

The SOS chromotest : Direct assay of the expression of gene *sfiA* as a measure of genotoxicity of chemicals (1).

Philippe QUILLARDET *,
Olivier HUISMAN **, Richard D'ARI **
and Maurice HOFNUNG * ◊.

* Unité de Programmation Moléculaire
et Toxicologie Génétique,
CNRS LA 271, INSERM U. 163,
Institut Pasteur, 28, rue du Dr. Roux,
75015 Paris, France.

** Institut de Recherche en biologie moléculaire,
Institut Jacques Monod, CNRS,
Université Paris 7 (tour 43),
2, Place Jussieu,

Summary.

We used a gene fusion, placing the lacZ gene encoding β -galactosidase under the control of the sfiA promoter, to construct a new tester strain for genotoxic agents. The assay is performed in a few hours and involves simple enzymatic assays. The dose response curves contain a linear portion which enables to define the SOS Inducing Potency (SOSIP) of compounds. For the compounds tested SOSIPs extend over 7 decades and correlate generally well with the mutagenic potency assayed in the Salmonella/microsome assay (Mutatest) and in a phage induction assay (Inductest). Sensitivities (lowest amount detected) are comparable in the SOS Chromotest and Mutatest but lower in the Inductest.

Our results suggest that at least part of the response in the Mutatest depends on the induction of an SOS function, and that most of the genotoxins are inducer of the SOS system -i.e. can lead to activation of the RecA protease.

Résumé.

Nous avons utilisé une fusion génétique qui met l'expression du gène lacZ de la β -galactosidase sous le contrôle du promoteur sfiA, pour construire une nouvelle souche bactérienne. Celle-ci permet d'examiner, en quelques heures, la capacité d'un produit à endommager le DNA, grâce à un simple dosage colorimétrique. Les courbes « dose-réponse » comportent une région linéaire qui permet de définir le pouvoir inducteur (SOSIP) des produits. Sur une série de produits examinés, nous avons observé des valeurs de SOSIP qui s'étendent sur 7 décades et qui sont étroitement corrélées avec le pouvoir mutagène évalué dans le test d'Ames (Mutatest) et entre le SOSIP et un test d'induction du prophage λ (Inductest). La sensibilité (plus faibles quantités détectées) est comparable dans le SOS Chromotest et le Mutatest mais généralement plus faible dans l'Inductest.

Nos résultats suggèrent qu'au moins une partie de la réponse observée dans le Mutatest dépend de l'induction d'une fonction SOS et que la plupart des produits génotoxiques sont inducteurs du système SOS, c'est-à-dire qu'ils peuvent conduire à l'activation de la protéase RecA.

clés : agents endommageant le DNA / induction SOS / test enzymatique colorimétrique / fusion d'opérons / gènes lacZ::sfiA.

Key-words : DNA damaging agents / SOS induction / colorimetric enzyme assay / operon fusion / lacZ::sfiA genes.

Introduction.

Several consequences of genotoxic action such as mutagenesis or phage induction are currently taken as end points in bacterial genetic toxicology tests. Thus, the mutagenic capacity of a genotoxic agent may be assessed by means of the *Salmonella*/microsome assay (Mutatest) [1, 2, 3] and its phage inducing capacity by means of Inductest [4, 5, 6, 7]. Such consequences are often not due to the primary action of the agent but rather, at least in part, to the response of the cell to this action.

In *E. coli* some of these responses, induced by DNA damaging treatments involve a set of functions, *i.e.* inducible error-prone repair, prophage λ induction, cell division inhibition, known as the SOS functions [8, 9]. Expression of the SOS functions depends on the RecA protease. Upon activation the RecA protease is able to cleave the LexA protein, the general repressor of the SOS functions as well as the λ cI protein, the repressor of phage λ [9, 10].

Chemicals : abbreviations and origin.

Aflatoxin (AF) B1, G1, B2 and G2, diethyl nitrosamine (DEN), 1,3-propane sultone and β -propiolactones were from SERVA ; ethylmethane-sulfonate (EMS) and methylmethane sulfonate (MMS) from EASTMAN ; Benzo-a-pyrene (B(a)P), N-methyl-N'-nitrosoguanidine (MNNG), dimethyl sulfate (DMS) and diethyl sulfate (DES) from ALDRICH ; dimethyl-nitrosamine (DMN) from EGA ; 4-nitroquinoline-1-oxide (4NQO) from FLUKA ; caffeine and mytomycin C (MMC) from SIGMA ; dimethylsulfoxide (DMSO) and sodium chloride from MERCK ; aspirin from UPSA ; neocarzinostatin (NCS) from KAGAHU ANTIBIOTICS RESEARCH ; 2-nitro-benzofuran (R5144) ; 2-nitro-5-methoxybenzofuran (R5255), 2-nitronaphtho (2-1-b) furan (R6597), nitro-2-methoxy-8-naphtho (2-1-b) furan (R6998), 2-nitro-7-methoxynaphtho (2-1-b) furan (R7000), nitro-2-methoxynaphtho (2-1-b) furan (R7100), 7-methoxynaphtho (2-1-b) furan (R7197) and naphtho (2-1-b) furan (R7216) from R. Royer (Paris).

Results and Discussion.

We have taken advantage of an operon fusion placing *lacZ*, the structural gene for β -galactosidase, under control of the *sfiA* gene [11], one of the SOS genes involved in cell division inhibition, to devise a simple and direct colorimetric assay of this SOS response to DNA damage (fig. 1). We call this assay the « SOS Chromotest ».

The assay (fig. 1) (described in more detail in P. Quillardet, O. Huisman, R. d'Ari and M. Hofnung, in press) consists in incubating the tester strain with increasing concentrations of the agent to be tested. After time for protein synthesis to occur β -galactosidase activity is assayed. The classical microsomal activation preparation [3] can be included in the incubation mixture. The chemicals tested may at certain concentrations inhibit protein synthesis, which would lead to an underestimation of β -galactosidase induction. To correct this, the strain was made constitutive for alkaline phosphatase synthesis [12]. This enzyme, non inducible by DNA damaging agents, is assumed to reflect general protein synthesis. It is assayed in parallel with β -galactosidase. The ratio of the two activities (β -galactosidase/alkaline phosphatase) is taken as a measure of the specific activity of β -galactosidase. To compare different experiments and different compounds, we normalized this activity ratio to the value in the absence of test compound. This is especially important when comparing experiments with and without activating preparation which influences alkaline phosphatase activity. The normalized value is called the induction factor I (P. Quillardet, O. Huisman, R. d'Ari and M. Hofnung, in press).

The SOS Chromotest has several practical advantages. It is easy to perform, requiring only a single strain and simple colorimetric enzyme assay. It is fast, giving a response within several hours. Furthermore, it does not require survival of the tester strain.

Remarkably, dose response curves present a linear portion in the low dose range (fig. 2). The slope of this linear region defines a single parameter, the « SOS Inducing Potency » or SOSIP, representing the increase in induction factor per nanomole of compound tested.

We compared the responses obtained in the SOS Chromotest and in two other tests, the Mutatest [3] and an Inductest [5], with a wide range of known genotoxins.

A comparison of mutagenic potency measured by the Mutatest, and SOSIP measured by the SOS Chromotest, revealed a striking quantitative correlation over seven orders of magnitude (fig. 3, left panel). This result suggests that the Mutatest may in fact essentially detect SOS mutagenesis. The mutagenic potency, like the SOSIP, would directly reflect the level of expression of some SOS function under *lexA* control. Likely candidates are the *umuC* or *mucC* genes products (G. Walker this issue) known to be indispensable for SOS mutagenesis [13], under *lexA* control, and inducible by DNA damaging agents [14].

We also found a generally good correlation between the SOSIP measured in our test and the inducing potency determined in the Inductest

(fig. 3, right panel). This is not surprising since both are known to depend on activation of the RecA protease. However several compounds

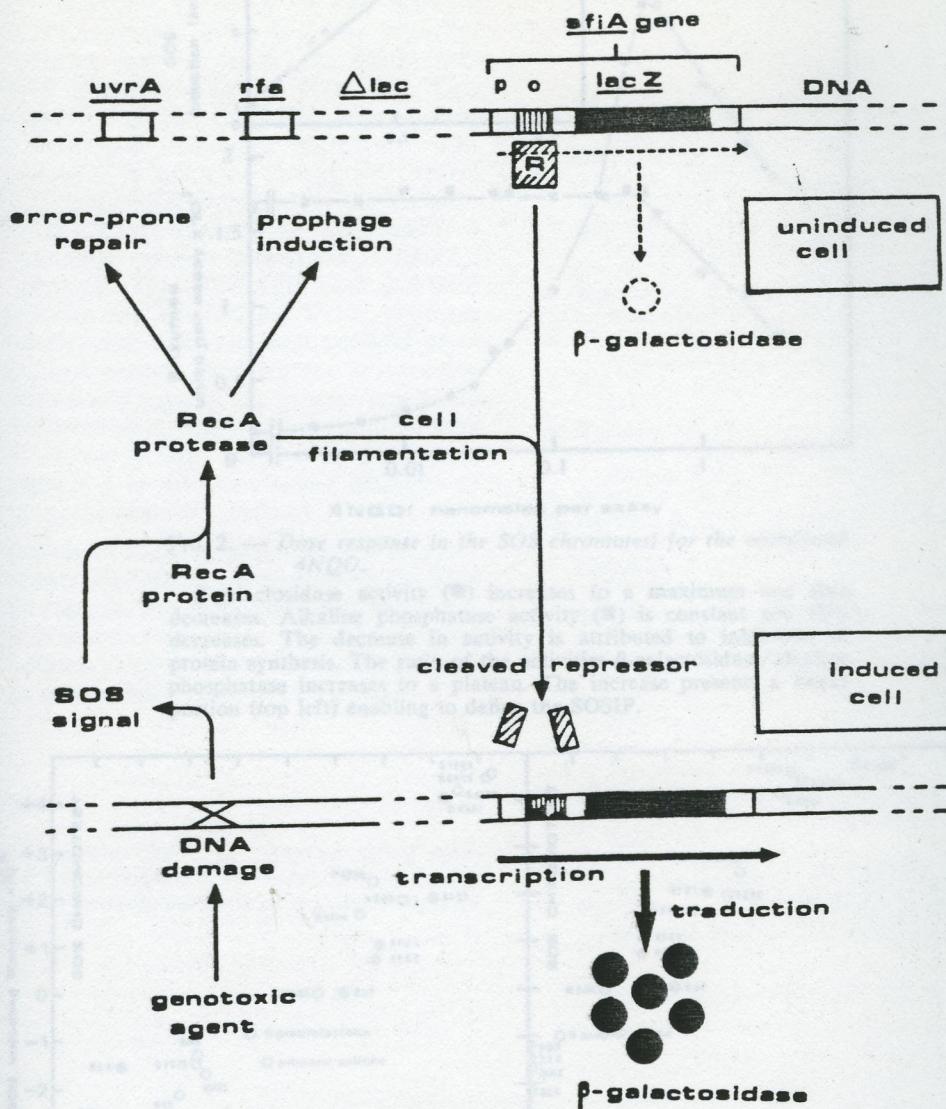


FIG. 1. — Principle of the SOS Chromotest.

The tester strain carries a *sfiA::lacZ* operon fusion and a deletion of the normal *lacZ* gene (Δlac) so that β -galactosidase expression strictly reflects *sfiA* expression. The strain is *uvrA* (excision repair deficient) and *rfa* (increased permeability). It has been rendered constitutive for the synthesis of alkaline phosphatase (*Pho^c*) to allow an easy monitoring of general protein synthesis.

Incubation of the agent to be tested is performed for two hours. Kinetic experiments showed that the Induction factor, which is the normalized value of the ratio β -galactosidase activity over alkaline phosphatase activity, reaches a plateau before this time for all the compounds for which they were performed. This plateau is stable for at least one hour.

Upper part of the figure : uninduced cell. The LexA repressor prevents expression of the *sfiA::lacZ* operon fusion.

Lower part of the figure : induced cell. The LexA repressor has been cleaved by the activated RecA protease, resulting in induction of the expression of the *sfiA::lacZ* operon fusion.

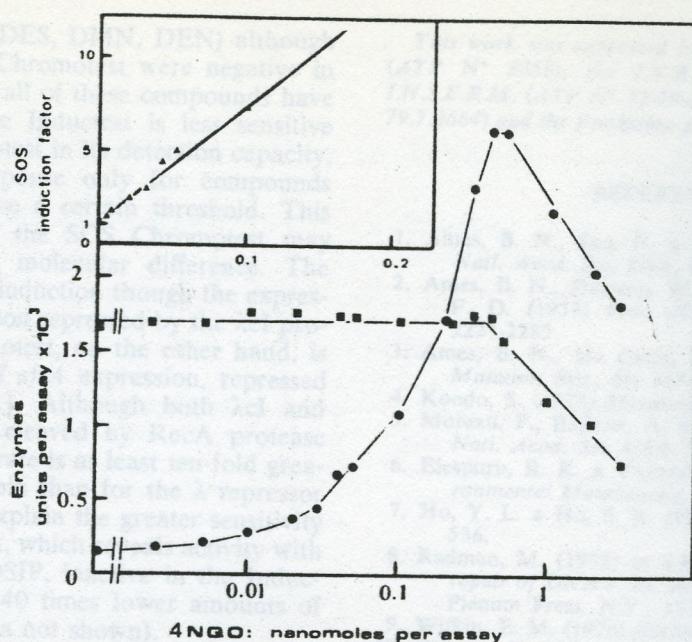


FIG. 2. — Dose response in the SOS chromotest for the compound 4NQO.

β -galactosidase activity (●) increases to a maximum and then decreases. Alkaline phosphatase activity (■) is constant and then decreases. The decrease in activity is attributed to inhibition of protein synthesis. The ratio of the activities β -galactosidase/alkaline phosphatase increases to a plateau. The increase presents a linear portion (top left) enabling to define the SOSIP.

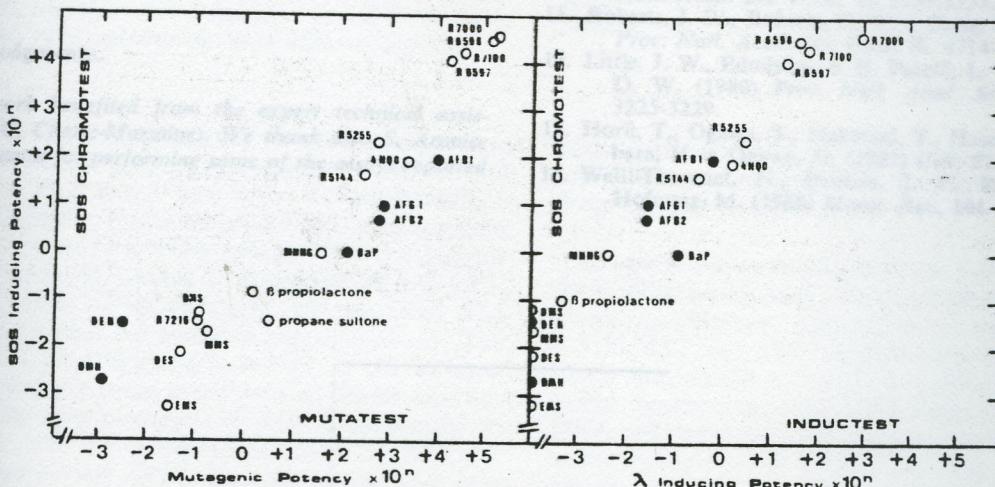


FIG. 3. — Correlation between the SOS inducing potency (SOSIP) and the mutagenic potency in the Mutatest (strain TA100), and between the SOSIP and the phage inducing potency for a series of genotoxins.

For each compound (except for the benzo and naphtofurans [18]) experiments were performed in parallel to determine the SOSIP the mutagenic potency in the Mutatest (strain TA100), and the phage inducing potency in the Inductest. The same stock solutions, and when needed the same activation mixtures, were used. Independent determinations varied by less than factor 3. Closed symbols : compounds tested with activation.

Closed symbols : compounds tested with activation.
Open symbols : compounds tested without activation.

Open symbols: compounds tested without activation.
 Exceptional compounds which are not detected in one of the tests are not represented (P. Quillardet, O. Huisman, R. D'Ari and M. Hofnung, in press).

(MMS, EMS, DMS, DES, DMN, DEN) although positive in the SOS Chromotest were negative in the Inductest. In fact all of these compounds have low SOSIP. Thus the Inductest is less sensitive than the SOS Chromotest in its detection capacity, giving a positive response only for compounds whose SOSIP is above a certain threshold. This greater sensitivity of the SOS Chromotest may reflect an underlying molecular difference. The Inductest assays SOS induction though the expression of phage production repressed by the λ cI protein. The SOS Chromotest, on the other hand, is based on induction of *sfiA* expression, repressed by LexA protein [11]. Although both λ cI and LexA repressors are cleaved by RecA protease [15, 16] the cleavage rate is at least ten-fold greater for the LexA protein than for the λ repressor [16, 17]. This could explain the greater sensitivity of the SOS Chromotest, which reveals activity with compounds of low SOSIP, inactive in the Inductest, and detects 4 to 40 times lower amounts of active compounds (data not shown).

In conclusion, in addition to pointing to its practical advantages, the results obtained with the SOS Chromotest suggest that mutagenic potency measured in the Mutatest reflects the level of induction of an SOS function and that most genotoxins may be inducers of the SOS response in bacteria.

Acknowledgments.

This work benefited from the expert technical assistance of C. Charie-Marsaines. We thank also S. Arnaise and N. Cantat for performing some of the assays reported here.

This work was supported by grants from the C.N.R.S. (ATP N° 3058), the I.N.R.A. (ATP N° 4185), the I.N.S.E.R.M. (ATP N° 72-79-104), the D.G.R.S.T. (ACC 79.7.0664) and the Fondation pour la Recherche Médicale.

REFERENCES.

1. Ames, B. N., Lee, F. & Durston, W. (1973) *Proc. Natl. Acad. Sci. USA*, **70**, 782-786.
2. Ames, B. N., Durston, W. E., Yamasaki, E. & Lee, F. D. (1973) *Proc. Natl. Acad. Sci. USA*, **70**, 2221-2285.
3. Ames, B. N., Mc Cann, J. & Yamasaki, E. (1975) *Mutation Res.*, **31**, 347-364.
4. Kondo, S. (1974) *Mutation Res.*, **26**, 235-241.
5. Moreau, P., Bailone, A. & Devoret, R. (1976) *Proc. Natl. Acad. Sci. USA*, **73**, 3700-3704.
6. Elespuru, R. K. & Yarmolinsky, M. B. (1979) *Environmental Mutagenesis*, **1**, 65-78.
7. Ho, Y. L. & Ho, S. K. (1981) *Cancer Res.*, **41**, 532-536.
8. Radman, M. (1975) in « Molecular Mechanisms for repair of DNA », ed. Hanawalt, P. and Setlow, R. Plenum Press, N.Y., 355-358.
9. Witkin, E. M. (1976) *Bacteriol. Rev.*, **40**, 869-907.
10. Devoret, R. (1981) *Progress in Nucleic Acids Res.*, **29**, 252-263.
11. Huisman, O. & d'Ari, R. (1981) *Nature (London)*, **290**, 797-799.
12. Torriani, A. M. & Rothman, F. (1961) *J. Bacteriol.*, **81**, 835-836.
13. Kato, T. & Shinoura, Y. (1977) *Molec. Gen. Genet.*, **156**, 121-131.
14. Bagg, A., Kenyon, D. J. & Walker, G. C. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 5749-5753.
15. Roberts, J. W., Roberts, C. W. & Craig, N. L. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 4714-4718.
16. Little, J. W., Edmiston, S. H., Pacelli, L. Z. & Mount, D. W. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 3225-3229.
17. Horii, T., Ogawa, T., Nakatani, T., Hase, T., Matsubara, H. & Ogawa, H. (1981) *Cell*, **27**, 515-522.
18. Weill-Thevenet, N., Buisson, J. P., Royer, R. & Hofnung, M. (1982) *Mutat. Res.*, **104**, 1-8.