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# Effects of Anti-Neoplastic Antibiotics on DNA Replication and Repair*

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# DNA복제 및 회복에 미치는 수종항암 항생제의 영향에 관한 연구

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## 요 약

본 연구는 알킬화제이며 항암항생제인 Mitomycin C(MMC)와 Bleomycin (BLM)이 DNA 복제 및 회복에 미치는 영향을 규명하고, 아울러 MMC에 의한 "유발상해회복"이 포유동물세포에 유발되는지 밝히고자 수행하였다. 이를 위해 자기방사법에 의한 비주기 DNA합성율과 알카리 유출법에 의한 DNA단사절단율을 측정하였다. CHO세포에 MMC를 제 1 차($MMC_1$) 처리한 후 5시간 뒤에 제 2 차 ($MMC_2$)로 처리하여 얻은 결과는 다음과 같았다.

1. BLM은 비주기 DNA합성을 매우 적게 유발시켰으며, BLM 5$\mu$g/ml의 농도에서 부터 비주기 DNA합성율은 증가를 보이지 않았다. BLM은 처리후 1.5시간까지 DNA합성억제를 보였으며, 1.5시간후 DNA합성율이 증가되었으나, 대조군의 60% 수준까지 회복되었을 뿐이다.

2. MMC에 의해 유발된 비주기 DNA합성은 농도에 따라 비례하였으며, 배양후 시간의 간격에 따라 비례하여 감소하였다. 제1,2차 MMC를 처리한 세포의 비주기 DNA합성은 제 1 차만 처리한 세포의 비주기 DNA합성보다 많았다.

3. 알카리 유출법 결과는 MMC에 의하여 유발된 DNA 단백질 연결로 인한 DNA단사절단율이 농도에 비례함을 나타내었다. DNA 단백질 연결로 인한 DNA 단사절단율은 배양후의 시간에 비례해서 감소되었다.

이러한 결과는 MMC와 BLM 모두가 DNA 상해제임과 MMC에 의한 DNA 상해 부위의 복제율이 어떠한 유발기작에 의하여 촉진됨을 시사한다.

## INTRODUCTION

Damage to DNA in *E. coli* induces the coordinate expression of a set of diverse

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responses collectively called SOS function. This function includes the appearance of repair and mutagenic activities, inhibition of septation, induction of certain prophages, and production of large amounts of *rec* A protein (Witkin, 1976). Induction of SOS function appears to involve the protease activity of the *rec* A protein, which cleaves cellular repressors of SOS function including its own repressor, the *lex* A gene product (Craig *et al.*, 1980; Little *et al.*, 1979).

Several observations suggest that SOS-like activities are also induced in mammalian cells in response to DNA damage (Radman, 1980; Hanawalt *et al.*, 1979). Analogous to Weigle reactivation of bacteriophage, the survival of certain UV-irradiated viruses is enhanced by exposing the mammalian host cells to DNA-damaging agents prior to infection (Radman, 1980; Hanawalt *et al.*, 1979). Some studies indicate that this enhanced reactivation is accompained by viral mutagenesis. Manifestations of the SOS response in mammalian cells are the enhancement of post-replication repair in Chinese hamster and human cells after UV irradiation or treatment with chemical carcinogens. (D'Ambrosio *et al.*, 1976, 1978).

This approach was criticized by Painter (1978), but much of the criticism was overcome by D'Ambrosio *et.al.* (1978) and Hanawalt *et al.* (1979). D'Ambrosio *et al.* (1976, 1978) were in time agreement with the studies on replication of irradiated viral DNA in conclusion that damage to cellular DNA speeds-up the establishment of strand continuity on damaged DNA templates. In mutagenesis studies, split-dose experiments with and without inhibitor of protein synthesis gave positive evidence of an inducible mutagenic repair in yeast (Eckardt, 1978), but negative results in V79 Chinese hamster cells (D'Ambrosio *et al.*, 1978).

In the present work, we therefore studied whether excision repair of mitomycin-C (MMC), an antineoplastic antibiotic and also an alkylating agent, inflicted DNA damage is enhanced or not by a preceding of inducing treatment of MMC in Chinese hamster ovary cells. MMC is a very effective cross-linker of DNA both *in vivo* and *in vitro* (Tomasz *et.al.*, 1974). We found that the excision repair was enhanced by the inducing treatment of MMC.

## MATERIALS AND METHODS

Monolayer cultures of Chinese hamster ovary (CHO) cells were grown at 37°C under humidified 5% $CO_2$ flow condition using Eagle's minimum essential medium (MEM; Grand Island Biological Co., Grand Island, N.Y.) supplemented with 10% fetal calf serum, penicillin G (1,000 units/ml) and streptomycin(100 $\mu$g/ml).

Exponentially growing cells grown for more than 24 hours were treated with mitomycin C (MMC;Kyowa, Tokyo), bleomycin (BLM; Nihon Kayaku), methyl methanesulfonate (MMS;Eastman Kodak) or UV-light (1.42 J/m$^2$). Chemical treatment was performed at

37°C for 30 minutes, and the cells were washed twice with phosphate buffered saline (PBS) and replaced with the fresh growth medium. ***De novo*** protein synthesis inhibitor, cycloheximide (Sigma) was treated to cells for 30 minutes prior to the treatment of the inducing dose of MMC($MMC_1$)), or the challenge dose of MMC ($MMC_2$). Protease (Sigma; 0.5 mg/ml) treatment to the harvested cells was performed in the final lysing step for an hour. The experiment involving the unscheduled DNA synthesis (UDS) were carried out as described by Cleaver and Thomas (1981) to determine the effects of MMC on inducible repair mechanism (Fig.1).

CHO cells ($10^5$ cells/ml) grown on coverslips in plastic petri dishes were exposed to MMC. The cells were washed and then labeled with $^3$H-thymidine (The Radiochemical Centre Ltd., Amersham, England 60 Ci/mM at a final concentration of 10 $\mu$Ci/ml for an hour at 37°C. $^3$H-thymidine incorporation was terminated by washing the cells with PBS containing 100 $\mu$g/ml of unlabeled thymidine (Cold-TdR) three times followed by incubation in the cold-TdR medium for 30 minutes to eliminate the nucleotide pools. Cells were fixed in glacial acetic acid: methanol (1:3) and soaked overnight in 4% perchloric acid at 4°C and then dehydrated. For autoradiography, coverslips were mounted on the slide with cells uppermost. Autoradiograms were prepared by using Kodak NTB liquid nuclear track emulsion. All slides were stained with 4% Giemsa. Silver grains over the nuclei of evenly and lightly labeled cells were counted under oil emulsion lens.

DNA single strand breaks induced by MMC were measured by alkaline elution technigue as described by Kohn *et al.* (1976). CHO cells were labeled with $^3$H-thymidine at a final concentration of 0.2 $\mu$Ci for two days. The radioactive medium was removed and the cells were washed with PBS and then exposed to $MMC_1$ or $MMC_2$. After MMC treatment, cells were incubated for an hour in fresh medium. And collected with cold PBS-Merchant solution (NaCl 150 mM, $K_2HPO_4$ 4.28 mM, $KH_2PO_4$ 0.7mM). For the alkaline elution, cell suspension was diluted with cold PBS-Merchant solution. The cells were lysed on the polyvinyl chloride filter at room temperature with 12 ml of lysing solution (0.2% sodium laurylsarcosine, 2M NaCl, 0.04M $Na_2$ EDTA, pH 10.0). The 3 ml lysing solution containing 1.5 mg protease is allowed to stand for 30 minutes in the final lysing step followed by flowing slowly through the filter without suction.

The filters were then washed with 0.02M $Na_2$EDTA (pH10.3) and eluted in the dark with a solution consisting of 0.02 M $H_4$EDTA plus tetrapropylammonium hydroxide (1% in water) added in the amount required to give the final pH 12.1–12.2. The pumping rate was about 0.05 ml/min. Fractions were collected at 60 minutes intervals. The filters were treated with 0.4 ml of 1 M HCl at 60°C for an hour followed by 2.5 ml of 0.4 M NaOH at room temperature for 30 minutes and mixed with scintillation fluid. The radioactivity of the incorporated $^3$H-thymidine were determined using a Packard Tri-Carb scintillation spectrometer. Fraction of total cell DNA counts remaining on filter was plotted against elution time.

DNA replication experiments were carried out as described by Painter (1979). Cells were grown for 24 hours in 35 mm plastic petri dishes containing 0.01 $\mu$Ci/ml $^{14}$C-thymidine (52 mCi/mM). The cells were then washed twice with serum-free medium and exposed to chemicals. After treatment with mutagen the cells were washed three times. At various times, cultures were pulse labeled with 10 $\mu$Ci/ml of $^{3}$H-thymidine (40-60 Ci/mM) for 10 minutes. After labeling, the radioactive medium was thoroughly removed and the cells were quickly washed with ice-cold 0.15 M sodium chloride-0.015 M sodium citrate (SSC) three times.

The cells were then scrapped off with rubber policeman in SSC and collected on Whatman GF/C glass filter that had been presoaked with cold 4% perchloric acid and dehydrated stepwise treatment with 75%, 95% and 100% ethanol and dried completely. The filters were placed in scintillation vials and to which were added 10 ml of the scintillation fluid. The radioactivities of the incorporated $^{3}$H-thymidine and $^{14}$C-thymidine were determined using a Packard Tri-Carb scintillation spectrometer. The $^{3}$H/$^{14}$C ratios for mutagen-treated cells were expressed as percentage of control incorporation into DNA as a function of time of incubation after the end of treatment, and were used for the calculation of the rate of DNA synthesis.

## RESULTS

The effects of MMC and BLM together with positive control MMS and UV-light on unscheduled DNA synthesis in CHO cells are shown in Fig. 1. This agent induces excision repair, which can be estimated by the amount of unscheduled DNA synthesis, i.e., the average number of grains per nucleus. The amount of unscheduled DNA synthesis induced by MMC was increased with dose increased, whereas BLM induced very little amount of unscheduled DNA synthesis.

Fig. 2 shows the effects of BLM on the rate of DNA synthesis in CHO cells at various times after treatment. Irradiation with 10 J/m$^2$ UV light to replicating CHO cells caused an inhibition of DNA synthesis. This inhibition effect was not recovered as time goes. One mM MMS caused an immediate decline of DNA synthesis and then leveled off. Five to 50 $\mu$g/ml of BLM caused minimum rates of DNA synthesis at 1.5 hours and recovered their rates but never recovered to more than 60% of control These results indicate MMC and BLM are both effective DNA damaging agents.

Fig. 3 represents the time dependence of unscheduled DNA synthesis induced by MMC. The amount of unscheduled DNA synthesis was decreased linearly with post-incubation time and reached to control level at 5 hours.

Fig. 4 represents the effect of MMC on inducible repair in CHO cells. If cells were treated with 2 $\mu$g MMC at 0 hours followed by treatment with 2 $\mu$g $MMC_2$ at 5 hours, the amount of unscheduled DNA synthesis was greater than that of unscheduled DNA synthesis

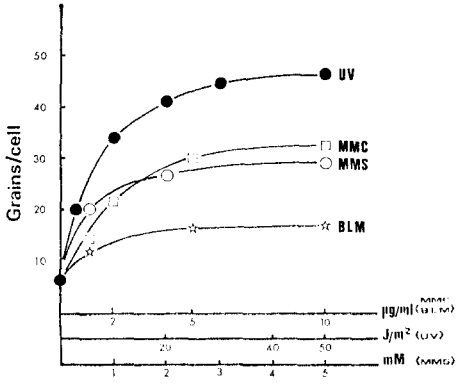


**Fig. 1.** Dose response of unscheduled DNA synshesis induced by MMC, BLM, MMS and UV-light in CHO cells.

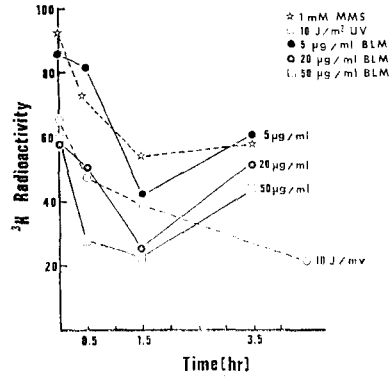


**Fig. 2.** Rates of DNA synthesis in CHO cells at various times after treatment with BLM, MMS and UV-light.

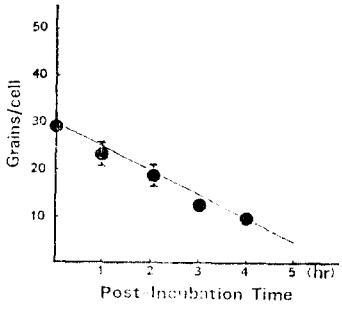


**Fig. 3**

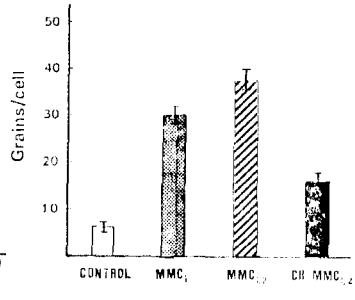


**Fig. 4.**

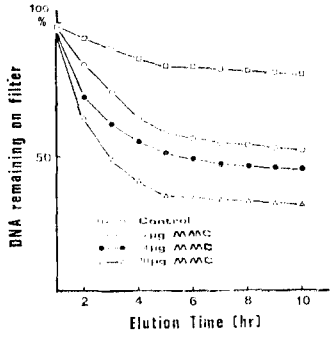


**Fig. 5.**

**Fig. 3.** Effects of post-incubation time on the unscheduled DNA synthesis induced by MMC in CHO cells.

**Fig. 4.** Effect of MMC on the inducible repair as deteed by unscheduled DNA in CH Ocells.

**Fig. 5.** Alkaline elution prfiles of CHO cells treated with various of concentrations of MMC.

from cells treated with 2 $\mu$g $MMC_1$ alone. Similar inducible repair in mammalian cells was observed after treating cells with $UV_1$ and $UV_2$ (D'Ambrosio, 1978; D'Ambrosio, 1976).

The amount of UDS in cells treated with cycloheximide was less than that from cells treated with MMC alone or both $MMC_1$ and $MMC_2$ (Fig. 4). This result suggests that *de novo* protein synthesis is required for inducible repair of MMC-damaged DNA. Incubation of cells with cycloheximide (5 $\mu$g/ml) until 30 minutes before pulse chase had no effect on the size of parental DNA (D'Ambrosio and Setlow, 1976).

Single strand breaks associated with DNA-protein crosslinks induced by various concentration of MMC in CHO cells are shown in Fig. 5. It represents the alkaline elution

profiles and increased elution of DNA single strand breaks associated with DNA-protein cross-links in MMC-treated cells. The measurement of single strand breaks was based on the relative percentage of DNA remaining on the filter at each elution time. A 30 minutes exposure to MMC caused a dose dependent decrease in the percentage of DNA remaining on filter and increased the rate of $^{3}$H-DNA elution from filter, suggesting that more single strand breaks associated with DNA-protein cross-links were induced with increasing doses of MMC. Increased elution represents the percentage of $^{3}$H-DNA eluted from filter in experimental group subtracted by that in the control. This stands for relative amount of single strand breaks in experimental groups over the control. The increased elution pattern also showed that the relative amount of single strand breaks were increased as dose of MMC were increased while the amount of UDS induced by above 2 $\mu$g MMC leveled off.

Fig. 6 shows the effect of post-incubation time in the rejoining process of single strand breaks associated with DNA-protein cross-links in cells treated with MMC. The results showed that the amount of single strand breaks associated with DNA-protein cross-links was reduced as a function of post-incubation time. The results indicate that single strand breaks associated with DNA-protein crosslinks induced by MMC were reduced as breakage sites were rejoined possibly by excision repair process. Especially, the frequency of single strand breaks in cells post-incubated for 5 hours approximately reached at that of control group. This result was exactly consistent with that of UDS experiment.

Fig. 7 shows the effect of MMC on inducible repair mechanism in CHO cells. The elution rate of DNA single strand breaks associated with DNA-protein cross-links in $MMC_1$ and $MMC_2$-treated cells is slightly lower than that in MMC lone-treated cells. These results can be interpreted that excision repair is enhanced by $MMC_1$. The elution rate of DNA single strand breaks associated with DNA-protein cross-links in cells treated

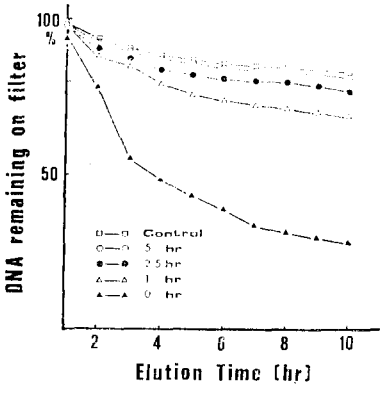


**Fig. 6.** Alkaline elution profiles of CHO cells at various times after treatment with MMC.

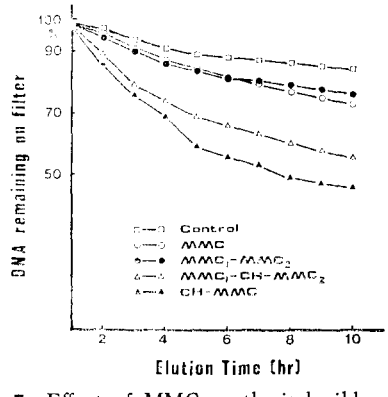


**Fig. 7.** Effect of MMC on the inducible repair as detected by alkaline elution in CHO cells.

with cycloheximide between the treatment of $MMC_1$ and $MMC_2$ is significantly higher than those in cells treated with MMC alone and than those in cells treated with $MMC_1$ and $MMc_2$, but too much lower than that in cells treated with cycloheximide prior to treatment of MMC alone. These results clearly indicate that MMC induces an inducible repair mechanism in CHO cells.

## DISCUSSION

It has been attempted to determine whether or not mammalian cells show the inducible repair mechanism by ultraviolet(UV) light, X-ray, and chemicals. In two mouse cell lines (L5178Y and 3T3), alkaline sucrose sedimentation studies showed that the DNA pulse-labeled shortly after irradiation contained gaps, and that such gaps could not be detected in DNA pulse-labeled several hours after irradiaton, despite the fact that dimers must have been present on the template strands (Lehmann, 1972). A possible explanation is that gaps were still being formed, but were filled in rapidly and were therefore not detected (Lehmann, 1972). DNA synthesized in human cells within the first hour after UV irradiation is made in segments of lower molecular weight than in nonirradiated cells (Buhl *et al.*, 1973). The rate at which small DNA was joined together into parental size in cells treated with a small dose of N-acetoxy-acetylaminofluorene (N-AAAF) or UV-light, several hours before a larger dose of N-AAAF or UV-light was appreciably greater than in absence of the small dose (D'Ambrosio *et al.*, 1976). Postreplication repair in synchronous Chinese hamster cells was enhanced by irradiation of cells in $G_2$ or S-phase with a small dose of UV irradiation at least 1.5 hrs before a three-fold larger dose of UV (D'Ambrosio *et al.*, 1978).

In the present work, we studied whether or not excision repair of MMC inflicted DNA damage is enhanced by a preceding of inducing treatment of MMC. The results of UDS experiment and alkaline elution experiment show that excision repair is enhanced.

The results of alkaline elution experiment are not exactly consistent with those of UDS experiment, but it is possible to be interpreted that excision repair is enhanced by MMC, considered the result of post-incubaion. Although the elution rate of single strand breaks associated with DNA-protein cross-links in cells post-incubated for 5 hours is slightly greater than that in control group, the higher elution rate of the $MMC_{1,2}$ treated cells than that of the MMC alone treated cells support that an inducible repair mechanism may be induced by $MMC_1$.

The interpretation of the apparent effect of an inducing dose upon the rate of daughter strand closure has been questioned by Painter (1978), who suggests that this response can be accounted for by the damaging effects of the inducing exposure. However, much of Painter's criticism was overcome by D'Ambrosio *et al.* (1978). D'Ambrosio *et al.* reported that there was significantly greater enhancement when the first dose was given in $G_2$

than when it was given in the S-phase 0.5-1.5 hours before the test dose. These data indicate that enhancement of post replication repair does not require active DNA replication and qualitatively is independent of when in the cell cycle the cells are irradiated (D' Ambrosio *et al.*, 1978).

A number of indirect studies support the idea that some SOS-inducing signals are related to DNA metabolism. The concept of an inducing signal arises from the belief that some kind of metabolic changes must initiate the chain of events that result in the SOS phenomena. The carcinogen-induced enhancement of the survival of UV-irradiated SV40, or UV- irradiated herpes virus is correlated with the inhibition of host-cell DNA synthesis, suggesting that the inhibition is an inducing agent (Sarasin and Hanawalt, 1978).

In the SOS system, it is not known what the inducing signal is, nor whether several different signals might serve this function. DNA damage results in the production of unusual species of DNA such as gapped DNA or oligonucleotides, and it is likely that these unusual molecules would activate the regulatory system. However, changes in nucleotide pools, nucleoid structure or degree of superhelicity might also result from DNA damage, and might have the same or a similar effect (Tomkins, 1975).

In *E. coli*, mutations in the SSB gene, which codes for SSB, a major singlestranded DNA-binding protein, also lead to defects in SOS induction in bacterial system. It seems likely that alterations in SSB function would affect the metabolism of inducing molecules, particularly single-stranded DNA, possibly allowing a signal molecule to be degraded when wild-type function would stabilize it instead (Lieberman and Witkin, 1981).

MMC appears every bit as reactive as typical alkylating agent, and produces the usual spectrum of products among which interstrand DNA cross-links figure only as a component (Moore, 1977; Tomasz *et al.*, 1974; Szybalski, 1964; Iyer *et al.*, 1963). The alkylating agent, MMC which blocks the replication fork to result in the gapped DNA or oligonucleotides. The single strand breaks associated DNA-protein cross-links induced by MMC are worthy of being taken into consideration as the inducing signal in mammalian cells although not clarified by molecular biological or biochemical methods. Presumably, the results of the enhanced repair in this investigation may be due to the inducible repair mechanism induced by MMC.

## ABSTRACT

Alkaline elution profiles showed that the frequency of DNA single strand breaks associated with DNA-protein crosslinks in cells treated with both an inducing dose of MMC ($MMC_1$) and a challenge dose of MMC ($MMC_2$) was slightly less than that in cells treated with MMC alone. The amount of unscheduled DNA synthesis in cells treated with both $MMC_1$ and $MMC_2$ was greater than that in cells treated with MMC alone. This enhancement of excision repair detected by UDS autoradiography and alkaline elution,

was not observed, when cells were incubated with cyclohexmide between the two treatments of $MMC_1$ and $MMC_2$.

These results suggest that MMC-damaged DNA from Chinses hamster cells is repaired by excision repair mechanisms that require *de novo* protein synthesis for enhancement, and that an inducible repair mechanism may exist in CHO cells.

## REFERENCES

Buhl, S.N., R.B. Setlow, and T.D. Regan, 1973. Recovery of the ability to synthesize DNA in segments of normal size at long times after ultraviolet irradiation of human cells. *Biophysical J.* **13**: 1265-1275.

Cleaver, J.E., and G.H. Thomas, 1981. Measurement of unscheduled DNA synthesis by autoradiography (E.C. Friedberg and P.H. Hanawalt, eds), DNA repair; In: A Laboratory Manual of Research Procedures. Vol. I, Part B. Marcel Dekker, N.Y., pp. 227-287.

Craig, N.L., and J.W. Roberts, 1980. *E. Coli rec* A protein directed cleavage of phage λ repressor requires polynucleotide. *Nature* **283**:26-30.

D'Ambrosio, S.M., and R.B. Setlow, 1976. Enhancement of postreplication repair in Chinese hamster cells. *Proc. Natl. Acad. Sci.* USA **73**:2396-2400.

D'Ambrosio, S.M., and R.B. Setlow, 1978. Defective and enhanced postreplication repair in classical and variant Xeroderma Pigmentosum cells treated with N-Acetoxy-2-Acetylaminofluorene. *Cancer Res.* **38**:1147-1153.

D'Ambrosio, S.M., P.M. Aebersold, and R.B. Setlow, 1978. Enhancement of postreplication repair in ultraviolet irradiated Chinese hamster cells by irradiation in $G_2$ or S-phase. *Biophysical J.* **23**: 71-78.

DasGupta, U.B., and W.C. Summers, 1978. Ultraviolet reactivation of herpes simplex virus is mutagenic and inducible in mammalian cells. *Proc. Natl. Acad. Sci,* USA **75**:2378-2381.

Eckart, F., E. Moustacchi, and R.H. Hay, 1978. On the inducibility of error-prone repair in yeast: In DNA Repair Mechanism. (P.C. Hanawalt, E.C. Friedberg, C.F. Fox editors). Academic Press, ICN-UCLA Symposia on Molecular and Cellular Biology Vol. IX: 421-423.

Hanawalt, P.C., P.K. Cooper, A.K. Ganesan, and C.A. Smith, 1979. DNA Repair. *Annu. Rev. Biochem.* **48**:783-836.

Iyer, V.N., and W. Szybalski, 1963. A molecular mechanism of mitomycin action: Linking of complementary DNA strands. *Microbiology* **50**:355-362.

Kohn, K.W., L.C. Erickson, R.A.G. Ewig, and A. Friedman, 1976. Fraction of DNA from mammalian cells by alkaline elution. *Biochemistry* **15**:4627-4636.

Lehman, A.R., 1972. Postreplication repair of DNA in ultraviolet-irradiated mammalian cells: No gaps in DNA synthesized late after ultraviolet irradiation. *Eur. J. Biochem.* **31**:438-445.

Lieberman, H.B., and E.M. Witkin, 1981. Variable expression of the ssb-1 allele in different strains of *Escherichia coli* K-12 and B: Differential expression of its effects on DNA replication, DNA repair and ultraviolet mutagenesis. *Mol. Gen. Genet.* **183**:348-355.

Little, J.W., and J.E. Harper, 1979. Identification of the *lex* A gene product of *Escherichia coli*

K-12. *Proc. Natl. Acad. Sci.* USA **76**:6147-6151.

Moore, H.W., 1977. Bioactivation as a model for drug design bioreductive alkation. *Science* **187**: 527-532.

Painter, R.B., 1978. Does ultraviolet light enhance postreplication repair in mammalian cells? *Nature* **275**:243-245.

Painter, R.B., 1979. DNA synthesis inhibition in mammalian cells as a test for mutagenic carcinogens. Workshop on Short Term Test for Chemical Carcinogens. 1979 in Vancouver, B.C.

Radman, M., 1980. Is there SOS induction in mammalian cells? *Photochem. Photobiol.* **32**:823-830.

Sarasin, A.R., and P.C. Hanawalt, 1978. Carcinogens enhance survival of UV-irradiated simian virus 40 in treated monkey kidney cells: Induction of a recovery pathway? *Proc. Natl. Acad. Sci.* USA **75**:346-350.

Sarasin, A., and A. Benoit, 1980. Induction of an error-prone mode of DNA repair in UV-irradiated monkey kidney cells. *Mut. Res.* **70**:71-81.

Szybalski, W., 1964. Mitomycins and porfiromycin: Chemical mechanism of activation and cross-linking of DNA. *Science* **15**:55-58.

Tomasz, M., C.M. Mercado, J. Olson, and N. Chatterjie, 1974. The mode of interaction of mitomycin C with deoxyribonucleic acid and other polynucleotides *in vitro*. *Biochemistry* **13**:4878-4887.

Tomkins, G.M., 1975. The metabolic code. *Science* **189**:760-763.

Witkin, E.M., 1676. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. *Bacteriol. Rev.* **40**:869-707.