Induction of SOS Genes by a Low Dose of Gamma Radiation, 10 Gy, in *Salmonella enterica* Serovar Typhimurium

Sangyong Lim, Minho Joe, Hoseong Seo and Dongho Kim*

Research Division for Biotechnology, Korea Atomic Energy Research Institute, Jeongeup 580-185, Korea

Abstract - In a previous study, a relatively high dose of gamma radiation (1 kGy) did not fully induce typical SOS genes such as *sulA, recA, recN,* and *din* in *Salmonella* Typhimurium (*S.* Typhimurium) (Lim *et al.* 2008, Gene expression profiles following high-dose exposure to gamma radiation in *Salmonella enterica* serovar Typhimuium. *J. Radiat. Ind.* 3:111-119). In this study, we examined changes in the transcriptional repertoire of *S.* Typhimurium after a dose of 10 Gy using DNA microarrays. It was found that more than half (~65%) of the 26 up-regulated genes belong to the SOS regulon: ten genes are typical SOS genes, and seven genes are *Salmonella* prophage genes, which are known to be activated by LexA cleavage. Among 29 down-regulated genes, the function of five genes with the most decreased expression is associated with carbohydrate transport and energy production. This suggests that upon exposure to gamma radiation cells may cease growing by reducing the metabolic activity, and repair DNA damage using a DNA repair system such as the SOS response system. The difference in expression of the SOS genes between a high (1 kGy) and low (10 Gy) dose of radiation shows the possibility that cells may opt for one of multiple regulatory circuits in response to the specific gamma radiation dose.

Key words : DNA microarrays, Ionizing radiation, *Salmonella* Typhimurium

INTRODUCTION

Living organisms, under aerobic conditions, inevitably produce reactive oxygen species (ROS) including superoxide radicals ($O2^{\cdot -}$) and hydrogen peroxide (H_2O_2) through aerobic metabolism, thereby suffering oxidative stress because ROS can damage cellular components such as nucleic acids and proteins (Imlay 2003). *Escherichia coli* (*E. coli*), a model organism for studying many of life's essential process, activates antioxidant enzymes such as superoxide dismutases (SOD) and catalases, to eliminate ROS (Storz and Imlay 1999). It is known that OxyR, a major oxidative regulator, controls not only the *katG* gene encoding catalase but also almost 40 genes, which protect the cell from hydrogen peroxide toxicity (Chiang and Schellhorn 2012). Aside from antioxidant systems, *E. coli* can induce a DNA repair network, called an SOS response, because ROS creates DNA damage (Farr and Kogoma 1991). Two proteins play key roles in the regulation of the SOS response: a repressor called LexA, and an inducer called RecA. About 40 genes are under control of LexA/RecA (Janion 2001; Michel 2005).

Since ionizing radiation (IR) can also generate different types of ROS (Riley 1994), exposure of bacteria to IR can probably trigger both oxidative stress and SOS response at the same time. Recently, we defined the genes affected by IR in *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) through a DNA microarray analysis (Lim *et al.* 2008) and investigated proteome changes induced by IR in

* Corresponding author: Dongho Kim, Tel. +82-63-570-3140, Fax. +82-63-570-3149, E-mail. fungikim@kaeri.re.kr

E. coli through two-dimensional gel/mass spectrometry (Lim *et al.* 2011). Although prophage genes, which are known to be repressed by LexA (Bunny *et al.* 2002), were induced by IR in *S.* Typhimurium treated with 1 kGy IR, the up-regulation of typical SOS genes such as *recA*, *sulA*, and *umuD* was not observed (Lim *et al.* 2008). In *E. coli* subjected to high doses of radiation (3 kGy), antioxidant enzymes, SOD and peroxidase, were induced, but none of the SOS genes were detected (Lim *et al.* 2011).

In this study, we analyzed changes in gene expression in *S.* Typhimurium using a microarray analysis after a relatively low dose of radiation of 10 Gy.

MATERIALS AND METHODS

1. Bacterial strain and growth conditions

Salmonella enterica serovar Typhimurium LT2 was cultivated aerobically at 37˚C in Luria-Bertani medium containing 1% bacto-tryptone, 0.5% yeast extract, and 1% NaCl. A stationary-phase culture that had been grown overnight with shaking was used as a stock culture.

2. Irradiation

Two 250-ml Erlenmeyer flasks containing 50 ml of fresh medium were inoculated with the stock culture at 1 : 100 and were then incubated aerobically until an exponential phase for 4 hr. After the cultures were transferred to a 50-ml conical tube (BD Biosciences, USA), one was irradiated at room temperature, and the other was not irradiated. Each culture was re-transferred to a 250-ml Erlenmeyer flask, incubated for a further 20 min, and the cells were harvested by centrifugation for RNA isolation. Gamma radiation was performed using a ^{60}Co gamma irradiator (IR-222, AECL, Ottawa, Ont., Canada). The source strength was approximately 1100 kCi at a dose rate of 10 Gy min^{-1}; the applied dose of irradiation was 10 Gy.

3. Oligonucleotide microarrays

The microarray was constructed by MYcroarray, Inc. (Ann Arbor, MI, USA) using the genome information for the sequenced *S.* Typhimurium LT2 (McClelland *et al.* 2001). A total of 4,391 gene-specific probes (45 nucleotides in length) were designed, which cover ~96.2% of 4,463 protein coding genes on the LT2 chromosome. The microarray used in this study consists of 21,120 features. Of these, 17,564 spots were used to represent the 4,391 genes (four replicates of a probe), and the remaining 3,556 features were used for internal quality control. Three arrays per slide were generated through an *in situ* synthesis using proprietary light directed oligonucleotide synthesis technology (www.mycroarray.com).

4. Probe preparation and hybridization

Total bacterial RNA was isolated using a RiboEX reagent (GeneAll, S. Korea), treated with DNase, and purified using RNeasy® Mini kit (Qiagen, Germany) according to the manufacturer's instructions. The quality and integrity of the prepared total RNAs were confirmed through the use of an Agilent 2100 bioanalyzer (Agilent Technologies, Inc., Palo Alto, CA, USA), and by Gene Quant pro (Amersham Pharmacia Biotech, UK). Extracted RNAs were amplified and labeled using Agilent's Low Input Quick Amp WT Labeling Kit (Agilent Technologies, Inc.) according to the manufacturer's instructions. Briefly, 100 ng total RNA was mixed with a WT Primer Master Mix and incubated at 65˚C for 10 min. Following the addition of the cDNA Master Mix, the reaction mixture was incubated at 40˚C for 2 hours for reverse transcription, and then at 70˚C for 10 min to stop the reaction. Transcription Master Mix was added to the reaction mixture, and the mixture was incubated at 40˚C for 2 hours to generate labeled cRNA probes. Amplified and labeled cRNA was purified on a RNase mini column (Qiagen) according to the manufacturer's protocol and quantified using an ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA). After fragmentation of Cy 3- (control sample) and Cy-5 labeled cRNA (experimental sample), they were hybridized into the arrays. The hybridizations were conducted at 57˚C for 17 hours in an Agilent Hybridization oven and washed following the manufacturer's protocol (Agilent Technologies, Inc.).

5. Data acquisition and analysis

Scans were performed using an Agilent DNA microarray Scanner (Agilent Technologies, Inc.) with the Agilent Feature Extraction software 10.7 (Agilent Technologies, Inc.). The signal intensities were quantified using GeneSpringGX 7.3.1 software (Agilent Technologies, Inc.). The genes were

Table 1. Genes with increased expression following gamma irradiation

Gene number	Gene symbol	Gene description	Log2 ratio[a] (10 Gy/0 Gy)
STM0192	*fhuC*	iron-hydroxamate transporter ATP-binding	1.14
STM0313	***dinP***	DNA polymerase IV	2.10
STM0405	*tgt*	queuine tRNA-ribosyltransferase	1.68
STM0598	*entA*	2,3-dihydroxybenzoate-2,3-dehydrogenase	1.03
STM0821	***dinG***	ATP-dependent DNA helicase DinG	1.55
STM0895		hypothetical protein (Fels-1)	4.86
STM0928	*nanH*	neuraminidase (Fels-1)	1.68
STM1019		hypothetical protein (Gifsy-2)	1.52
STM1071	***sulA***	SOS cell division inhibitor	2.00
STM1162	***dinI***	DNA damage-inducible protein I	1.52
STM1641	*hrpA*	ATP-dependent RNA helicase HrpA	1.41
STM1882	***yebG***	DNA damage-inducible protein YebG	1.56
STM1937	*tyrP*	tyrosine-specific transport protein	1.51
STM1998	***umuD***	DNA polymerase V subunit UmuD	2.39
STM2061	***sbmC***	DNA gyrase inhibitor	1.14
STM2621		hypothetical protein (Gifsy-1)	1.45
STM2636		integrase-like protein (Gifsy-1)	1.25
STM2684	***recN***	recombination and repair protein	2.99
STM2727		hypothetical protein (Fels-2)	1.30
STM2728		hypothetical protein (Fels-2)	2.98
STM2788		tricarboxylic transport	4.10
STM2829	***recA***	recombinase A	1.42
STM2965	*yqcC*	putative cytoplasmic protein	1.25
STM4032		putative acetyl esterase	1.22
STM4055	*sodA*	superoxide dismutase	1.10
STM4238	***dinF***	DNA-damage-inducible SOS response protein	1.25

[a]The ratio is the fold increase in mRNA level in irradiated culture compared to control culture.
[b]SOS genes (Fernandez de Henestrosa *et al.*, 2000) induced by irradiation are indicated in bold.

filtered by removing flag-out genes in each experiment. Normalization of the gene expression through a LOWESS regression was applied for three datasets obtained from three biological replicates. The genes were considered to be differentially expressed when the logarithmic gene expression ratios had more than a 2-fold difference in the expression level. The statistical significance of the data was determined through a Student's t test. *P*-values of less than 0.01 were taken as statistically significant.

RESULTS AND DISCUSSION

To examine the changes in gene expression of *S.* Typhimurium following a dose of 10 Gy, we used oligonucleotide microarrays that present 4,391 ORFs from *Salmonella* Typhimurium LT2 chromosome (McClelland *et al.* 2001). The total RNA was purified from a sample taken 1 h after irradiation, and used to make cDNA that was labeled and hybridized into the microarray. As a control for the irradiation-independent changes, a total RNA preparation from a non-irradiated sample was also performed (see Materials and Methods). Of all the genes showing a statistically significant expression ratio ($P < 0.01$) of at least a 2-fold change relative to a non-irradiated counterpart, 26 genes were induced and 29 genes were reduced by an irradiation dose of 10 Gy. The lists of the genes up- and down-regulated by irradiation are presented in Tables 1 and 2, respectively.

Among the genes whose expression was induced by irradiation, ten SOS genes are represented, i.e., *dinF*, *dinG*, *dinI*, *dinP*, *recA*, *recN*, *sbmC*, *sulA*, *umuD*, and *yebG*, (Table 1), which are involved in DNA lesion repair and the prevention of premature cell division (Fernandez de Henestrosa *et al.* 2000). Different types of prophages such as Gifsy and Fels are found in most strains of *S.* Typhimurium (Bossi and Figueroa-Bossi 2005). The prophage is maintained in a state of latency most of the time, but the SOS response can lead to a prophage induction, giving rise to active viruses (Bunny *et al.* 2002; Waldor and Friedman 2005). Of the 26 up-regulated genes, seven genes belong to prophage genes, STM0895, STM0928, STM1019, STM2621,

Table 2. Genes with reduced expression following gamma irradiation

Gene number	Gene symbol	Gene description	Log2 ratioa (10 Gy/0 Gy)
STM0384	*psiF*	hypothetical protein	−1.24
STM0853	*yliH*	biofilm formation regulatory protein BssR	−1.15
STM1089		putative inner membrane protein	−1.03
STM1090	*pipC*	pathogenicity island-encoded protein C	−1.47
STM1172	*flgM*	anti-sigma28 factor FlgM	−1.01
STM1246	*pagC*	virulence membrane protein PagC precursor	−1.66
STM1271	*yeaR*	putative cytoplasmic protein	−1.48
STM1285	*yeaG*	putative serine protein kinase	−1.04
STM1601	*ugtL*	hypothetical protein	−1.17
STM1613		putative PTS system enzymeIIB component	−1.05
STM1782	*ychH*	hypothetical protein	−1.38
STM2256	*napB*	citrate reductase cytochrome c-type subunit	−2.36
STM2258	*napG*	quinol dehydrogenase periplasmic component	−2.23
STM2314	*cheW*	putative chemotaxis signal transduction protein	−1.06
STN2753		putative dehydrogenase	−1.19
STM2945	*sopD*	secreted effector protein	−1.04
STM3197	*glgS*	glycogen synthesis protein GlgS	−1.30
STM3239	*yhaO*	putative transport protein	−1.85
STM3352	*oadA*	oxaloacetate decarboxylase	−1.40
STM3598		putative L-asparaginase	−1.57
STM3599		anaerobic C4-dicarboxylate transporter	−1.04
STM3600		putative sugar kinase	−2.03
STM3601		putative phosphosugar isomerase	−1.99
STM3675	*sgbH*	3-keto-L-gulonate-6-phosphate decarboxylase	−1.01
STM3780	*gatY*	putative fructose-1,6-bisphosphate aldolase	−1.13
STM3594	*yigG*	hypothetical protein	−1.32
STM4073	*ydeW*	putative transcriptional repressor	−1.22
STM4277	*nrfA*	cytochrome c552	−2.32
STM4279	*nrfC*	putative formate-dependent nitrite reductase	−2.50

[a]The ratio is the fold increase in mRNA level in irradiated culture compared to control culture.
[b]Genes that belong to carbohydrate metabolism and energy production are indicated in bold.

STM2636, STM2727, and STM2728 (Table 1). However, the induction mechanism of *Salmonella* prophage genes differs from that used by typical SOS genes. An increase in the expression of the SOS genes begins when the DNA is damaged. RecA protein forms filaments on a single strand of DNA and acquires protease activity, thereby inducing the self-cleavage of the LexA repressor and its inactivation during DNA damage (Janion 2001; Michel 2005). In contrast, the repressors of *Salmonella* prophage genes are not cleaved upon induction; rather, they are inactivated by the binding of small antirepressor proteins, whose expression is under the control of the LexA repressor (Lemire *et al*. 2011). This difference may uncouple the prophage induction from the SOS response at a relatively high IR dose: a dose of 1 kGy clearly triggered the activation of the prophage genes, but not the SOS genes (Lim *et al.* 2008). Further research is necessary to investigate the regulatory circuits of prophage genes after low and high doses of IR.

Interestingly, nine (~31%) of the down-regulated genes are associated with ‘carbohydrate transport and metabolism’ and ‘energy production and conversion’ (Table 2). In addition, we found that all of five genes, STM2256, STM2258, STM3600, STM4277, and STM4279, which decreased most in expression after IR, belong to these categories. This shows that the cellular metabolic activity wanes when the SOS response is induced. It is natural that the replication of chromosomal DNA should be blocked until DNA damage is repaired, which occurs upon IR exposure. Thus, cells may adopt a state of reduced metabolic activity and cease growing for DNA repair while the SOS repair system operates. It is known that the SOS response does not occur in the absence of *cya*, which encodes adenyl cyclase that synthesizes cyclic AMP (cAMP) from ATP (Janion 2001). Because cAMP and CAP (catabolite gene activation) protein are involved in the regulation of many catabolic genes (Saier 1998), cAMP may be a signal molecule that mediates between an induction of the SOS system and a reduction of the metabolic activity.

In conclusion, we found that the induction of SOS response is manifest in cells exposed to a low dose of IR compared with cells exposed to a high dose of IR (Lim *et al.* 2008). However, the *Salmonella* prophage genes, which belong to the SOS-regulon (Bunny *et al.* 2002; Waldor and Friedman 2005), are strongly induced regardless of the IR dose. Since the regulatory mechanism of prophage genes is a little bit different from that of typical SOS genes (Lemire *et al.* 2011), the *Salmonella* prophage genes seem to be good candidates for an IR response study.

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