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EXPERIMENTAL ARTICLES

The Role of Thiol Redox Systems in the Response of *Escherichia coli* to Far-UV Irradiation

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Abstract—*Escherichia coli* mutants deficient in glutathione (*gshA*), glutaredoxin (*grxA*), thioredoxin (*trxA*), and thioredoxin reductase (*trxB*) synthesis were studied with respect to their resistance to far-UV (UV_{254}) exposure. The *trxA, trxB*, and *grxA* mutants subjected to a short-term UV exposure were found to be more resistant to UV irradiation than the parent cells. Under the same conditions, the *trxA* and *trxB* mutants demonstrated a high level of induction of the *sulA* gene, a component of the SOS regulon. The mutagenic effect of long-term UV exposure of all the mutants with redox deficiencies was more pronounced than in the case of the parent strain, and the *trxA* and *trxB* mutants were found to be the least viable microorganisms. Pretreatment of the cells with low concentrations of the thiol-oxidizing agent diamide enhanced the *sulA* gene expression; however, high concentrations of diamide inhibited *sulA* expression. The data obtained indicate that the thiol redox systems of *E. coli* are involved in its response to far-UV irradiation.

Key words: Escherichia coli, UV irradiation, glutathione, thioredoxin reductase, gene expression.

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In the course of their life cycle, bacteria may be exposed to UV radiation; its biological activity is wavelength-dependent. One of the main results of bacterial exposure to UVC ($\lambda < 290$ nm, also called shortwave or far UV) is DNA damage which induces mutagenesis and cell death. *E. coli* and some other bacteria use a variety of molecular mechanisms for protection against shortwave UV irradiation. These mechanisms, among which the SOS regulatory system is of great importance, can repair damaged DNA. Two regulatory proteins, LexA and RecA, regulate the SOS response which involves induction of over 30 genes; their products are involved in DNA replication and repair, as well as in control of cell division. The LexA repressor inhibits the expression of these genes. When DNA damage occurs, the RecA protein binds to single-strand DNA at the replication fork and cleaves the LexA repressor, thereby relieving the repression of the SOS response genes [1].

Much attention was recently paid to redox regulation of cellular activity. Modification of sulfhydryl groups in signal proteins plays an important role in this regulation; it involves reactive oxygen species (ROS) and such thiol-containing molecules as glutathione (GSH), glutaredoxins (Grxs), thioredoxins (Trxs), and peroxiredoxins (Prxs). Molecular mechanisms involved in the redox regulation of eukaryotic cells are the best studied. The mechanisms responsible for redox regulation in bacteria are, however, far less understood [2, 3]. Our data and the results obtained by other authors on the modulation of expression of some SOS regulon genes by exogenic low-molecular-weight thiols suggest the possibility that the above-listed thiols are involved in the regulation of the SOS response [4]. These compounds are believed to activate the SOS response by acting as reductants in Fenton-like reactions. These reactions lead to an increase in the content of reactive oxygen species, which, in turn, are capable of inducing DNA damage. Another possible pathway may involve reactions of thiols with the RecA protein that participates in the regulation of the SOS response. The presence of the "crucial" SH groups in the RecA protein (modification of these groups may result in significant changes in the RecA activity) points to the latter pathway [5]. It was also demonstrated that *E. coli* cells produce dozens of proteins associated with thioredoxin, including RecA [6].

The goal of this work was to study the role of glutathione, thioredoxin, and glutaredoxin in the resistance of *E. coli* cells to far UV irradiation and in the regulation of the SOS response.

MATERIALS AND METHODS

The *Escherichia coli* strains used in this work are listed in the table. Strains NM131, NM141, NM151, NM161, and NM171 were previously obtained by us [7] by nonspecific transduction by bacteriophage P1 of

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Escherichia coli strains used in this work

Strain	Genotype	Source or reference
DM4000	*hisG4 argE3 thr-1⁻ ara-14 xyl-5 mtl-1 rpsL31 tsx-33 ilv TS sulA::Mud1(bla lac) cam (sulA::lacZ)*	M. Volkert*
RI89	MC1000 *phoR Δara-714 leu*$^+$	J. Beckwith**
RI336	RI89 *gshA*::Tn*10*Kan^r	J. Beckwith**
RI363	RI89 *ΔtrxA*::Kan^r	J. Beckwith**
RI319	R189 *trxB*::Kan^r	J. Beckwith**
WP812	DHB4 *grxA*::Kan^r	J. Beckwith**
NM131	RI89 *sulA::lacZ*	[7]
NM141	R336 *sulA::lacZ*	[7]
NM151	RI363 *sulA: lacZ*	[7]
NM161	RI319 *sulA::lacZ*	[7]
NM171	WP812 *sulA::lacZ*	[7]

Notes: * University of Massachusetts, United States.
** Harvard University, United States.

a *sulA::lacZ* fusion from *E. coli* DM4000 to strains RI89, RI319, RI336, and RI363 [8].

Bacteria were grown on M9 minimal medium [9] supplemented with 0.4% glucose, 0.2% casamino acids, and thiamine (10 μg/ml). Overnight bacterial cultures were centrifuged, resuspended in 100 ml of the medium, and incubated at 37°C in 250-ml flasks on a shaker (150 rpm). Exponential phase cultures (75 ml) were placed in a specially designed thermostatically controlled chamber equipped with a magnetic stirrer and intense aeration. The cell suspension from the chamber was pumped continuously in a circulation mode through a UV-irradiated quartz tube. An SVD-120 mercury-quartz lamp was used as a source of UV radiation. The required wavelength was obtained by means of an interference filter with maximum transmission at 254 nm.

Bacterial growth was estimated from the optical density of the culture at 670 nm (OD_{670}). The number of viable cells was determined by counting the colonies grown on petri dishes with agarized LB medium inoculated with dilutions of the cultures.

The mutation frequency was determined by counting the rifampicin-resistant colonies. For this purpose, 1 ml of the cell suspension with an OD_{670} of 0.4 (which corresponds to the middle of the logarithmic growth phase) was mixed with 3 ml of 0.8% agar and plated onto agarized (1.5%) LB medium supplemented with rifampicin (125 μg/ml). Rifampicin-resistant colonies were counted after 24-h incubation at 37°C. The results were normalized to the number of inoculated cells, which was determined by plating serial dilutions of the culture onto agarized LB medium.

The contents of reduced (GSH) and oxidized (GSSG) glutathione were determined on a spectrophotometer [10] in the order described in [11]. Protein content was determined by the Lowry method. Expression of the *sulA* genes was determined by measuring the β-galactosidase activity in the strains carrying a fusion between the *sulA* promoter and the structural gene coding for β-galactosidase [9].

Each result presented in the paper is an average of at least three independent measurements ± mean-square error. The data were statistically analyzed using the Student's t test, with the $P < 0.05$ as the minimum requirement for a statistically significant difference.

In our study, the following reactants were obtained from Sigma, United States: agar, casamino acids, thiamine, deoxycholate, glutathione reductase, mercaptoethanol, DNTB, EDTA, NADPH, GSH, GSSG, NEM, ONPG, and antibiotics. All other reactants used in this work were of analytical grade.

RESULTS

To elucidate the effect of mutations in the genes responsible for the synthesis of thiol redox systems on the viability of *E. coli* cells exposed to far-UV radiation (UV_{254}), *E. coli* strains deficient in the synthesis of glutathione (*gshA*; RI336), thioredoxin reductase (*trxB*; RI319), thioredoxin (*trxA*; RI363), and glutaredoxin (*grxA*; WP812) were used. During our preliminary experiments, the intensity of UV radiation and the duration of exposure causing moderate or severe damage were determined. At a given intensity of UV radiation and the short-term exposure to UV_{254} (6 min), a significant decrease in cell viability was detected only in the case of parent cells (RI89, wt) and mutants deficient in glutathione synthesis. Under the same conditions, the *trxB*, *trxA*, and *grxA* mutants were found to be UV-resistant. However, after a longer exposure (15 min), the decrease in the viability of the *trxB* and Δ*trxA*

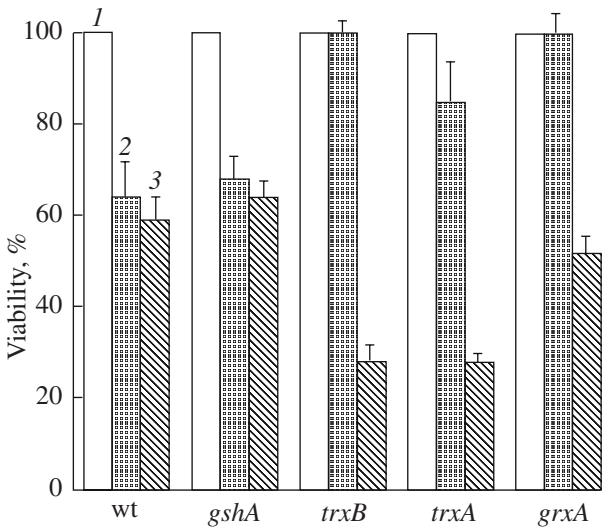

Fig. 1. Viability of the *E. coli* mutants with redox deficiencies on far-UV (UV_{254}) irradiation exposure: viability of unexposed cells (taken for 100%) (*1*); cell viability after 6-min irradiation (*2*); cell viability after 15-min irradiation (*3*). Strains NM131 (parent type, wt); NM141 (*gshA*); NM161 (*trxB*), NM151 (*trxA*), and NM171 (*grxA*) were used.

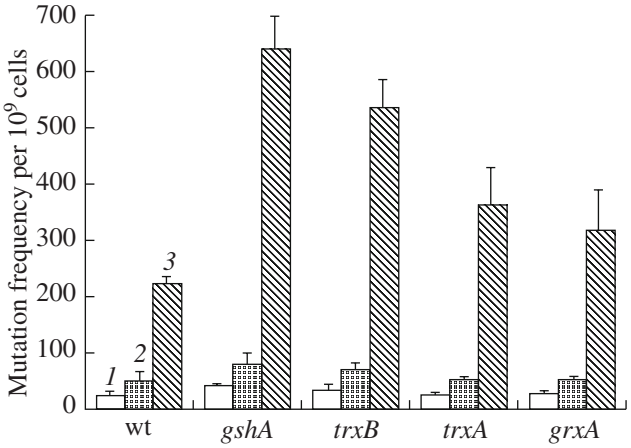

Fig. 2. Mutagenic effect of UV irradiation on the *E. coli* mutants with redox deficiencies: mutation frequency in unexposed cells (*1*); mutation frequency after 6-min irradiation (*2*); mutation frequency after 15-min irradiation (*3*). The samples were taken 60 min after exposure. Designations: see Fig. 1.

mutants was more pronounced than in the case of other mutants (Fig. 1). It was demonstrated that the inhibitory effect of the 6-min exposure to UV radiation on the growth rate of parent cells was more pronounced, since all the mutants were more resistant to UV radiation. The *trxB* mutants were most resistant to UV light (data not presented).

Short-term exposure to UV radiation (6 min) had virtually no effect on the mutation frequency in the strains under study. Longer exposure (15 min) resulted in an increase in the mutation frequency in all strains; however, in the case of the *gshA* and *trxB* mutants, this index was 3 and 2.5 times higher, respectively, than in the parent cells under the same conditions (Fig. 2). It should be noted that the higher mutation frequency observed in the *trxB* and *trxA* mutants was accompanied by low viability of the mutants as compared to the parent cells.

To study the role of thiol redox systems in the induction of the SOS response under UV irradiation, several strains carrying mutations in the genes involved in one of the major redox systems and a *sulA::lacZ* fusion were constructed [7]. In *E. coli*, the *sulA* (*sfiA*) gene is a component of the SOS regulon controlled by the *recA* and *lexA* genes. UV irradiation (254 nm) is one of the main inducers of the SOS response in bacteria; therefore, an increase in the *sulA::lacZ* expression was observed in all the studied strains. After a 60-min exposure, the indices of the *sulA::lacZ* induction (the ratio between the levels of β-galactosidase activity in irradiated and unexposed cells) were distributed as follows: the highest induction was observed in the *trxA* and *trxB* mutants (11.6 and 9.8, respectively), whereas the lowest induction was detected in the *gshA* mutants (4.6). In the parent cells and the *grxA* mutants, these indices were 7.0 and 5.8, respectively (Fig. 3). After a 15-min exposure to near-UV, inhibition of the *sulA::lacZ* expression was observed in all the studied strains.

In the *E. coli* cytoplasm, the concentration of glutathione was several times higher than the levels of thioredoxin and glutaredoxin. Due to this phenomenon, as well as because of the low concentration of its oxidized form (glutathione disulfide, GSSG), glutathione is the major intracellular redox buffer. In unexposed cells, the highest concentration of intracellular GSH was observed in *E. coli* mutants deficient in the thioredoxin reductase synthesis (*trxB*); the lowest GSH concentration was observed in the mutants deficient in thioredoxin synthesis (*trxA*). The glutathione content in *grxA* mutants was similar to that in parent cells (Fig. 4a). After a 6-min exposure, the intracellular glutathione concentration in all the studied strains increased in all strains. In strains *grxA* and *trxA*, this increase was 1.3- and 2.4-fold, respectively. The content of glutathione in the *trxB* cells was the highest even after exposure to UV light (Fig. 4a). Determination of the extracellular glutathione content in the studied strains indicated that in the parent cells and *trxB* mutants, the UV-induced increase in the glutathione concentration in the cytosol was possibly be due to a decrease in the glutathione export to the medium (Fig. 4b). A decrease in the GSH/GSSG ratio was observed in all UV-exposed strains, except for the *trxA* mutants (data not presented). A notable increase in the content of intracellular low-molecular-weight thiols and, at the same time, a decrease in the proportion of extracellular thiols have been previously detected in the

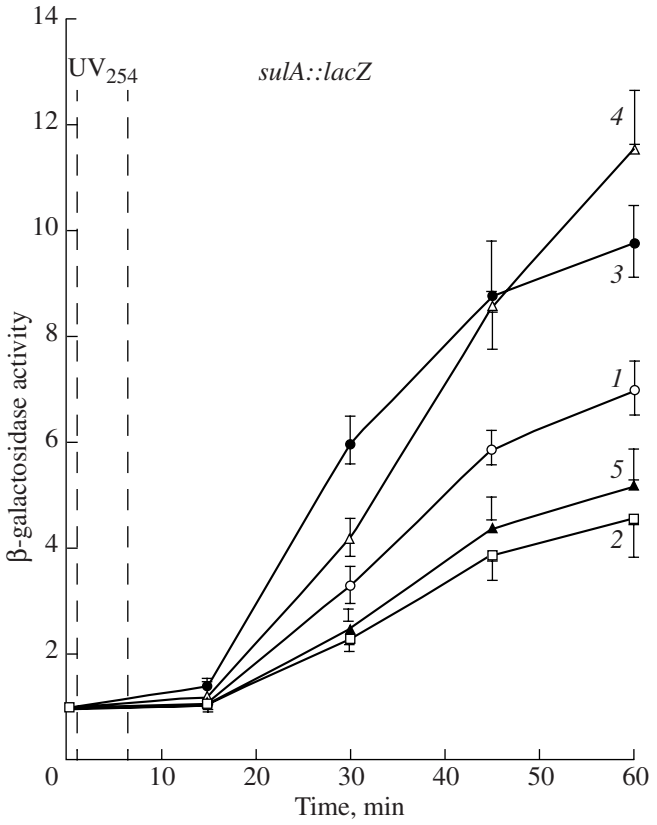

Fig. 3. Induction of the *sulA::lacZ* fusion by UV_{254} irradiation: wt, parent cells (*1*); *gshA* (*2*); *trxB* (*3*); *trxA* (*4*); and *grxA* (*5*). The levels of β-galactosidase activity were expressed in relative units. The β-galactosidase activity unit corresponds to the level of β-galactosidase activity before exposure.

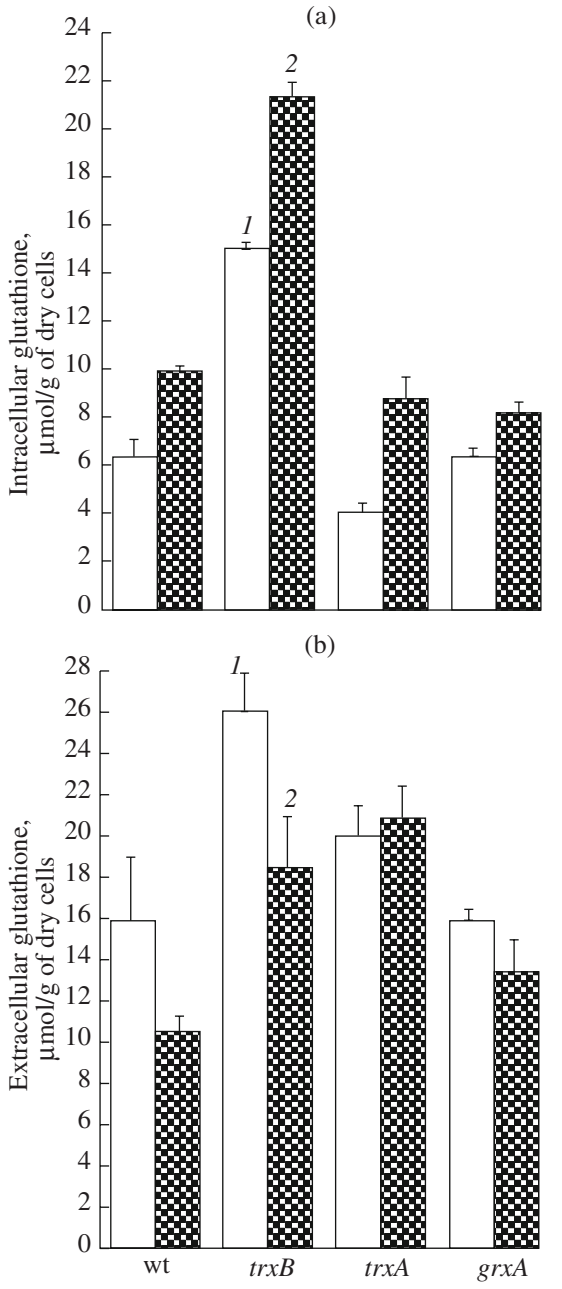

Fig. 4. Effect of UV_{254} irradiation on the contents of intracellular (a) and extracellular (b) glutathione (GSH) in the *E. coli* cells with redox deficiencies: control (unexposed cells) (*1*); cell exposed to far-UV radiation (UV_{254}) for 6 min (*2*). The samples were taken 50 min after exposure. Designations: see Fig. 1.

E. coli cells exposed to near-UV radiation or other stress factors [2, 12].

It was demonstrated that the cell-penetrating thiol-oxidizing agent diamide caused a significant decrease in the content of reduced glutathione and formed disulphide bonds in the cytoplasmic proteins [13, 14]. This phenomenon is known as "disulphide stress". In our experiment, the effect of diamide was concentration-dependent. Pretreatment of the parent cells with 0.08 mM of diamide caused a 34% increase in the UV-induced expression of *sulA::lacZ*; that is, its effect was similar to that on the *trxB* mutation under the same conditions. At higher concentrations, diamide had a strong toxic effect accompanied by inhibition of growth and expression of the SOS response.

To test the hypothesis that diamide action links with depletion of glutathione, the UV-induced expression of *sulA::lacZ* in the GSH-deficient *E. coli* mutants was measured. Pretreatment of these cells with 0.08 mM diamide caused a 64% increase in the *sulA::lacZ* expression. Hence, diamide at low concentrations had a similar (stimulatory) effect on both gsh^+ and gsh^- cells. It was demonstrated that the level of intracellular glutathione did not decrease significantly under the influence of diamide (0.08 mM). An addition of 1 mM GSH to the *E. coli* culture deficient in glutathione synthesis had no significant effect on the UV-induced *sulA::lacZ* induction. On the whole, the data obtained indicate that the effect of diamide under the described conditions is most probably due to the formation of disulfide bonds in proteins, rather than to depletion of intracellular GSH.

DISCUSSION

In this work, it was demonstrated that the presence of mutations in the *E. coli* genes encoding the thiol redox systems resulted in significant changes in the response of these bacteria to far-UV irradiation. The extent and direction of these changes depended on the mutation type and parameters of UV exposure. The *trxA* and *trxB* mutants differ from the other studied strains in their viability, mutagenicity, and *sulA::lacZ* expression. It has been previously demonstrated that, under the influence of H_2O_2, *trxB* mutants (deficient in thioredoxin reductase synthesis) exhibited the properties that distinguished them from other *E. coli* mutants with redox deficiencies [7, 15]. These bacteria were most resistant to cytostatic and cytotoxic effects of H_2O_2; they were even more resistant to peroxide stress than the parent cells. The basic levels of catalase activity and intracellular glutathione in the *trxB* mutants were high. After oxidative stress, these strains were capable of rapidly restoring a normal level of GSH. At the same time, these bacteria are characterized by a high frequency of induced mutation. The results of assessment of the H_2O_2-induced expression of the *katG* and *sulA* genes suggest that differences in resistance to oxidative stress between *trxB* and other strains are determined primarily by the ability of the *trxB* mutants to induce the genes of the OxyR and SOS regulons [7, 15]. The data obtained indicate that *trxB* mutants exhibited most of the above-described properties after exposure to UV radiation (Fig. 1–4).

How do *E. coli* mutants with redox deficiencies modify their response to UV irradiation? First of all, let us consider the possible mechanism by which thioredoxin (Trx) and thioredoxin reductase (TR) regulate the SOS response. Thioredoxins are a family of small proteins containing a redox active Cys-X1-X2-Cys sequence which can be reversibly oxidized and reduced. Due to its capacity for reducing disulfides in proteins, Trx is involved in redox regulation. Oxidized thioredoxin is reduced by the NADPH-dependent thioredoxin reductase. Unlike glutathione, the intracellular concentration of thioredoxin is very low. However, its reducing activity relative to transcriptional factors is 1000 times higher than that of GSH; that is why thioredoxin is more specific as a reducing agent for redox-regulated signal reactions. The pathways for redox regulation mediated by thioredoxin include reduction of disulfide bonds in the regulatory proteins or formation of protein conjugates. It is noteworthy that the results of proteomic analysis indicate that *E. coli* cells possess 80 thioredoxin-containing proteins, many of which are involved (directly or indirectly) in the regulation of cellular functions. This group includes the RecA protein which plays a key role in the regulation of the SOS response [6]. It is also well known that RecA contains several "crucial" SH groups, and their modification may result in significant changes in the RecA activity [5].

The size and structure of glutaredoxins (Grxs) are similar to those of thioredoxin. These proteins also contain the redox active Cys-X1-X2-Cys sequence. Unlike the Trx/TR system, glutaredoxin does not have a specific reductase for reduction of its oxidized form; this function is fulfilled by the GSH/glutathione reductase system. An important trait of glutaredoxins is their ability to reduce mixed disulfides [16]. The involvement of glutaredoxin in reversible S-glutathionylation makes it an important component of the redox regulation system of *E. coli*. For instance, glutaredoxin is involved in the regulation of the stress response in bacterial cells. In *E. coli* cells, low H_2O_2 concentrations activate the OxyR transcriptional factor by intramolecular disulfide bond formation and a conformational transition in the regulatory domain. As a consequence, the protein acquires the capacity to activate transcription of the genes responsible for *E. coli* adaptation to high H_2O_2 concentrations. The reverse process, OxyR inactivation, occurs via reduction of disulfide bonds under the influence of glutaredoxin 1 in the presence of GSH [17]. It is noteworthy that glutaredoxin 1 synthesis (encoded by the *grxA* gene) in *E. coli* cells was observed among the SOS response genes induced by far-UV irradiation [18].

It was shown that, in single *E. coli* mutants with redox deficiencies, the intensities of disulfide bond formation in the cytoplasm are as follows (per unit): *trxB,* 9.7; *trxA*, 1.4; *gshA*, 1.4; *grxA*, 0.9; and wt, 1.0 [14]. Taking into account our results and the results obtained by other authors, it may be suggested that moderate or acute (in the case of *trxB*) disulfide stress is responsible for the characteristic behavior of the latter three strains.

In *E. coli* cells, there is a certain balance between the levels of thioredoxin, glutaredoxin 1, and glutathione whose functions overlap or duplicate each other. For instance, the *trxA* mutation results in an increase in the Grx1 content; the contents of glutaredoxin and thioredoxin in the mutants deficient in glutathione are 5.5 and 1.9 times higher, respectively; and the *grxA* mutants contain high amounts of thioredoxin [19, 20]. These facts do not allow unambiguous interpretation of the role of these thiols in response of *E. coli* to UV irradiation.

On the whole, the results obtained indicate that thiol redox systems play a certain role in the *E. coli* resistance to far-UV irradiation, as well as in the induction of SOS response.

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