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Ntg1p, the base excision repair protein, generates mutagenic intermediates in yeast mitochondrial DNA

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ABSTRACT

Mitochondrial DNA is predicted to be highly prone to oxidative damage due to its proximity to free radicals generated by oxidative phosphorylation. Base excision repair (BER) is the primary repair pathway responsible for repairing oxidative damage in nuclear and mitochondrial genomes. In yeast mitochondria, three N-glycosylases have been identified so far, Ntg1p, Ogg1p and Ung1p. Ntg1p, a broad specificity N-glycosylase, takes part in catalyzing the first step of BER that involves the removal of the damaged base. In this study, we examined the role of Ntg1p in maintaining yeast mitochondrial genome integrity. Using genetic reporters and assays to assess mitochondrial mutations, we found that loss of Ntg1p suppresses mitochondrial point mutation rates, frameshifts and recombination rates. We also observed a suppression of respiration loss in the *ntg1-Δ* cells in response to ultraviolet light exposure implying an overlap between BER and UV-induced damage in the yeast mitochondrial compartment. Over-expression of the BER AP endonuclease, Apn1p, did not significantly affect the mitochondrial mutation rate in the presence of Ntg1p, whereas Apn1p over-expression in an *ntg1*-Δ background increased the frequency of mitochondrial mutations. In addition, loss of Apn1p also suppressed mitochondrial point mutations. Our work suggests that both Ntg1p and Apn1p generate mutagenic intermediates in the yeast mitochondrial genome.

1. Introduction

Maintenance of mitochondrial DNA fidelity is critical for maintaining respiratory capacity of the cell. Higher eukaryotic cells lose viability with loss of respiratory function, making efficient repair and replication of mitochondrial DNA crucial for cell survival. Reactive oxygen species, generated in the mitochondria as inevitable byproducts of oxidative phosphorylation, are thought to be responsible for most of the oxidative damage to the mitochondrial genome [1]. These oxidative lesions are repaired primarily by the base excision repair pathway (BER) [1,2].

In the classical model for BER in the nucleus, an oxidative lesion is recognized and excised by a DNA N-glycosylase, generating an apurinic/apyrimidic (AP) site in the process. The AP site may be processed by the associated AP lyase activity possessed by some N-glycosylases, to generate a nicked product with a 5′-phosphate and a 3′-unsaturated aldehyde. The product of AP lyase activity is processed further by the 3′-repair diesterase activity of AP endonucleases to generate a 3′-OH group [3]. The abasic sites generated spontaneously or by N-glycosylase activity, can also be directly processed by AP endonucleases to generate a 5′-sugar phosphate flap. This intermediate is further processed by the combined action of

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DNA pol δ, pol β or both, along with PCNA and FEN1 to generate a 5′-phosphate and a 3′-hydroxyl group. The DNA polymerase uses the 3′-OH group to insert a correct base at the damaged site. Finally, a DNA ligase seals the nick resulting in repair of the damaged base [1,3].

In yeast mitochondria, the BER pathway is thought to repair oxidative lesions, although not all the nuclear BER proteins have been found to localize or function in the mitochondrial compartment. The nuclear yeast N-glycosylases, Ogg1p and Ntg1p, and the nuclear AP endonuclease, Apn1p, have been demonstrated to localize to the mitochondria and may also be the chief components of the BER machinery in this cellular compartment [4–6]. Ntg1p is a broad specificity N-glycosylase with associated AP lyase activity that may play a role in the repair of damage induced by oxidation, alkylation, and UV light [6–8]. Ntg1p expression is low under normal growth conditions but is induced in response to DNA damaging agents [9]. Yeast *OGG1* codes for a DNA glycosylase with associated AP lyase activity that also excises oxidative lesions [10,11].

Apn1p possesses a number of biochemical activities, including AP endonuclease activity and a 3′–5′ exonuclease activity [12]. In addition to its classical role in BER, Apn1p can also incise oxidatively damaged DNA on the 5′-end of damaged bases independently of N-glycosylase activity, via the nucleotide incision repair pathway. This activity of Apn1p is thought to generate a 3′-hydroxyl group and a 5′-dangling nucleotide, which on further processing by FEN1 and PCNA in eukaryotes or PolI in bacteria, can provide an appropriate substrate for DNA polymerase to fill the gap [13]. Loss of Apn1p results in an increased nuclear point mutation rate and affects viability of the cells after exposure to alkylating agents such as MMS or oxidizing agents such as hydrogen peroxide [14,15]. However, *APN1* deletion has no effect on the rates of spontaneous mitochondrial mutations, although MMS treated *apn1-Δ* cells display an approximately six-fold increase in mitochondrial point mutation rates [5]. Underexpression of the nuclear DNA N-glycosylase Mag1p in an *apn1-Δ* cell reduces spontaneous nuclear mutation rates. Conversely, over-expression of Mag1p in an *apn1-Δ* cell increases the nuclear mutation rates, implying that Mag1p gives rise to potentially mutagenic AP sites in the nucleus [16].

Deletion of the other N-glycosylase *OGG1* leads to a 2-fold increase in the frequency of mitochondrial petite mutants [4] and a 12-fold increase in mitochondrial point mutations [17]. In both cases, deletion of *NTG1* suppresses this increased respiration loss and point mutation rate. Additionally, deletion of *NTG1* does not significantly affect the percentage of spontaneously arising mitochondrial petites [4] nor does it lead to a significant increases in the rate of mitochondrial or nuclear point mutations [17].

Recently however, Doudican et al. [18] showed that an *NTG1* deletion results in a two-fold increase of mitochondrial point mutations. Under conditions of oxidative stress, combinatorial deletion of the mitochondrial helicase, *PIF1*, and *NTG1* resulted in increased loss of respiration competence implying that a critical balance exists between DNA damage, repair and recombination pathways in the mitochondria [18]. The conflicting reports on the contributions of Ntg1p in repairing oxidative damage prompted us to investigate the role of Ntg1p in maintaining mitochondrial genome stability. Using both standard assays and genetic reporters generated in our lab, we could calculate the rates of spontaneous mitochondrial point mutations, frameshift mutations and mitochondrial DNA recombination. In addition, we determined the frequency of respiration loss due to ultraviolet light (UV). Finally, we analyzed the effect of increased expression, as well as deletion of downstream components of the BER pathway on the *ntg1-Δ* strain. Our findings suggest that Ntg1p and Apn1p may generate mutagenic intermediates in the mitochondrial genome.

2. Materials and methods

2.1. Growth media

YPD and YPG rich medium contained 1% yeast extract, 2% Bacto peptone and 2% dextrose or 2% glycerol, respectively. YPG 0.1% dextrose medium contained 2% glycerol and 0.1% dextrose. Synthetic growth media consisted of 0.17% yeast nitrogen base (Becton Dickinson), 2% dextrose, 0.5% ammonium sulfate and was supplemented with appropriate amino acids as described in [19]. Synthetic dextrose medium containing 40 μg/L copper sulfate was used for inducing expression from the *CUP1* promoter on the pRK2 expression vector. Expression of the *kanMX* gene was selected on YPD plates containing 200 mg/L geneticin. Erythromycin resistant mutants were selected on YG medium supplemented with 50 mM sodium phosphate buffer (pH 6.5) and 4 g/L erythromycin (Sigma) [20]. Canavanine resistant mutants were selected on synthetic medium lacking arginine supplemented with 60 μg/mL canavanine sulfate (Sigma).

2.2. Strains and plasmids

Strains used in this study are listed in Table 1. *S. cerevisiae* strains used in this study are isogenic with DFS188 (*MATa ura3-52 leu2-3, 112 lys2 his3 arg8::hisG*), a derivative of D273-10B. The *ntg1-Δ* strain was constructed by transformation of wild-type *MATa*, *MATα* or reporter strains with a 1.6 kb fragment containing the *kanMX* gene flanked on either side by 50 base pairs 5′ and 3′ to *NTG1* start and stop, respectively. Oligonucleotides: 5′-CCTCTTTAATGATTCATCATATATTTGTATCTATATAT ACTTTATAGCCCcgtacgctgcaggtcgac-3′ and 5′-GAATAAAAAGTATACATATTAAC AACTAGGCCTGCTTTCTTTTCTTTTCatcgatgaattcgagctcg-3′ were used to amplify the *kanMX* gene from pFA6a-*kanMX4* [21]. The resulting 1.6 kb PCR product was transformed into strains DFS188, CAB152, CAB162, CAB183-1 and CAB193-1 (Table 1). Transformants were selected on rich medium containing geneticin. The deletions were confirmed by PCR. To construct the *mip1-D347A ntg1-Δ* strain (NPY097), diploids generated by mating an *ntg1-Δ MATα* strain to an isogenic *mip1-D347A MATa* strain were induced to sporulate. Tetrads were dissected, and *MATa* haploid strains carrying the *mip1-D347A* allele and the *ntg1-Δ* allele were selected. The recombination reporter was introduced into DFS188 *ntg1-Δ* and *mip1-D347A ntg1-Δ* cells by cytoduction from NPY66 as described previously [22]. Haploid *ntg1-Δ* and *mip1-D347A ntg1-Δ* cells carrying the recombination construct were selected to generate NPY101 and NPY100, respectively.