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Loss of DNA Repair Methyltransferase Gene *MGT1* Alters Mitochondrial Dynamics and Accelerates Aging in *Saccharomyces cerevisiae*

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Abstract

The *MGT1* gene encodes O^6-methylguanine-DNA methyltransferase, a highly conserved enzyme that directly reverses alkylation damage by transferring methyl groups from guanine to a catalytic cysteine. While its nuclear function in genome maintenance is well characterized, its contribution to mitochondrial homeostasis remains elusive. Here, we investigated the $\Delta mgt1$ mutant of *Saccharomyces cerevisiae*. Remarkably, the mutant displayed higher colony formation on non-fermentable carbon sources, indicating altered respiratory efficiency. DAPI-based imaging revealed increased and dispersed mitochondrial DNA (mtDNA), with quantitative analysis confirming elevated mtDNA content. Despite this, $\Delta mgt1$ cells exhibited a markedly shortened chronological lifespan, suggesting a link between *MGT1* deficiency and genomic instability, mitochondrial dysregulation, and accelerated cellular aging. Bioinformatic analyses highlighted interactions with key DNA repair proteins, including Msh6p, Mlh1p, Mre11p, Exo1p, and Ogg1p, suggesting integration into broader genome-stability networks. These findings reveal a previously unrecognized mitochondrial dimension of *MGT1* function and raise the intriguing possibility that the plant homolog *AtAGT1* may similarly modulate mitochondrial integrity, stress resilience, and lifespan in higher eukaryotes.

Introduction

Alkylating agents such as methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), and N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) are potent genotoxic compounds capable of inducing O^6-methylguanine (O^6-meG) and other alkylated lesions

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in nuclear DNA (Saffhill et al. 1985). These lesions, if unrepaired, can lead to G : C to A : T transition mutations, genomic instability, and ultimately carcinogenesis. The enzyme O^6-alkylguanine-DNA alkyltransferase, AtAGT1 in *Arabidopsis thaliana* is responsible for repairing O^6-meG through a direct reversal mechanism in which the alkyl group is transferred to a cysteine residue of the enzyme, irreversibly inactivating it (Pegg 2011). In mammals, this enzyme is known as *MGMT*, while in *Saccharomyces cerevisiae* it is encoded by the *MGT1* gene, producing the homologous protein Mgt1p (Sassanfar and Samson 1990, Hwang et al. 2009).

The yeast *MGT1* gene is located on chromosome IV and encodes a 567 bp protein essential for preventing alkylation-induced mutagenesis (Sassanfar and Samson 1990). Mgt1p is targeted to the peroxisome in a Pex5p-dependent manner (David et al. 2022) and is evolutionarily conserved across diverse eukaryotes, including fungi, plants, and animals (Table 1). Functional parallels between Mgt1p in yeast, *MGMT* in mammals, and *AGT1* in *Arabidopsis thaliana* highlight a conserved role of this enzyme in maintaining genome integrity across both nuclear and organellar DNA (Gerson 2004, Sharma et al. 2009).

Table 1. Evolutionary Conservation of MGT1/MGMT-Type O^6-Methylguanine DNA Alkyltransferase.

Kingdom/Clade	Model Organism	Gene Name/ Locus	Pathway	Core Function
Algae	*Chlamydomonas reinhardtii* (Green Algae)	*CrAGT* (Gene ID: Cre07.g342750)	Direct Reversal Repair	Repairs O^6-methylguanine and other alkylation damage; protects chloroplast & mitochondrial DNA integrity
Fungi	*Saccharomyces cerevisiae* (Yeast)	*MGT1*	Direct Reversal / Mismatch Repair	O^6-methylguanine DNA methyltransferase; prevents G:C → A:T transition mutations; maintains genome stability
Plantae	*Arabidopsis thaliana*	*AtAGT1* (At1g14150)	Direct Reversal / Base Excision Repair	Removes alkyl groups from O^6-alkylguanine in nuclear & mitochondrial DNA; functional homolog of MGT1/MGMT
	Arabidopsis thaliana	*AtAGT2* (At5g16770)	Stress-Responsive DNA Repair	Induced under genotoxic conditions; contributes to alkylation tolerance
Animalia – Invertebrates	*Caenorhabditis elegans*	*agt-1*	Direct Reversal DNA Repair	Repairs alkylated guanine; prevents point mutation accumulation
	Drosophila melanogaster	*agt*	DNA Damage Response	Maintains genome stability under alkylating stress
Animalia – Vertebrates	*Danio rerio* (Zebrafish)	*mgmt*	Direct Reversal Repair	Conserved MGMT function in developmental systems
	Mus musculus (Mouse)	*Mgmt*	Alkylation Damage Repair Pathway	Protects somatic and stem cells from alkylating mutagens; knockout associated with increased cancer susceptibility
	Homo sapiens (Human)	*MGMT*	Direct Reversal DNA Repair	Clinically significant DNA repair enzyme; regulates mutation rate and chemotherapeutic drug resistance

In humans, reduced *MGMT* expression— often caused by promoter methylation or genetic polymorphisms is strongly associated with tumor progression, particularly in glioblastoma, colorectal cancer, and lung cancer (Fong et al. 1990, Peterson 2001, Sharma et al. 2009, Butler et al. 2020). Loss of *MGMT* function increases the cellular burden of O^6-meG lesions, triggering mismatch repair (MMR) responses that can ultimately lead to double-strand breaks and apoptosis (Peng and Pei 2021). Likewise, deletion of *MGT1* in yeast results in phenotypes including G1/S cell-cycle arrest, increased heat sensitivity, reduced chemical resistance, and altered competitive fitness (Paulovich et al. 1987, Breslow et al. 2008).

Although *MGT1* is well characterized for its nuclear DNA repair role, its potential involvement in mitochondrial genome stability and metabolic regulation remains unclear. Yeast mitochondria are major sites of reactive oxygen species (ROS) production, and oxidative stress is known to influence mtDNA copy number, mitochondrial dynamics, and cellular aging (Dan Dunn et al. 2015). Disruptions in DNA repair pathways may therefore indirectly affect mitochondrial function, energy metabolism, and lifespan (Boesch et al. 2011, Natter and Kohlwein 2013). Because yeast cells lack tissue-level compensatory mechanisms present in multicellular organisms, deletion of genes like *MGT1* may reveal mitochondrial or metabolic alterations that are otherwise masked in higher eukaryotes.

However, no comprehensive study has examined whether *MGT1* deletion affects mitochondrial respiration, mtDNA abundance, or cellular longevity in yeast. Therefore, this study investigates mitochondrial respiratory capacity, mtDNA distribution and copy number, and chronological lifespan in *Δmgt1* yeast cells to understand the broader cellular consequences of *MGT1* deletion and its potential implications for genome stability and aging.

Materials and Methods

The isogenic deletion mutants (Δ*mgt1*) and diploid wild-type (DP-WT) yeast strain BY4743 (mat a/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0) were collected from EUROSCARF to utilize in this research. Liquid YPD (1% yeast extract, 2% dextrose, and 2% peptone) and Solid YPD (2% agar) were used as media to grow cells at 30°C. The optical densities of the yeast cells were determined with a spectrophotometer with a 600 nm wavelength (OD_{600}). The cell cultures control strain (BY4743 WT) and mutant strains (Δ*mgt1*) were prepared using YPD liquid media. All wild-type and mutant strains were grown and stored in aliquots to ensure a seamless testing process. For each sample, 7 ml of liquid YPD was taken into a 15 ml falcon tube and 5 μl of yeast cells from the main stock was added to the culture. The falcon tubes were then incubated at 30°C with shaking at 180 rpm. After 24 hrs, 3 ml glycerol was added to the culture and was stored at -20°C in the refrigerator for subsequent experimental use, while long-term stocks were kept at -80°C. To make working plates,

YPD agar media was prepared, autoclaved, and checked for contamination. A 30 µl aliquot of yeast culture from primary stock was taken, spread on solid YPD media, and incubated for 24 hrs.

Strains with mitochondrial abnormalities lack the efficiency to utilize non-fermentable carbon sources (López-Otín et al. 2013). An experiment was conducted to determine whether our mutant is respiratory deficient. Fresh cultures of yeast strains, *Δmgt1* and BY4743 WT, were made from the working plates. Two types of media- YPD (2% dextrose) and YPG (3% glycerol) were prepared in both solid and liquid form. Sterile Petri dishes were used to plate the solid media, while four liquid cultures were prepared using liquid media with fresh yeast cultures: YPD-BY4743 WT, YPD-*Δmgt1*, YPG-BY4743 WT, and YPG-*Δmgt1*. Following 48 hrs of incubation at 30°C with shaking at 180 rpm, 3 ml of yeast cultures were collected and adjusted to an optical density (OD_{600}) of 0.2. Serial dilutions were then performed to achieve OD_{600} values of 0.02, 0.002, and 0.0002. Subsequently, 5-10 µl of the diluted cultures were carefully spotted onto YPD and YPG agar plates. After incubation, the growth of the cultures on each plate was thoroughly examined the following day.

Another experiment was conducted to examine the distribution of mtDNA. Fresh cultures of the *Δmgt1* and BY4743 WT strains were prepared by incubating them overnight in liquid YPD until an OD_{600} of 0.8-2.0 was reached. Yeast cells were combined with two volumes of 100% ethanol in a 1.5 ml microcentrifuge tube and left to sit for 1 hour at 25°C. The cells were then washed three times with PBS buffer. Subsequently, 200 µl of a 1xPBS/ 1 : 2000 DAPI solution was added to the microcentrifuge tube, following the method described by Hasek 2006. Finally, the yeast cells were taken on microscope slides and visualized under a fluorescent microscope (Nikon Eclipse 50i). Fluorescence intensity was measured using ImageJ/Fiji software, employing the Corrected Total Cell Fluorescence (CTCF) formula: CTCF = Integrated Density - (Area of selected cell × Mean fluorescence of background readings) (El-Sharkawey 2016).

To investigate chronological lifespan, we prepared fresh YPD cultures of *Δmgt1* and BY4743 WT strains. On the first day, these cultures were added to 500 ml flasks at a concentration of 1.5×10^7 cells/ml. We maintained a five-part flask volume ratio to one-part medium volume to ensure proper growth conditions. Incubation was done at 30°C with constant shaking at 180 rpm for 15 days. At 72 hrs, the culture's Optical Density (OD_{600}) was measured using 3.0 ml culture and the formula (0.4 × 1000/OD). Serial dilutions were performed at 1 : 100 to obtain the desired number of yeast cells. Then, 10 to 30 µl of this diluted culture of *Δmgt1* and BY4743 WT was plated on solid YPD media. After 72 hrs of incubation, colony-forming units (CFUs) were counted for both yeast strains; the method was adapted from Parrella and Longo 2008. Earliest CFU count was taken at day three of the plate culture to ensure 100% survival of the colony and the method of subsequent plating and CFU count was repeated every 72 hours to monitor the cell survival rate for 15 days. Data for variants *Δmgt1* and BY4743 WT were organized in an Excel sheet for clarity and analysis. A survival graph was generated in Excel to

visually represent survival trends over time. To assess survival outcomes, we utilized the OASIS (Online Application for Survival Analysis) software, where Kaplan-Meier and log-rank tests were conducted to calculate survival percentages and evaluate statistical significance (p-value <0.05).

A bioinformatic analysis was conducted to identify interactions between *MGT1* and other genes. We utilized the online tool "STRING: Functional Protein Association Networks" (https://string-db.org/) to explore protein-protein interactions involving *MGT1* in *Saccharomyces cerevisiae*. The software generated a list of genes interacting with *MGT1*, accompanied by a visual network representation. We analyzed the interaction data using FunSpec (http://funspec.med.utoronto.ca/) to gain further insights. This tool associates genes with functional roles based on the MIPS (Munich Information Center for Protein Sequences) classification system. To ensure statistical rigor, we applied a stringent p-value threshold of less than 0.01 for the results.

Results and Discussion

Petite mutation hampers the respiration efficiency in the cell by affecting the mitochondrial mechanism (Bernardi 1979). Yeast with petite mutation cannot grow entirely on non-fermentable carbon sources or grow poorly. Although extensive information is available about the role of the *MGT1* gene in DNA damage repair, its influence on mitochondrial function remains unexplored. Therefore, we performed a glycerol assay to identify whether our mutant strain showed petite characteristics. Cells (control BY4743 WT strain and $\Delta mgt1$) were cultured in a non-fermentable carbon source, glycerol, to understand whether the *MGT1* null mutation hampers respiratory efficiency or not. The result demonstrated that the $\Delta mgt1$ mutant formed more colonies than the BY4743 WT strain in both YPD and YPG media (Fig. 1). The result indicates that the *MGT1* null mutation does not hamper cellular respiration.

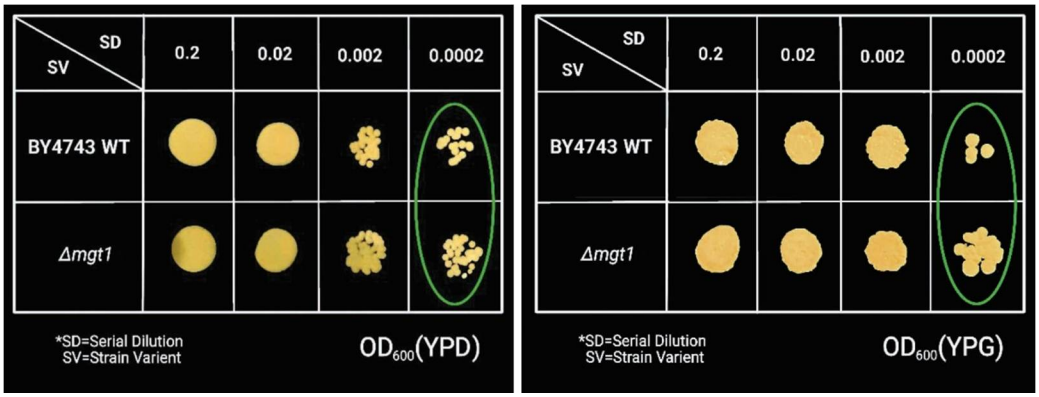

Fig. 1. Respiration status of the mutant: (a) cell cultured in YPD medium (2% dextrose), (b) cell cultured in YPG medium (3% glycerol) after serial dilution. The $\Delta mgt1$ mutant formed more colonies than the BY4743 WT strain in both YPD and YPG media, marked with oval shapes.

Altered mitochondrial DNA copy number and distribution are directly related to many diseases (Filograna et al. 2021). In our experiment, the mutant strain showed higher colony formation in glycerol assay and which might suggest mitochondrial function instability in the *Δmgt1* mutant. Therefore, we checked mutants' mtDNA pattern and distribution. Yeast mitochondria are punctate. It is difficult to extract mitochondrial DNA without damaging it. To determine the distribution and abundance of yeast mitochondria without hampering the mtDNA, we used DNA-binding fluorescence dye DAPI (4',6-diamidino-2-phenylindole) and fluorescence microscope (Kapuscinski 1995, Swayne et al. 2007). The prominent central structure indicates nuclear DNA (nDNA) and numerous smaller spots represent mtDNA. The results showed that in wild-type cells, mitochondrial DNA (mtDNA) was predominantly localized near the cell periphery. In contrast, in *Δmgt1* mutants, mtDNA was numerous and distributed widely across the cytoplasm, appeared as bright fluorescence cluster (Fig. 2). This distribution pattern suggests a high abundance of mtDNA. Five individual cells from both of the microscopic photographs of BY4743 WT and *Δmgt1* strains were used to analyse the fluorescence intensity using the ImageJ/Fiji software (Fig. 3). The quantitative analysis confirmed that *Δmgt1* strains showed significantly higher fluorescence intensity than BY4743 WT cells (p-value 0.0000384956<0.05). The Mean Corrected Total Cell Fluorescence (CTCF) of BY4743 WT and *Δmgt1* were 5179629.64 and 13615419.52, respectively.

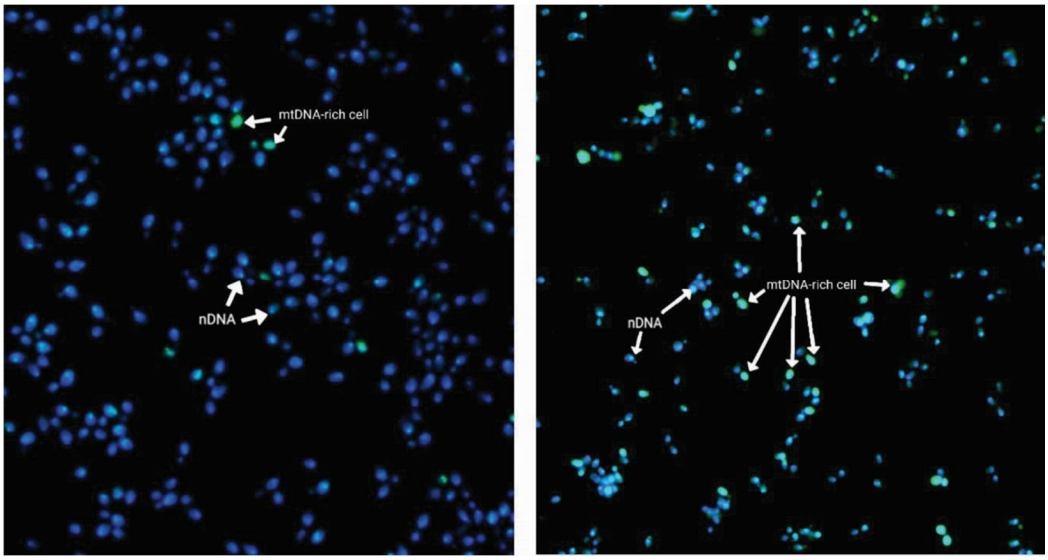

Fig. 2. Mitochondrial DNA distribution of the mutant: Fluorescence photo micrographs of the yeast strains; (a) BY4743 WT and (b) *Δmgt1*. The fluorescence microscopy was performed after DAPI staining. In wild-type cells, mtDNA was mainly near the cell periphery, whereas in *Δmgt1* mutants, mtDNA was significantly abundant ($p < 0.05$) compared to BY4743 WT and dispersed throughout the cytoplasm. The arrows indicated nuclear DNA (nDNA) and dense mitochondrial DNA (mtDNA-rich cell) in the yeast cells.

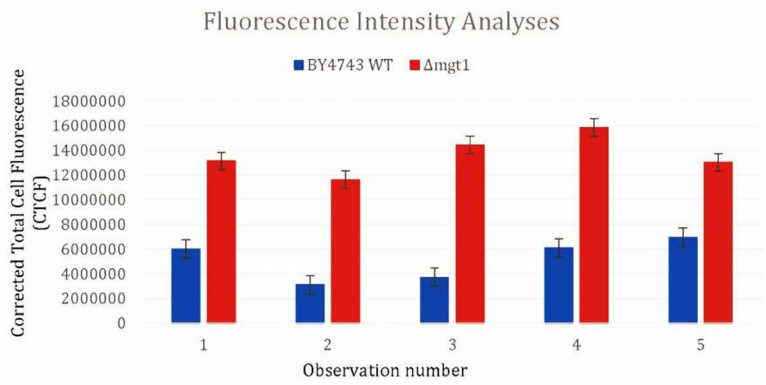

Fig. 3. Fluorescence intensity analyses: The bar chart shows Corrected Total Cell Fluorescence (CTCF) of BY4743 WT strain (blue bars) and *Δmgt1* (red bars). CTCF was measured for five individual cells in separate observations for both the yeast strains. Data visualization denotes consistent higher fluorescence intensity in the *Δmgt1* mutant compared to the BY4743 WT strain.

Alteration in the mitochondria, mtDNA, or production ATP affects a cell's lifespan and aging (Bratic and Larsson 2013). In our experiments, deletion of the *MGT1* gene led to changes in mitochondrial activity, such as elevated mtDNA copy number. This finding highlights the need to conduct lifespan analysis experiments. So, we conducted a chronological lifespan (CLS) analysis over 15 days, evaluating survival every 3 days using colony-forming unit (CFU) counts on YPD-agar plates to compare the longevity of different strains. The CLS graph showed a decline in percent survival for both strains over the 15-day period, but the *Δmgt1* mutant exhibited a steeper decline compared to the wild type (Fig. 4). Statistical analysis using Kaplan-Meier and the log-rank test confirmed that the mutant strain had significantly lower survival rates than the diploid wild-type (BY4743 WT) strain throughout the study (p-value <0.05).

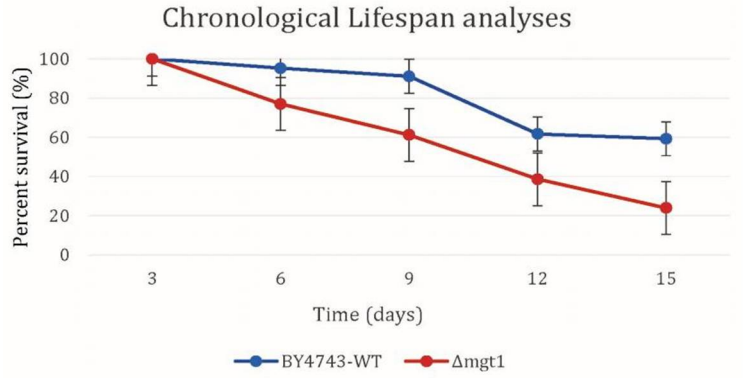

Fig. 4. Chronological Lifespan (CLS) analyses: The CLS analysis in yeast shows the lifespan curve for both the control BY4743 WT strain (blue line) and the *Δmgt1* mutant strain (red line). The average lifespan of each population was determined by counting colony-forming units (CFU), and the resulting data were analyzed with a log-rank test (p-value < 0.05). The *Δmgt1* mutant exhibited a notably shorter lifespan compared to the BY4743 WT cells.

A bioinformatic study was carried out to comprehend how *MGT1* functions are involved with other entities. The String software was used to obtain the list of genes that showed protein-protein interaction with Mgt1p as well as the interaction maps. MIPS (Munish Information Center for Protein Sequences) categorization was used to examine the functional interaction of Mgt1 protein with other proteins using the 'Fun-Spec' analysis software.

The protein Mgt1p is predicted to interact with other proteins (Fig. 5, Table 2). The Mgt1p is associated with Mismatch Repair (MMR) pathway (Msh6p, Mlh1p, Exo1p), Base Excision Repair (BER) pathway (Ogg1p, Mag1p), DNA Double-Strand Break (DSB) repairpathway(Mre11p),and Ubiquitination-deubiquitination machinery (Ufd4p, Ubr1p).

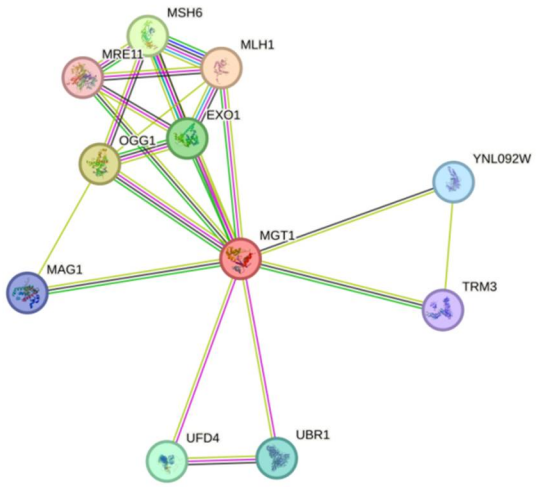

Fig. 5. Interaction network: Proteins interacting with Mgt1p were analyzed using the String software. The analysis revealed direct associations of *MGT1* with *MSH6, MLH1, MAG1, TRM3, MRE11, EXO1, YNLO92W, OGG1, UFD4, and UBR1*.

Oxygen intake is essential for mitochondrial glycerol metabolism, which drives ATP production via oxidative phosphorylation (Himms-Hagen 1968). Therefore, yeast growth on non-fermentable carbon sources such as glycerol, lactate, or ethanol is widely used as an indicator of mitochondrial function (Parrella and Longo 2008). To evaluate whether the *Δmgt1* strain exhibited a respiratory defect, we performed a glycerol growth assay. The *Δmgt1* strain produced a higher number of colonies than the wild-type under both conditions of YPG (3% glycerol) and YPD (2% dextrose) (Fig. 1). While impaired growth can signal mtDNA instability, increased growth may reflect elevated mtDNA copy number. Supporting this, fluorescence imaging revealed that *Δmgt1* cells contained significantly higher mtDNA-associated fluorescence than wild-type cells ($P < 0.05$), visualized as multiple distinct puncta along the cell periphery and cytoplasm (Fig. 2). These observations strongly suggest that mtDNA copy number is increased in *Δmgt1* cells.

Table 2. Interaction network of MGT1 with other genes using MIPS classification.

MIPS functional classification					
Gene name	Category	p-value	In Category from Cluster	Gene quantity from cluster (k)	Total gene quantity (f)
	DNA repair [10.01.05.01]	7.422e-08	*MGT1 MSH6 MAG1 OGG1 MRE11 EXO1*	6	159
	ATP binding [16.19.03]	0.0001909	*MSH6 UBR1 UFD4 MLH1*	4	191
	DNA damage response [32.01.09]	0.0002352	*MGT1 MAG1MRE11*	3	77
MGT1	Chemical agent resistance [32.05.01.03]	0.0005724	*MGT1 MAG1*	2	22
	Polynucleotide degradation [01.03.16]	0.0006263	*MRE11 EXO1*	2	23
	Meiotic recombination [10.01.05.03.01]	0.001717	*MLH1 MRE11*	2	38
	DNA binding [16.03.01]	0.001926	*MSH6 MAG1 MLH1*	3	158
	Modification by ubiquitination, deubiquitination [14.07.05]	0.005977	*UBR1 UFD4*	2	79

An increase in mtDNA copy number is frequently associated with mitochondrial stress responses and has been reported in mitochondrial dysfunction, neurodegenerative disease, and several cancers (Sun et al. 2018, Filograna et al. 2021). Alternatively, high ATP demand promotes mtDNA amplification to sustain mitochondrial efficiency (Chabi et al. 2003, Miller et al. 2003, Kelly et al. 2012, Castellani et al. 2020) and as part of an early cell-cycle arrest response (Lee et al. 2000). The *Δmgt1* mutants were previously reported to show temperature-induced cell-cycle arrest (Paulovich et al. 1987). In the experiment, *Δmgt1* formed more colonies in both culture media (YPG and YPD), and fluorescence microscopy visualized increased mtDNA content, which may suggest high ATP demand in cells. Additionally, the elevated mtDNA signal may reflect compensatory mitochondrial biogenesis in response to genomic stress. These mitochondrial alterations are closely linked to cellular aging phenotypes, where increased mitochondrial respiration and elevated mtDNA copy numbers are hallmarks of age-associated metabolic remodeling (Bratic and Larsson 2013). To determine whether loss of *MGT1* influences aging, we assessed chronological lifespan. The *Δmgt1* mutant exhibited a significantly shortened lifespan compared to the wild-type (Fig. 4). This is consistent with previous findings that deletion of essential DNA repair genes can severely compromise long-term survival (Sassanfar and Samson 1990). During aging, DNA repair efficiency declines (Yehuda et al. 2001), and loss of direct reversal pathways increases the likelihood of persistent alkylation damage and transition mutations, which may progress to double-strand breaks and cell death (Peng and Pei 2021). Thus, the reduced lifespan of *Δmgt1* is likely due to cumulative genomic instability and insufficient repair capacity during aging.

Bioinformatics analysis (Fig. 5, Table 2) further supports the involvement of Mgt1p in interconnected DNA repair, stress response, and mitochondrial pathways. Mgt1p

interacts directly with DNA repair proteins including Msh6p, Mag1p, Ogg1p, Mre11p, And Exo1p, and participates in cellular responses to DNA damage. Additionally, Msh6p, Ubr1p, Ufd4p, And Mlh1p are involved in ATP binding, suggesting that *MGT1* deletion may alter energy homeostasis. Increased cellular ATP demand can elevate mitochondrial respiration and ROS production, thereby contributing to oxidative stress and elevated mtDNA content. Mgt1p is also subject to ubiquitin-mediated degradation by Ubr1p and Ufd4p, and the gene *MGT1* exhibits indirect interactions with *MSH3*, *RAD14*, and *MSH2*, which regulate cell cycle progression and replication fidelity (Kadyrova et al. 2016). Furthermore, *ATP11*, *IMP2*, *MSS1*, and *PTH1*, which contribute to mitochondrial function and ATP synthesis, also interact with *MGT1* (SGD n.d.). Together, this interaction network supports the hypothesis that deletion of *MGT1* disrupts mitochondrial dynamics, genome maintenance, and cell cycle regulation, ultimately leading to reduced lifespan in the $\Delta mgt1$ strain.

Deletion of *MGT1* in *Saccharomyces cerevisiae* led to a significant increase in mitochondrial DNA (mtDNA) copy number, indicating a compensatory response to oxidative or metabolic stress. Despite this increase, the $\Delta mgt1$ strain exhibited a markedly reduced lifespan, suggesting that the absence of *MGT1*-mediated DNA repair results in genomic instability, altered mitochondrial homeostasis, and accelerated cellular aging. These findings highlight the critical link between nuclear genome maintenance and mitochondrial integrity. Further investigation into the signaling pathways connecting *MGT1* deficiency to mitochondrial remodeling and reduced cellular lifespan will provide deeper insights into the molecular mechanisms governing mitochondrial function and cellular aging. Understanding these mechanisms in yeast may also offer valuable clues for plant systems, where the homologous gene *AtAGT1* plays a similar role in maintaining genomic stability and mitochondrial health.

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