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DNA microarray analyses reveal a post-irradiation differential time-dependent gene expression profile in yeast cells exposed to X-rays and γ-rays

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Abstract

Ionizing radiation (IR) is the most enigmatic of genotoxic stress inducers in our environment that has been around from the eons of time. IR is generally considered harmful, and has been the subject of numerous studies, mostly looking at the DNA damaging effects in cells and the repair mechanisms therein. Moreover, few studies have focused on large-scale identification of cellular responses to IR, and to this end, we describe here an initial study on the transcriptional responses of the unicellular genome model, yeast (*Saccharomyces cerevisiae* strain S288C), by cDNA microarray. The effect of two different IR, X-rays, and gamma (γ)-rays, was investigated by irradiating the yeast cells cultured in YPD medium with 50 Gy doses of X- and γ-rays, followed by resuspension of the cells in YPD for time-course experiments. The samples were collected for microarray analysis at 20, 40, and 80 min after irradiation. Microarray analysis revealed a time-course transcriptional profile of changed gene expressions. Up-regulated genes belonged to the functional categories mainly related to cell cycle and DNA processing, cell rescue defense and virulence, protein and cell fate, and metabolism (X- and γ-rays). Similarly, for X- and γ-rays, the down-regulated genes belonged to mostly transcription and protein synthesis, cell cycle and DNA processing, control of cellular organization, cell fate, and C-compound and carbohydrate metabolism categories, respectively. This study provides for the first time a snapshot of the genome-wide mRNA expression profiles in X- and γ-ray post-irradiated yeast cells and comparatively interprets/discusses the changed gene functional categories as effects of these two radiations vis-à-vis their energy levels.

Keywords: Changed gene expression; Irradiation; Microarray; *Saccharomyces cerevisiae*

Today one of the major public health challenges we face is how to "precisely" determine the biological effects of low or ultra-low doses of environmental pollutants. With the functional genomics era, biological science has seen the advent of various investigative technologies, aimed at determining these very effects. However, it is easier said than done, and not only do we need the technology, it is imperative to have a test organism to examine or validate the deleterious effects of a particular pollutant. The unicellular model yeast (*Saccharomyces cerevisiae*) is one of the most ideal organisms being used to deduce the biological processes in human cells, and consequently serves as an honorary mammal [1]. This is mostly due to the availability of the complete genome sequences of human and yeast, which has in turn generated methods for genome-wide transcriptional analyses, such as microarrays [2,3]. Genome-wide expression analysis using DNA microarrays is a widely used method to explore environmentally induced changes in the remodeling of gene expression in a particular cell, organ, tissue, or organism. Moreover, microarray analyses in response to a variety of

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environmental factors, including DNA-damaging agents have made yeast a valuable model organism [3–9].

Ionizing radiation (IR), a genotoxic stress factor in our environment, is a major and highly notorious stressor of plants and animals, including humans. Among the well-known effects of IR are DNA damage, including single- and double-strand breaks (DSB), base damage, and DNA–protein cross links, caused either directly or indirectly via the reactive oxygen species (ROS), ultimately leading to cell death. Moreover, the possible affects of IR, dramatically enhanced by the nuclear accidents in our industry and the problems associated with emissions, wastes, cancers, and mutations, have captured the imagination of the public. Therefore, its (IR) study is of not only academic interest but essential to our understanding of the mechanisms underlying the IR response(s) in cells. By employing a model organism such as yeast, it is possible to screen for potential sets of genes and regulatory pathways that are employed by yeast cells to respond to external stimuli-radiation in our study. These can then be applied to study/hypothesize similar effects in organism of interest, in our case humans. Moreover, there are hardly any studies which investigate the effects of X- or gamma (γ)-rays in humans at the molecular level mostly due to the limited use of human materials.

Recently, using the yeast (*S. cerevisiae* D7) model, two studies reported the microarray-based transcriptional analysis as a powerful tool to detect the biological effects of varying doses of radiation [10,11]. Our present study on yeast responses to damaging irradiation doses utilizes this DNA microarray technology to reveal the genome-wide transcriptomics profiles after exposure to X-rays and γ-rays in the indicator yeast strain *S. cerevisiae* S288C. The aim of the study is simple and straightforward—to identify the molecular changes associated with the IR in yeast cells at the mRNA expression level using high-throughput microarray approach and discuss those changes in context of the applied irradiations.

Materials and methods

Strains and culture conditions. Yeast *S. cerevisiae* strain S288C (α *SUC2 mal mel gal2 CUP1*) was inoculated in 600 ml of YPD medium (1% Bacto-yeast extract (Difco Laboratories), 2% polypeptone (NIHON SEIYAKU), and 2% glucose) and cultured at 25 °C, with shaking, to an optical density of 1.0 at 660 nm. The cultures were centrifuged (5000 rpm, 10 °C, 2 min) and resuspended in 300 ml of sterile 0.9% NaCl solution. The 30 ml test suspensions were transferred to FALCON tube (50 ml) and kept at 0 °C, and cell viabilities were determined.

Irradiations and sampling. The 30 ml test suspension tubes were exposed to X-ray irradiation (dose: 50 Gy). X-rays were produced by a generator (SHIMADZU, PANTAC HF-320S) operated at 200 kVp and 20 mA. The X-rays were filtered with 0.5 mm aluminum and 0.5 mm copper. The dose rate was 2.003 Gy/min. The 50 ml test suspensions were irradiated by γ-rays from a ^{60}Co (cobalt) gamma source (111 TBq) at a dose of 50 Gy (dose rate of 1.609 Gy/min). All procedures were carried out at room temperature (RT). The unirradiated and irradiated test suspensions were centrifuged (5000 rpm, 4 °C, 2 min) and washed with sterile distilled water. The cells were re-suspended in each 600 ml of YPD medium, and cultured at 25 °C, with shaking in the dark condition. The cells were harvested at 20, 40, and 80 min post-irradiation (PI), and surviving cell viabilities were determined. Experiments were repeated three times for each irradiation set as independent experiments.

Viability of yeast. Cell viabilities of control and irradiated samples were determined by serial dilutions in sterile distilled water, and subsequently by plating on YPD agar medium. Each of the plates was incubated for 48 h at 25 °C and the colony number was counted.

DNA microarray. Microarray experiments were carried out as previously described [6]. Briefly, total RNA was extracted by the hot-phenol method, and poly(A) + RNA was purified from about 400 mg of total RNA with an Oligotex-dT30 mRNA purification kit (TaKaRa, Otsu, Shiga, Japan). The cDNA labeling reaction was performed as instructed (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). The control sample cDNA was labeled with Cy3, and treated sample cDNA was labeled with Cy5. After neutralization with 10 µl of 2 M acid-free Hepes, the Cy3- and Cy5-labeled cDNA solutions were filtered using an Autoseq™G-50 spin column (Amersham Biosciences). The Cy3- and Cy5-labeled cDNA solutions were dried and precipitated into pellets. These pellets were dissolved with 15 µl RNase-free water. Both cDNA solutions were mixed followed by addition of 10 µl of 20× SSC (saline-sodium citrate) hybridization buffer and heated at 90 °C for 5 min. After cooling on ice, 1 µl of 10% SDS solution was added. The solution was spread on the DNA chip (Ver. 2.0, DNA Chip Research, Inc., Yokohama, Japan) and carefully covered with a glass slip. Hybridization was carried out at 60 °C for 24–36 h in a hybridization chamber maintaining humidity. After hybridization for the desired time period, the cover glass was removed by sinking in the solution of 2× SSC/0.1% SDS for 15 min. The slide was gently incubated with 2× SSC/0.1% SDS, twice with 0.2× SSC/0.1% SDS, and with 0.2× SSC for 20 min per each wash in turn, followed by rinsing with 0.05× SSC, and centrifuging at 600 rpm for 20 s; the chip was dried at RT. Three independent microarray analyses were carried out.

Scanning and data analysis. A Scan Array 4000 laser scanner (GSI Lumonics, Billeria, MA, USA) was used to measure hybridized spots' signals and array images were analyzed using Gene Pix 4000 analysis application program (Molecular Devices, Sunnyvale, CA, USA). The area surrounding each spot was used to calculate local background value, which was subtracted from the total element signal value. The signal subtracting background value was used to determine the differential expression, by a ratio value (Cy5 intensity/Cy3 intensity) for each element. A correlation factor was applied to normalize systematic differences in the intensity of spots. The defective spots were eliminated from the data sets, and excluded as non-specific hybridizations, with a cut off point of 2 standard deviations (SD) of the median of the negative control spots. GeneSpring Ver. 4 software (Silicon Genetics, CA, USA) was used for the clustering and statistical analysis. If the logarithm (n, 2) value of the normalized Cy5/Cy3 ratio is more than 1 ($\log n > 2$, 2) then it means "up-regulated gene", and is less than −1 ($\log n < 0.5$, 2) then means "down-regulated gene" [12]. For the reliability of data, the ratio values greater than twofold or less than 0.5-fold over the control were considered to be induced (up-regulation) or suppressed (down-regulation), respectively. Information for the gene classification was retrieved from the Munich International Center for Protein Sequence (MIPS) comprehensive yeast genome database website (http://mips.gsf.de/genre/proj/yeast/index.jsp).

Results and discussion

Gene expression patterns by microarray analysis

After irradiation of X- and γ-rays, the *S. cerevisiae* cell viability was reduced from an initial concentration of 1.6×10^7 cfu/ml to 7.3×10^6 cfu/ml and 1.7×10^7 cfu/ml to 5.7×10^6 cfu/ml, respectively. Total RNA extracted from PI yeast cell cultures was subjected to cDNA microarray analysis as mentioned in the Materials and methods. The yeast cDNA microarray used here represents a total of

5989 unique open-reading frames (ORFs). The genes/ORFs that were expressed more than twofold or less than 0.5-fold in at least two out of the three independent experiments were selected as the induced or repressed genes, respectively. A time-course analysis revealed, after 20, 40, and 80 min PI, up-regulation of 126 (20 min), 50 (40 min), and 19 (80 min), and 24 (20 min), 44 (40 min), and 57 (80 min) genes by X- and γ-rays, respectively. Similarly, after 20, 40, and 80 min PI, down-regulation of 83 (20 min), 112 (40 min), and 12 (80 min) and 19 (20 min), 31 (40 min), and 27 (80 min) genes by X- and γ-rays, respectively, was observed. Functional categorization of these changed genes was performed using the MIPS database [13], which is presented graphically in Fig. 1 (for X-ray) and Fig. 2 (γ-ray), respectively. The detailed list along with the functional categories of these genes is shown in Tables 1 (1-1, 1-2, and 1-3 for 20–80 min, respectively) and 2 (2-1, 2-2, and 2-3 for 20–80 min, respectively).

What kind of gene functions are effected by X- and γ-rays

At first glance, the data (Figs. 1 and 2) suggest differences between these two irradiation rays, a large number of changed gene expressions by X-rays compared to almost half that number for γ-rays. Moreover, the yeast cells exposed to X-rays showed early (20 min) response at the mRNA level compared to a relatively late (40–80 min) response under γ-rays. Interestingly, a gradual decrease in the changed gene expressions is evident for the X-rays, especially in the case of up-regulated genes. On the other hand, an almost opposite effect is visible with γ-rays, a gradual increase in gene function changes, particularly in the case of down-regulated genes. Additionally, the highly induced and suppressed categories were relatively higher for X-rays than γ-rays. For example, the categories, "cell cycle and DNA processing" and "cell rescue defense and virulence" were very highly

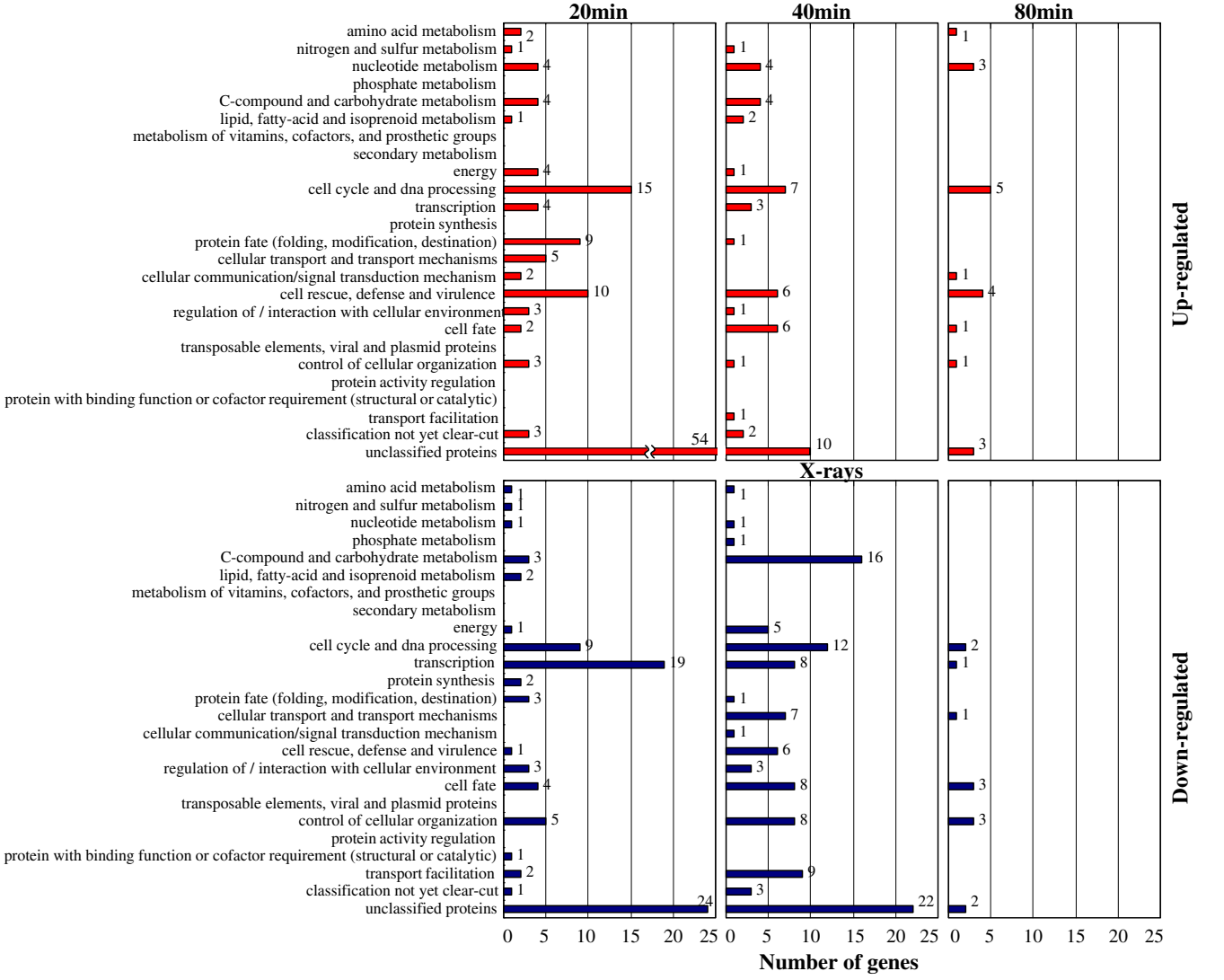

Fig. 1. Functional classification of genes (ORFs) whose expression changed upon exposure to X-rays. Both the up- and down-regulated genes are classified with respect to their functions in a time-dependent manner during post-irradiation culture of yeast cells.

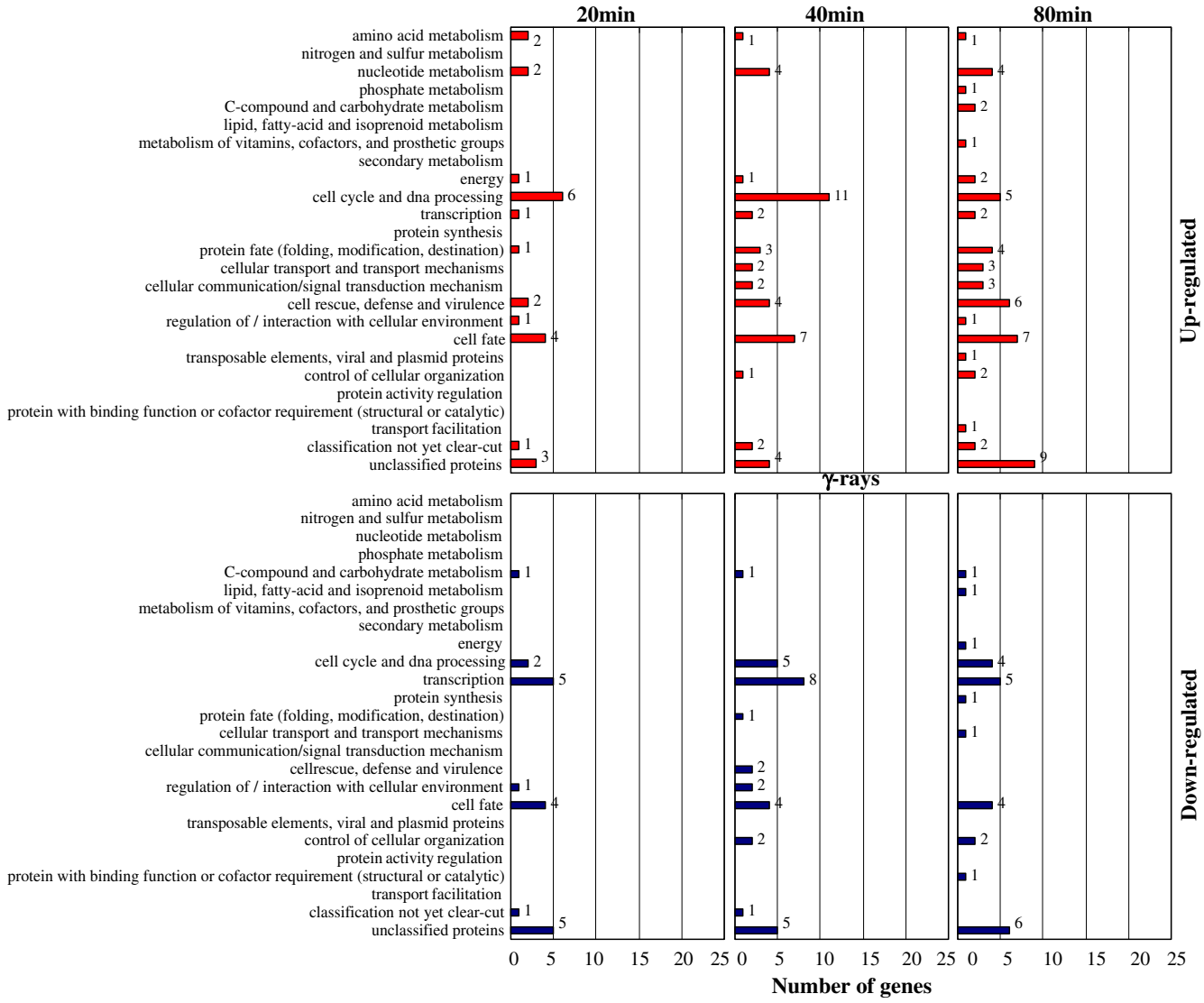

Fig. 2. Functional classification of genes (ORFs) whose expression changed upon exposure to γ-rays. Both the up- and down-regulated genes are classified with respect to their functions in a time-dependent manner during post-irradiation culture of yeast cells.

induced by X-rays over that seen for γ-rays, which were induced much later (40–80 min PI). On the contrary, in the down-regulated gene list, ''transcription'' and ''cell cycle and DNA processing'' categories were strongly suppressed over that seen for γ-rays in a similar fashion as seen during the up-regulation of gene category mentioned above. These three observations suggest that X-rays have a potent effect on yeast cells vis-à-vis γ-rays by causing large changes in different sets of genes very early in the PI yeast cell culturing period. As expected, among the changed genes, we could also identify DNA repair- and damage-related genes. For X-rays, a total of 12, 13, and 10 (up-regulated) and 3, 4, and 3 (down-regulated) genes were identified from 20, 40, and 80 min PI yeast culture, respectively. For γ-rays, a total of 11, 21, and 12 (up-regulated), and 4, 5, and 5 (down-regulated) genes were identified from 20, 40, and 80 min PI yeast culture, respectively. This analysis indicates that γ-rays have only a slightly larger set of gene numbers (especially at 40 min PI) for this subcategory compared with X-rays.

Can we distinguish between X- and γ-rays based on functional categories?

Rather than just looking at the functional categories independently for each irradiation rays, we were also interested in comparing the changed functional categories between X- and γ-rays. This should help define some hypothetical similarities and differences in ''effects'', if any, between X- and γ-rays on yeast cells. In the up-regulated category list, we can find ''metabolism, energy, cell cycle and DNA processing, cellular transport and transport mechanisms, protein fate, and cell rescue defense and virulence'' strongly induced within 20–40 min by X-rays (Fig. 3A). Interestingly, most of the above categories,

Table 1

Table 1-1: Common genes and functional classification after irradiation to X-ray (20 min)

Amino acid metabolism
ARG3, LYS1 LYS4

Nitrogen and sulfur metabolism
DAL80 UGA3

Nucleotide metabolism
RNR2, RNR4, RNY1, TIR1 URA7

C-compound and carbohydrate metabolism
GCY1, HSP12, PFK261, ARA1 GAL10, MLS1, PMT5

Lipid, fatty-acid and isoprenoid metabolism
HSP12 GAS1, FAA3

Energy
CYC7, NDE2, FRDS1, QCR8 MLS1

Cell cycle and dna processing
HUG1, RNR2, TFS1, RNR4, YCL048W, RAD53, HSP12, POL1, RAD5, HOS4, TRX2, RFA1, ECO1, TOP2, REC1
CLB1, CLN2, CDC5, CDC50, SIC1, SDA1, PSF1, APC4, ASE1

Transcription
YPR015C, GSP2, HOS4, DAL80 SWI5, ASH1, YAP6, HHF2, SAS10, SET2, UGA3, RCL1, HST3, SIK1
YGR067C, SYF2, NOP58, DBP10, CCA1, TRM1, HTB2, NTO1, HTA1

Protein synthesis
NOP7, IFM1

Protein fate (folding, modification, destination)
HSP26, YOR285W, SOM1, ATG8, YPT53, SDP1, HOS4, TRX2, ERP5
CSN9, APC4, PMT5

Cellular transport and transport mechanisms
SOM1, GSP2, ATG8, YPT53, ERP5

Cellular communication/signal transduction mechanism
RAD53, SDP1

Cell rescue, defense and virulence
HUG1, DDR2, CRS5, CYC7, HSP26, HOR7, HSP12, GTT1, TIR1, TRX2 YHR048W

Regulation of / interaction with cellular environment
TFS1, CRS5, HSP12 ASH1, CSN9, PMA1

Cell fate
YCL048W, RFA1 CLN2, SWI5, ASH1, AMA1

Control of cellular organization
NDE2, SOM1, MDM35 CLN2, URA7, ECM37, IEM1, GAS1

Protein with binding function or cofactor requirement (structural or catalytic)
PSF1

Transport facilitation
PMA1, YHR048W

Classification not yet clear-cut
YCR062W, EMP46, PLM2 SCW10

Unclassified proteins
YNL194C, YHR087W, YHR095W, RTN2, MOH1, YOL085C, YPL014W, SRF4, YNL143C, YGR069W, TDR048C, YKR040C
PHM7, YHR140W, YNL195C, YBR085C-A, YDR034W-B, YJL163C, YLR269C, YKL044W, YLR252W, FMP45, YHR138C
FMP33, YAL018C, YKL151C, UBR2, SRL3, KRE26, SPG4, DCS2, YNL208W, YBR230C, YJL016W, YOL150C, YKL107W
YJL067W, YFR035C, YNR005C, YKL162C-A, YHR035W, YLR296W, EMI5, YMR290W-A, YGL217C, YLR297W, YNL319W
YMR046W-A, RDR1, YJR018W, YOL047C, YLR408C, YIL086C, YOL162W
YDR222W, YOR309C, FYV7, YCR051W, YGR228W, YGL176C, YCR049C, YOR199W, YDL096C, YDL176W, HGH1, DSE3
YBL046W, THG1, YOR154W, YOL024W, SET4, YML119W, RIO1, YJR030C, YLR364W, YGR290W, FYV13, YOR200W

Table 1-2: Common genes and functional classification after irradiation to X-ray (40 min)

Amino acid metabolism
PUT1

Nitrogen and sulfur metabolism
YJR149W

Nucleotide metabolism
RNR2, RNR4, RNR3, RNR1 ISF1

Phosphate metabolism
PHO89

C-compound and carbohydrate metabolism
HSP12, MAE1, SNF2, SHC1
GSY1, GLC3, GSY2, DSE4, EMI2, HXT2, GUT2, HXT8, HXT4, MTH1, MAL31, HXT7, HXT10, YHR210C, MAL11, CHS1

(continued on next page)

Table 1 (*continued*)

Lipid, fatty-acid and isoprenoid metabolism	
HSP12 , ERG4	
Energy	
FRDS1	GSY1, GLC3, GSY2, GLG1, ALD4
Cell cycle and dna processing	
HUG1, RNR2, RNR4, RNR3, RNR1, HSP12, RAD51	
EGT2, SPO16, DSE2, RCK1, SIC1, CIN5, YLL017W, REC107, RME1, SAE2, SLT2, CHS1	
Transcription	
SNF2 , HCM1, RRN7	HHF1, HHT2, HHF2, CIN5, HTA1, HAP4, HHT1, RME1
Protein fate (folding, modification, destination)	
YOR285W	YLR327C
Cellular transport and transport mechanisms	
HXT2, HXT8, HXT4, NCE103, MAL31, HXT7, HXT10	
Cellular communication/signal transduction mechanism	
CMK2	
Cell rescue, defense and virulence	
HUG1, AQR1, HSP12, GTT1, YGP1, YFR022W	PIR1, HSP30, SLT2, HAL5, CUP1-2, CUP1-1
Regulation of / interaction with cellular environment	
HSP12	HSP30, PHO89, SLT2
Cell fate	
SNF2, SHC1, RAD51, YSW1 , SPS100, HBT1	EGT2, DSE1, SPO16, PFS1, HO, BOI1, SLT2, CHS1
Control of cellular organization	
SHC1	DSE1, PFS1, DSE2, ALD4, GUT2, GYP7, BOI1, HAL5
Transport facilitation	
AQR1	HXT2, HXT8, HXT4, MAL31, HXT7, HXT10, PHO89, MAL11, YLR004C
Classification not yet clear-cut	
PLM2, YCR062W	SCW11, MRK1, KIN82
Unclassified proteins	
YGR079W, SPO19, YJL037W, YDR317W, YLR297W, YHR140W, YLR050C, FMP52, YJL022W, YKR015C	
YER067W, YIL057C, YER066C-A, FMP48, YNL144C, BOP2, YOR019W, YLR049C, PRM10, YBR285W, FMP43	
YFR017C, OSW2, YLR198C, YPL158C, YNL046W, YLR257W, YMR254C, YOR263C, FYV10, YLR194C, DSE3	

Table 1-3: Common genes and functional classification after irradiation to X-ray (80 min)

Amino acid metabolism	
YHR033W	
Nucleotide metabolism	
RNR4, RNR3, RNR2	
Cell cycle and dna processing	
HUG1, RNR4, RNR3, RNR2, RAD51	HOF1, RSC2
Transcription	
HHT2	
Cellular transport and transport mechanisms	
SEC15	
Cellular communication/signal transduction mechanism	
GPG1	
Cell rescue, defense and virulence	
HUG1, YGP1, GTT1, HYR1	
Cell fate	
RAD51	HOF1, HO , SEC15
Control of cellular organization	
ECM4	HOF1, RSC2, SEC15
Unclassified proteins	
YLR050C, MOH1, AVO2	YOR315W, YOR366W

Genes marked in red show the up-regulation in proportion to concentration. All the other genes are down-regulated.

including "metabolism, cell cycle and DNA processing, protein fate, cell rescue defense and virulence, and cell fate," were also induced, though at relatively low levels and around 40–80 min PI by γ-rays irradiation (Fig. 3B). This analysis suggests that the effect of X- and γ-rays is similar in terms of changed gene expression, albeit at totally different time periods PI. It can be asked, does this time-lag constitute a difference in effects? Furthermore, it is evident that the yeast cells in the PI-culture period are trying to recover cellular functions by increased metabolism, cellular transport, along with an expected increase in the cellular defense mechanism. That this is more pronounced in the case of X-rays is logical considering their more potent effect over γ-rays on yeast cells as discussed above.

Table 2

Table 2-1: Common genes and functional classification after irradiation to γ -ray (20 min)

Amino acid metabolism
ARG3, ARO9 LYS4

Nucleotide metabolism
RNR2, RNR4

C-compound and carbohydrate metabolism
DSE4

Energy
FRDS1

Cell cycle and dna processing
HUG1, TFS1, RNR2, RFA1, RNR4, RAD51 EGT2, PST1

Transcription
YPR015C HHF2, HHF1, HTA1, ASH1, HHT2

Protein fate (folding, modification, destination)
HSP42

Cell rescue, defense and virulence
HUG1, DDR2

Regulation of / interaction with cellular environment
TFS1 ASH1

Cell fate
RFA1, SPS100, HBT1, RAD51 EGT2, HO, ASH1, PST1

Classification not yet clear-cut
PLM2 PCL9

Unclassified proteins
YHR087W, YOL150C, YOR055W YNL058C, YIL096C, FYV7, YNL057W, YDR413C

Table 2-2: Common genes and functional classification after irradiation to γ -ray (40 min)

Amino acid metabolism
ARG3

Nucleotide metabolism
RNR3, RNR2, RNR4, RNR1

C-compound and carbohydrate metabolism
DSE4

Energy
FRDS1

Cell cycle and dna processing
HUG1, RNR3, RNR2, RNR4, RNR1, RAD51, TRX2, RAD53, DUN1, ECO1, RFA1 EGT2, SIC1 , RME1, NIS1, CLN2

Transcription
YPR015C, RGM1 HHT2, HHF2, HHF1, HTB2, RME1, HTA1, HHT1, SWI5

Protein fate (folding, modification, destination)
TRX2, UBC5, UMP1 CSN9

Cellular transport and transport mechanisms
UBC5, VPS55

Cellular communication/signal transduction mechanism
RAD53, DUN1

Cell rescue, defense and virulence
HUG1, GTT1, TRX2, UBC5 PIR1, HSP30

Regulation of / interaction with cellular environment
HSP30, CSN9

Cell fate
RAD51, MFA2, UBC5, HBT1, PFS1, DFG10, RFA1 EGT2, DSE1, SWI5, CLN2

Control of cellular organization
PFS1 DSE1, CLN2

Classification not yet clear-cut
PLM2, YCR062W SCW11

Unclassified proteins
YKR077W, YLR040C, YCR061W, YLR365W DSE3, YLR049C, YIL057C, YPL158C, YOR263C

Table 2-3: Common genes and functional classification after irradiation to γ -ray (80 min)

Amino acid metabolism
BNA2

(*continued on next page*)

Table 2 (*continued*)

Nucleotide metabolism	RNR2, RNR3, RNR4, THI12
Phosphate metabolism	PHO89
C-compound and carbohydrate metabolism	SHC1, YEL070W DSE4
Lipid, fatty-acid and isoprenoid metabolism	TAH18
Metabolism of vitamins, cofactors, and prosthetic groups	BNA2
Energy	FRDS1, RSF1 TAH18
Cell cycle and dna processing	HUG1, RNR2, RNR3, RNR4, RAD51 EGT2, DSE2, CLB1, HEK2
Transcription	YJL103C, RSF1 DED1, HHT1, HTA1, HHF2, HHT2
Protein synthesis	DED1
Protein fate (folding, modification, destination)	UBC5, UMP1, ATG1, SRT1
Cellular transport and transport mechanisms	UBC5, ATG1, VPS55 GEA1
Cellular communication/signal transduction mechanism	COS111, GPG1, RSF1
Cell rescue, defense and virulence	HUG1, GTT1, RTA1, UBC5, TRX3, HOR7
Regulation of / interaction with cellular environment	PHO89
Cell fate	AFR1, MF(ALPHA)2, UBC5, SHC1, RAD51, SAG1, ATG1 PRY3, EGT2, HO, DSE1
Transposable elements, viral and plasmid proteins	YCL074W
Control of cellular organization	SHC1, SRT1 DSE1, DSE2
Protein with binding function or cofactor requirement (structural or catalytic)	HEK2
Transport facilitation	PHO89
Classification not yet clear-cut	PLM2, KIN82
Unclassified proteins	FMP16, YLR149C, YGR146C, YHR138C, YFR017C, YOL085C, MOH1, PRM7, YJL163C YOR315W, YMR003W, YDR179W-A, YEL048C, YER152C, YAL066W

Genes marked in red show the up-regulation in proportion to concentration. All the other genes are down-regulated.

However, there is one important aspect that deserves to be briefly discussed here, which is related to the energy (linear energy transfer) levels of these rays. The X-rays used in this study have a total effective energy in the range of 60 keV (kilo electron volt) to 100 keV, whereas the γ-rays have an energy equivalent of 1.173 MeV (mega electron volt) and 1.333 MeV, respectively. Does this large difference in energy levels affect the observed delay in gene expression by γ-rays over X-rays? Do we need to ask this question, to which we reply yes, we need to examine this possibility to explain the possible varying biological effectiveness of different radiation energies? It is generally accepted that considerable differences exist for the biological effectiveness of different photon qualities, usually indicating an increasing biological effect with decreasing photon energy (see [14] and references therein). A recent paper on DNA DSB misrejoining in primary human fibroblasts exposed to ultrasoft carbon K-shell characteristic X-rays, 29 kVp X-rays, and ^{60}Co γ-rays also investigated the varying efficiencies of different photon energies [14]. Their results revealed a modest variation in the number of induced DSBs along with a significant variation in the level of DSB misrejoining with photon quality thereby confirming the dependence of relative biological effectiveness on type of end point investigated [14]. In line with the preceding results and discussion, we suggest that the differences in gene expression profiles observed between low energy X-rays and high energy γ-rays may be due to these energy (linear energy transfer) differences.

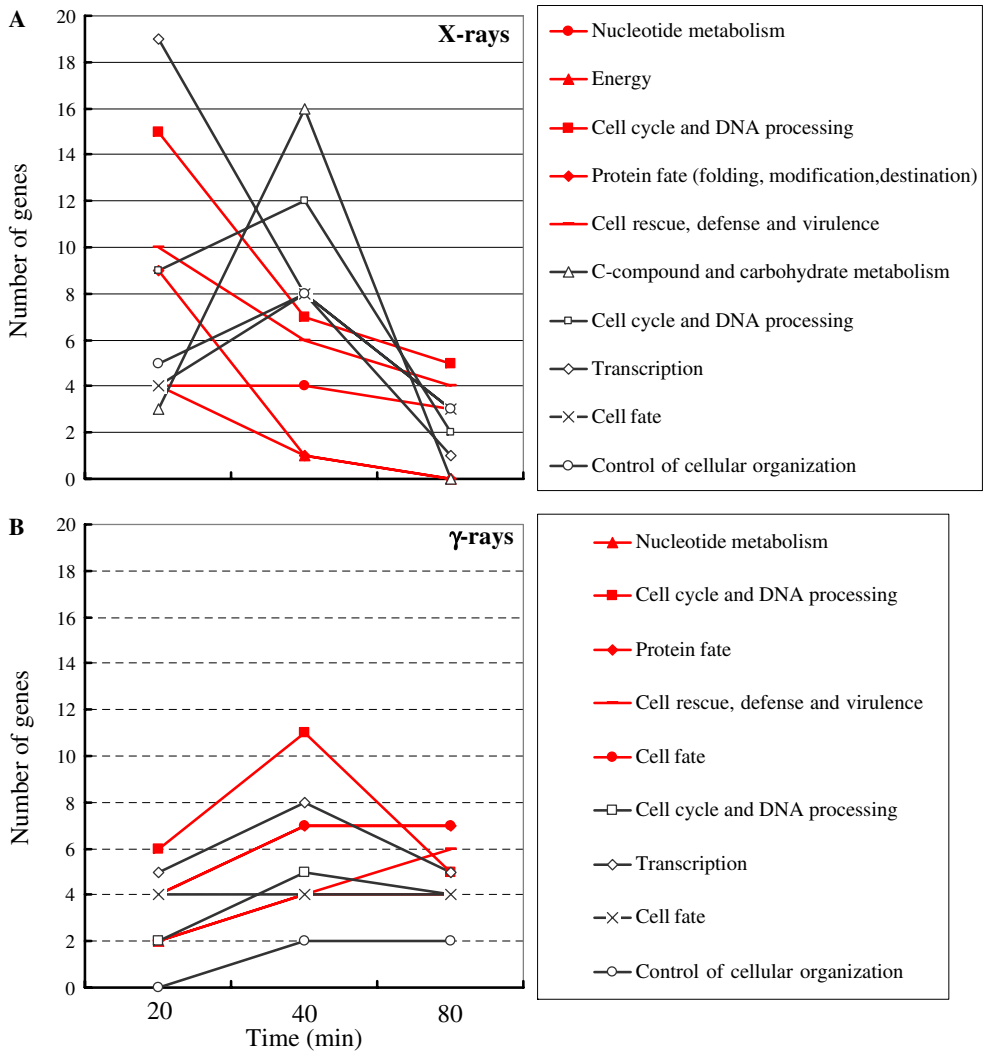

Fig. 3. Graphic representation of functional categories highly induced or reduced by X- and γ-rays. The selected categories are presented as number of genes in each category in a time-course manner. (A) X-rays and (B) γ-rays.

Furthermore, we interpret our present results as that the X-rays have low energy they are dissipated in the yeast cells causing them to respond early during PI-culture period, over the late and relatively low overall changed gene response by the highly penetrating and pass through γ-rays. It must be emphasized here that although we hypothesize this scenario based on the physics behind these irradiation mechanism and energy components therein, we are still very much in the dark as to the mechanisms by which biological organisms react to differences in physical energy levels derived from X- and γ-rays. This question raised here deserves further attention, and we aim to look at these aspects in future studies on radiation effects in living organisms, not only at the level of the genome but also at more functional levels including proteins and metabolites. The study by Kuhne et al. [14] is an excellent example of one of the directions of future research.

In conclusion, the present study on yeast model under exposure to harmful irradiations by microarray approaches will be beneficial in providing an idea of the radiation-induced biological damages and responses in the environment.

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