Письма в ЭЧАЯ. 2011. Т. 8, № 1(164). С. 100–108

MODELING ULTRAVIOLET-INDUCED SOS RESPONSE IN TRANSLESION SYNTHESIS-DEFICIENT CELLS OF *ESCHERICHIA COLI* BACTERIA

O. V. Belov

Joint Institute for Nuclear Research, Dubna

A quantitative analysis is performed of SOS response induced by ultraviolet radiation in *Escherichia coli* bacterial cells with the disordered function of translesion synthesis. The dynamics of the concentration of the basic SOS proteins is estimated for the *recA*, *umuD*, and *umuC* mutants of *E. coli*. The estimation is based on the model approaches developed earlier.

Выполнена количественная оценка SOS-ответа, индуцированного ультрафиолетовым излучением в бактериальных клетках *Escherichia coli* с нарушением нормальной функции translesion-синтеза. Динамика концентрации ключевых белков бактериальной SOS-системы рассчитана для *recA*-, *umuD*- и *umuC*-мутантов *E. coli*. Количественная оценка выполнена на основании разработанных ранее математических подходов.

PACS: 87.18.Cf

INTRODUCTION

A mutation process in *Escherichia coli* bacterial cells induced by various physical and chemical factors is controlled by several genes of the specific DNA repair system. It is known that in *E. coli*, at least 40 genes are coordinately induced in response to DNA damage in a process known as the SOS response [1–3]. The main genes among them are *lexA*, *recA*, *umuDC*, *uvrABCD*, *sulA*, and *polB*. After the induction of the SOS system, the *recA*, *umuD*, and *umuC* genes become the most important in the whole SOS gene complex. The RecA and LexA proteins (the products of the *lexA* and *recA* genes) are the main regulators of SOS response [1]. Cells react to DNA damage when RecA forms nucleoprotein filaments with single-stranded DNA produced as a consequence of damage and mediates the cleavage of LexA. The cleavage of the LexA repressor induces the expression of the SOS genes. The gene products regulated as part of SOS response include those involved in DNA repair, induced mutagenesis, the regulation of cell division, and other functions [1].

The *umuD* and *umuC* genes are regulated as part of SOS response, and the functions of their gene products are needed for most of mutagenesis resulting from exposure to DNA-damaging agents such as UV light [1–3]. Posttranslational RecA-mediated proteolytic cleavage of UmuD to UmuD′ is required for DNA damage-induced mutagenesis, while uncleaved

UmuD is implicated in a DNA damage checkpoint [4]. Both UmuD and UmuD′ are able to form the UmuD_2, UmuDD′, and UmuD'_2 dimmers, which form complexes with UmuC [5, 6]. The main complex responsible for the realization of induced mutagenesis in *E. coli* cells is $\mathrm{UmuD}'_2\mathrm{C}$ (DNA polymerase V). DNA damage-induced mutagenesis results from errors introduced during the process of replicative bypass of a DNA lesion, which requires DNA polymerase III, $\mathrm{UmuD}'_2\mathrm{C}$, and RecA. This process is known as translesion synthesis (TLS) [7, 8]; it leads to the fixation of primary DNA lesions as mutations. The cellular mechanisms which temporarily block DNA replication and cell cycle progression after exposure to DNA-damaging agents have been shown to play an important role in mediating resistance to these agents in eukaryotes [1, 9, 10]. The inhibition of growth following DNA damage allows DNA repair to occur prior to continued DNA replication and chromosome segregation, thereby ensuring the fidelity of these processes. Considering the most important role of the *recA*, *umuD*, and *umuC* genes in the process of induced mutagenesis, these genes were chosen as the subject of this research.

Many attempts to model various stages of induced mutation process in wild-type bacterial cells were made in recent years. But models able to describe mathematically induced mutagenesis in mutant bacterial cells have not yet been developed. As of today, the most detailed mathematical description of mutation process in bacterial cells is performed in [11], where the ultraviolet induction of error-prone repair in *E. coli* bacterial cells is considered. In [11], the whole sequence of events leading to the fixation of the primary DNA lesion as a point mutation is modeled. The dynamic changes of the basic SOS protein concentrations and the process of TLS by the modified replication complex are also described quantitatively. For this reason, the model developed in [11] was chosen for modeling induced mutagenesis in *E. coli* bacterial cells containing mutations in the key genes responsible for TLS. In the present work, ultraviolet radiation was chosen as a damaging factor, too. The main aim of this research is to estimate mathematically the influence of mutations in the key genes responsible for TLS on the kinetics of other proteins of the SOS system in *E. coli* bacterial cells.

1. MATHEMATICAL MODEL

In our previous work, we developed a model of UV-induced mutagenesis in wild-type *E. coli* cells [11]. This model is based on the mathematical description of the basic ways of TLS in bacterial cells from the occurrence of the primary DNA lesion to the final reaction (a gene mutation). The modeling of the kinetics of inducing a SOS signal (which is single-stranded DNA) was performed with the following dimensionless equations [11, 12]:

$$\begin{aligned}
&\text{for} \quad \tau < \tau_2, \qquad y_0(\tau, \Psi) = \Psi \, \exp(-p_1\tau) \int_0^{\tau} \frac{\exp(p_1\xi)d\xi}{p_2\Psi + \exp(p_3\xi)}, \\
&\text{for} \quad \tau \geqslant \tau_2, \qquad y_0(\tau, \Psi) = \Psi \, \exp(-p_1\tau) \int_0^{\tau_2} \frac{\exp(p_1\xi)d\xi}{p_2\Psi + \exp(p_3\xi)}, \qquad (1)
\end{aligned}$$

$$\tau_2 = \frac{1}{p_3} \ln\left(\exp(p_4)\,(1 + p_2\Psi) - p_2\Psi\right),$$

where y_0 is the normalized intracellular concentration of an inducing signal; Ψ is the fluence of UV radiation energy; τ is the normalized time, and τ_2 is the normalized time of replication termination. p_1, p_2, p_3, and p_4 are the normalized parameters of the equations [11]. Thus, for the induced signal dynamics, we obtained two equations: for the time before the replication cycle finishing ($\tau < \tau_2$) and for the time after replication termination ($\tau \geqslant \tau_2$).

The dynamics of the basic SOS proteins in wild-type cells depending on time and the fluence of UV radiation energy was estimated quantitatively by the following system of ordinary differential equations:

$$\frac{dy_1}{d\tau} = \frac{y_{01}(1+p_5^{h_1})}{1+\left(\dfrac{y_1}{\gamma_1 N_A}\right)^{h_1}} - p_6 y_1 y_3 - y_1,$$

$$\frac{dy_2}{d\tau} = \frac{y_{02}(1+p_7^{h_2})}{1+\left(\dfrac{y_1}{\gamma_2 N_A}\right)^{h_2}} + p_1 y_3 - p_8 y_0 y_2 - y_2,$$

$$\frac{dy_3}{d\tau} = p_8 y_0 y_2 - p_1 y_3,$$

$$\frac{dy_4}{d\tau} = \frac{y_{04} p_9 \left(1+p_{10}^{h_4}\right)}{1+\left(\dfrac{y_1}{\gamma_4 N_A}\right)^{h_4}} + p_{11} y_6 y_{10} + p_{12} y_6 y_7 + p_{13} y_6 - p_{14} y_4 y_3 - p_{15} y_4^2 -$$

$$- p_{16} y_4 y_8 - p_{17} y_4 y_6 - p_{18} y_4 y_{11} - p_{19} y_4, \qquad (2)$$

$$\frac{dy_5}{d\tau} = \frac{y_{05} p_{20} \left(1+p_{21}^{h_5}\right)}{1+\left(\dfrac{y_1}{\gamma_5 N_A}\right)^{h_5}} - p_{22} y_5 y_7 - p_{23} y_5 y_8 - p_{24} y_5 y_9 - p_{25} y_5,$$

$$\frac{dy_6}{d\tau} = p_{14} y_3 y_4 + p_{16} y_4 y_8 + p_{18} y_4 y_{11} - p_{26} y_6^2 - p_{17} y_4 y_6 - p_{11} y_6 y_{10} - p_{12} y_6 y_7 - p_{13} y_6,$$

$$\frac{dy_7}{d\tau} = p_{15} y_4^2 - p_{22} y_5 y_7 - p_{12} y_6 y_7 - p_{27} y_7,$$

$$\frac{dy_8}{d\tau} = p_{26} y_6^2 - p_{16} y_4 y_8 - p_{23} y_5 y_8 - p_{28} y_8,$$

$$\frac{dy_9}{d\tau} = p_{17} y_4 y_6 + p_{16} y_4 y_8 + p_{12} y_6 y_7 - p_{24} y_5 y_9 - p_{29} y_9,$$

$$\frac{dy_{10}}{d\tau} = p_{22} y_5 y_7 - p_{11} y_6 y_{10} - p_{30} y_{10},$$

$$\frac{dy_{11}}{d\tau} = p_{23} y_5 y_8 - p_{18} y_4 y_{11} - p_{31} y_{11},$$

$$\frac{dy_{12}}{d\tau} = p_{24} y_5 y_9 + p_{18} y_4 y_{11} + p_{11} y_6 y_{10} - p_{32} y_{12}.$$

The initial conditions for wild-type *E. coli* cells measured as number of protein molecules per cell are the following:

$$\begin{aligned} y_1(0) &= y_{01} = 1300, & y_2(0) &= y_{02} = 7200,\\ y_3(0) &= y_{03} = 0, & y_4(0) &= y_{04} = 180,\\ y_5(0) &= y_{05} = 15, & y_6(0) &= y_{06} = 0,\\ y_7(0) &= y_{07} = 135, & y_8(0) &= y_{08} = 0,\\ y_9(0) &= y_{09} = 0, & y_{10}(0) &= y_{010} = 93,\\ y_{11}(0) &= y_{011} = 0, & y_{12}(0) &= y_{012} = 0. \end{aligned}$$

Here y_i $(i = 1, \ldots, 12)$ are the normalized intracellular concentrations of the LexA, RecA, RecA*, UmuD, UmuC, UmuD′, $UmuD_2$, $UmuD'_2$, UmuDD′, $UmuD_2C$, $UmuD'_2C$, and UmuDD′C proteins, respectively; p_j $(j = 5, \ldots, 32)$ are the normalized velocity constants of the protein–protein interactions. The procedure of determining the values of these parameters is described in detail in [11].

2. RESULTS

Calculations for *recA* Mutant Strains. In the case of a mutation in the *recA* gene, the RecA protein is absent in the cell. Thus, in Eqs. (2) we have to let the initial concentration of the RecA protein y_{02} equal zero. The absence of the RecA protein leads to the absence of the RecA protease, which is responsible for the dissociation of the LexA repressor and for the cleavage of the UmuD protein. In this case, we observe the constant level which equals to the initial intracellular level for all the basic SOS proteins after UV irradiation. Thus, a mutation in the *recA* gene leads to the full absence of SOS response, and an increase in mutagenesis stipulated by errors during translesion synthesis is not observed in this case.

Calculations for *umuD* Mutant Strains. As the cells with a mutation in the *umuD* gene cannot produce the normal form of the UmuD protein, the levels of all the SOS protein complexes containing the products of the *umuD* gene are equal to zero at any moment during all the time when SOS response is observed in wild-type cells. Thus, in Eqs. (2), let the parameters y_{04}, y_{07}, and y_{10} characterizing the initial levels of UmuD, $UmuD_2$, and $UmuD_2C$ proteins be equal to zero.

A mutation in *umuD* does not influence the production of LexA, RecA, and RecA* proteins (Fig. 1, *a*–*c*), but significantly influences the UmuC protein kinetics (Fig. 1, *d*). In the absence of the UmuC protein interactions with various *umuD* gene products such as UmuD, UmuD′, $UmuD'_2$, UmuDD′, and $UmuD_2$, we have obtained an increase in the UmuC intracellular level and very slow dissociation of the *umuC* gene product. A decrease in the UmuC protein level in the case of *umuD* mutation is determined only by its nonspecific degradation.

The maximal levels of the UmuC protein intracellular concentration in *umuD* mutant strains in comparison with wild-type strains are presented in Table 1.

The results were calculated for various fluences of UV radiation energy. According to the obtained results, for an energy fluence of 1 $J \cdot m^{-2}$ we have a 2.14-fold increase of the maximal intracellular level of the UmuC protein in *umuD* mutant cells in comparison with wild-type cells. For 5 $J \cdot m^{-2}$, we obtained a 2.92-fold increase of the maximal level. Energy

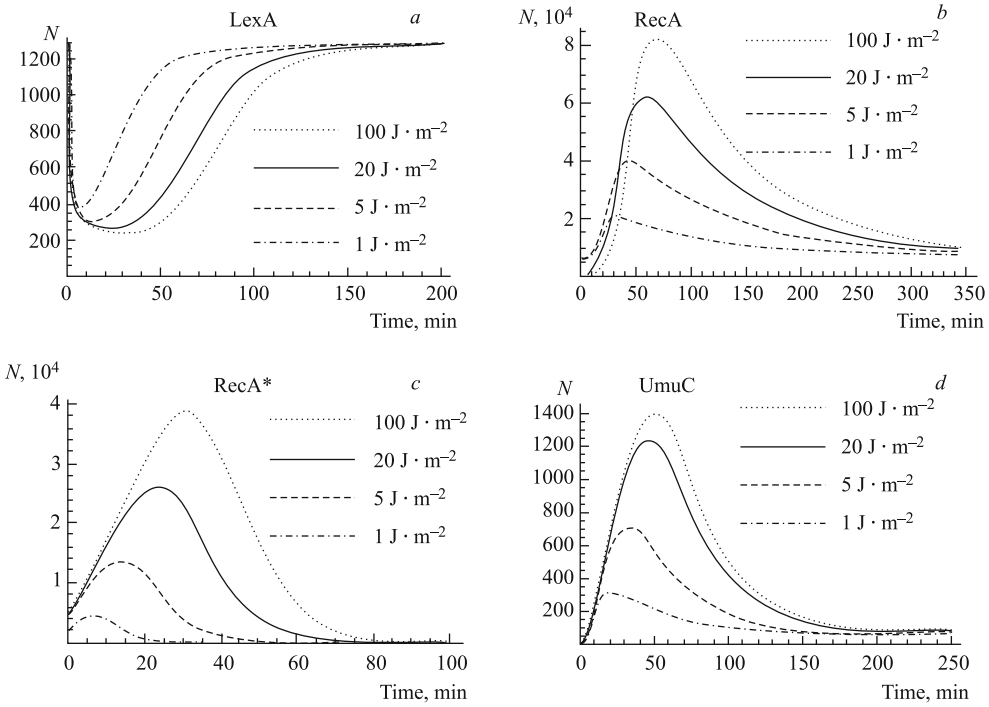

Fig. 1. Changes in the concentration of the LexA (*a*), RecA (*b*), RecA* (*c*), and UmuC (*d*) SOS proteins in *umuD* mutants of *E. coli* for various UV radiation fluencies. N is the number of protein molecules per cell

Table 1. **A comparison of the maximal level of the UmuC protein in wild-type cells and in *umuD* mutant strains of *E. coli***

UV radiation energy fluence, $J \cdot m^{-2}$	Wild-type cells, molecules per cell	*umuD* mutant strains, molecules per cell
1	140	300
5	240	700
20	340	1200
100	385	1400

fluences of 20 and 100 $J \cdot m^{-2}$ give a 3.53 and 3.64-fold increase, respectively. Thus, we have an absence of SOS response like in the case of a mutation in the *recA* gene, and the mutagenic branch of DNA repair is suppressed in *umuD* mutant cells. But all the other functions of the LexA, RecA, RecA*, and UmuC proteins remained in the cell.

Calculations for *umuC* Mutant Strains. It is known that a mutation in the *umuC* gene also leads to the suppression of translesion synthesis because the $UmuD'_2C$ protein (the main compound of a modified replication complex) is not synthesized like in the case of mutations in the *recA* and *umuD* genes. Cells with *umuC* mutations are unable to synthesize two other important regulatory complexes of the SOS system: $UmuD_2C$ and UmuDD′C.

To calculate the kinetics of SOS proteins in *umuC* mutant cells, we have put the initial concentration y_{05} of the UmuC protein zero. The initial intracellular level of the $UmuD_2C$ regulatory complex was also assumed zero. The initial concentration y_{07} of the $UmuD_2$ protein was estimated from the mass balance in the case of wild-type cells [11]. The dimensional equation for the basal level of $UmuD_2$ is

$$X_{07} = \frac{\varepsilon X_{04}^2}{a_1 X_{05} + \delta_7}, \tag{3}$$

where $X_{04} = 2.99 \cdot 10^{-22}$ mol and $X_{05} = 2.49 \cdot 10^{-23}$ mol are the dimensional initial concentrations of the UmuD and UmuC proteins, respectively, $\varepsilon = 7.73 \cdot 10^{19}$ mol$^{-1} \cdot$ min^{-1} is the dimensional rate constant in the reaction of the UmuD protein dimerization, $a_1 = 6.18 \cdot 10^{20}$ mol$^{-1} \cdot$ min^{-1} is the dimensional rate constant in the interaction between the *umuC* gene product and $UmuD_2$ protein, $\delta_7 = 0.015$ min^{-1} is the rate constant in the nonspecific degradation of the $UmuD_2$ protein [11]. In *umuC* mutant strains, the initial level X_{05} of the UmuC protein is zero. Thus, in this case, the following formula for X_{07} is true:

$$X_{07} = \frac{\varepsilon X_{04}^2}{\delta_7}. \tag{4}$$

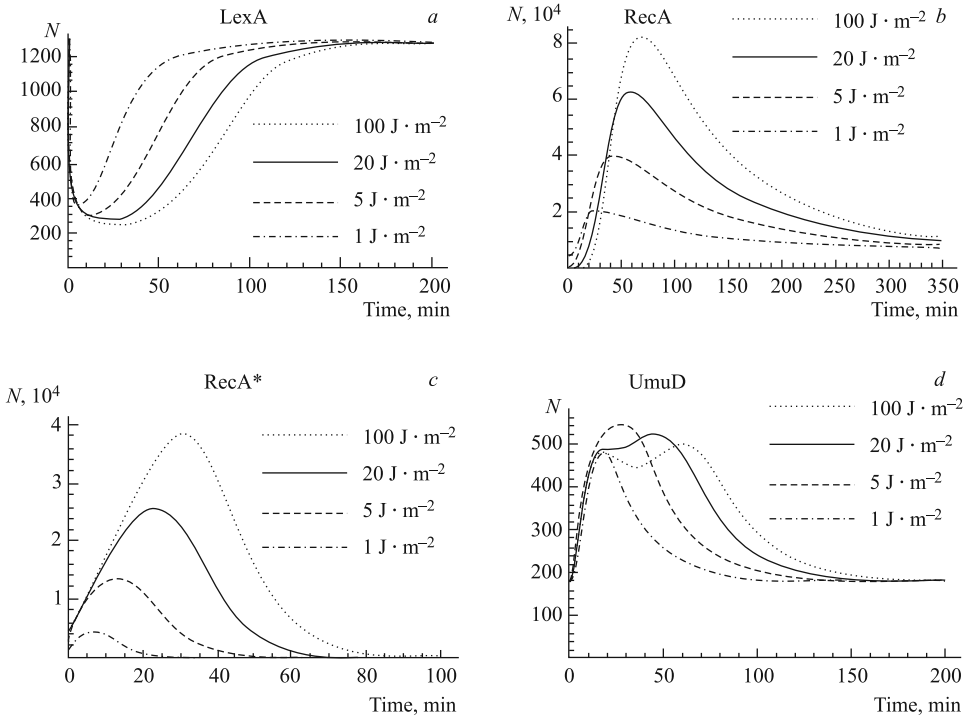

Fig. 2. Changes in the concentration of the LexA (*a*), RecA (*b*), RecA* (*c*), and UmuD (*d*) SOS proteins in *umuC* mutants of *E. coli* for various UV radiation energy fluencies Ψ. N is the number of protein molecules per cell

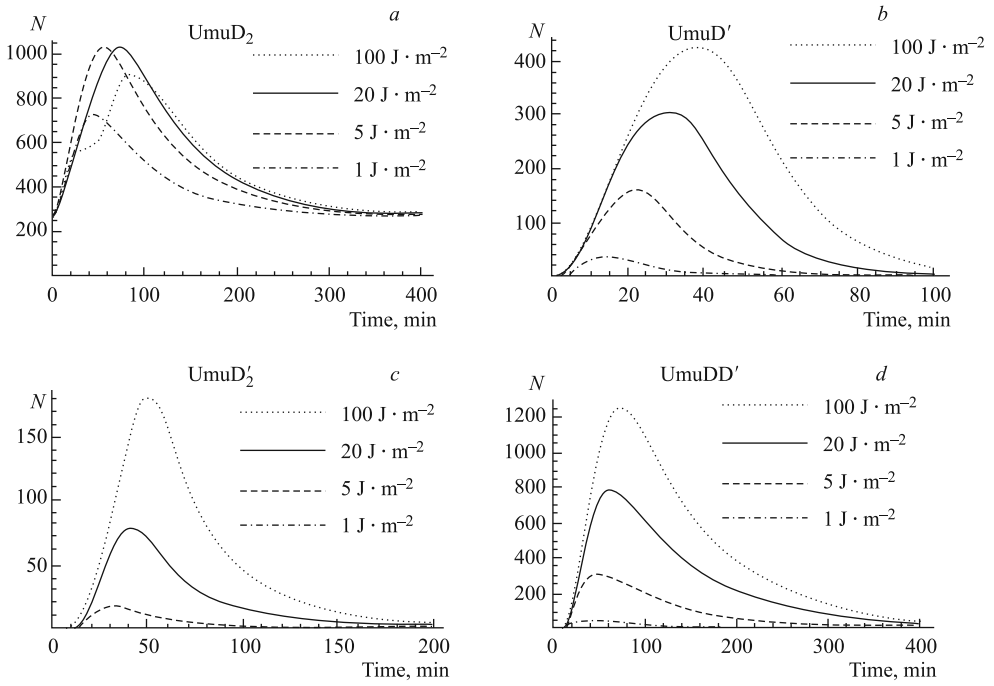

Fig. 3. Changes in the concentration of the $UmuD_2$ (*a*), UmuD′ (*b*), $UmuD'_2$ (*c*), and UmuDD′ (*d*) SOS proteins in *umuC* mutants of *E. coli* for various UV radiation energy fluencies Ψ. N is the number of protein molecules per cell

Table 2. **A comparison of the maximal level of the *umuD* gene products in wild-type cells and in *umuC* mutant strains of *E. coli***

Protein	Ψ, J · m^{-2}	Wild-type cells		*umuC* mutant strains	
		Protein molecules per cell	Time of the maximum, min	Protein molecules per cell	Time of the maximum, min
UmuD′	1	32	13.6	37	14.7
	5	131	19.5	159	21.2
	20	249	28.2	305	29.7
	100	367	37.0	429	37.2
$UmuD'_2$	1	0	—	0	—
	5	4	24.2	18	31.5
	20	14	32.4	79	41.6
	100	25	40.0	181	50.4
UmuDD′	1	16	28.7	45	37.4
	5	55	37.3	311	47.0
	20	84	57.5	790	60.8
	100	99	77.5	1251	72.5
$UmuD_2$	1	333	51.2	727	41.4
	5	449	66.1	1031	54.2
	20	471	90.0	1039	73.0
	100	433	102.7	914	88.7

The parameters X_{04}, ε, a_1, and δ_7 have the same values as for wild-type cells; thus, the dimensional value for the initial $UmuD_2$ concentration is $X_{07} = 4.61 \cdot 10^{-22}$ mol, and for the dimensionless value we have the following expression: $y_{07} = X_{07}N_A \approx 277$ molecules per cell. Here, N_A is the Avogadro constant.

The results calculated for *umuC* mutants are presented in Figs. 2, 3. For the LexA, RecA, RecA*, and UmuD proteins, we obtained the same results as for a wild-type strain. The level of the active form of the *umuD* gene product is slightly higher than in wild-type cells (Fig. 3, *b*). For the dimerized *umuD* gene products, we obtained a significant increase in their maximal intracellular concentration and longer time of the degradation of these proteins (Fig. 3, *a*, *c*, *d*). Such effects could be explained by the absence of the interaction of the $UmuD_2$, UmuDD′, and $UmuD'_2$ proteins with molecules of the UmuC protein. A decrease in the pool of dimerized SOS proteins is caused only by subunit exchanges between various *umuD* gene products and by their nonspecific degradation. A comparison of the maximal level of the *umuD* gene products in wild-type cells and in *umuC* mutant strains of *E. coli* is presented in Table 2.

CONCLUSIONS

A mathematical model describing the dynamics of the basic SOS proteins in *E. coli* cells containing mutations in the key genes responsible for translesion synthesis was developed. Thus, from the results shown above, we can conclude that mutations in the *umuD* and *umuC* genes do not affect the kinetics of the LexA, RecA, and RecA* proteins. But in this case TLS is suppressed because the main regulatory complex $UmuD'_2C$ cannot be synthesized. In this work, for the first time the dynamics of the dimerized *umuD* gene products in *umuC* mutant strains is predicted. The obtained results show that the levels of the $UmuD_2$, UmuDD′, and $UmuD'_2$ proteins are much higher in *umuC* mutant strains than in wild-type cells. As the functions of these dimerized products are not changed in *umuC* mutant strains, the obtained results can be used for predicting some specific cell reactions for which these *umuD* gene products are responsible.

The scientific significance of this research is that another important step has been made towards the complex mathematical description of all aspects of the induced mutation process in bacterial cells. The mathematical model developed in [11] and extended in the present work to include mutant strains has a considerable potential for modifications and can be improved as soon as new experimental data come up.

Acknowledgements. The author thanks the Director of the Laboratory of Radiation Biology, the Joint Institute for Nuclear Research (Dubna), Prof. E. A. Krasavin for his support and suggestions on the manuscript.

REFERENCES

1. *Friedberg E. C., Walker G. C., Siede W.* DNA Repair and Mutagenesis. Washington: ASM Press, 1995. 698 p.
2. *Koch W. H., Woodgate R.* The SOS Response // DNA Damage and Repair / Eds. Nickoloff J. A., Hoekstra M. F. V. 1. DNA Repair in Prokaryotes and Lower Eukaryotes. Humana Press Inc., 1997. P. 107–134.

3. *Smith B., Walker G. C.* Mutagenesis and More: *umuDC* and the *Escherichia coli* SOS Response // Genetics. 1998. V. 148. P. 1599–1610.

4. *Opperman T. et al.* A Model for a *umuDC*-dependent Prokaryotic DNA Damage Checkpoint // Proc. Nat. Acad. Sci. USA. 1999. V. 96. P. 9218–9223.

5. *Bruck I. et al.* Purification of a Soluble UmuD′C Complex from *Escherichia coli* // J. Biol. Chem. 1996. V. 271. P. 10767–10774.

6. *Woodgate R. et al.* UmuC Mutagenesis Protein of *Escherichia coli*: Purification and Interaction with UmuD and UmuD′ // Proc. Nat. Acad. Sci. USA. 1989. V. 86. P. 7301–7305.

7. *Rajagopalan M. et al.* Activity of the Purified Mutagenesis Proteins UmuC, UmuD′, and RecA in Replicative Bypass of an Abasic DNA Lesion by DNA Polymerase III // Proc. Nat. Acad. Sci. USA. 1992. V. 89. P. 10777–10781.

8. *Walker G. C.* Skiing the Black Diamond Slope: Progress on the Biochemistry of Translesion Synthesis // Proc. Nat. Acad. Sci. USA. 1998. V. 95. P. 10248–10350.

9. *Elledge S. J.* Cell Cycle Checkpoints: Preventing an Identity Crisis // Science. 1996. V. 274. P. 1664–1672.

10. *Paulovich A. G., Toczyski D. P., Hartwell L. H.* When Checkpoints Fail // Cell. 1997. V. 88. P. 315–321.

11. *Belov O. V., Krasavin E. A., Parkhomenko A. Yu.* Model of SOS-Induced Mutagenesis in Bacteria *Escherichia coli* under Ultraviolet Irradiation // J. Theor. Biol. 2009. V. 261. P. 388–395.

12. *Aksenov S. V.* Dynamics of the Inducing Signal for the SOS Regulatory System in *Escherichia coli* after Ultraviolet Irradiation // Math. Biosci. 1999. V. 157. P. 269–286.

Received on April 27, 2010.