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YCF and *YAP* gene expressions in yeast cells after irradiation combined with mercury treatment

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Background: All aerobically growing organisms suffer from exposure to oxidative stress, caused by partially reduced forms of molecular oxygen, known as reactive oxygen species (ROS). These are highly reactive and capable of damaging cellular constituents such as DNA, lipids and proteins. Consequently, cells from many different organisms have evolved mechanisms to protect their components against ROS. It is known to have some genes for resistance to heavy metals and ionizing radiation (IR). ***Materials and Methods:*** *Saccharomyces cerevisiae* is an ideal model organism for deducing biological processes in human cells. In this work, cell viability and gene expression was investigated in yeasts treated with IR, $HgCl_2$, and IR combined with $HgCl_2$. Cell viability was measured by colony forming unit (CFU) method in an YPD medium. Gene expression was analyzed by the Real-time PCR. ***Results:*** The viability was lower at the higher dose. At a dose above a certain level, the viability came down to zero. The combined treatment decreased the viability, as well. Metal resistance genes were expressed in the cells treated with $HgCl_2$. In a similar way, irradiation also triggered the expression of some radiation resistance genes. *YCF* and *YAP* genes were induced consecutively with the $HgCl_2$ concentration, and also with a higher total dose under a lower dose rate condition. These two genes were, however, expressed differently under the 0.2 mM $HgCl_2$ treatment condition. In the cells treated with 0.1-0.2 mM $HgCl_2$, the viability was higher than with any other concentrations. ***Conclusion:*** The results demonstrated that the higher dose induced more expression of oxidative stress resistance genes related to cell survival mechanism. Combined treatment of radiation with mercury chloride resulted in synergistic effects leading to a higher expression of the genes than treatment of a single stressor alone.
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Keywords: *Metalloid Stress, ionizing radiation, YCF gene, YAP gene, yeast, ROS.*

INTRODUCTION

Saccharomyces cerevisiae is an ideal model organism for deducing biological processes in human cells. Completions of the human and yeast genome sequences have considerably increased the opportunities to address a human gene function using yeast. All aerobically growing organisms suffer from exposure to oxidative stress, caused by partially reduced forms of molecular oxygen, known as reactive oxygen species (ROS). These are highly reactive and capable of damaging cellular constituents such as DNA, lipids and proteins. Consequently, cells from many different organisms have evolved mechanisms to protect their components against ROS. ROS can also be formed by exposure of cells either to ionizing radiation or redox-cycling chemicals present in the environment like heavy metals [1]. Many diseases and most of the genes associated with the repair of radiation -induced damage in mammalian cells were initially characterized in yeast. Combined action of ionizing radiation and other agents is of potentially great importance, because there are many occasions when interactions might occur in our environment. Ionizing radiation (IR) is used for therapy, diagnosis, prevention of diseases, and sterilization of foods. Exposure of cells to DNA-damaging agents like IR results in complex response mechanisms to maintain a genetic integrity after DNA damage. These include a cell cycle delay, repair of DNA damage, transcriptional responses, and a programmed cell death [2, 3]. Metal ions are essential to life. However, some metals such as mercury are harmful, even when present at trace amounts. As a mercury ion, it induces an oxidative stress or predisposes cells to an oxidative stress, with considerable damage to proteins, lipids and DNA. The protective effect of Ycf1p against the toxicity of

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mercury is especially pronounced when yeast cells are grown in a rich medium or in a minimal medium supplemented with glutathione. Secretor vesicles from *S. cerevisiae* cells induce the expression of Ycf1p and a transport by this protein of mercury-glutathione adducts into the vacuole [4]. Ycf1 is positively regulated by distinct, but related bZIP transcriptional activators, Yap1 [5, 6]. *YCF1* gene encodes an ABC transporter protein that shares a strong sequence similarity with a human cystic fibrosis trans-membrane conductance regulator and a multidrug resistance-associated protein. Yap (yeast activator protein) induced activities of several enzymes involved in an oxygen detoxification. Yap1 also controls the expression of certain ABC transporter protein encoding genes, namely *YCF1*, involved in the removal or sequestration of cadmium [7, 8]. In the absence of a stress, Yap1p is present in both the cytoplasm and the nucleus. Upon treatment with an oxidative stress, the protein is concentrated in the nucleus. Metalloid exposure triggers a nuclear accumulation of Yap1p and stimulates an expression of antioxidant genes [8-10]. In yeast, the physiological response to changing environmental conditions is controlled by a cell type. The previous study on the cell viability after treatment of radiation and mercury resulted in different response to the same stress condition in both cell types (haploid and diploid) [11]. Diploids are more resistant than haploids to a radiation stress, and to heavy metals such as mercury and cadmium, as well [12, 13]. Haploid cells were used in this experiment, namely *S, cerevisiae* strain W303-1A *MAT*a {*leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*}. W303 strain contains a mutant allele of *YBP1*, *ybp1-1*, encoding four amino acid substitutions. The *YBP1* gene was also resistance to an oxidative stress [14]. The expression of the *YAP* and *YCF* genes was investigated by a Real-time PCR and the cell survival induced by radiation and mercury stresses in *S. cerevisiae* was analyzed.

MATERIALS AND METHODS

Strain and Culture

S. cerevisiae strain W303-1A *MAT*a {*leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*} was grown on a rotary shaker in a YPD medium at 30 ºC for 60 h.

Stress induction

A metalloid stress was introduced by liquefied $HgCl_2$. Treatment of $HgCl_2$ was done in the concentration of 0.1- 0.9 mM. For an oxidative stress induced by IR, 100, 400, 800 and 1200 Gy hr^{-1} were applied to obtain a total dose of 400 and 800 Gy, respectively. A combined stress was induced with a medium containing $HgCl_2$ after the cells were irradiated with IR.

CFU (colony forming unit)

Cell viability was measured by means of CFU (colony forming unit). The culture approached an optical density (600 nm) equal to 0.6, which corresponds to a density of 2.9×10^7 cells ml^{-1}. The cells were diluted and spread on the plate treated with $HgCl_2$ or without $HgCl_2$. Experiments were performed three times and the cell survival rates were calculated from an average of the three experimental data.

RNA isolation

S. cerevisiae was grown on a complex medium of YPD to the logarithmic growth phase. Total RNA was extracted using the Ribo-Pure Yeast (Ambion) following the protocol provided with the kit. DEPC-treated water was used in the process.

Real-time PCR

The cDNA for the Real-time analysis was prepared with ycf_RT_R, yap_RT_R and act_RT_R primers by RT-PCR reactions. The Real-time PCR analysis was performed using the SYBR Premix Ex Taq (TaKaRa), with the prepared cDNA (2 μg reaction^{-1}) and primers; ycf_F2/R2, yap_F2/R1, act_F3/R2 (table 1).

Table 1. Primer sequences used in PCR and Real-time PCR.

Primers	Sequence of primers		
ycf_F2	5'-	AGTAATAAGGTGAGCGCGTTATCCATCGCA	-3'
ycf_R2	5'-	GCCCTCCTTAAACTTATGGCGTCAGAGTTG	-3'
yap_F2	5'-	CGAAAATGAACCAGGTATGTGGAACAAGGC	-3'
yap_R1	5'-	CGAAACCAAGTCGGCCAAAACCGGAGAAGG	-3'
act_F3	5'-	TTGAACACGGTATTGTCACCA	-3'
act_R2	5'-	AAACAATACCAGTTCTACCGG	-3'
ycf_RT_R	5'-	TTAATTTTCATTGACCAAACCAGC	-3'
yap_RT_R	5'-	CTAATTGAACGTCTTCTGCA	-3'
act_RT_R	5'-	ACTTGTGGTGAACGATAGATGGACCACTTT	-3'

RESULTS AND DISCUSSION

Cell viability measurement

Metalloid and ionizing radiation could induce a kind of oxidative stress. Total doses of ionizing radiation treatments were 100, 400, and 800 Gy for each dose rates applied. The cell viability reduced more with the higher dose rates (800 and 1200 Gy hr^{-1}) than with the lower dose rates (100 and 400 Gy hr^{-1}). The viability of the irradiated cells was prone to be inversely proportional to a total dose (table 2). Treatment of $HgCl_2$ was done in the concentration range from 0.1 to 0.9 mM. The cell survival rates significantly reduced in a concentration higher than 0.2 mM. The half lethal concentration estimated from the survival curve was 0.253 mM. The cells were almost extinct when the concentration of the $HgCl_2$ exceeded 0.3 mM. The cells treated with low concentration of $HgCl_2$ showed higher survival rate (figure 1). The combined treatment of 100 Gy with 0.2 mM or 0.25 mM of $HgCl_2$ significantly reduced the survival rates of the cells, while the combination of 100 Gy with 0.3 mM resulted in an extinction of the cells (figure 2). The combination of ionizing radiation 800 Gy with 0.3 mM $HgCl_2$ caused a complete inactivation of the cells. Simultaneous treatment of the two stressors resulted in a lower viability than that of one stressor only. In other word, the combined treatment affected, in terms of viability, the cells more than a single stress treatment.

Table 2. Cell viability (%) of *S. cerevisiae* cells irradiated with ionizing radiation.

		Dose rate (Gy hr^{-1})			
		100	400	**800**	**1200**
Total Dose (Gy)	0	100.0	100.0	100.0	100.0
	100	74.8	49.5	59.1	47.7
	400	65.6	73.6	50.4	48.9
	800	55.0	50.4	37.4	38.8

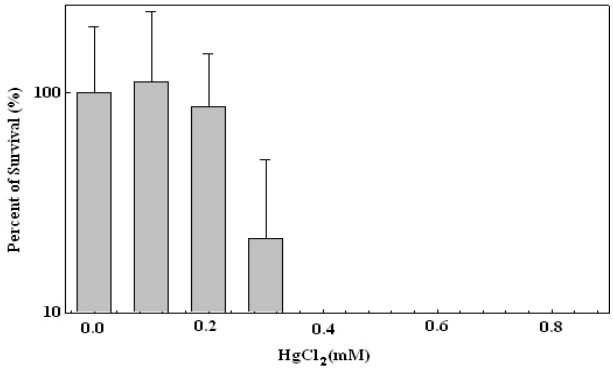

Figure 1. Cell viability of *S. cerevisiae* treated with various concentrations of mercury chloride (II).

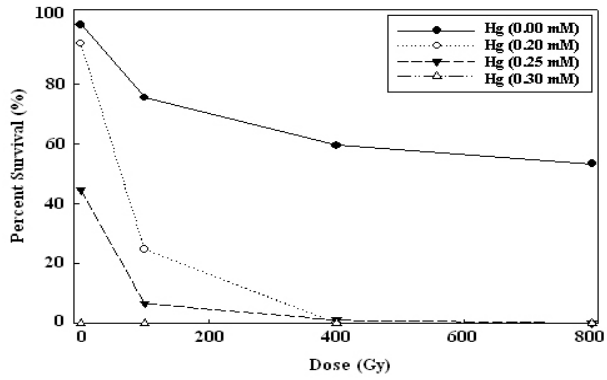

Figure 2. Cell survival rate of *S. cerevisiae* after treatment of mercury chloride (II) combined with ionizing radiation (dose rate; 400 Gy hr^{-1}).

Gene expression

The expression of the *YCF* gene increased at a higher total dose. The expression of a particular gene was dependent on kinases, the mean expression levels exhibited a ≥2-fold change in an expression in the mutant compared to a wild -type after exposure to ionizing radiation [3]. For the mechanism of protection against a radiation-induced lethality, several gene groups are implicated indirectly through the mechanisms of mRNA and protein stability and/or trafficking. Identification of genes controlling DNA-damage responses in yeast has often led to the discovery of functionally related genes in mammalian cells, including many DNA repair genes [2]. The 0.0 mM, 0.25 mM and 0.3 mM treated cells revealed the same expression of the *YCF* gene. The expression of *YCF1* is induced by the presence of cadmium or by limiting levels of adenine. The response of *YCF1* to cadmium is mediated by an up-regulation of Yap1p, a transcriptional factor which positively controls the expression of numerous genes involved in an adaptation to an oxidative stress. A Yap1p-regulatory element was identified in the *YCF1* promoter region. The results from the cells treated only with $HgCl_2$ were compared to those from the cells treated with ionizing radiation and $HgCl_2$ at the same time (figure 2). As for the cells with radiation treatment only, the *YCF* gene expression was lower in the cells irradiated with 400 Gy than that of the non-irradiated control cells. In particular, the cells irradiated with 400 Gy showed a lower expression of *YCF* gene than the cells irradiated with 800 Gy. The *YCF* gene expression, however, showed a quite different pattern in the 0.2 mM $HgCl_2$ treated cells. The *YCF* gene expression was higher in the cells with the simultaneous treatment of radiation and $HgCl_2$ than the cells treated with $HgCl_2$ only, while the *YAP* gene expression was higher in the cells treated with $HgCl_2$ only. The cells after the combined treatment of 0.2 mM $HgCl_2$ with 400 Gy resulted in a higher expression of the *YCF* gene than the cells after the combined treatment of 0.2 mM $HgCl_2$ with 800 Gy. The 800 Gy irradiated cells revealed a lower *YCF* gene expression than the 400 Gy irradiated cells at the 400 Gy hr^{-1} dose rate. In case of 800 Gy hr^{-1} dose rate, the *YCF* gene was expressed in the cells irradiated with 400 Gy and 800 Gy, as well. A lower expression level was found in the 0.2 mM $HgCl_2$ treated cells than in the non-treated cells (figure 3). However, the expression level increased with the concentration of $HgCl_2$. The combined treatment resulted in synergistic effects which were consistent with reports of other workers for cadmium [16], heating [17], etc. Various oxidants activate Yap1p and this protein is required for an oxidative stress tolerance as well as for a resistance to heavy metals or cytotoxic agents [4, 5, 15]. Transcription factor Yap1p plays a major role in conferring a cellular resistance to mercury and also in regulating the *GSH1* expression in response to this metal. This finding is consistent with the epistatic relationship described above and is presumably due to a direct or indirect enhancement of the *YCF1* gene transcription by yAP-1. The expression of *YCF1* positively correlates with changes in the *YAP1* gene dosage. The observed yAP-1 dependent changes in *YCF1* mRNA are brought about through an action of yAP-1 on the *YCF1* promoter region [6]. Mercury chloride (II) treated cells except for 0.2 mM showed a higher *YAP* gene expression for 800 Gy than 400 Gy total irradiation dose. The expression of the *YAP* gene increased under the condition of high total dose combined with 0.3 mM $HgCl_2$ treatment. For the 0.2 mM $HgCl_2$ treated cells, the expression of the *YAP* gene increased at the high dose rates and with a low total dose (figure 4). Exceptionally, both genes in the 0.2 mM $HgCl_2$ treated cells were expressed more when irradiated with 400 Gy than the 800 Gy total dose. The results meant that a lower total dose increased the expression of the genes.

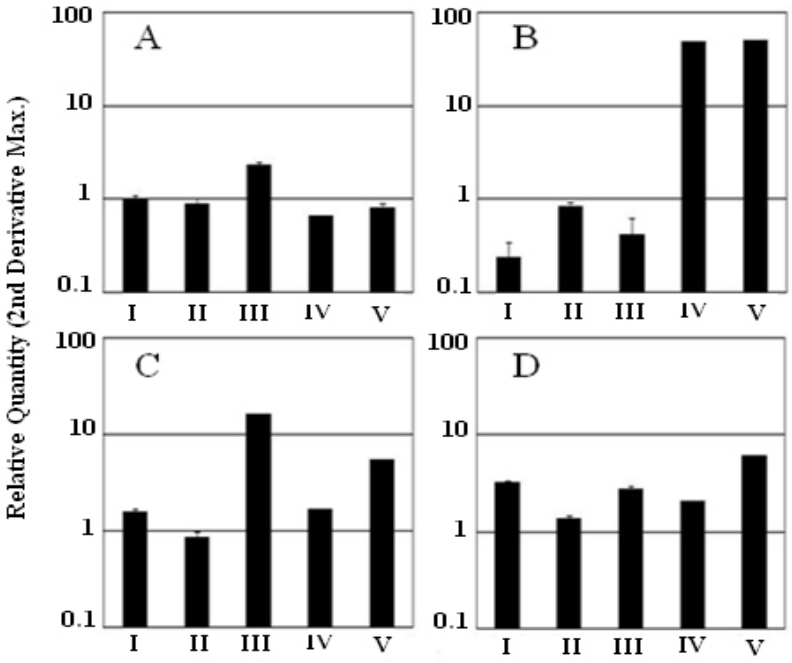

Figure 3. Gene expression of *YCF* assessed by the Real-time PCR.
A. non-treated cells; B. 0.2 mM mercury chloride (II) treated cells; C. 0.25 mM mercury chloride (II) treated cells; D. 0.3 mM mercury chloride (II) treated cells. Lane I was for a sham control cells. Lane I was for the cells irradiated with 400 Gy radiation at 400 Gy hr^{-1}. Lane III was for the cells irradiated with 800 Gy at 400 Gy hr^{-1}. Lane IV was for the cells irradiated with 400 Gy at 800 Gy hr^{-1}. Lane V was for the treated with 800 Gy at 800 Gy hr^{-1}.

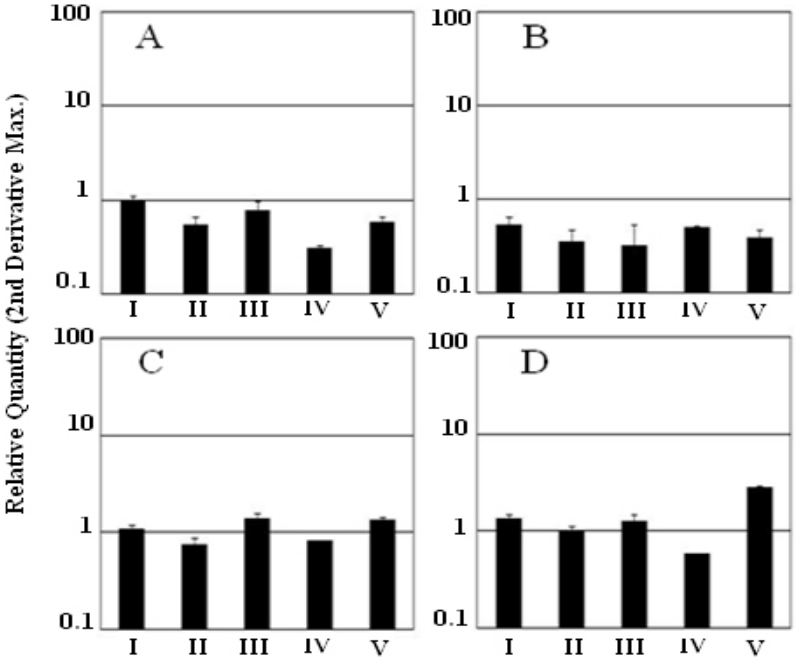

Figure 4. Gene expression of *YAP* assessed by the Real-time PCR.
A. non-treated control cells; B. 0.2 mM mercury chloride (II) treated cells; C. 0.25 mM mercury chloride (II) treated cells; D. 0.3 mM mercury chloride (II) treated cells. Lane I was for a sham control cells. Lane II was for the cells irradiated with 400 Gy radiation at 400 Gy hr^{-1}. Lane III was for the cells irradiated with 800 Gy at 400 Gy hr^{-1}. Lane IV was for the cells irradiated with 400 Gy at 800 Gy hr^{-1}. Lane V was for the treated with 800 Gy at 800 Gy hr^{-1}.

CONCLUSION

The cell viability was reduced significantly by the treatment of $HgCl_2$ combined with IR. The combined treatment had a greater impact on the cell survival. In case of combined treatments, the *YAP* and *YCF* genes expression induced by a metalloid stress increased with $HgCl_2$ concentration and with the total dose of ionizing radiation, as well. The combined treatment resulted in synergistic increase in gene expressions, and the increase was found to be dose-dependent.

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