molecular Probes, 1:5,000) to detect the loading control.

Cell synchronization

Cells were arrested in G1 using α -factor (US Biological) as described [14]. After release, budding analysis revealed synchronous transversal of the cell cycle, with S-phase entry after about 40 min.

Results and discussion

Screen for deletion mutants with reduced UV mutability

In order to identify deletion mutants that show altered induced mutability, clones of the yeast gene deletion collection in the haploid strain BY4741 were surveyed as follows. Clones were expanded on YPD as an ordered array, replica-plated onto canavanine containing plates and UV-irradiated (Fig. 1A). Additional replica were placed on 4-NQO-containing plates and on untreated control plates. Low probability to form canavanine resistant mutants on UV-irradiated and 4-NQO-containing plates was semi-quantitatively confirmed for 108 deletion mutants (Supplemental Table 1). Only 2 deletion mutants showed this phenotype for 4-NQO alone.

Certain genes were eliminated because of their known role in established DNA repair pathways and, most likely, induced mutants were undetectable in our screening because of their high UV sensitivity. Additionally, many of the identified genes had no obvious direct connection with the known molecular mechanisms of UV mutagenesis and were more likely to affect the efficiency of the forward mutation-based screening system. We reasoned that many mutations that enhance toxicity towards canavanine could limit the yield of resistant mutants since cells may loose viability on the selection plate before a mutationally inactivated arginine permease takes effect. Increased uptake of canavanine or defective detoxification may be underlying causes. For example, a number of *VPS* gene deletions (for vacuolar protein sorting) were isolated (Supplemental Table 1). In an older study, 7 genes required for normal frequency of UV-induced canavanine resistance mutants had been isolated by conventional mutant screening

(*umr1-umr7*) [21]. Enhanced canavanine toxicity was detectable in some but not all *umr* mutants.

After evaluating canavanine sensitivity in a gradient assay and quantitatively characterizing UV-induced forward mutation frequencies (data not shown), the number of potential deletion mutants reducing UV mutagenesis was reduced to 32 (Supplemental Table 1, underlined gene names). Obviously, this screening system was subject to substantial inherent variability, e.g. the amount of cells treated was initially not controlled. Also, a high spontaneous background may have mitigated the detection of UV-induced mutants. We successfully re-isolated deletions of *REV3*, *REV7* and *RAD6*, known to be critically involved in induced mutagenesis, however, we failed to isolate deletions of other known genes with similar role, such as *REV1* or *RAD18*. We conclude that the applied screen was appropriate but non-exhaustive.

To demonstrate that the mutation phenotype was independent of strain and mutational system used, we transferred selected gene deletions into an unrelated genetic background (Y300) and measured UV mutability in the *trp1-1* reversion system. According to molecular processes affected by these genes, we chose the following 9 genes out of 32 as most likely to be involved in the general process of mutagenesis: *HNT3*, *RSC1*, *ARP5*, *INO2*, *SDS3*, *LDB19*, *LDB7*, *SWI6*, *DOA4*. Of these, only two genes were found to be reproducibly required for normal levels of UV mutagenesis both in forward and reverse mutation systems, irrespective of genetic background: *SWI6* (Fig. 1B-D) and *DOA4* (data not shown). The remaining candidate genes await further characterization and it is possible that the use of the more specific reversion assay has eliminated genes that do not have an effect on all types of mutational events.

SBF transcription factor is required for normal UV mutagenesis by stimulating *REV7* expression

Here, we present a more detailed characterization of the influence of the Swi6 transcription factor component on induced mutagenesis. (A study of the ubiquitin-recycling protein Doa4 will be presented elsewhere.) Swi6 forms a complex with the DNA-binding protein Swi4 termed SBF (for Swi4-Swi6 cell cycle box binding factor) or, independently, with Mbp1 forming MSB (for Mlp1 binding factor) [35-39]. Both transcription factors are primarily involved in the regulation of genes preferentially expressed at the G1/S transition [40-44]. No evidence for S-phase specific expression of the respective transcripts have been reported for most genes essential for the mutagenic process, such as *RAD6*, *RAD18*, *REV1*, *REV3*, *REV7*, *CDC7*, or *CDC8*, making them unlikely targets of SBF or MSB regulation. However, when transcript levels of *REV1*, *REV3*, *REV7*, and *RAD6* were compared to wild type, we unexpectedly found a reduced level of *REV7* but not of other transcripts in *swi6* deletion mutants; the same was true for *swi4* but not *mbp1* deletion strains (Fig. 2A, quantified in B, and data not shown). The results of Northern blot analysis were verified by RT-PCR (Fig. 2C, quantified in 2E). At the same time, we showed that *REV7* transcript has no detectable UV inducibility (Fig. 2D,E). We conclude that SBF transcription factor is required for constitutive *REV7* expression.

In order to confirm this result at the level of protein expression, chromosomal *REV7* was C-terminally tagged with 3xHA [33]. As predicted by previous RNA analysis, deletion of *SWI6* resulted in reduced Rev7-HA levels that were frequently difficult to detect at all (Fig. 2F). Additionally, in synchronized cells released from α -factor arrest in G1, we explored possible cell-cycle stage-dependent expression levels. The protein levels of Rev7 were found to be relatively constant throughout the cell cycle and no evidence for higher levels in G1/S were obtained (Fig. 2G).

In order to prove that reduced Rev7 levels are responsible for the reduced UV-mutability of a *swi6* mutant, we overexpressed plasmid-borne *REV7* from a galactose-inducible promoter.

Since RNA analysis suggested that the SBF complex, consisting of Swi4 and Swi6, controls *REV7* expression, we also analyzed UV survival and mutation frequencies in *swi4* with and without overexpression of *REV7* (Fig. 3A,B). *REV7* overexpression did not change the mutability of the wild type and transformation with the empty vector plasmid had no detectable effect in any strain (data not shown). In contrast, *REV7* overexpression alleviated the defects in UV reverse mutagenesis that were evident in both *swi4* and *swi6* mutants (Fig. 3B). Even when Rev7 was overexpressed, the strains did not quite reach wild-type mutation frequencies. One has to assume that additional mechanisms are responsible for the reduced mutability of *swi4* or *swi6* mutants that are independent of Rev7 levels. Also, the somewhat enhanced UV sensitivity of the mutant strains is not corrected (Fig. 3A).

In summary, we have identified the SBF transcription factor as required for UV mutagenesis. The underlying mechanism involves regulation of expression of *REV7*, required for processivity of polymerase ζ . This is a somewhat unexpected finding since SBF regulates predominantly, though not exclusively, genes with increased expression at the G1/S boundary but there is no evidence for significant fluctuations of *REV7* transcript or protein during cell cycle progression. However, cell-cycle dependent regulation may still be revealed under suboptimal growth conditions or during meiosis. It is interesting to note that the mammalian Rev7 homolog appears to have additional roles that are cell-cycle stage-specific [45].

Other yeast genes involved in mutagenesis express transcripts that fluctuate during cell cycle, e.g. *POL30*, *POL32*, *DBF4*, but they lack an obvious SBF binding site in their promoters. This sequence is found in the promoter of the UV-inducible *RNR4* gene whose inactivation leads to depressed UV mutation frequencies [26,30]. However, no difference in Rnr4-GFP-fusion protein levels was noted between wild-type and *SWI6*-deleted cells (data not shown).

As indicated by the absence of SBF binding sequence in its promoter region, *REV7* does not appear to be a direct target of SBF [41]. A number of transcription factors are themselves regulated by SBF but *REV7* promoter has not yet been identified as one of their targets [42]. It remains to be seen if on yet another level of regulation a transcription factor cascade regulated by SBF can provide the direct link to *REV7*.

For the first time, this study has identified a transcription factor as critical for DNA-damage induced mutagenesis in eukaryotes. If conserved, these novel aspects of regulation of error-prone bypass may open up important avenues for advancing human health by modifying the efficiency of translesion synthesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Identification and characterization of yeast deletion mutants with altered mutability. (A) Replica of clones of the systematic gene deletion collection were transferred to plates containing canavanine and exposed to UV-C radiation or 4-NQO. Compared to the untreated control, many canavanine-resistant mutants emerged in most replica. Those with an unexpectedly low number represent candidate genes that may be required for induced mutagenesis. (B) Survival of colony-forming cells, (C) frequency of canavanine resistant mutants and (D) of revertants of *trp1-1* among surviving cells following treatment of stationary-phase cells with UV. Responses of wild-type (WT) (filled symbols) and *swi6* Δ :*KanMX4* cells

(open symbols) were compared in two different genetic backgrounds, BY4741 (○●) and Y300 (□■). Representative single-experiment data are shown.

Fig. 2.

Level of *REV7* transcript and protein in wild-type, *swi6*, *swi4* and *mbp1* deletion mutants. (A) Transcript levels of *REV7* and *PDA1* (control) as determined by Northern blotting. (B) Quantified *REV7* transcript levels of A, normalized for *PDA1* loading controls. (C) Transcript levels of *REV7* and *PDA1* determined by RT-PCR. (D) Transcript levels of *REV7* after UV treatment. Control lanes (marked C) correspond to mock reactions that included all components except reverse transcriptase. (E) Quantified *REV7* transcript levels of C, D, normalized for *PDA1*. (F) Analysis of Rev7-HA protein levels in wild type and *swi6Δ*. An isogenic *rev7Δ* mutant was included to verify band identity. Pgk1 was used as loading control. (G) Absence of cell-cycle stage-dependent regulation of Rev7. Cells were released from α -factor arrest in G1. Unspecific bands (*, **) can serve as loading controls.

Fig. 3.

(A) Survival of colony-forming cells and (B) frequency of *trp1-1* revertants among surviving cells following treatment of stationary-phase WT (Y300, ○●), *swi6*Δ (Δ) and *swi4*Δ cells (□■) with UV. Cells were transformed with Rev7 overexpression plasmid and induced with galactose (closed symbols). These data were compared to untransformed cells (open symbols).