ОБЪЕДИНЕННЫЙ
ИНСТИТУТ
ЯДЕРНЫХ
ИССЛЕДОВАНИЙ

Дубна

E19-99-74

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INDUCTION OF THE SOS RESPONSE IN ULTRAVIOLET-IRRADIATED *ESCHERICHIA COLI* ANALYZED BY DYNAMICS OF LEXA, RECA AND SULA PROTEINS

Submitted to «Journal of Biological Physics»

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1999

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E19-99-74

Анализ индукции SOS-ответа бактерий *Escherichia coli*,
облученных ультрафиолетовым светом,
при помощи динамики белков LexA, RecA и SulA

SOS-ответ у бактерий *Escherichia coli* проявляется после ДНК-повреждающих воздействий, например, после облучения ультрафиолетовым светом. Регуляция SOS-ответа осуществляется через взаимодействие двух регуляторных белков, LexA и RecA. В клетках, облученных ультрафиолетовым светом, эксцизионная репарация является основной системой восстановления повреждений ДНК. При помощи расчета динамики внутриклеточных концентраций белков LexA, RecA и SulA исследована индукция SOS-ответа бактерий *Escherichia coli* с нормальной и дефектной системой эксцизионной репарации. Белок SulA отвечает за SOS-индуцибельную задержку клеточного деления. Результаты расчетов показывают, что эксцизионная репарация оказывает влияние на динамику индукции LexA, RecA и SulA, модулируя динамику распределения RecA-белка между нормальной и SOS-активной формами.

Работа выполнена в Отделении радиационных и радиобиологических исследований ОИЯИ.

Препринт Объединенного института ядерных исследований. Дубна, 1999

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E19-99-74

Induction of the SOS Response
in Ultraviolet-Irradiated *Escherichia coli* Analyzed
by Dynamics of LexA, RecA and SulA Proteins

The SOS response in *Escherichia coli* is induced after DNA-damaging treatments including ultraviolet light. Regulation of the SOS response is accomplished through specific interaction of the two SOS regulator proteins, LexA and RecA. In ultraviolet light treated cells nucleotide excision repair is the major system that removes the induced lesions from the DNA. Here, induction of the SOS response in *Escherichia coli* with normal and impaired excision repair function is studied by simulation of intracellular levels of regulatory LexA and RecA proteins, and SulA protein. SulA protein is responsible for SOS-inducible cell division inhibition. Results of the simulations show that nucleotide excision repair influences time-courses of LexA, RecA and SulA induction by modulating the dynamics of RecA protein distribution between its normal and SOS-activated forms.

The investigation has been performed at the Department of Radiation and Radiobiological Research, JINR.

Preprint of the Joint Institute for Nuclear Research. Dubna, 1999

Introduction

The SOS response in *Escherichia coli* bacteria is a set of inducible physiological reactions that help a cell to survive after the treatment with various DNA-damaging agents, such as ultraviolet and ionizing radiation and some chemicals [1]. Induction of the SOS response is due to increased expression of about 20 unlinked bacterial genes that are members of the SOS regulatory system. The SOS response includes enhanced capacity for DNA repair, transient inhibition of cell division, increased frequency of mutagenesis and more. The SOS system acts by sensing an inducing signal that appears in a cell after the DNA-damaging treatment. RecA protein, the positive regulator of the SOS system, is activated after interaction with signal molecules. LexA protein, negative regulator, is the transcriptional repressor of the genes of the SOS system. LexA is proteolytically cleaved by activated RecA (RecA*) [2] which results in increased expression of the SOS genes and manifestation of the SOS response.

The most immediate consequence of DNA damage is generation of the inducing signal. It is believed that the critical component of the signal is free single-stranded DNA (ssDNA). ssDNA was shown to be necessary for activation of RecA [3, 4]. Regions of ssDNA, also termed gaps, were shown to generate after ultraviolet light (UV) irradiation when replication forks "bump" into UV-induced lesions, and resume DNA synthesis downstream from the lesion leaving a gap [5].

Previously, induction of the SOS response has been studied in several ways: by measuring LexA level using LexA-specific antibodies [5]; by monitoring dynamics of phage λ induction in lysogens [6]; by measuring level of specific β-galactosidase activity in fusions of *lacZ* gene to various SOS genes [7, 8]. However, these methods are not sufficient to understand the overall regulation and induction of the SOS response in that they measure the SOS induction indirectly. For example, intermediate steps are involved in the induction of phage λ. In *lacZ* fusions, dynamics of SOS-controlled gene expression is studied. The more direct evidence might be provided by studying the dynamics of the LexA and RecA regulatory proteins, and other SOS proteins.

Earlier, we proposed a dynamic model for interacton of LexA and RecA [9]. Differential equations of the model included terms describing LexA-mediated regulation of both LexA and RecA production. In these terms we used the first-order approximation for kinetics of LexA binding to operator DNA, which is equivalent to the assumption of noncooperative LexA binding. The model was found to be consistent with available experimental data on LexA dynamics in UV-irradiated *E. coli* but was not entirely consistent with the data on RecA dynamics. Some experimental evidence suggests that LexA exhibits a weak cooperativity when binding to operator DNA [10]. In the present paper we extend our earlier model in that we now introduce cooperativity parameters into the equations to account for the cooperativity of LexA binding to operator DNA. With the model modified this way we analyse the SOS response dynamics in UV-irradiated *E. coli* by simulating levels of regulatory proteins LexA, RecA, RecA* and SulA protein that is responsible for division inhibition in the conditions of the SOS response and that is regulated by LexA [11]. We perform simulations for different UV doses and two *E. coli* strains: wild-type and *uvr* mutant which is defective in nucleotide excision repair (NER). NER is the major cellular system for repair of UV-induced lesions [12]. To calculate the inducing signal level in wild-type and *uvr* mutant bacteria we use the equations derived previously [13]. By simulating the RecA* level in wild-type and *uvr* mutant bacteria we show that NER influences the dynamics of the SOS response induction.

Model

LexA-RecA interaction

Induction of the SOS response is triggered by the inducing signal but before the SOS response will be induced, specific interaction between LexA and RecA takes place. Regulation of the SOS response includes repression of *lexA* and *recA* genes by LexA, activation of RecA into RecA* form by its binding to ssDNA (the inducing signal) and proteolysis of LexA by RecA*. Increased expression of SOS genes then occurs because of the drop in LexA level. When DNA damage is repaired, LexA repressor accumulates again to its normal constitutive level and turns off expression of the SOS genes. Dynamical equation description of the SOS induction can be given considering levels of regulatory LexA and RecA proteins and inducing signal as variables:

$$\dot{\mathbf{X}} = \mathbf{V}(\mathbf{X}, X_0)$$

where $\mathbf{X} = (X_1, X_2, X_3)$ is vector of protein levels (X_1 denotes LexA, X_2 denotes RecA and X_3 denotes RecA*), X_0 is the signal level and $\mathbf{V}(\mathbf{X})$ is the vector of rates of changes in levels $\mathbf{X}$. These rates can be expanded as follows:

$$\begin{aligned}
\frac{dX_1}{dt} &= \alpha_1 \left(\frac{X_{10}[1 + (X_{10}/\gamma_1)^{h_1}]}{1 + (X_1/\gamma_1)^{h_1}} - X_1 \right) - \beta_1 X_1 X_3, \\
\frac{dX_2}{dt} &= \alpha_2 \left(\frac{X_{20}[1 + (X_{10}/\gamma_2)^{h_2}]}{1 + (X_1/\gamma_2)^{h_2}} - X_2 \right) - \beta_2 X_2 X_0 + v_0 X_3, \\
\frac{dX_3}{dt} &= \beta_2 X_2 X_0 - v_0 X_3.
\end{aligned} \tag{1}$$

Equations (1) with $h_1 = 1$ and $h_2 = 1$ are as derived previously [9, 13]. Equations (1) are the material balance equations. The positive terms in Equations (1) describe increase in $\mathbf{X}$ due to protein synthesis and RecA* conversion back to normal RecA. The negative terms describe decrease in $\mathbf{X}$ because of dilution due to bacterial growth and activation of RecA into RecA*. Level of the inducing signal X_0 occurs in the terms that contribute into RecA activation.

Table 1: Estimation of parameter values (See text for definition of parameters)

Parameter	Value	Reference
f	$N_A V = 6.02 \times 10^8$ M^{-1} l	-
X_{10}, X_{20} (wild-type)	2.2×10^{-6} M, 1.2×10^{-5} M	[5]
X_{10}, X_{20} (*uvr* mutant)	1.5×10^{-6} M, 1.8×10^{-5} M	[5]
α_1, α_2	$(\ln 2)/60 = 0.0116$ min^{-1}	[13]
β_1	8.7×10^4 M^{-1} min^{-1}	this work
β_2	3.7×10^5 M^{-1} min^{-1}	[14]
γ_1, γ_2	2.0×10^{-8} M, 2.0×10^{-9} M	[15]
v_0	0.24 ± 0.01 min^{-1}	this work
h_1, h_2	2.4 ± 0.2, 2.0	this work
v_1	0.7 min^{-1}	[16]
l_0	900 nt	[17]
t_0	0.17 min	[17]
T_0	40 min	[18]
α_4	$(\ln 2)/10 = 0.0693$ min^{-1}	[19]
γ_4	8.0×10^{-10} M	[20]
h_4	2.0	this work

In Equations (1), X_{10} and X_{20} (M) are the uninduced levels of LexA and RecA proteins, respectively; α_1, α_2, β_1 and β_2 (min^{-1}) are the rate constants; γ_1 and γ_2 (M) are LexA equilibrium dissociation constants for operators of *lexA* and *recA* genes; v_0 (min^{-1}) is the rate of RecA* conversion back to normal RecA form, that is equal to the rate of disappearance of ssDNA gaps because of gap repair processes. Estimates of parameter values together with the references are summarized in Table 1. Values of parameters β_1, v_0 and h_1 were fitted to experimental data (see the Results section).

Cooperativity of LexA binding to DNA

In Equations (1), we introduce novel parameters h_1 and h_2 that describe the cooperativity of LexA binding to operator DNA of *lexA* and *recA* genes. Parameters h_1 and h_2 appear in the terms describing LexA-controlled synthesis of LexA and RecA. We assume the rate of

protein synthesis be proportional to the fraction of operator DNA that is free from LexA and that is therefore available for RNA polymerase to initiate transcription. Consider the equilibrium of the following one-step reaction scheme $nL + D_f \Leftrightarrow D_o$ where L is free LexA; D_f is free operator DNA; D_o is operator DNA bound by LexA; n is the maximum number of LexA monomers capable of binding to the operator DNA. The equilibrium dissociation constant for this reaction is $\gamma^n = ([D_f][L]^n)/[D_o]$ where γ is an apparent LexA monomer dissociation constant for operator DNA. The fraction of free operator DNA is then described by the following semiempirical equation:

$$F = \frac{[D_f]}{[D_o] + [D_f]} = \frac{1}{1 + ([L]/\gamma)^h} \qquad (2)$$

where $1 \leq h \leq n$. Parameter h is the measure of cooperativity and is termed the Hill coefficient. When $h = 1$ then there is no cooperativity in the system. When $h = n$ then the system has the absolute cooperativity.

In our earlier model [9] we assumed $h = 1$ in Equation (2) which is equivalent to the absence of cooperativity of LexA binding to operator DNA. This assumption implies that binding of a single molecule of LexA is sufficient to block transcription of an SOS gene. The model in this simpler form was found to be consistent with available experimental data on LexA dynamics in UV-irradiated *E. coli* but was not entirely consistent with RecA dynamics.

There is an experimental evidence implying that LexA exhibits some weak cooperativity when binding to multiple operator sites in front of some SOS genes [10]. *recA* gene has one, whereas *lexA* gene has two operator site capable of binding LexA molecules. All operator sites of SOS genes have a dyad symmetry and each LexA monomer binds each half of the operator [21]. LexA exists predominantly as a monomer in solution [22] but binds to each operator site as a dimer [23]. We propose an extension of our earlier model of LexA control over transcription of the SOS genes in that (i) we assume that two LexA molecules bind to two operator half-sites with absolute cooperativity, and (ii) there is a certain degree of cooperativity between LexA molecules binding to two adjacent operator sites. According to the as-

sumption (i), the Hill coefficient for LexA binding to operator of *recA* gene is $h_2 = n = 2$ (single operator site of *recA* gene can bind $n = 2$ LexA molecules at most). According to the assumption (ii) the Hill coefficient for LexA binding to the operator of its own gene *lexA* is $1 \leq h_1 \leq n = 4$ (two operator sites of the *lexA* gene can bind $n = 4$ molecules of LexA at most). The value of h_1 is unknown from the literature and was determined from fitting simulation of LexA level to experimental data.

Inducing signal

To be able to assay dynamics of LexA and RecA we have to know the dynamics of the inducing signal because its level controls the LexA-RecA interaction. Earlier, we proposed a model for calculation of the level of inducing signal as a function of time after UV irradiation in wild-type *E. coli* [13]. In this model ssDNA is the critical component of the signal, and the concentration of ssDNA in a cell is taken as a measure of the level of the signal. First, we calculated the number of gaps present in a cell at any given moment t, because all ssDNA is supposed to generate in the form of gaps. We then found level of signal $[X_0(t)]$ by multiplying the number of gaps by an average length of a gap and by a factor $f = N_A V$ (where N_A is Avogadro number and V is the mean *E. coli* cell volume), to express $X_0(t)$ in moles of nucleotides (nt) per litre. Number of gaps present in a cell at any given moment is determined by the ongoing replication of damaged DNA that generates gaps opposite lesions; removal of a portion of lesions in front of a replication fork by NER; the counteracting process of repair of gaps. In this way we obtained level of inducing signal (in terms of moles of nt of ssDNA per litre) for wild-type cells in the form

$$X_0(t) = \frac{50 l_0 D}{f T_0} e^{-v_0 t} J(t) \tag{3}$$

where for times $t < t_1$ function $J(t)$ for a wild-type strain reads as

$$J(t) = \int_0^t \frac{e^{v_0 \xi} d\xi}{25 D t_0 / T_0 + e^{v_1 \xi}} \tag{4}$$

and for all times $t \geq t_1$ function $J(t) = J(t_1)$ is a constant. In Equations (3) and (4) v_1 (min^{-1}) is the rate constant of lesions removal by NER; l_0 (nt) is the mean length of a gap; t_0 (min) is the delay time at a dimer; T_0 (min) is time for replication completion under normal growth conditions; D ($\mathrm{J\ m}^{-2}$) is the UV dose; t_1 (min) is the time when replication terminates. Estimates of parameter values together with the references are summarized in Table 1. Time of replication termination t_1 calculates as

$$t_1 = \frac{1}{v_1} \ln \left[e^{v_1 T_0} \left(1 + \frac{25 D t_0}{T_0} \right) - \frac{25 D t_0}{T_0} \right]. \tag{5}$$

Equations (3), (4) and (5) are for wild-type *E. coli* with normal NER function. In case of *uvr* mutant with impaired NER, we let $v_1 = 0$. Then integral (4) can be gained in a closed form and reads

$$J(t) = \frac{T_0 (e^{v_0 t} - 1)}{v_0 (25 D t_0 + T_0)}. \tag{6}$$

Time for replication termination in *uvr* mutant calculates as

$$t_1 = T_0 + 25 D t_0. \tag{7}$$

Level of inducing signal is calculated using Equation (3) with function $J(t)$ given by Equation (4) and t_1 given by Equation (5) for a wild-type strain, and by Equation (6) and Equation (7) for a *uvr* mutant strain.

Induction of SulA protein

To better understand dynamics of the SOS response regulation and induction, we consider the SulA dynamics in UV-induced *E. coli*. Gene *sulA* coding for SulA which is the DNA damage-inducible cell division inhibitor, is a member of the SOS system [11]. Promoter of the *sulA* gene is repressed by LexA that binds to its single operator site. As in our dynamical model for LexA-RecA interaction, we assume that the rate of SulA production is proportional to the fraction of operator DNA of the *sulA* gene that is free from LexA. This fraction is given by

Equation (2), with appropriate value for the Hill coefficient. We write the following differential equation for the SulA concentration (X_4):

$$\frac{dX_4}{dt} = \alpha_4 \left(\frac{X_{40}[1 + (X_{10}/\gamma_4)^{h_4}]}{1 + (X_1/\gamma_4)^{h_4}} - X_4 \right) \tag{8}$$

where X_{40} (M) is the uninduced steady-state SulA level; α_4 (min^{-1}) is the rate constant for SulA degradation; γ_4 (M) is the LexA equilibrium dissociation constant for operator of the *sulA* gene; and h_4 is the Hill coefficient describing cooperativity of LexA molecules binding to the operator of the *sulA* gene. According to our model of cooperative LexA binding to operator DNA, $h_4 = n = 2$ (single operator site of *sulA* gene can bind $n = 2$ LexA molecules at most). Estimates of parameter values together with the references are summarized in Table 1.

Results and discussion

Dimensionless equations. To assay the dynamics of LexA, RecA and SulA numerical solution of Equations (1) and (8), with $X_0(t)$ given by Equations (3), (4) and (5) (wild-type cells) and (6), (7) (*uvr* mutant) is required. For correct numerical solution we rewrite the equations by introducing the following dimensionless variables

$$u_0 = \frac{X_0}{50 l_0/(T_0 \alpha_1 f)}, \quad u_1 = \frac{X_1}{X_{10}}, \quad u_2 = \frac{X_2}{X_{20}}, \quad u_3 = \frac{X_3}{X_{20}}, \quad u_4 = \frac{X_4}{X_{40}}, \tag{9}$$

dimensionless parameters

$$p_1 = \frac{X_{10}}{\gamma_1}, \quad p_2 = \beta_1 \frac{X_{20}}{\alpha_1}, \quad p_3 = \frac{X_{10}}{\gamma_2}, \quad p_4 = \beta_2 \frac{50 l_0}{T_0 \alpha_1^2 f}, \quad p_5 = \frac{v_0}{\alpha_1}, \quad p_6 = \frac{\alpha_4}{\alpha_1}, \quad p_7 = \frac{X_{10}}{\gamma_4}, p_8 = \frac{25 t_0}{T_0}, \quad p_9 = \frac{v_1}{\alpha_1}, \quad p_{10} = v_1 T_0 \tag{10}$$

and dimensionless time $\tau = t\alpha_1$. Equations for normalized levels of LexA, RecA and SulA [Equations (1) and (8)] then assume the form

$$\begin{aligned}
\frac{du_1}{d\tau} &= \frac{1 + p_1^{h_1}}{1 + (p_1 u_1)^{h_1}} - u_1(1 + p_2 u_3), \\
\frac{du_2}{d\tau} &= \frac{1 + p_3^{h_2}}{1 + (p_3 u_1)^{h_2}} - u_2(1 + p_4 u_0) + p_5 u_3, \\
\frac{du_3}{d\tau} &= p_4 u_0 u_2 - p_5 u_3, \\
\frac{du_4}{d\tau} &= p_6 \left(\frac{1 + p_7^{h_4}}{1 + (p_7 u_1)^{h_4}} - u_4 \right).
\end{aligned} \tag{11}$$

Initial conditions for Equations (11) are

$$u_1(0) = 1.0, \quad u_2(0) = 1.0, \quad u_3(0) = 0.0, \quad u_4(0) = 1.0. \tag{12}$$

Equations (3), (4), (5), (6) and (7) for normalized level of the inducing signal assume the form

$$u_0(\tau) = D e^{-p_5 \tau} J(\tau) \tag{13}$$

where for times $\tau < \tau_1$ function $J(\tau)$ for a wild-type strain reads as

$$J(\tau) = \int_0^\tau \frac{e^{p_5 \xi} d\xi}{p_8 D + e^{p_9 \xi}} \tag{14}$$

and for all times $\tau \geq \tau_1$ function $J(\tau) = J(\tau_1)$ is a constant. Parameter τ_1 calculates as

$$\tau_1 = \frac{1}{p_9} \ln \left[e^{p_{10}} (1 + p_8 D) - p_8 D \right]. \tag{15}$$

For *uvr* mutant, function $J(\tau)$ is

$$J(\tau) = \frac{e^{p_5 \tau} - 1}{p_5 (1 + p_8 D)} \tag{16}$$

and parameter τ_1 is

$$\tau_1 = \frac{p_{10}}{p_9}(1 + p_8 D)\,. \qquad (17)$$

Dimensionless parameter D is UV dose normalized by 1 J m^{-2}. Inserting numerical values of parameters listed in Table 1 into dimensionless parameters definitions (10), we obtain the following values of dimensionless parameters:

$$p_1 = 110.0(75.0), \quad p_3 = 1100.0(750.0), \quad p_4 = 5140.0,$$
$$p_6 = 5.97, \quad p_7 = 2750.0(1880), \quad p_8 = 0.106,$$
$$p_9 = 60.3, \quad p_{10} = 28.0. \qquad (18)$$

Values in parenthesis refer to *uvr* mutant cells.

Fitting parameters to data. To perform all numerical calculations we have used *Scientist for Windows ver. 2.01* program purchased from *MicroMath Scientific Software*, running on an IBM compatible computer. Numerical integration of function (14) was performed using an adaptive quadrature algorithm developed by *MicroMath* that is similar to that described in [24]. Numerical integration of differential equations (11) was performed using EPISODE package implemented in *Scientist*, which is described in [25]. Fitting of parameters was carried out by least squares using Powell's variant of Levenberg-Marquardt algorithm [26], also implemented in *Scientist*.

Values of parameters β_1, v_0 and h_1 were not available in the literature. We determined values of their dimensionless equivalents p_2, p_5 and h_1 by fitting simulation of normalized LexA level (u_1) to experimental data on measurement of LexA content in wild-type *E. coli* UV-irradiated with the dose 5 J m^{-2} [5] ($D = 5$). Data in [5] were obtained as percentage of LexA remaining in UV-irradiated cells, which is equivalent to our definition of u_1.

Preliminary experiments with simultaneous fitting of the three parameters p_2, p_5 and h_1 showed that estimates of parameters p_2 and h_1 are highly dependent on each other (correlation coefficient 0.97-0.99). This means that a change in one of these parameters may be compensated for by a change in the other. This results in the inability

to fit both p_2 and h_1 with necessary accuracy. Thus we have to fix either p_2 or h_1 and then fit the remaining two parameters to the data. In our numerical simulations we fixed $p_2 = 90$ and varied p_5 and h_1. Parameter p_2 (its dimensional equivalent β_1) is the rate constant for RecA*-dependent LexA cleavage. From the experimental data, the range for p_2 is from about 30 to 200 [5, 27]. Variation in p_2 affects the initial part of the LexA curve, when most extensive cleavage of LexA occurs. Later on, when inducing signal declines variation in p_2 may probably be of less importance. In contrast, parameter h_1 which describes the cooperative LexA binding to its own operator and hence the rate of LexA production, is likely to be important during the whole time course of LexA variation. From numerical experiments on fitting p_5 and h_1, it becomes apparent that fixing greater values for p_2 produces smaller sum of squared deviations (SSD) but at the same time worse agreement with the initial part of LexA curve. We fixed $p_2 = 90$ based on the visual inspection of simulated LexA curve so that the initial part of the curve agrees with the first few data points within the experimental error of 5 % [27], and SSD is tolerable. Fitting parameters p_5 and h_1 with p_2 fixed produced the following values along with standard deviations:

$$p_2 = 90.0, \quad p_5 = 21 \pm 1, \quad h_1 = 2.4 \pm 0.2. \qquad (19)$$

SSD equals 0.0129. Using Equations (10) and (19), we calculate values of parameters β_1 (dimensional equivalent to p_2) and v_0 (dimensional equivalent to p_5) (see Table 1).

Simulation of normalized LexA level (u_1) according to Equations (11) with initial conditions (12), parameters (18) and (19) along with experimental data [5] is shown in Figure 1. Also, we show simulation of u_1 done with our earlier model [9, 13] that is equivalent to the model used here, with $h_1 = h_2 = 1.0$. SSD for this simulation equals 0.0505 which is about four times greater. As judged both by visual inspection of the simulation and by the minimum SSD the modified model which accounts for coperativity of LexA binding to operator DNA, is better consistent with experimental data than the earlier model that does not include the cooperativity parameters [9, 13].

The Hill coefficient describing cooperativity of LexA binding to its

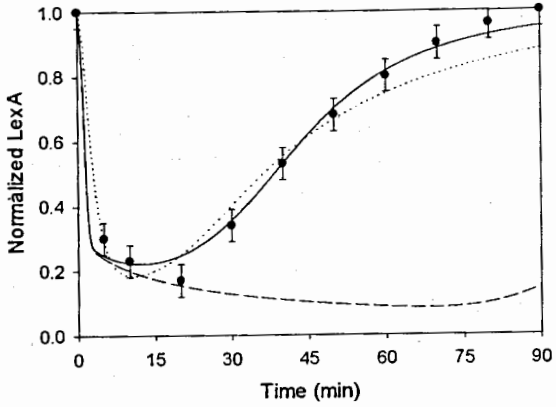

Figure 1: Simulation of LexA level in wild-type (solid and dotted line) and *uvr* mutant cells (dashed line) UV-irradiated with dose 5 J m^{-2} ($D = 5$) normalized to uninduced LexA level. Solid line and dashed line refer to simulations done with the modified model with LexA cooperativity. Dotted line refers simulation done with the earlier model without LexA cooperativity [9, 13]. Circles refer to experimental data on relative LexA content in wild-type cells [5]. Experimental errors are 5% [27]

own operator (h_1) was determined to have value 2.4. In view of maximal possible value for h_1 being 4, this indicates of a weak to moderate cooperativity which is consistent with the qualitative data [10].

Dynamics of LexA, RecA and SulA proteins. We used Equations (11) with initial conditions (12) and parameters (18) and (19), and Equations (13), (14), (15), (16) and (17) to simulate normalized levels of LexA (u_1), RecA ($u_2 + u_3$) and SulA (u_4) proteins in wild-type and mutant cells. In Figure 1, simulation of LexA level after UV irradiation with dose 5 J m^{-2} ($D = 5$), normalized to uninduced LexA level, is shown for both wild-type and *uvr* mutant cells. For about 10 min after UV irradiation LexA level in both wild-type and *uvr* mutant

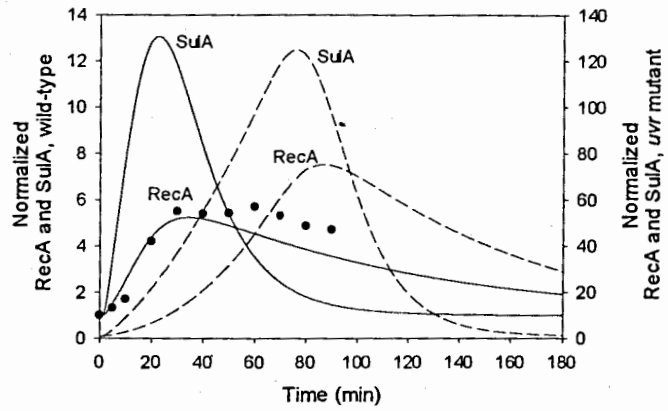

Figure 2: Simulation of RecA and SulA levels in wild-type (solid line) and *uvr* mutant cells (dashed line) UV-irradiated with dose 5 J m^{-2} ($D = 5$) normalized to the uninduced levels. Circles refer to experimental data on normalized specific activity of RecA-β-galactosidase hybrid protein [7]

cells decreases with the same rate. Then, in wild-type cells normal NER function slows down the signal generation rate and LexA level starts to restore. It restores to its uninduced level by 90 min. In contrast, in *uvr* mutant cells with a defect in NER LexA level continues to decrease slowly. This probably reflects the signal persistence. When the persistent signal generation ceases at about 70 min because of the completion of DNA replication round, LexA level starts to restore.

In Figure 2, simulation of RecA and SulA levels after UV irradiation with the dose 5 J m^{-2} ($D = 5$), normalized to uninduced RecA and SulA levels, is shown for both wild-type and *uvr* mutant cells. Also, experimental data on RecA dynamics in wild-type cells are shown [7]. This data set refers to normalized specific activity of RecA-β-galactosidase hybrid protein. Our simulation agrees well with the initial part of experimental curve, but goes systematically lower in the remaining part. This discrepancy might be due to differences in

production rate or turnover number of the hybrid protein in comparison with the RecA protein. The simulation of RecA level in wild-type cells is in general consistent with the data. In wild-type cells, RecA and SulA peak at 34 and 24 min after UV irradiation, respectively, and then decline to uninduced levels remarkably slower than LexA restores to its uninduced level. In *uvr* mutant cells, induction of RecA and SulA is much slower than in wild-type cells. RecA and SulA peak at 86 and 76 min after UV irradiation, respectively. Peak levels of RecA and SulA are about 14 and 10 times higher than in wild-type cells, respectively (note the differences in scales). Thus, UV induction of RecA and SulA in *uvr* mutant cells is continued for longer times and is more pronounced than in wild-type cells. For both RecA and SulA, the delay of rising to their peak levels in *uvr* mutant cells in comparison to wild-type cells is the same (about 52 min). This shows that defect in NER (*uvr* mutant cells) might delay the maximal induction of SOS proteins coordinately. Our simulation of RecA and SulA levels provides the correct dynamical information about RecA and SulA specific activity. Conventional methods based on usage of *lacZ* operon or protein fusions give only the specific activity of β-galactosidase that mimics RecA [7] or SulA [8] specific activity with more or less adequacy. This inadequacy is due to a systematic error because of, for example, variable position of the operon fusion within *recA* or *sulA* gene which affects the level of β-galactosidase, or different turnover number of the hybrid β-galactosidase protein. All this may influence the dynamics of the specific activity of β-galactosidase and make it irrelevant for investigating the true levels of proteins (note differences between simulation and experimental data for RecA level in Figure 2).

Dynamics of activated RecA. RecA responds to inducing signal by converting to the activated form (RecA*) which directly stimulates LexA cleavage reaction. Thus, in addition to RecA dynamics, to investigate SOS induction it is important to calculate the dynamics of RecA distribution between activated and normal forms. It is convenient to calculate the fraction of RecA* which is defined as $u_3/(u_2+u_3)$. This value shows how much of the total RecA is activated. Simulated fraction of RecA* after UV irradiation with the dose 5 J m^{-2} ($D=5$) is

shown for both wild-type and *uvr* mutant cells in Figure 3. The initial rate of increase in RecA* fraction is identical in both wild-type and *uvr* mutant cells. In wild-type cells, fraction of RecA* rises to 85 % within 5 min and then decreases to zero by 50 min. In *uvr* mutant cells, fraction of RecA* rises to 95% within 5 min and remains this high until 60 min and then decreases to zero by 110 min. The nearly

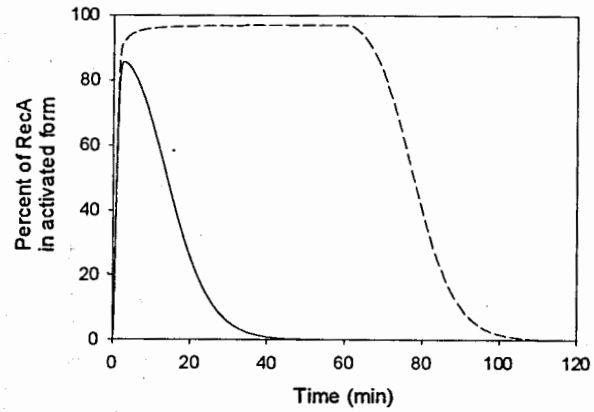

Figure 3: Simulation of fraction of RecA* (expressed as percent of RecA that is in activated form relative to all RecA) in wild-type (solid line) and *uvr* mutant cells (dashed line) UV-irradiated with dose 5 J m^{-2} ($D=5$)

constant fraction of RecA* in *uvr* mutant cells indicates that the rate of RecA activation during progressive generation of inducing signal is equal to the rate of RecA accumulation in a cell. RecA accumulation occurs because of protein synthesis and RecA* reversal back to normal RecA. This shows that very large fraction of RecA is activated after UV irradiation. The fraction of activated RecA is by 10 % more in *uvr* mutant cells than in wild-type cells. Our simulation shows that the distribution of RecA between normal and activated forms is highly skewed towards activated form in *uvr* mutant cells. The effect of NER is that its normal function in wild-type cells gradually reverses RecA*

to normal RecA during the SOS response. In contrast, a defect in NER in *uvr* mutant cells lets almost all RecA to remain in activated form for a considerable time. This would allow more LexA cleavage and prolonged expression of the SOS response (see Figure 2).

Dose-response for RecA and SulA induction. To investigate the induction of the two SOS proteins, RecA and SulA, over the range of UV doses, we calculated the peak levels for RecA and SulA induction after UV irradiation with doses from 1 to 30 J m^{-2}. Peak levels of normalized RecA and SulA versus UV dose for wild-type and *uvr* mutant cells are shown in Figure 4. Usually, the response function is

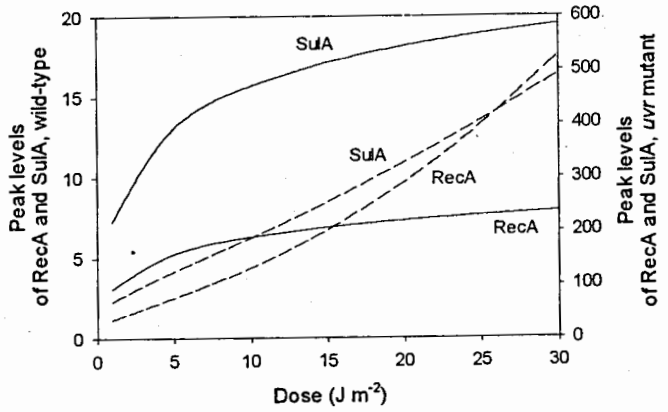

Figure 4: Dose-response curves for normalized peak levels for RecA and SulA in UV-irradiated wild-type (solid line) and *uvr* mutant cells (dashed line)

the specific β-galactosidase activity measured after incubation of UV-irradiated cells for a given period of time (often 2 or 3 hours) [8, 28]. After 2 or 3 hours of incubation, some turn-off of RecA or SulA induction might have occured. Our definition of a response function as the peak level of specific RecA and SulA activity during the induction provides with adequate description of some of the SOS responses

(RecA and SulA induction) as a function of UV dose.

In wild-type cells, peak levels of both RecA and SulA increased rapidly until about 5 J m^{-2} and then continued to increase with a slower rate. This threshold-like behaviour might reflect some kind of saturation of the SOS response in along with increase in the UV dose. Normal NER function might have reached the maximal efficiency of its operation and is able to counteract the increasing number of UV-produced lesions. Over the 1 to 30 J m^{-2} range of UV doses peak levels of RecA were about 2.5 times lower than the peak levels of SulA. Peak levels of SulA increased as about much as the peak levels of RecA along with increase in the UV dose.

In *uvr* mutant cells, peak levels of RecA and SulA were more than 10-fold higher and increased almost linearly with the dose showing that defect in NER makes cells respond to the increasing inducing signal by increasing maximal operation of the SOS responses. Interestingly, for doses higher than about 25 J m^{-2} peak levels of RecA became higher than peak levels of SulA. In general, more than 10-fold higher doses were required in wild-type cells for RecA or SulA to reach the same peak levels as in *uvr* mutant cells.

Thus, from the results shown above we may conclude that the SOS response induction is profoundly affected by NER. The key regulator of the SOS response appears to be the RecA protein in activated form. The rate of LexA cleavage which is mediated by activated RecA is so fast that very small amounts of inducing signal (equivalent to even a single UV-induced lesion) are sufficient to lower its level about 10-fold (data not shown). In wild-type cells with normal NER the proportion of RecA in activated form is progressively lowered with time by NER slowing down the rate of inducing signal generaton. In *uvr* mutant cells with impared NER the proportion of activated RecA is kept at about 95 % for a long time which results in higher levels of induction of RecA, SulA and other SOS proteins and, in general, longer induction.

The author would like to thank Prof. E.A. Krasavin for discussions and anonymous referee for carefully reading the manuscript and providing helpful suggestions that improved the quality of the manuscript.

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Received by Publishing Department
on March 24, 1999.