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SOS Chromotest methodology for fundamental genetic research

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Abstract

The present mini-review summarizes data in selected fields of basic genetics which were exclusively obtained in agreement with the principles of SOS Chromotest methodology and with *Escherichia coli* PQ37 *sfiA::lacZ* as a tester strain.

Keywords: DNA repair; SOS response; NO; Genotoxicity

It should come as no surprise that from a historical point of view, genetics became a science only after mathematical methods of analysis were applied in 1866 by G. Mendel [4]. Over 100 different assay systems were more or less generally accepted for the evaluation of genotoxic/mutagenic properties of the chemicals, but not all could evaluate these properties quantitatively. This was the case for the evaluation of gene expression as well, until a simple and rapid method, the SOS Chromotest with *Escherichia coli* PQ37 *sfiA::lacZ* as a tester strain, was developed in 1982 [7]. Since that time the SOS Chromotest has been used by a large number of laboratories. Over 130 publications (1997) show that the idea and the method were very fruitful.

I first became aware of the SOS Chromotest in 1984 in the laboratory of Maurice Hofnung. He gave me as a gift the tester strain *E. coli* PQ37 for my work. After that I received a series of unique *E. coli* strains of different genotypes (for SOS Chromotest methodology) developed by Quillardet and Hofnung [6]. I would like to point out that all these diagnostic tester strains were very stable; indeed, these strains were used in my experimental work on genetics during that entire period.

Since the SOS Chromotest first appeared in Russia "within the walls" of the Academy of Sciences, it was quite natural that the method be applied as a powerful tool to study the problems of basic genetics.

The present paper briefly reviews some selected data from my work with *E. coli* PQ37 (and related strains) from three alternative directions. These are: (1) the influence of structure-substitution-position on empirical relationships between SOS genotoxicity and several in vivo mutagenicity endpoints (PAHs and ethyleneimines); (2) the antagonistic relationships between alternative DNA repair inducible pathways in *E. coli* (SOS and adaptive response to alkylating agents—Ada) and mechanisms of the phenomenon; and (3) endogenous genotoxicants: The contribution of nitric oxide (NO) and intracellular iron ions.

1. A comparative study of mutagenic and SOS-inducing activities of polycyclic aromatic hydrocarbons (PAHs)

1.1. Biphenyls, phenanthrenequinones and fluorenones

A total of 23 chemicals—biphenyls, phenanthrenequinones and fluorenones—were tested for mutagenicity towards *Salmonella typhimurium* strains TA1538, TA1535 and TA98 [17]. SOS-inducing activity of the same chemicals was studied in terms of the SOS-inducing potency (SOSIP) [6] in *E. coli* PQ37, using an automated BIOSCREEN system controlled by a dedicated computer program for the SOS Chromotest (manufactured by Labsystems Oy, Finland). The program also contained a module to interpret and report the results. With respect to the manual method, the important modification was the use of a kinetic measurement principle in order to avoid the problems of interpretation of uncontrolled optical changes during the assays. Results of the experiments are summarized in Table 1.

The results summarized in Table 1 show that the presence of a nitro group and its location in the chemical structure had a dramatic effect on mutagenicity and the SOS response.

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Table 1
Mutagenic and genotoxic effects of phenanthrenequinones, 9-fluorenones and biphenyls

Chemicals tested		Phenanthrenequinones (PhQ)					9-Fluorenones (Flu)			Biphenyls (Bph)		
		PhQ	2-Nitro PhQ	4-Nitro PhQ	2,7-Dinitro PhQ	2,4,7-Trinitro PhQ	Flu	7-Nitro-flu-4-carboxy-lic acid	2,7-Dinitro-flu-4-carbox-amide	2,2′-Bph-dicarboxylic acid	2,4,4′-TrinitroBph--2′-carbox-ylic acid	2,4,4′-TrinitroBph-2,6-dicarbox-ylic acid
Revertants/nmole[a]		89±12		457±10		300 ± 13	626 ± 28			108 ± 1	357 ± 26	170 ±5
Number of His^+ rev./plate in the Ames test (TA1538), 1.5 nmole/plate[b]		23	436	12	767	473	21	23	1382	13	826	302
SOS Chromo-test	SOSIP	–	8.0	–	3.6	5.1	–	0.52	1.10	–	–	–
	Induction factor	–	6	–	4	5	—	3	5	–	–	–

[a] Spontaneous level was 13 revertants/plate. [b] Revertants/nmole were calculated on the basis of linear regression of the dose-response curve ± standard error of the slope.

When the nitro group was absent, the genotoxic activity of the substance was very low, if even present. The most active compounds were those that possessed either 1-nitro group in the para position, or in addition, other groups in other positions. In the chemical structure of nitro-PAH there are several features that are important for genotoxicity, including increased electrophilicity required for reactivity towards nucleophilic groups in the DNA and susceptibility to reduction by bacterial nitroreductase and esterification by transacetylase. The common features of these chemicals are the presence in their structure of a biphenylic nucleus, and carbonyl and nitro groups. It was shown that plasmid *pKM101* did not increase the susceptibility of TA98 to all chemicals tested: The *mucA* and *mucB* gene products that seem to be functionally homologous with *umuD* and *umuC* bacterial gene products had no influence on frameshift mutagenesis.

Thus it seems that in this group of chemicals there are striking position and substituent-specific effects. Similar comparisons between the biphenyl derivatives indicated that the inactive or weakly active mono- and dinitrobiphenyls were not converted to more mutagenic forms by the presence of carboxylic acid groups.

A comparison of the mutagenic potential of some frameshift mutagens with their SOS-inducing ability indicated that only 5 out of 14 mutagens were SOS inducers at the same time.

There was a close correlation between mutagenicity and SOS-inducing activity of fluorenones and phenanthrenequinones. None of the biphenyls tested induced the SOS response and this property did not depend upon the mutagenic activity of the chemicals.

The same negative correlation between mutagenic activity in the *Salmonella* assay in the frameshift strains TA98 and TA1538 and SOS repair activity in *E. coli* PQ37 was shown in azo dyes, unsubstituted acridines and most of the aromatic amines. Therefore, only those frame-shift mutagens that interact with the DNA chemically by means of covalent binding with the carbonyl group of the mutagens, for example, induce the SOS response in *E. coli* PQ37.

In line with this, the main advantage of the SOS Chromotest with strain PQ37, apart from its practical benefits, may be its ability to respond to a covalent change in the structure of DNA. It is particularly valid in the detection of chemicals that give rise to base-pair substitutions through SOS induction. In the case of positive results the Ames test may be omitted, especially for the base-pair mutagens. But if the chemical tested induces frameshift mutations, then each negative result in the SOS Chromotest should indicate the necessity of additional Ames tests with the appropriate bacterial strains of *S. typhimurium*.

1.2. Ethyleneimines

It is well established that chemical characteristics of organic compounds usually change in the borders of homologous rows. It seemed that was the case for the biological activity of the chemicals. We initially studied the interrelationships between the chemical structure and mutagenic (Mutatest)/SOS-inducing activities in the series of ethyleneimines (EIs)—ethyleneimine (EI) and its oligomers—di-tri-tetramers with the general formula: $(CH)_2$ N–$(CH_2$–CH_2–$NH)_n$H, where $n = 0, 1, 2, 3, \ldots$ [11]. An increase in n meant an increase in amino groups that can be activated due to protonization. We examined the rates of His^+ revertants in the Mutatest and Trp^+ revertants in 3 isogenic *E. coli* B/r WP2 strains with the defects in DNA repair systems. In the comparative experiments the value of IF in an automated variant of the SOS Chromotest was calculated. Table 2 shows the data obtained.

All the compounds tested selectively induced base pair substitutions in both *S. typhimurium* TA1535 and TA100;

Table 2
Mutagenic activity of EI and oligomers. A comparison with the IF value in the SOS Chromotest

Compounds	His$^+$ rev. in *S. typhimurium* tester strains[a]			Rate of Trp$^+$ rev/10^6 viable cells in *E. coli* % viable cells to the control (brackets)			IF (max) in *E. coli* PQ37
	TA1535	TA100	TA1538	WP2	WP2*uvrA*	CM561*lexA*	
Monomer	189 ± 14	291 ± 5	35 ± 3	1.2 (43.0)	3.0 (15.2)	0.02 (17.6)	2.0
Dimer	650 ± 90	557 ± 76	35 ± 5	3.6 (31.5)	–	0.02 (15.0)	4.0
Trimer	924 ± 97	2619 ± 426	39 ± 3	13.0 (46.0)	56.0 (0.9)	–	16.0
Tetramer	229 ± 7	1118 ± 180	48 ± 4	0.05 (90.0)	60.0 (0.5)	0.02 (0.5)	9.0
NaN_3, 10 μg/pl	1215 ± 35	1141 ± 122	–	–	–	–	–
2NF, 5μg/pl	–	–	308 ± 45	–	–	–	–
Spontaneous level	2 ± 1	34 ± 4	34 ± 4	0.05	0.05	0.01	–

[a] Mean value in 3 dishes.

plasmid *pKM101* had no influence on the rate of mutations. TA1538 was not mutable by the EIs tested. The mutagenic potencies increased in the order EI < dimer < trimer. The tetramer of EI was less active than the trimer for both test systems. In *E. coli* a striking influence of *uvrA* and *lexA* mutations was observed. *E. coli* EM561*lexA* was not mutable, while WP2*uvrA* was much more mutable as compared to the isogenic wild type (Table 2). Both *E. coli* mutants were much more sensitive to the cytotoxic activity of EIs. It appeared that potentially lethal lesions induced by EIs were repaired by UVR ABC and LexA pathways, while the mutagenic process was controlled by a LexA-regulated SOS response. This conclusion is in agreement with the data in the SOS Chromotest.

Thus, for most of the chemicals studied there existed a striking quantitative correlation between the responses in the SOS Chromotest and the Mutatest. Among the EIs the mutagenic potency increased in *S. typhimurium* TA1535 and TA100 (*pKM101*) from monomer to dimer and trimer proportionally according to the number of active NH^+ and NH_2^+ groups found in DNA after the interaction of the chemical with the DNA target. The same tendency was observed in the values of inducing factors (IF) of these chemicals.

Therefore, raising the total positive charge of a mutagen does increase its local concentration in the area of the biological target, DNA, and, as a consequence, causes an increase in the rate of its interaction with the target. Experimental evidence of this idea has been obtained in our work.

2. A phenomenon of interrelationships between the alternative DNA repair pathways in *E. coli*: SOS and Ada responses

Several inducible pathways exist in *E. coli* to counteract the effects of environmental stress or mutagenic damage. The regulatory genes of these pathways usually encode protein activators, and the systems are consequently under positive control. The phenomenon of the adaptive response (AR), an inducible highly specific and accurate DNA repair process, was first described in *E. coli* [8]. The AR is activated by exposure of the cells to sublethal doses of alkylating agents N-methyl-N-nitrosourea (MNU), N-methyl-N-nitro-N-nitrosoguanidine, etc., resulting in increased resistance to mutagenic and cytotoxic effects of these agents. AR activates the expression of several genes via positive regulation mediated by the Ada protein. The molecular mechanism of this gene activation has been described in detail [2]. The Ada protein transfers the methyl group from damaged DNA to two of its own cysteine residues; and thereafter, it becomes a transcriptional activator. The methylated Ada protein is able to bind specific sequences of DNA in the promoter region of its own operon *ada/alkB*, *alkA*, *alkB* and at least one other gene *aidB* [2]. The *alkA* gene encodes a glycosylase, involved in the removal of methylated bases from DNA. The function of *alkB* is unknown. We first presented data that *aidB* plays the role of a unique "hazard gene": It repairs high toxic adducts after the treatment of the cells with bifunctional alkylating agents such as the derivative of MNU-ACNU [15]. In *alkA* and *alkB* promoters the Ada-binding sequences have been identified.

In *E. coli* DNA damage induces the alternative DNA repair pathway—SOS response [18]. The SOS regulon consists of approximately 30 unlinked genes whose expression is coordinately regulated by the LexA repressor protein. Many of these LexA-regulated genes encode proteins that function to repair DNA lesions in an accurate manner. However, in the event that a DNA lesion in *E. coli* cannot be repaired accurately, an error-prone repair pathway exists. This pathway, termed translesion DNA synthesis, is the mechanistic basis of SOS mutagenesis [10]. Genetic control of the error-prone SOS response is not associated with the alternative error-free Ada-response.

Chemicals of different classes, including DNA alkylating agents, induce the SOS response. Therefore, the Ada and SOS responses in *E. coli* are induced simultaneously by

the same agents, but induction of the alternative pathways is initiated by the different products of DNA alkylation. We reported [13] that SOS repair, which occurs in *E. coli* PQ37 previously induced for the Ada response by MNU pretreatment, was inhibited. The experimental SOS inducing agents (in the automated version of the SOS Chromotest) were chosen due to different primary molecular mechanisms of the reactions with DNA [12]. MNU and MNNG are typical monofunctional alkylating agents, while mitomycin C is the alkylating agent generating bulky lesions in DNA, which are repaired by UVR ABC-dependent DNA excision repair. 4NQO is a typical UV mimetic agent. Initially we checked that adaptation decrease the cytotoxic and mutagenic effects of MNU in the adapted cells [13]. Table 3 shows that the levels of SOS induction sharply decreased in the adapted cells, as well.

As the SOS response inhibition due to the Ada response development was nonspecific, we proposed that there might be a common substrate or substrates linked to SOS induction and affected by the MNU adaptation. We suspected that the substrates were DNA single-strand breaks: (1) the mutual stage in repair of DNA lesions produced by chemicals tested; and (2) the key component in the molecular complex for SOS induction. Indeed, in response to DNA damage, the RecA protein binds to single-stranded DNA, forming a nucleoprotein filament. This filament is central to the role of the RecA protein within the cell. It acts as a coprotease to facilitate autodigestion of the LexA repressor and inactivates the transcriptional repressor activity of LexA, thus leading to induction of the SOS regulon [10]. We proposed that the appearance of DNA single-strand breaks in the course of DNA repair and their interaction with proteins of the Ada response represented a critical stage in the inhibition of SOS response development. Negative results in SOS inhibition by MNU pretreatment of *E. coli* cells in the experiment with mitomycin C (Table 3) were in agreement with our proposal. In fact, the intact UVR ABC complex is needed for the repair of DNA damage induced by the agent, while the *E. coli* PQ37 tester strain is a *uvrA* mutant. Later we extended our findings by demonstrating interrelationships between the alternative SOS and OxyR responses [14] (data not shown).

Thus, we first presented evidence (at the level of gene expression) for interrelationships between the alternative inducible DNA repair pathways in *E. coli.*

Table 3
Influence of *E. coli* PQ37 adaptation by MNU on *sfiA* gene expression in the SOS Chromotest

SOS inducer	Max. mutagens conc., nmole		IF	SOSIP/nmole
MNU		A*	7.00	0.09
		B**	2.00	0.001
MNNG	485.44			
Mitomycin C	100	A	5.00	0.07
		B	2.00	0.01
4NQO	2.99			
	2.63	A	3.00	3.36
		B	3.00	2.71
		A	21.00	57.9
		B	7.00	39.1

*A: control cells, without MNU pretreatment for adaptation. **B: cells were pretreated with a sublethal dose of MNU.

3. Endogenous genotoxicants: Contribution of NO and intracellular iron ion

NO is a unique diffusible molecular messenger that was identified as a biological mediator in 1987. NO is formed intracellularly from the amino acid L-arginine by a family of enzymes, the NO synthases, and plays a role in many physiological functions [5]. In addition, NO is generated in large quantities during host defense and immunological reactions where it contributes to cytotoxicity against tumor cells, bacteria, viruses and other invasive microorganisms. The main mechanisms of antimicrobial NO action include interference with cell division and energy production via inhibition of DNA synthesis and electron transport proteins due to nitrosylation of protein SH groups and nitrosative deamination of DNA [5]. NO may also interact with oxygen-derived radicals to produce other toxic substances, such as peroxynitrite [1]. Peroxynitrite is a powerful oxidant that exhibits unique chemical reactivity, such as protein nitration, DNA strand break, guanine nitration, etc., which may then bring about not only cytotoxic effect but also mutagenesis.

Mammalian cells as well as bacteria such as *E. coli* can generate a stress response providing a defense of the cells against various reactive species. Nitric oxide radicals, like the superoxide anions O_2^-, trigger the oxidative stress response by activation of the *soxRS* regulon, which is controlled by the redox-sensitive transcriptional regulator SoxR in *E. coli* [16].

We first studied the SOS DNA repair response as a reaction of *E. coli* cells to treatment with NO and NO-donating agents S-nitrosothiols (GSNOs) and dinitrosyl iron complexes with the thiol-containing ligands cysteine or glutathione (DNICs). The roles of iron ions and peroxynitrite in SOS induction were examined as well.

To quantify SOS response induction by NO and NO donors we used *E. coli* PQ37. To elucidate the role of iron ions in *sfiA* gene activation, EPR spectroscopy was used. It is based on the ability of DNICs to be transformed into paramagnetic monomeric forms in the presence of free thiols as a result of its attaching to protein SH groups. This leads to the appearance of the typical EPR signal with anisotropic g-factor ($g_\perp = 2.041$ and $g_{\perp\perp} = 2.014$). Increasing the DNIC concentration leads to a corresponding increase in the EPR signal in the cells.

We first reported [3] the ability of NO and NO-producing agents to activate the SOS response in *E. coli* PQ37 (Ta-

Table 4
NO donors activate s*fiA* gene expression in *E. coli* PQ37; influence of iron and *o*-phenanthroline (OP)

Treatment	Induction ratio (IR)	*sfiA::lacZ* expression after incubation with 0.1 mmole OP	
		β-galactosidase, Miller units	IR* after removal of OP by centrifugation**
Control (no treatment)	0	0.6	0
4-NQO, 2.63 nmole (positive control)	7.3	0.9	2.1
DNIC, 0.5 mmole	2.1	0.9	1
GSNO, 0.5 mmole	2.0	0.7	1
Fe^{2+}-citrate (1:5), 0.5 mmole	0.8	–	–
H_2O_2, 0.03 mmole	3.0	0.7	0.3
$ONOO^-$, 0.2 mmole	0	–	–

*IR (induction ratio) was calculated according to the equation: $IR = (\beta\text{-Gal})_t - (\beta\text{-Gal})_c/(\beta\text{-Gal})_c$. **IR was determined for cells washed after incubation with 0.1 mmole OP.

ble 4). Table 4 shows that DNICs with cysteine and glutathione were the most potent *sfiA* gene inducers. GSNOs mediated a similar response, but at much higher concentrations. Exogenous peroxynitrite contributes slightly to *sfiA* activation. Analysis of NO donor effects to the SOS response revealed a strong influence of intracellular iron. Pretreatment of the cells with the chelating agent OP prevents induction of the SOS response by all agents tested. The EPR study shows the appearance of an EPR DNIC-type signal after incubation of the cells with GSNO, because of mutual transformation between GSNO and DNIC in the presence of accessible iron inside the cells; pretreatment of the cells with OP leads to a decrease in this signal [9].

Thus, intracellular iron ions play a dual role in the process of the interaction of NO-donating agents with *E. coli* cells. On the one hand, they transform and stabilize GSNO in the form of less toxic DNICs, and, on the other hand, they play a substantial role in the process of SOS signal formation by NO. *S. typhimurium* tester strain TA1535 provided an appropriate tool to study base pair substitution in NO mutagenicity. In our experiments reversion produced by DNIC, GSNO and NO was moderately enhanced (data not shown).

Thus, NO functions as a weak endogenous mutagen and potent genotoxicant, activating both the SOS and the SoxRS regulons in *E. coli*. Iron ions are absolutely indispensable for NO regulatory functions in the cell.

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