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Saccharomyces cerevisiae Ogg1 prevents poly(GT) tract instability in the mitochondrial genome

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Abstract

Reactive oxygen species can attack the mitochondrial genome to produce a vast array of oxidative DNA lesions including 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dGuo). We assess the role of the *Saccharomyces cerevisiae* 8-oxo-dGuo DNA glycosylase, Ogg1, in the maintenance of a poly(GT) tract reporter system present in the mitochondrial genome. Deletion in the poly(GT) tract causes the reporter system to produce arginine-independent (Arg^+) colonies. We show that the mitochondrial form of Ogg1 is functionally active at processing 8-oxo-dGuo lesions and that Ogg1-deficient cells exhibit nearly six-fold elevated rate of Arg^+ mutants under normal growth condition, as compared to the parent. Overexpression of Ogg1 completely suppressed the high rate of Arg^+ mutations to levels lower than the parental, suggesting that Ogg1 function could be limited in the mitochondria. Further analysis revealed that the Arg^+ mutations can be prevented if the cells are grown under anaerobic conditions. These findings provide in vivo evidence that oxidative stress induces the formation of lesions, most likely 8-oxo-dGuo, which must be repaired by Ogg1, otherwise the lesions can trigger poly(GT) tract instability in the mitochondrial genome. We also demonstrate that overproduction of the major apurinic/apyrimidinic (AP) endonuclease Apn1, a nuclear and mitochondrial enzyme with multiple DNA repair activities, substantially elevated the rate of Arg^+ mutants, but which was counteracted by Ogg1 overexpression. We suggest that Ogg1 might bind to AP sites and protect this lesion from the spurious action of Apn1 overproduction. Thus, cleavage of AP site located within or in the vicinity of the poly(GT) tract could destabilize this repeat.

Keywords: DNA repair enzymes; Repeat sequences; Yeast; Mitochondria; Genetic instability

1. Introduction

Haploid cells of the budding yeast *Saccharomyces cerevisiae* consist of nearly 50 copies of the mitochondrial genome (mtDNA), which is ∼75–85 kb in size and 82% rich in AT sequences, and mainly encodes subunits of the electron transport system, as well as RNAs for assembling the mitochondrial translational machinery [1,2]. The mtDNA is constantly exposed to endogenous reactive oxygen species (ROS) produced, for example, during the four-electron reduction of oxygen to water in the oxidative phosphorylation process. It is estimated that each mitochondrion produce 10×10^6 ROS molecules per cell per day [3,4]. These ROS can damage the mtDNA to generate a repertoire of 70 known modifications, including isomers, of which 8-oxo-dGuo is a major lesion that can lead to G to T transversion mutations [5]. In fact, the mtDNA accumulate mutations one order of magnitude greater than that of the nuclear DNA, consistent with the vulnerability of the mitochondria to ROS-induced DNA damage [6,7]. In mammalian cells, the mtDNA is continuously replicated even in terminally differentiated cells, such as nerve cells [8]. Thus, somatic mtDNA mutations are likely to cause severe effects on cellular function, as well as being involved in the pathogenesis of a variety of diseases including cancer and degenerative diseases, such as Parkinson's, Alzheimer's, and Huntington's [9–12].

To date, several DNA repair enzymes have been found in the mitochondria that contribute to the maintenance of mtDNA integrity [13–16]. For example, inactivation of *S. cerevisiae* Ogg1, an enzyme that repairs 8-oxo-dGuo, leads to increased

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frequency of mitochondrial petite mutants due to deletions in the mtDNA [17]. However, these Ogg1-deficient cells do not appear to exhibit elevated point mutations in the large ribosomal RNA gene (21S rRNA) that give rise to erythromycin resistant colonies [17,18]. Thus, it would appear that unrepaired 8-oxo-dGuo lesions might cause deletions rather than base substitutions in the mitochondrial genome. In this study, we set out to investigate if Ogg1-deficiency would interfere with the stability of poly(GT) tracts installed within the mitochondrial genome. Essentially, the poly(GT) tract serves as a sensitive reporter system consisting of a derivative *ARG8m* that functions as a mitochondrial gene, instead of a nuclear gene, and encoding an enzyme (acetylornithine aminotransferase) of the arginine biosynthetic pathway [19–21]. The derivative *ARG8m* gene contains either poly(GT) or poly(AT) tracts creating +1 or +2 frameshifts causing the cells to become phenotypically Arg^- [21]. Alterations in the poly(GT) or poly(AT) tracts restore the reading frame of the *arg8m* alleles to give rise to Arg^+ colonies [21]. For this study, we exploited only the poly(GT) tract with +2 frameshift as it is 25-fold more unstable in the mtDNA, undergoing a rate of $\sim 40.0 \times 10^{-7}$ changes in the tract per cell division when compared to the poly(AT) tracts, which changes at a rate of $\sim 1.6 \times 10^{-7}$ per cell division [21]. Using this system, we show that Ogg1-deficient cells exhibit nearly six-fold elevated rate of Arg^+ mutants, as compared to the parent. These Arg^+ mutants were completely suppressed by overexpression of the Ogg1 enzyme, suggesting that unrepaired 8-oxo-dGuo lesions can trigger instability of GT tract repeats in the mitochondrial genome. Interestingly, overproduction of the major apurinic/apyrimidinic (AP) endonuclease Apn1, a nuclear and mitochondrial enzyme with multiple DNA repair activities including the ability to incise AP sites, did not prevent the poly(GT) tract instability of the Ogg1-deficent cells, and instead potentiated the destabilization resulting in higher levels of Arg^+ mutants. However, the Apn1-induced GT tract instability was counteracted by the simultaneous overexpression of Ogg1. Because Ogg1 can bind to AP sites with high affinity, we suggest that it might serve to protect this lesion from the spurious action of Apn1 overproduction. Thus, cleavage of AP site within the poly(GT) tract could destabilize the repeat.

2. Materials and methods

2.1. Yeast strains

The *S. cerevisiae* strains used in this study were CAB 193 (isogenic to DFS188; Mat a, *ura3-52 leu2-3, 112, lys2 his3 arg::hisG*; except carrying poly(GT) repeat in +2 reading frame) and CAB 152 (also isogenic to DFS188, except carrying poly(AT) repeat in +2 reading frame) (kindly provided by Dr. E. Sia, University of Rochester, New York, USA). The following isogenic strains were derived from CAB 193 and constructed using the one-step gene targeting approach [22]. RVY6 (*ogg1A:HIS3*), RVY7 (*apn1Δ::LEU2*), RVY8 (*ntg1Δ::LEU2*), RVY9 (*ogg1Δ::HIS3 ntg1Δ::LEU2*), and RVY10 (*ogg1Δ::HIS3 apn1Δ::LEU2*). Yeast cells were grown in either complete yeast peptone dextrose (YPD) or minimal synthetic (SD) medium, to which nutritional supplement were added at 20 μg/ml [23,24]. Standard genetic analysis and transformation were carried out as described previously [23,24]. The *Escherichia coli* DH5α strain was used for plasmid maintenance.

2.2. Construction of the plasmid pOGG1-GFP

The plasmid pOGG1 (kindly provided by Dr. S. Boiteux, CNRS-CEA, Fontenay aux Roses, France), which contains the entire *S. cerevisiae OGG1* gene, was used as the template to amplify by polymerase chain reaction (PCR) the full length of the *OGG1* gene using the primers OGG1-F1 (5′-AAAGTTATTAG ACCTGAATTCACGACTACTCATAGA AAACG-3′) and OGG1-F2 (5′-CTATGACTTTTTAGGGGTACCTATTTTTGCTTCTTTGATG-3′) bearing the restriction sites (underlined) for *Eco*R1 and *Kpn*1, respectively. This procedure yielded a 1.6 kb fragment which was digested with *Eco*R1 and *Kpn*1 and subcloned into the Yeplac195 yeast expression vector containing the *GFP* gene downstream of the cloning site. Thus, the *OGG1* gene was fused to the N-terminal of GFP in order to create the plasmid pOGG1-GFP.

To obtain a higher expression level of the Ogg1-GFP fusion protein, the fragment containing the *OGG1* gene attached to the N-terminal of *GFP* was amplify by PCR using the primers OGG1-F2 (5′-CCGATTTTATTTATCAAGCTT-ATGTCTTATAAATTCGGC-3′) and GFP-Sal1 (5′-AGAACTAGTCGACCC-TTATTTGTATAG-3′) bearing the restriction sites (underlined) for *Hin*dIII and *Sal*1, respectively. The 1.9 kb fragment was subcloned into the multicopy vector pYES2.0, bearing the galactose-inducible promoter *GAL1*, to produce the plasmid pYES-OGG1-GFP.

2.3. Preparation of mitochondrial fractions from yeast

Mitochondrial extracts were prepared from the purified organelle as previously described [13].

2.4. Western blot

The proteins were separated on 12.5% SDS-PAGE gel, transferred onto a nitrocellulose membrane (8 cm × 10 cm) (Amersham Life Science) which was then blocked with buffer TSEM (10 mM Tris–HCl, pH 7.45, 150 mM NaCl, 0.1% Tween, 1 mM EDTA, 5% powdered milk) for 1 h. After the blocking, the membrane was probed with 10 ml of buffer TSEM containing monoclonal GFP antibodies at a dilution of 1:5000 for 16 h at 4 °C. Following the probing, the membrane was washed three times for 15 min with TSEM before adding 10 ml of the anti-mouse IgG conjugated to horseradish peroxidase at a dilution of 1:2500 (BIO/CAN Scientific Inc., Ont., Canada) for 1 h at room temperature. Finally, the membrane was washed again three times for 15 min with TSEM and immunoreactive polypeptides were detected by chemiluminescence (Dupont-NEN).

2.5. Preparation of DNA substrate and assay for Ogg1 activity

A 30 base synthetic oligonucleotide containing a unique 8-oxo-dGuo residue at position 11 (5′-TGACTGCATA$^{8\text{-oxo-dGuo}}$CATGTAGACGATGTGCAT-3′) was 5′-^{32}P-end-labeled (provided by Dr. Murat Saparbaev, France). The specific activity of the labeled oligonucleotide was determined by running an aliquot on a 10% polyacrylamide gel and quantitating the amount of incorporated radioactivity. The oligonucleotide was ethanol precipitated, resuspended in TE (10 mM Tris–HCl, pH 7.5, and 1 mM EDTA), and annealed with an equimolar amount of the complementary oligonucleotide (5′-ATGCA CATCGTCTACA TGCCTATGCAGTCA-3′) by heating at 83 °C and slow-cooling to room temperature. The 8-oxo-dGuo cleavage reactions were carried out in buffer containing 25mM Tris–HCl, pH 7.4, 50 mM KCl, 2 mM EDTA, 1 pmol oligonucleotide substrate, and the indicated concentration of mitochondrial extracts or purified bacteria fpg protein in a final volume of 12.5 μl. Incubation were carried out at 37 °C for 30 min and stopped by the addition of 5 μl formamide loading buffer. Samples were prepared for gel electrophoresis by heating at 65 °C for 5 min. Reaction products were analysed on 10% denaturing polyacrylamide gels.

2.6. Measurement of mutations rates of mitochondrial DNA

The rate of mutation to Arg^+ was performed as previously described [21]. Briefly, 10–15 independent colonies of the indicated strains were grown in 1 ml of SD medium supplemented with 20 μg/ml of the appropriate auxotrophic