Published online 30 March 2015 *Nucleic Acids Research, 2015, Vol. 43, No. 8* ***4109–4120***
doi: 10.1093/nar/gkv217

Suppression of the *E. coli* SOS response by dNTP pool changes

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Received November 26, 2014; Revised February 13, 2015; Accepted March 02, 2015

ABSTRACT

The *Escherichia coli* SOS system is a well-established model for the cellular response to DNA damage. Control of SOS depends largely on the RecA protein. When RecA is activated by single-stranded DNA in the presence of a nucleotide triphosphate co-factor, it mediates cleavage of the LexA repressor, leading to expression of the 30^{+}-member SOS regulon. RecA activation generally requires the introduction of DNA damage. However, certain *recA* mutants, like *recA730*, bypass this requirement and display constitutive SOS expression as well as a spontaneous (SOS) mutator effect. Presently, we investigated the possible interaction between SOS and the cellular deoxynucleoside triphosphate (dNTP) pools. We found that dNTP pool changes caused by deficiencies in the *ndk* or *dcd* genes, encoding nucleoside diphosphate kinase and dCTP deaminase, respectively, had a strongly suppressive effect on constitutive SOS expression in *recA730* strains. The suppression of the *recA730* mutator effect was alleviated in a *lexA*-deficient background. Overall, the findings suggest a model in which the dNTP alterations in the *ndk* and *dcd* strains interfere with the activation of RecA, thereby preventing LexA cleavage and SOS induction.

INTRODUCTION

Exposure of *Escherichia coli* to agents or conditions that damage DNA or impair DNA replication results in the induction of the SOS response. The expression of the SOS regulon genes is controlled by LexA and RecA proteins (1–3). Binding of the RecA protein to single-stranded DNA (ssDNA) at or near replication blockage sites in the presence of a nucleoside triphosphate causes a conformational change in RecA (active RecA). RecA then promotes cleavage of LexA protein, the repressor of the SOS regulon (4,5). Inactivation of the repressor enables the expression of more than 30 SOS genes (6–8). The early phase of SOS is characterized by generally error-free repair and maintenance processes. However, if the DNA damage level remains too high to be processed by these pathways, error-prone pathways are activated, mediated by error-prone DNA polymerases, causing elevated mutation levels (6).

In *E. coli* three DNA polymerases are expressed as part of the inducible SOS response: DNA polymerase II, DNA polymerase IV and DNA polymerase V (9). DNA polymerases IV and V are members of the Y family of polymerases. Both lack intrinsic proofreading activity and are considered low-fidelity DNA polymerases. Pol IV is encoded by the *dinB* gene, and polymerase V is encoded by the *umuDC* operon. Previous studies (10,11) have indicated that both Pol IV and Pol V have significant access to the replication fork under SOS-induced conditions, although most mutagenesis results from the action of Pol V. Active RecA also promotes autocatalytic cleavage of UmuD protein to UmuD′ and then participates in forming the active form of DNA polymerase V, UmuD′$_2$C-RecA-ATP, also called the mutasome (12–14).

In addition to ssDNA binding, RecA co-protease function also requires binding of a nucleoside triphosphate co-factor (4,15). Various (d)NTP species have been shown to have different efficiencies in promoting RecA activity *in vitro*, dATP being the most effective, while other nucleotides can inhibit RecA activity (15–18). Interestingly, and possibly related to this, it has also been observed that addition of nucleosides or free bases to the growth medium can affect the level of SOS induction: addition of adenine promotes SOS activity *in vivo*, while addition of cytidine and guanine is inhibitory (19,20). These data have suggested the possibility that changes in the nucleotide pools may affect the activity of RecA protein (20,21).

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In addition, the cellular dNTPs are important both as they serve as DNA precursors and determinants of DNA replication fidelity. Specifically, the relative ratios among the individual dNTPs affect the mispairing rate of DNA polymerases, while the absolute dNTP levels determine the correction of polymerase errors by exonucleolytic proofreading (next-nucleotide effect) (22,23). In this respect, SOS mutagenesis, like normal DNA replication, would be expected to be sensitive to changes in the dNTP levels, as was demonstrated in the case of mutagenesis by ultraviolet (UV) light (24).

In this study, we have investigated how distinct nucleotide pool changes may affect SOS induction and mutagenesis in *recA730* strains. In *recA730* strains, carrying the RecA E38K mutation (25), RecA protein is constitutively active without the need for the introduction of DNA damage (26,27). As a result, the SOS system is expressed constitutively, resulting in—among others—a spontaneous mutator effect (26,27) due to persistent presence of the PolV mutasome (12–14). In the work described here, dNTP pool alterations were achieved by employing *ndk* and *dcd* mutants of *E. coli,* defective in nucleoside diphosphate kinase and dCTP deaminase, respectively. These mutants have been shown to display altered cellular dNTP pools combined with distinct mutator phenotypes (23,28–31). Our results show, unexpectedly, that the dNTP alterations in these mutants strongly suppress SOS expression and associated mutagenesis.

MATERIALS AND METHODS

Bacterial strains and media

The *E. coli* strains used in this study are listed in Table 1. Strain MC4118 was described in Maliszewska-Tkaczyk *et al*. (32) and strain JW2502 in Baba *et al*. (33). Strain constructions by P1 transduction were done using P1*virA*. The F′ *prolac* episomes used in the mutagenesis experiments of Tables 2 and 3 were introduced into the strains of the NR9338 series by conjugation. Strains used in the β-galactosidase assay were derivatives of NR9338 carrying plasmid pSK1002 (34). MC4118 is a *lac*$^+$ derivative of strain MC4100 created by the method of Diederich *et al.* (35) as described in Fijalkowska *et al.* (36). Solid and liquid media were prepared using standard recipes (37). Solid minimal medium (MM) contained 0.5% glucose or 0.4% lactose as a carbon source and 5 μg/ml of thiamine. For experiments with *dcd* strains the solid media contained additionally 50 μg/ml of thymidine to improve colony growth on the plates (larger colony sizes). Liquid media, used for generation of mutant frequencies and extraction of cellular dNTP pools (see below), did not contain any added thymidine. Antibiotics, when required during strain constructions, were added at 30 μg/ml (kanamycin), 12.5 μg/ml (tetracycline), 50 μg/ml (ampicillin) or 10 μg/ml (chloramphenicol). LB-Rif plates used for the scoring of rifampicin-resistant mutants contained 100-μg/ml rifampicin.

Mutant frequency measurements

Mutant frequencies for each strain were determined using a total of 12–20 cultures (1 ml of Luria-Bertani Broth (LB)), started from a single colony (one colony per tube), and growing them to saturation at 37°C. The number of *lac* revertants was determined by plating 100 μl of undiluted cultures on MM plates containing lactose as carbon source. The number of Rif^R mutants in each culture was determined by plating 100 μl of undiluted cultures on LB-Rif plates. The viable cell count in the cultures was determined by plating 100 μl of a 10^{-6} dilution on LB or MM plates containing glucose as carbon source. The plates were incubated for 24–48 h at 37°C. Mutant frequencies were calculated by dividing the number of mutants per plate by the average number of total cells. Sporadic jackpot cultures were removed from the analysis. Statistical analysis was performed using the software program Statistica.

Beta-galactosidase assay

Bacterial cultures containing plasmid pSK1002 (34) were grown at 37°C in LB medium. Overnight cultures were diluted 1:2000 in fresh medium and grown at 37°C with shaking to an $OD_{600} = 0.4$. At this point, cells were treated with a DNA-damaging agent (20 Jm^{-2} of UV, 0.02% of methylmethane sulfonate (MMS) or 0.5 μg/ml of mitomycin C) and grown for additional 2 h. β-galactosidase activity was determined as described (37). The values of β-galactosidase specific activity were calculated as nmoles of o-nitrophenyl-β-D-galactoside hydrolyzed per minute per mg of total protein.

Microarray studies

Bacterial strains were grown in LB. The strains used were of the MC4118 series (see Table 1). Overnight cultures were diluted 1:100 in fresh LB and grown at 37°C with shaking to an $OD_{600} = 0.5$. RNA was isolated using the RNeasy-Bacteria Protect Mini kit (Qiagen). cDNA labeling was performed with the use of FairPlay III Microarray Labeling Kit (Agilent Technologies) and CyDye Cy5 and Cy3 mono-reactive dyes (GE Healthcare). The gene expression levels were determined using the *E. coli* Gene Expression Microarray system (Agilent Technologies). Two-color hybridizations with dye swap between duplicates were performed according to the Agilent Two-Color Microarray-Based Analysis protocol. Each dye swap combination was repeated three times. For feature extraction, Axon GenePix 4000B scanner and GenePix Pro 6.1 software (Molecular Devices) were used. Statistical analysis using Student's *t*-test was performed using Acuity 4.0 software (Molecular Devices). Differences in gene expression level were expressed as $\log_2$ ratio value in Figure 1. The complete data set was submitted to the GEO repository (series record GSE62898) (http://www.ncbi.nlm.nih.gov/gds).

dNTP pool measurements

dNTPs were extracted using the procedure described by Diaz *et al.* (38) with some modifications. Bacterial cultures were grown at 37°C in LB medium. Strains used were of the MC4118 series (see Table 1). Each overnight culture was diluted 1:2000 in fresh medium and grown at 37°C with shaking to an $OD_{600} = 0.3$. Cells from 100 ml of culture were

Table 1. *Escherichia coli* strains used in this work

Strain	Relevant genotype and/or construction	Source or reference
A. Used for construction		
NR9338	*ara thi* Δ(*pro-lac*) *sulA366*	(27)
MC4118	Δ(*argF-lac*)*169 sulA366*	(32)
NR11531	NR9338, but *recA730 srl*::Tn*10*	(27)
JW2502	Δ*ndk*::*kan*	(33)
NR11814	Δ*ndk*::*cam*	(23)
BW1040	*dcd-12*::Tn*10dkan*	(40)
CC101	F'CC101 (A·T→C·G)	(39)
CC103	F'CC103 (G·C→C·G)	(39)
CC105	F'CC105 (A·T→T·A)	(39)
NR9405	NR9338, but *lexA51 malB*::Tn*9*	this work
B. Used for measurements		
EC9524	NR9338 [pSK1002]	this work
EC9525	NR11531 (*recA730*) [pSK1002]	this work
EC9529	NR9338 *dcd-12*::Tn*10dkan* [pSK1002]	this work
EC9526	NR9338 Δ*ndk*::*cam* [pSK1002]	this work
EC9527	NR11531 (*recA730*) *dcd-12*::Tn*10dkan* [pSK1002]	this work
EC9528	NR11531 (*recA730*) Δ*ndk*::*cam* [pSK1002]	this work
EC9642	NR9338, but F'CC101	this work
EC9656	EC9642 *dcd-12*::Tn*10dkan*	this work
EC9668	EC9642 Δ*ndk*::*kan*	this work
EC9783	EC9642 *recA730 srl*::Tn*10*	this work
EC9786	EC9642 *recA730 srl*::Tn*10 dcd-12*::Tn*10dkan*	this work
EC9789	EC9642 *recA730 srl*::Tn*10* Δ*ndk*::*kan*	this work
EC9792	NR9338 *lexA51 malB*::Tn*9* F'CC101	this work
EC9795	EC9792 *dcd-12*::Tn*10dkan*	this work
EC9798	EC9792 Δ*ndk*::*kan*	this work
EC9801	EC9792 *recA730* srl::Tn10	this work
EC9804	EC9792 *recA730 srl*::Tn*10 dcd-12*::Tn*10dkan*	this work
EC9807	EC9792 *recA730 srl*::Tn*10* Δ*ndk*::*kan*	this work
EC9644	NR9338, but F'CC103	this work
EC9658	EC9644 *dcd-12*::Tn*10dkan*	this work
EC9670	EC9644 Δ*ndk*::*kan*	this work
EC9784	EC9644 *recA730 srl*::Tn*10*	this work
EC9787	EC9644 *recA730 srl*::Tn*10 dcd-12*::Tn*10dkan*	this work
EC9790	EC9644 *recA730 srl*::Tn*10* Δ*ndk*::*kan*	this work
EC9793	NR9338 *lexA51 malB*::Tn*9* F'CC103	this work
EC9796	EC9793 *dcd-12*::Tn*10dkan*	this work
EC9799	EC9793 Δ*ndk*::*kan*	this work
EC9802	EC9793 *recA730 srl*::Tn*10*	this work
EC9805	EC9793 *recA730 srl*::Tn*10 dcd-12*::Tn*10dkan*	this work
EC9808	EC9793 *recA730 srl*::Tn*10* Δ*ndk*::*kan*	this work
EC9646	NR9338 F'CC105	this work
EC9660	EC9646 *dcd-12*::Tn*10dkan*	this work
EC9672	EC9646 Δ*ndk*::*kan*	this work
EC9785	EC9646 *recA730 srl*::Tn*10*	this work
EC9788	EC9646 *recA730 srl*::Tn*10 dcd-12*::Tn*10dkan*	this work
EC9791	EC9646 *recA730 srl*::Tn*10* Δ*ndk*::*kan*	this work
EC9794	NR9338 *lexA51 malB*::Tn*9* F'CC105	this work
EC9797	EC9794 *dcd-12*::Tn*10dkan*	this work
EC9800	EC9794 Δ*ndk*::*kan*	this work
EC9803	EC9794 *recA730 srl*::Tn*10*	this work
EC9806	EC9794 *recA730 srl*::Tn*10 dcd-12*::Tn*10dkan*	this work
EC9809	EC9794 *recA730 srl*::Tn*10* Δ*ndk*::*kan*	this work
EC9428	MC4118, but *lac*$^+$ (Amp^R)	this work
EC9461	EC9428 *dcd-12*::Tn*10dkan*	this work
EC9487	EC9428 Δ*ndk*::*cam*	this work
EC9477	EC9428 *recA730 srl*::Tn*10*	this work
EC9471	EC9428 *recA730 srl*::Tn*10 dcd-12*::Tn*10dkan*	this work
EC9503	EC9428 *recA730 srl*::Tn*10* Δ*ndk*::*cam*	this work
EC9681	MC4118 but *lac*$^+$ (Amp^R) *lexA51 malB*::Tn*9*	this work
EC9684	EC9681 *dcd-12*::Tn*10dkan*	this work
EC9682	EC9681 Δ*ndk*::*kan*	this work
EC9685	EC9681 *recA730 srl*::Tn*10*	this work
EC9686	EC9681 *recA730 srl*::Tn*10 dcd-12*::Tn*10dkan*	this work
EC9687	EC9681 *recA730 srl*::Tn*10* Δ*ndk*::*kan*	this work

collected by filtration on 0.2-μm polycarbonate filters (Sterlitech) and washed with 10 ml of cold saline. Nucleotides were extracted from the filters by incubation in 7 ml of cold 60% methanol for 2 h at −20°C. The lysate was lyophilized and the obtained residue resuspended in 1 ml of water, followed by extraction using 0.5 ml of chloroform. The aqueous phase was collected and re-lyophilized, and the residue resuspended in 0.1-ml water. Determination of dNTPs was done by ion-pairing reverse-phase chromatography, as described in Ahluwalia *et al.* (22), with minor modifications. Peaks for the individual dNTPs and ATP (ADP) were identified based on retention times of nucleotide standards and confirmed by the UV spectra of the peak. Quantitation of each dNTP was by peak area at 260 nm, corrected for the differential extinction coefficients of the various nucleotides at this wavelength and expressed per OD_{600} of the corresponding culture at the time of harvest.

RESULTS

The SOS mutator effect of *recA730* strains is abrogated by a *ndk* nucleoside diphosphate kinase deficiency

The studies described in this paper flow from our previous investigations of the mutator effect associated with a defect in nucleoside diphosphate kinase (*ndk* gene product). Defective *ndk* strains display distinct changes in their dNTP pool levels (elevation of dCTP and reduction in dATP) and are also mutators (23,28–29). The mutator effect has a defined specificity in terms of the specific base-substitution mutations it promotes, and positive correlations between the dNTP pool changes and the elevated base substitutions have been drawn (23).

As part of the above studies, we were interested in the possible interaction of *ndk* with the *E. coli* SOS system. In particular, we focused on the spontaneous SOS mutator effect resulting from constitutive SOS induction in the *recA730* mutant (26,27). This *recA730* mutator effect, resulting from the error-prone action of the Pol V mutasome on undamaged DNA (12–14), is characterized by its own specificity of mutation, promoting preferentially transversion mutations (27). However, whether any dNTP pool changes may contribute to the spontaneous SOS mutator effect has not been established, and this is an important open question. One recent study on mutagenesis induced by UV light, which is also mediated by SOS induction, revealed dNTP pool elevations in *E. coli* upon UV irradiation and, importantly, these dNTP changes were shown to contribute to the mutagenic effects of the UV irradiation (24).

Based on these considerations, we undertook a study of a *recA730 ndk* double mutant strain, focusing on its mutator effects. It was thought that any observed changes in the *recA730* mutator effect upon addition of the *ndk* defect might provide additional insights into the underlying causes of the mutator effect. Unexpectedly, as shown in the data of Table 2A, a negative interaction between the two individual mutators was observed. For these measurements, we used the *lacZ* reversion system developed by Cupples and Miller (39) which permits scoring of specific base-pair substitution mutations in the *lacZ* gene. Data for three different *lac* alleles are shown, reverting by A·T→C·G, G·C→C·G or A·T→T·A transversion mutation, specifically. These alleles were chosen because they have been used productively to demonstrate the magnitude of the *recA730* mutator effect (27). In addition, the A·T→T·A transversion is highly diagnostic for the *ndk* mutator effect (23).

The Table 2A shows that *recA730* is a mutator for each of the three *lac* alleles (17-, 12- and 16-fold enhancement, respectively). The mutator effect of the single *ndk* strain for the A·T→T·A transversion is also clearly seen (9-fold). Interestingly, in the *recA730 ndk* double mutant all frequencies are reduced compared to the level of the single *recA730* strain: 3-fold for the A·T→C·G, 8-fold for the G·C→C·G and nearly 2-fold for the A·T→T·A transversion. The case of the A·T→T·A transversion is particularly illuminating, as both the *recA730* and *ndk* are strong mutators (16- and 9-fold, respectively) for these events. Nevertheless, when the two are combined, no amplification of the mutant frequency is observed and, in fact, the resulting frequency is even less than a simple additive effect. It appears that the frequency simply returns to that of the single *ndk*, further implying a loss of the SOS mutator effect.

Effect of the *dcd* mutator on the SOS mutator effect

E. coli dCTP deaminase, encoded by the *dcd* gene, catalyzes the formation of dUTP from dCTP, an important step in the ultimate production of dTTP (40). In a dCTP deaminase-deficient strain, dCTP accumulates and the dTTP pool may be reduced (23,30). Like *ndk* mutants, *dcd*-defective strains

Table 2. Mutant frequencies (*lac*$^+$ revertants per 10^8 cells) mediated by indicated base-pair substitutions in *recA730*- and *ndk*-related strains

	Strain	A·T→C·G	G·C→C·G	A·T→T·A
A.	wild-type	0.31 (0.18–0.82)	0.16 (0.02–0.34)	2.4 (1.5–6.9)
	recA730	5.2 (3.4–12.7)	2.0 (1.1–4.1)	38 (34–46)
	ndk	0.37 (0.23–0.81)	0.05 (0.04–0.54)	22 (15–32)
	recA730 ndk	1.6 (1.0–4.2)	0.26 (0.19–2.1)	21 (16–28)
B.	*lexA51*	0.31 (0.07–0.37)	0.25 (0.11–0.51)	1.2 (0.53–1.9)
	lexA51 recA730	11 (6.7–15.8)	2.7 (1.2–4.9)	36 (31–46)
	lexA51 ndk	0.38 (0.03–0.73)	0.18 (0.04–0.40)	17 (11–23)
	lexA51 recA730 ndk	11 (9.2–15)	2.9 (1.9–4.7)	69 (56–90)

Shown are average values and confidence intervals for 12 independent cultures for each strain. (A) The strains used were the *recA730*, *ndk* and *recA730 ndk* derivatives of EC9642 and (F′CC101), EC9644 (F′CC103) and EC9646 (F′CC105), which revert to *lac*$^+$ by A·T→C·G, G·C→C·G and A·T→T·A, respectively. (B) The corresponding *lexA51* (*lexAdef*) strains were derivatives of EC9792 (F′CC101), EC9793 (F′CC103) and EC9794 (F′CC105) (see Table 1 and the Materials and Methods section).

Table 3. Mutant frequencies (*lac*$^+$ revertants per 10^8 cells) mediated by indicated base-pair substitutions in *recA730*- and *dcd*-related strains

	Strain	A·T→C·G	G·C→C·G	A·T→T·A
A.	wild-type	0.75 (0.26–1.4)	0.32 (0.02–0.34)	2.7 (1.5–6.3)
	recA730	6.9 (6.3–8.7)	2.5 (0.76–5.6)	36 (33–41)
	dcd	1.1 (0.47–2.8)	0.47 (0.16–0.59)	10 (7.3–13)
	recA730 dcd	4.2 (2.1–10)	1.3 (0.85–1.9)	28 (25–34)
B.	*lexA51*	0.36 (0.08–0.49)	0.16 (0.03–0.35)	4.8 (3.6–5.8)
	lexA51 recA730	8.4 (4.9–20)	3.9 (2.0–7.5)	47 (38–51)
	lexA51 dcd	1.4 (0.85–2.1)	0.50 (0.12–0.48)	11 (7.5–16)
	lexA51 recA730 dcd	19 (13–27)	7.5 (4.7–15)	48 (37–55)

Shown are average values and confidence intervals for 12 independent cultures for each strain. (A) The strains used were the *recA730*, *dcd* and *recA730 dcd* derivatives of EC9642 and (F′CC101), EC9644 (F′CC103) and EC9646 (F′CC105), which revert to *lac*$^+$ by A·T→C·G, G·C→C·G and A·T→T·A, respectively. (B) The corresponding *lexA51* (*lexAdef*) strains were derivatives of EC9792 (F′CC101), EC9793 (F′CC103) and EC9794 (F′CC105) (see Table 1 and the Materials and Methods section).

are characterized by a mutator phenotype, which has been correlated with the specific dNTP pool changes occurring in this strain (23,31). When we combined *recA730* with the *dcd* null allele, we likewise observed a negative interaction between the two individual alleles (see Table 3A), although the antimutagenic effect is slightly less than observed for the combination with *ndk*. Nevertheless, in all cases the mutant frequency for the *recA730 dcd* double is less than the sum of the two individual mutant frequencies and, in fact, is reduced from the level of the single *recA730* strain (1.6-, 1.9- and 1.3-fold, for the three reversions, respectively). For the A·T→T·A transversion, the 13-fold mutator effect for *recA730* combined with the 3.7-fold mutator effect for *dcd* does not lead to any further increase in frequency, but rather a reduction compared to the single *recA730* strain. Thus, the *dcd* defect clearly exerts an antimutagenic effect on the *recA730* mutator.

Effect of *ndk* and *dcd* on rifampicin-resistant mutations

In addition to the *lac* reversion system described above, we also investigated combined mutational effects using the *rpoB* forward assay. In this assay, rifampicin-resistant mutants are scored at a large number of sites in the *rpoB* gene (41). The data in Table 4A show that, for rifampicin-resistant mutants, *recA730* is a strong mutator (18-fold). The *ndk* and *dcd* single-mutant strains also display a mutator phenotype for this target, 6.5-fold for *ndk* and 2.5-fold for *dcd*. Importantly, when combining *recA730* with *ndk* a 2-fold reduction was observed compared to the single *recA730* mutant, and a 1.5-fold reduction was seen for the *recA730 dcd* combination. Thus, the data of Tables 2–4 clearly indicate a negative effect of the *ndk* and *dcd* defects on the SOS mutator effect.

Effect of *ndk* and *dcd* on *umuDC* expression

As the *recA730* SOS mutator effect is strictly dependent on the mutagenic action of Pol V, the product of the *umuDC* operon, one simple possibility for the observed antimutator effect would be reduced activity or expression of Pol V in the double mutant strains. One way to test this is by using the *umuC*::*lacZ* gene fusion, which has been used previously as a direct indicator for Pol V expression and, indirectly, as an indicator for overall SOS expression (34). The data

Table 4. Mutability of *recA730*- and *ndk*- or *dcd*-related strains as measured in the *rpoB* forward target (frequency of rifampicin-resistant mutants)

	Strain	Rif^R per 10^8 cells
A.	wild-type	3.5 (2.4–4.6)
	recA730	63 (51–73)
	ndk	23 (19–33)
	recA730 ndk	30 (24–37)
	dcd	8.7 (5.0–11.5)
	recA730 dcd	41 (33–51)
B.	*lexA51*	5.7 (4.4–7.2)
	lexA51 recA730	79 (65–95)
	lexA51 ndk	42 (32–52)
	lexA51 recA730 ndk	115 (103–163)
	lexA51 dcd	9.3 (6.4–12.5)
	lexA51 recA730 dcd	88 (58–100)

Shown are average values and confidence intervals for 12 independent cultures for each strain. (A) Strains used were EC9428 (wt), EC9477 (*recA730*), EC9487 (*ndk*), EC9503 (*recA730 ndk*), EC9461 (*dcd*), EC9471 (*recA730 dcd*) and (B) the *lexAdef* derivatives EC9681 (*lexA51*), EC9685 (*lexA51 recA730*), EC9682 (l*exA51 ndk*), EC9687 (*lexA51 recA730 ndk*), EC9684 (*lexA51 dcd*) and EC9686 (*lexA51 recA730 dcd*) (see Table 1 and the Materials and Methods section).

in Table 5 clearly show that *umuC* expression, as measured by units of β-galactosidase, while significantly elevated in the *recA730* strain, is strongly reduced from that level in the double mutants. This reduction provides a ready explanation for the observed reduction in mutator activity. Importantly, the data also show that the *ndk* and *dcd* deficiencies interfere with *umuC* expression in a wild-type (*rec*$^+$) background when the cells are treated by the DNA damaging agents mitomycin C, MMS or UV light, which otherwise effectively induce the SOS response.

Expression of the entire SOS regulon is diminished in *ndk* and *dcd* backgrounds

To investigate whether the observed reduction in Pol V expression is unique to the *umuDC* operon or may reflect an general shutdown of the SOS system (*lexA* regulon), we performed microarray analysis of gene expression profiles in each of the single *ndk, dcd* and *recA730* strains, as well as the *recA730 ndk* and *recA730 dcd* doubles. The complete results are available at GEO repository (series record GSE62898).

Table 5. *UmuC* expression levels in *recA730* strains or after treatment with DNA-damaging agents

Treatment	Strain	β-galactosidase units
None	wild-type	16
	recA730	206
	recA730 ndk	34
	recA730 dcd	90
Mitomycin C	wild-type	3629
	ndk	1556
	dcd	1659
MMS	wild-type	999
	ndk	297
	dcd	754
UV	wild-type	1322
	ndk	969
	dcd	483

β-galactosidase level as determined using the *umuC*::*lacZ* gene fusion of plasmid pSK1002 as described in the Materials and Methods section. The strains used were EC9524 (wt), EC9525 (*recA730*), EC9528 (*recA730 ndk*), EC9527 (*recA730 dcd*), EC9526 (*ndk*) and EC9529 (*dcd*) (see Table 1).

In Figure 1 we display a subset of the data, focusing on a set of established members of the SOS regulon (7).

The data for *recA730* in comparison to the wild-type clearly show the induction of the SOS regulon. For example, the *recN*, *yebG* and *recA* expression levels are induced by around 14-fold, *dinI* by 12-fold and *umuD* and *umuC* by 6.5- and 4-fold, respectively. In contrast, the *dcd* and *ndk* defects do not cause any SOS induction. The *ndk* and *dcd* defects, however, do cause a significant reduction of SOS gene expression in the *recA730* background. The *ndk* deficiency has the strongest effect, reducing *recA* expression by as much as 6.5-fold, *dinI* by 5.2-fold and *umuD* and *umuC* by 3.8- and 2.5-fold, respectively, in comparison to the single *recA730*. The *dcd* deficiency reduces *recA* expression by 2-fold, *dinI* by 2.75 fold and *umuD* and *umuC* expression by 2.3- and 2.1-fold, respectively. Based on these data, we conclude that the *ndk* or *dcd*-mediated impairment of the SOS mutator effect is due to a general inhibition of the SOS regulon. Therefore, the source of the inhibitory effect likely resides at the level of the RecA–LexA interaction, which controls the expression of the SOS system through the RecA-facilitated cleavage of LexA.

Effect of *ndk* and *dcd* on the SOS mutator in a *lexA*(Def) background

We also investigated the ability of the *ndk* and *dcd* deficiencies to inhibit the *recA730* mutator effect in a *lexA*-deficient background (*lexA51*). The results are shown in Tables 2B, 3B and 4B. They indicate that when the LexA repressor is absent, the inhibitory effect of the *ndk* and *dcd* deficiencies is diminished or no longer observed. It appears that in several cases the mutator effects in the double mutants can be described as a simple additive effect of the individual mutator effects. These results are consistent with the RecA-mediated cleavage of LexA as the critical step affected by the *ndk* and *dcd* deficiencies. We have also monitored this issue by quantitative polymerase chain reaction, analyzing the expression of three individual SOS-inducible genes (*yebG*, *recN* and *recA*) (see Supplementary Figure S1). The results confirm the inhibition of each of these genes in the corresponding *ndk* and *dcd* backgrounds, as well as their recovery in their *lexA51* derivatives.

The SOS mutator effect depends on cleavage of both LexA, determining the expression level of the *umuDC* operon, and UmuD. The latter cleavage yields UmuD′, which is an essential component of the Pol V mutasome (14,42–43). Thus, in principle, the inhibitory effect exerted by the *ndk* and *dcd* deficiencies in the *lex*$^+$ background could be due to an inhibition of both cleavages. Nevertheless, the observed recovery of the mutator effect in the *lexA*(Def) background indicates that the cleavage of UmuD must not be a rate-limiting factor under these conditions and that sufficient UmuD′ is being produced. Differential cleavage of LexA and UmuD under conditions of RecA protein alterations has been reported (44,45), and this may be part of the explanation. Also, production of Pol V (UmuCD'$_2$) is subject to several regulatory steps (42), and the rate-limiting step may not simply reside at the RecA level. Note that, as expected, mutagenesis in the *recA730 ndk lexA51* mutants is still fully *umuDC* dependent (results not shown).

Measurement of dNTP pool changes

The active form of RecA responsible for the autocleavage of LexA has been determined to be a RecA homopolymer bound to ssDNA as an extended and dynamic nucleofilament (46,47). The active state of this nucleofilament also requires the presence of ATP (or dATP) nucleotide cofactor bound at the RecA monomer–monomer interfaces (47). As the *ndk* and *dcd* deficiencies are characterized by substantial changes in the levels of individual nucleotides, notably the dNTPs (23,28), it is reasonable to propose that such changes in the nucleotide levels may affect the activation state of RecA (and RecA730).

In Figure 2 we show the results of our dNTP pools measurements. The first important finding is that the constitutive induction of the SOS response in the *recA730* strain is not associated with any significant pool alteration compared to the wild-type strain. Second, distinct dNTP pool changes are seen in the *ndk* and *dcd* strains, consistent with previous reports (23). For both *ndk* and *dcd*, there was a large increase in the level of dCTP (12- and 10-fold enhancements, respectively). In the *ndk* strain, there was also a small increase in the dTTP pool (1.5-fold) and a decrease in dATP pool (2-fold). In the *dcd* strain, there also was a 3-fold reduction in the dTTP pool. Finally, and importantly, the *ndk*- and *dcd*-mediated dNTP pool alterations remain essentially unaffected by the *recA730* allele.

As ATP is generally considered the main *in vivo* activator for RecA protein (46), we also monitored the ATP content of the cells. Both our HPLC analysis used for the dNTP analysis described above and a series of separate ATP assays using the BacTiter-Glo^{TM} (Promega) reagent (see Supplementary Figure S2) revealed no significant changes in ATP level or the ATP/ADP ratios for any of the strains.

Name	*recA730* vs *wt*	*ndk* vs *wt*	*dcd* vs *wt*	*recA730 ndk* vs *recA730*	*recA730 dcd* vs *recA730*	Function/Description
recN	3,832	0,175	-0,022	-2,769	-0,944	protein used in recombination and DNA repair
yebG	3,824	0,619	0,477	-2,609	-1,25	DNA-damage-inducible protein
recA	3,8	0,365	0,449	-2,686	-1,004	DNA strand exchange, LexA coprotease
dinI	3,605	0,132	0,36	-2,378	-1,463	DNA-damage-inducible protein
umuD	2,699	0,021	0,126	-1,926	-1,23	DNA polymerase V subunit
sbmC	2,238	0,192	-0,007	-1,329	-1,133	DNA gyrase inhibitor
umuC	2,033	-0,003	0,061	-1,317	-1,09	DNA polymerase V subunit
dinD	1,998	0,538	0,057	-1,151	-1,016	DNA-damage-inducible protein
recX	1,956	0,387	0,325	-1,26	-1,013	recA inhibitor
uvrA	1,951	-0,063	0,108	-1,427	-0,565	excision nuclease subunit A
uvrB	1,747	0,321	0,254	-1,068	-0,645	excision nuclease subunit B
ruvA	1,726	0,043	0,144	-1,369	-0,689	Holliday junction helicase subunit B
dinB	1,66	0,387	0,308	-1,029	-0,763	DNA polymerase IV
sulA	1,612	0,482	0,293	-0,817	-0,882	inhibits cell division
ydjM	1,585	0,229	-0,877	-1,036	-0,846	inner membrane protein
lexA	1,469	0,39	-0,095	-1,082	-0,419	regulator for SOS
dinG	1,46	-0,143	0,032	-1,227	-0,579	ATP-dependent helicase
polB	1,444	0,647	0,482	-0,624	-0,438	DNA polymerase II
dinF	1,435	0,548	-0,027	-0,791	-0,443	DNA-damage-inducible protein
ruvB	1,42	0,14	0,141	-0,904	-0,565	Holliday junction helicase subunit A
cho	1,105	0,222	-0,15	-0,459	-0,704	endonuclease of nucleotide excision repair
rmuC	1,014	-0,416	-0,47	-1,036	-1,18	predicted recombination limiting protein
uvrD	0,938	0,058	0,308	-0,381	-0,006	DNA-dependent ATPase I and helicase II
ssb	0,865	-0,339	0,289	-0,856	-0,287	ssDNA-binding protein
ftsK	0,552	-0,198	-0,087	-0,476	-0,383	cell division protein
molR	0,48	0,3	0,002	-0,02	-1,051	molybdate metabolism regulator

Figure 1. Microarray analysis on *recA730* and related strains. Shown are the $\log_2$ values for the fold increase (or decrease) in mRNA expression determined using the Agilent *E. coli* Gene Expression Microarray System for the indicated comparisons. See the Materials and Methods section for details. Red colors indicate increased gene expression, green color reduced expression. Strains used were EC9428 (wt), EC9461 (*dcd*), EC9487 (*ndk*), EC9477 (*recA730*), EC9471 (*recA730 dcd*) and EC9503 (*recA730 ndk*) (see Table 1).

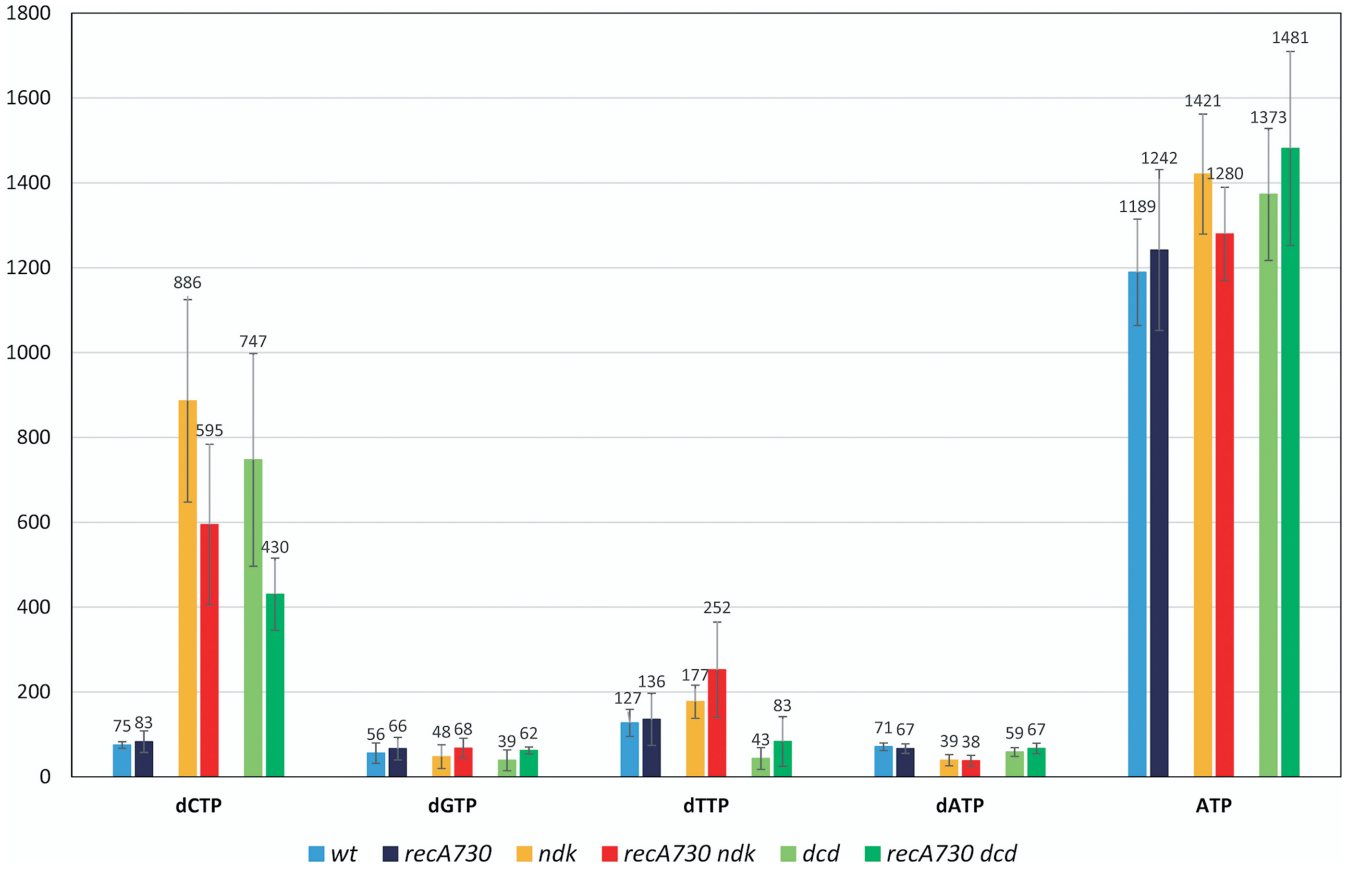

Figure 2. dNTP levels in *recA730* and related strains. dNTP levels were determined by HPLC analysis as described in the Materials and Methods section. The numbers represent the mean of three independent experiments and are expressed on the Y-axis as milliabsorbance units (A_{260}) measured by the HPLC instrument per OD_{600} of the bacterial cultures at harvest. Strains used were EC9428 (wt), EC9461 (*dcd*), EC9487 (*ndk*), EC9477 (*recA730*), EC9471 (*recA730 dcd*) and EC9503 (*recA730 ndk*) (see Table 1).

DISCUSSION

Suppression of SOS in *ndk* and *dcd* defective backgrounds

In the present work we describe several important observations regarding the activity of the *E. coli* SOS system under conditions of altered dNTP pool levels. Specifically, we show that SOS expression is inhibited in the *ndk* and *dcd* backgrounds characterized by dNTP pool changes. The findings relate primarily to the spontaneous SOS induction in the constitutive *recA730* mutant, but may also apply to other instances where the SOS system is induced, for example by DNA damaging agents in the wild-type strain (Table 5). The observation that the *ndk* or *dcd* deficiencies suppress the *recA730*-mediated SOS mutator effect was surprising when first made. As these deficiencies themselves produce distinct mutator effects (23,26,28–29,31), our experiments revealed a case where combined mutator effects lead to a reduction rather than an enhancement of effects. Previous studies on *ndk* and *dcd* strains have revealed their mutator effects to result from dNTP pool alterations by at least two mechanisms: (i) enhanced production of mispairing errors (due to the dNTP imbalances) and (ii) reduced exonucleolytic proofreading of any mispairings due to elevation of certain dNTPs (22–23,48). As the SOS mutator activity results from the mutagenic action of Pol V, either by producing increased levels of base–base mismatches during any of its DNA synthesis or, alternatively, by error-prone extension of mismatches created by Pol III holoenzyme (49), the SOS mutator effect would likewise be expected to be sensitive to the dNTP pool changes. Clearly, exactly the opposite effect was observed, and subsequent experiments showed that the negative effect of *ndk* and *dcd* results from a suppression of the entire SOS regulon.

How can the *ndk* and *dcd* deficiencies cause suppression of the SOS system? Our data show that the suppression results from a reduced ability of RecA730 to mediate LexA cleavage. While other explanations must also be considered (see below), the most direct hypothesis may be that the altered dNTP levels negatively impact the RecA filament. The precise structure and dynamics of the active RecA filament are still being investigated, but it is clear that it consists of an extended polymeric structure of possibly hundreds of RecA monomers long wound around ssDNA and, importantly, containing a nucleoside triphosphate cofactor, such as ATP (46,47), which is essential for its active status. Active filaments are dynamic, generally growing at the 3′ end and undergoing disassembly at the 5′ end. The filament also possesses a robust ATPase activity (16,50) that is critical for certain RecA functions, like homologous recombination, but which is not required for others, such as LexA cleavage

and SOS induction (51). Upon ATP hydrolysis, the ADP-containing filaments become inactive and tend to dissociate from the DNA. Generally, filament formation depends on a slow, rate-limiting nucleation step on available ssDNA, followed by a more rapid extension step (46). The nucleation step is rate-limiting, as the availability of ssDNA is controlled effectively by the *E. coli* single-stranded binding (SSB) protein, which keeps RecA away from the (limited) amount available under normal circumstances. Even after DNA-damaging treatments, such as UV-light irradiation, when increased amounts of ssDNA favor formation of the filament, nucleation still requires assistance of the RecFOR RecA-loading system (46).

The SOS constitutive nature of the *recA730* mutant (E38K) has been reported to result from an increased association rate of RecA730 protein with ssDNA, resulting in an increased ability to displace SSB protein (51,52) and permitting filament nucleation in the absence of DNA damage and without requiring assistance of the RecFOR RecA loading complex (52,53). However, importantly, filament formation by RecA730 is still fully dependent on the nucleotide cofactor (51). The (d)NTP cofactor requirement for the activation of RecA and RecA730 provides an attractive starting point for interpreting the inhibitory effects of the *ndk* and *dcd* deficiencies. Both deficiencies promote significant changes in the cellular dNTP levels, which may interfere with the ongoing activation of the filament. The primary *in vivo* cofactor for RecA is believed to be ATP. This is based on its effectiveness in activating RecA *in vitro* and, further, its significant overabundance compared to all other cellular (d)NTPs *in vivo*. Nevertheless, it is interesting to note that under several *in vitro* conditions dATP is slightly more effective than ATP in promoting several RecA activities (15,17,51,54–55). Our studies revealed no significant changes in the level of ATP (or that of the other rNTPs) in the *ndk*- or *dcd*-deficient strains, consistent with an earlier study on the *ndk* deficiency (28). Furthermore, the *dcd*-encoded dCTP deaminase is highly specific for the dCTP substrate (56) and is not likely to play a role in rNTP metabolism. Thus, we may assume that the inhibitory effects are mediated specifically by the observed changes in the level of the dNTPs. Both strains display a strong increase in the dCTP level (10- to 12-fold). In addition, the *ndk* strain showed a small increase in the dTTP level (1.5-fold) and a decrease in the dATP level (2-fold), while the *dcd* strain had a lowered dTTP pool (3-fold). Similar changes, both qualitatively and quantitatively, were noted in previous studies on these deficiencies in slightly different strain backgrounds (23).

Little *in vivo* information is available about the possible role of the cellular dNTPs in RecA activation, although a number of studies have addressed the role of alternative cofactors using *in vitro* experiments (15,17–18,57–59). These studies revealed that nucleotides other than ATP or dATP are able to function at least in some of the RecA-mediated reactions, however with reduced efficiency. For example, in early studies on *in vitro* cleavage of lambda repressor, which may resemble in part LexA cleavage (17), UTP and dUTP were able to stimulate cleavage of the phage repressor to some extent, while (d)GTP, (d)CTP and dTTP were ineffective. In competition with ATP at equimolar concentrations, (d)UTP, (d)GTP and (d)CTP behaved as competitive inhibitors for the ATP-mediated reaction, while dTTP was a very potent inhibitor. Similar results were obtained by Phyzicky and Roberts (15) in a study of lambda repressor cleavage by the conditionally constitutive RecA441 protein. *In vitro* studies (18,58–59) have suggested that certain alternative (d)NTP cofactors, such as UTP or (d)CTP can, like ATP, bind to RecA to yield a high-affinity DNA binding state, but they can only do so with reduced efficiency. Others, like dGTP or dTTP, were not able to generate such a state. It was suggested that a main difference between ATP-containing active complexes and those containing alternative (d)NTPs reflects their effect on the RecA-DNA binding: whereas ATP promotes strong RecA-DNA binding, other (d)NTPs promote only weak binding, leading to enhanced dissociation of the filament (58).

With regard to our present *in vivo* study, a more complicated situation is at hand, as one needs to consider the *in vivo* competition between ATP and any of the alternative (d)NTPs. ATP can be estimated to be present at a level ∼20- to 100-fold higher than of the individual dNTPs (Figure 2; see also (28)). Despite this, the extended nature of the nucleofilaments, which may be many hundreds of RecA monomers in length (46), may make the occasional insertion of alternative cofactors hard to avoid. If such insertions were to lead to premature dissociation or were to interfere otherwise with the functioning or the growth dynamics of the filament, they could become a rate-limiting factor for overall RecA activity. If correct, it would not be surprising if the currently observed dNTP pool changes, including the strong elevation of the dCTP pool (10-fold or greater), would cause a significant restriction on the activity of the SOS system. This is an attractive hypothesis that is open to further investigation in view of the increased availability of ways to manipulate the *in vivo* dNTP pools (e.g. (22,48,60)). The issue is also open to *in vitro* studies using (d)NTP mixtures mimicking *in vivo* concentrations. As *ndk* and *dcd* have in common the strong elevation of the dCTP concentration, one may surmise that the concentration of this particular nucleotide is most relevant for the observed SOS shutdown. This possibility finds further support in the earlier studies with the *recA441* allele (formerly *tif-1*). This allele contains two mutations, E38K (as in *recA730*) and I298V (61,62). The I298V substitution acts as a temperature-dependent suppressor of the E38K-mediated SOS induction, such that the constitutive SOS induction is only seen at higher temperature (e.g. 42°C). Importantly, this conditional phenotype is also influenced by the composition of the growth medium: the presence of adenine stimulates SOS induction, while addition of guanine and cytidine inhibits induction (19,63). Thus, not only do these observations point to the likely importance of the composition of the intracellular nucleotide pools, they also suggest that in particular cytosine (and guanine) nucleotides would be the stronger inhibitors of SOS induction. Careful measurements of the (d)NTP pools under these conditions may provide more insight into these issues. In related results (not presented here), we have observed that the *recA730* mutator phenotype is likewise sensitive to the medium composition (adenine stimulates while guanine and cytosine diminish its mutator phenotype) (see also (21)).

In addition to the direct effect of the dNTPs as components of the RecA filament, as proposed above, more indirect explanations for their effect should be considered. Overall, the dynamics of the RecA filament, encompassing its formation, growth and breakdown are complex, and are under the control of large number of gene products identified so far, such as RecX, DinI, RecBCD, RecFOR, RadA, XthA and others, which can work in positive or negative ways (46,64–67). Thus, it is formally possible that the dNTP alterations of the *ndk* and *dcd* mutants cause changes in the expression or activity of any of these proteins, and may negatively affect the stability of the filament in this manner. For example, increased activity of the UvrD helicase, one of whose activities is to remove RecA from DNA (68), was shown to suppress SOS expression (69). Also, the availability of ssDNA substrates may be altered in dNTP pool bias mutants, for instance by their effect on replication rate or fork progress, such that SOS induction might be affected. Finally, it is to be noted that analysis of two different constitutive *recA* mutants has shown their precise modes of loading onto the DNA to be distinct (66). Thus, depending on the precise rate-limiting steps for SOS induction in each case, the effect of dNTP alterations could be more or less pronounced and, hence, some caution is required when extrapolating from the *recA730* results to the general case of SOS induction.

Lack of dNTP pool changes in constitutively induced SOS cells

A second main result from our study is the observation that SOS induction *per se* is not associated with major changes in the dNTP pool levels. As dNTP pool alterations are contributing factors to mutagenesis, the lack of such changes may simplify further analysis of the SOS mutator effect. For example, in a previous study (27) we argued that the SOS mutator effect results primarily from the error-prone extension by Pol V of replication errors created by Pol III holoenzyme (HE) (rather than from errors made by Pol V itself). Inherent in this model was the assumption that the pattern of DNA replication errors in normal and SOS-induced cells is the same and that the preferential induction of transversion mutations in *recA730* strains simply reflects the preferential access of Pol V to transversion mismatches, which are the more likely impediments for continued DNA synthesis by Pol III holoenzyme (HE) (27). The lack of observed dNTP pool changes in the *recA730* strain is fully consistent with this aspect of the model.

In contrast, Gon *et al.* (24) reported dNTP elevations of several-fold for each of the four dNTPs after treatment of *E. coli* with UV light, which also effectively induces the SOS system. This dNTP increase was correlated with an increase in expression of the *nrdAB* genes encoding the ribonucleotide reductase (RNR), the main enzyme involved in dNTP synthesis (70), and a corresponding increase in the RNR protein level. This induction of RNR in response to UV light irradiation is consistent with previous reports on the inducibility of the *nrdAB* operon by DNA damage (71–73). As the *nrdAB* operon is not part of the SOS regulon, its induction by DNA-damaging treatments presumably reflects a LexA-independent pathway of gene expression control (73). Our microarray measurements likewise did not reveal any alterations in the *nrdAB* operon expression in the *recA730* strain. Further consistent with this, our microarray experiments with the *recA730* strain did not reveal altered expression among the large group of genes involved in nucleotide metabolism, although there were two exceptions, a 2-fold increase in *add* (adenosine deaminase) and a 4-fold increase in *gpt* (guanine phosphoribosyltransferase) (for complete results see GEO repository, series record GSE62898). Finally, the measured dNTP pools in the *recA730 ndk* and *recA730 dcd* double mutants were essentially unchanged from those in the single *ndk* and *dcd* mutants, further corroborating the lack of control of the SOS system on the dNTP pools.

The SOS response is an important system, which is regulated at a large number of different levels (6,43,46,74–75). These levels of regulation ensure the timely induction, progression and termination of the response. The current work suggests that the cellular (d)NTP concentrations are yet one more determining factor for the expression and control of this system.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS

We thank Drs M. Frazier and M. Longley of the National Institute of Environmental Health Sciences for their careful reading of the manuscript for this paper. We also thank Dr Marek Skoneczny of the Institute of Biochemistry and Biophysics for his assistance with the microarray analysis.

FUNDING

The Intramural Research Program of the National Institutes of Health, National Institute of Environmental Health Sciences [project number Z01 ES065086]; International PhD Projects Program of the Foundation for Polish Science [project number MPD/2009-3/2] "Studies of Nucleic Acids and Proteins - from Basic to Applied Research" and European Union Regional Development Fund. Funding for the open access charge: the National Institute of Environmental Health Sciences.
Conflict of interest statement. None declared.

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