KOREAN JOURNAL OF ZOOLOGY
Vol. 18, No. 1, 41—49 (1975)

Gamma-ray Induced DNA Repair Synthesis in Relation to Chromosome Exchanges in Mammalian Cells in Vitro*

Sang Dai Park
(Dept. of Zoology, Seoul National University)

哺乳動物細胞에 있어 감마線에 의한 DNA 回復合成과 染色體交換과의 聯關性

朴 相 大
(서울大 自然大 動物學科)

(Received October 20, 1974)

摘 要

DNA 回復合成과 染色體交換과의 聯關性을 추구하기 위해 감마線을 照射한 BHK-21과 KB 細胞의 DNA 回復合成의 線量反應과 時期를 調査하였다.

감마 線에 의한 DNA 回復合成率은 5 kR까지 照射線量에 比例하나 그후 50 kR까지는 變化가 없었다. DNA 回復合成의 初期 線量反應은 細胞에 따라 다르나 照射후 1~2時間까지 지속하였다. 감마線에 의한 染色體交換은 細胞에 따라 다른 感受性을 보였고 DNA 回復合成과의 聯關性을 보여주지 않았다.

INTRODUCTION

The possible involvement of primary damage induced in DNA and of its repair processes as the cause of chromosome aberrations has been a subject of repeated discussion (Scott *et al.*, 1974). Data so far presented for or against this relationship are still scanty and highly speculative. However, recent studies seem to suggest that there is no apparent relationship between the DNA repair synthesis and the chromosome aberrations in irradiated mammalian system (Park, 1972, Cleaver and Wolff, 1973 ; Painter and Wolff, 1973 ; Cleaver, 1974 ; Scott *et al.*, 1974).

The data reported here also strongly indicate that DNA repair synthesis seems not to be related to chromosome exchanges in mammalian cells irradiated with γ-rays.

* This work was supported, in part, by the Ministry of Education Research Grant for 1973.

MATERIALS AND METHODS

Two established mammalian cell lines, BHK-21 (Syrian hamster kidney) and KB (human oral carcinoma) were used for this study. Monolayer cultures of these cell lines were grown on cover glasses (22×29mm) in 5 cm plastic petri dishes (Millipore Corp.) using Eagle's MEM supplemented with 10% fetal calf serum for 48 hours at 37°C.

The experiments involving DNA repair synthesis were carried out two distinct procedures of unscheduled DNA synthesis, dose response and time dependence. For the determination of dose response of unscheduled DNA synthesis, cultures were γ-irradiated with gradual exposures from 0 to 50 kR using Gammator B (Radiation Machinery Co., 400 Ci ^{137}CsCl, 583R/min). The cultures were then incorporated with ^{3}H-thymidine (Amersham/Seale Corp., specific act., 20 Ci/mM) at a final concentration of 10 μCi/ml for an hour. For the time dependence experiments cultures irradiated with 2.5 kR of γ-rays were labeled with ^{3}H-thymidine and then incubated in the radioactive medium up to 4 hours. ^{3}H-thymidine labeling was terminated by washing the cultures in cold BSS containing unlabeled thymidine. Autoradiograms were prepared using stripping film (Kodak AR-10), as described previously (Kang and Park, 1969). Silver grains were counted over the nuclei of the lightly labeled cells, and any cell with 50 grains above background was considered as a DNA repair synthesizing cell.

For the determination of chromosome exchanges the cultures were irradiated from 0 to 500 R. Immediately following the irradiation, the irradiated media were changed with fresh media and the cultures were incubated for additional 24 hours to obtain the exchange type of aberrations. Colcemide was treated during the final 4 hours of incubation at a final concentration of 0.06 μg/ml. Cells were harvested using trypsin-EDTA and chromosome preparations were made by the air-drying technique. The exchange type of aberrations was scored according to the criteria of Kihlman (1971).

RESULTS

The dose response of the labeling index, labeling pattern and the average number of grains per cell in the lightly labeled cells in BHK-21 and KB cell lines irradiated with gradual exposures from 0 to 50 kR of γ-rays is shown in Table 1. The labeling indices were increased in direct proportion to the irradiated doses. The labeling pattern indicated that the increased labeling indices were mainly due to increases of lightly labeled cells. The heavily labeled cells were not significantly changed except at higher doses which showed a slight decrease in percentage.

The dose response of DNA repair synthesis represented as average grain counts over the lightly labeled cells was increased and directly proportional to dose employed, but reached almost a plateau after 5 kR (Fig. 1). The relative amount of DNA repair synthesis was found to be higher in BHK-21 than in KB cells.

Table 1. Dose response of unscheduled DNA synthesis in BHK-21 and KB cell lines fixed immediately following irradiation with various doses of γ-rays and ^{3}H-thymidine labeling.

Dose (R)	Cell line[c]	Labeling index (%)	Labeling pattern (% ± S.E.)[a]			Average grains/cell (mean ± S.E.)
			Unlabeled	Heavily labeled (S)	Lightly labeled (UDS)[b]	
Control	1	39.8	60.2±4.5	38.2±3.6	1.6±0.7	5.7±2.3
	2	20.1	79.9±5.2	19.1±2.5	1.0±0.6	5.5±2.3
500	1	50.2	49.8±4.1	39.0±3.6	11.2±1.9	13.5±3.7
	2	27.5	72.5±4.9	18.2±2.5	9.3±1.8	10.2±3.2
1,000	1	58.3	41.7±3.7	37.8±3.5	20.5±2.6	15.2±3.9
	2	35.6	64.4±4.6	18.0±2.4	17.6±2.4	13.8±3.7
2,500	1	68.3	31.7±3.3	36.0±3.5	32.3±3.3	25.6±5.0
	2	40.2	59.8±4.5	17.7±2.4	22.5±2.7	23.2±4.9
5,000	1	72.7	27.3±3.0	35.1±3.4	37.6±3.5	27.5±5.2
	2	43.5	56.5±4.3	18.8±2.5	24.7±2.9	25.4±5.0
10,000	1	85.9	14.1±2.2	30.4±3.2	55.5±4.3	33.2±5.8
	2	50.8	49.2±4.0	16.1±4.0	16.1±2.3	34.7±3.4
50,000	1	89.4	10.6±1.9	25.2±2.9	64.2±4.6	37.5±6.1
	2	57.2	42.8±3.8	12.9±2.1	45.3±3.9	30.5±5.5

a: Based on 1,000 cells analyzed.
b: Lightly labeled cells were considered as unscheduled DNA synthesizing cells which have less than 50 grains above background in the nuclei.
c: 1, BHK−21 ; 2, KB cell line.

Table 2 represents the time dependence of DNA repair synthesis carried out at a constant exposures of 2.5 kR of γ-rays and kept in a labeled medium for various time intervals. The labeling indices were increased with time. The labeling pattern showed that the percentage of heavily labeled cells sharply decreased at 0 and 1 hour after irradiation, but it gradually increased after 2 hours, whereas the lightly labeled cells increased sharply at 1 hour but

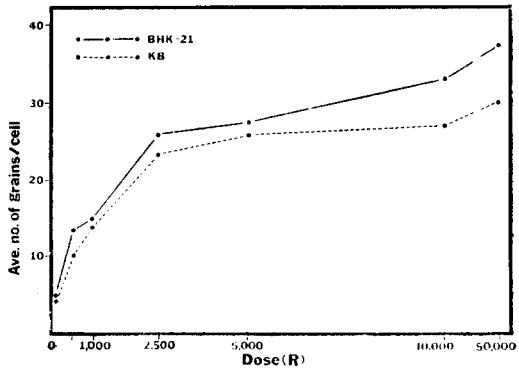

Fig. 1. Dose response of unscheduled DNA synthesis in BHK-21 and KB cell lines after various doses of γ-rays.

Table 2. Time depence of unscheduled DNA synthesis in BHK-21 and KB cell lines fixed at various time intervals following irradiation with 2.5 kR of γ-rays and ^{3}H-thymidine labeling.

Time after irradiation (h)	Cell line	Labeling index (%)	Labeling pattern (%±S.E.)			Average grains/cell (mean±S.E.)
			Unlabeled	Heavily labeled (S)	Lightly labeled (UDS)	
0*	1	39.8	60.2±4.5	38.2±3.6	1.6±0.7	5.7±2.3
(Control)	2	20.1	79.9±5.2	19.1±2.5	1.0±0.6	5.5±2.3
0	1	68.3	31.7±3.3	36.0±3.5	32.3±3.3	20.6±5.0
	2	40.2	59.8±4.5	17.7±2.4	22.5±2.7	13.2±4.9
1	1	80.2	19.8±2.6	34.9±3.4	45.3±3.9	29.5±5.4
	2	50.2	49.8±4.1	14.5±2.2	35.7±3.4	25.7±5.1
2	1	88.4	11.6±2.0	41.0±3.7	47.4±4.0	31.2±5.6
	2	58.7	41.3±3.7	21.8±2.7	36.9±3.5	26.2±5.1
3	1	92.5	7.5±1.6	44.5±3.9	48.0±4.0	31.6±5.6
	2	66.8	33.2±3.3	29.6±3.1	37.2±3.5	26.8±5.2
4	1	96.8	3.2±1.0	48.5±4.0	48.3±4.0	32.0±5.7
	2	72.7	27.3±3.0	34.9±3.4	37.8±3.5	27.0±5.2

*: Not irradiated.

reached a plateau soon after. The increased number of grains was found to be proportional to the increase in the percentage of lightly labeled cells.

The overall results suggest that γ-rays is more productive than X-rays for inducing DNA repair synthesis (Park, 1972), and that DNA repair synthesis induced by γ-rays is also dose dependent and cell line specific, and it continues up to 1~2 hours after irradiation.

Table 3. Frequency of chromosome aberrations in BHK-21 and KB cell lines fixed 24 hours following irradiation with various doses of γ-rays.

Dose (R)	Cell line	Total cells scored in mitosis	Normal metaphases (%)	Type of aberration (%±S.E.)				Breaks /cell	Breaks /cell/R
				Chromatid type		Chrome type			
				Deletions	Exchanges	Deletions	Exchanges		
Control	1	94	94.7	2.1±1.8	—	—	3.2±1.8	0.085	—
	2	100	95.0	6.0±2.4	—	—	—	0.060	—
25	1	106	79.7	3.7±1.8	1.5±1.1	2.8±1.6	4.9±2.1	0.166	0.0066
	2	125	75.6	4.0±1.8	—	2.2±1.3	5.4±2.1	0.136	0.0054
50	1	117	63.9	5.0±2.1	2.0±1.3	5.4±2.1	5.9±2.2	0.244	0.0045
	2	115	60.4	3.7±1.8	1.7±1.2	4.2±1.9	8.7±2.8	0.250	0.0050
100	1	124	49.9	3.2±1.6	3.0±1.6	10.5±2.9	13.6±3.3	0.378	0.0038
	2	117	45.2	2.0±1.0	2.0±1.4	9.3±2.8	15.7±3.7	0.399	0.0040
250	1	98	35.7	2.4±1.6	2.0±1.4	26.0±5.2	65.0±8.2	1.673	0.0067
	2	102	30.1	5.0±2.2	1.5±1.2	28.0±5.2	79.6±8.8	1.914	0.0077

Table 4. The rate of chromosome exchanges in BHK-21 and KB cell lines fixed 25 hours following irradiation with various doses of γ-rays.

Dose (R)	Cell line	Total cells scored in mitosis	Total exchanages (%±S.E.)	Exchanges[a] /cell	Exchanges /cell/R
Control	1	94	3.2±1.8	0.034	—
	2	100	—	—	—
25	1	116	6.4±2.3	0.021	0.0008
	2	125	5.4±2.1	0.043	0.0017
50	1	117	7.9±2.6	0.034	0.0007
	2	115	10.4±3.0	0.090	0.0018
100	1	124	16.6±3.7	0.100	0.0010
	2	117	17.7±3.9	0.151	0.0015
250	1	98	67.8±8.3	0.658	0.0026
	2	102	81.1±8.9	0.795	0.0032

a: Exchanges/cell=2 X (total exchanges—spontaneous exchanges)/total metaphases scored.

Table 5. Relationship between unscheduled DNA synthesis, modal chromosome number and chromosome exchanges in γ-irradiated BHK-21 and KB cells.

Cell line	Unscheduled DNA synthesis		Modal chromosome number	Chromosome[c] exchanges (exch./cell/R)
	Lightly[a] labeled cells (%±S.E.)	Average grains /cell (mean±S.E.)		
BHK-21	36.9±3.5	25.4±1.0	44	0.0013
KB	25.7±2.9	21.7±1.7	77	0.0021

a: Average percentage of lightly labeled cells irradiated with 500 R-50 kR of γ-rays (%±S.E.).
b: Average grains/cell in the lightly labeled cells irradiated with 500 R-50 kR of γ-rays (mean±S.E.).
c: Exchanges/cell/R in cells irradiated with 25-250 R of γ-rays.

Table 3 indicates the frequency of chromosome aberrations in these two cell lines irradiated from 0 to 500 R of γ-rays. As expected, the type of aberrations was mostly of the chromosome type. The rate of chromosome aberration was proportional to the dose. The increase of aberration rate was prominent in the chromosome type aberrations. In all dose ranges KB cells were shown to be higher in aberration rate than BHK-21. The rate of chromosome exchanges (Table 4) was also higher in the KB than in BHK-21 cells at all dose levels.

Table 5 represents the summary of this study. As in the previous X-irradiated experiments (Park, 1972), γ-ray induced DNA repair synthesis does not show a

correlation with chromosome exchanges. These data support the X-irradiation results and a parallel relationship between the results of the two experiments. The relatively higher rates of labeling indices and lightly labeled cells may be due to higher dose rate of γ-rays and higher concentration and specific activity of ^{3}H-thymidine.

DISCUSSION

The dose response of DNA repair synthesis induced by ionizing radiation was first described by Hill (1967). He found that unscheduled DNA synthesis in mouse L cells was increased up to 94% after 4 kR of X-rays but the rate was decreased after 8 kR. Spiegler and Norman (1970) reported that the rate of ^{3}H-thymidine incorporation into human lympocytes was dose dependent in 600~2400 rads. Brent and Wheatley (1971) reported in HeLa cells that dose response of repair replication was linearly proportional up to 5 kR and above this to 10 kR the extent of repair replication did not increase but appeared to decrease. Shaeffer and Merz (1972) reported that quantitatively different dose responses for unscheduled DNA synthesis were found in eight different mammalian cells irradiated from 0 to 10 kR. Data concerned with γ-ray induced DNA repair synthesis are very scarce since only two reports have so far appeared. Richold and Arlett (1972) have failed to detect repair replication and unscheduled DNA synthesis in Chinese hamster and human cell lines irradiated with 600~1500 rads of ^{60}Co source γ-rays. Clarkson and Evans (1972), however, reported that unscheduled DNA synthesis was demonstrated in human lymphocytes following 5 k rads of ^{60}Co γ-rays. The failure of Richold and Arlett (1972) might have been due to the lower doses of γ-rays and lower concentration of ^{3}H-thymidine. The present results support the data of Clarkson and Evans (1972) and the dose response is similar to that of X-ray induced DNA repair synthesis.

Studies on the time dependence of DNA repair synthesis are used as a means of determining the repair processes of induced DNA damage. Hill (1967) reported that the peak of unscheduled DNA synthesis induced by X-rays was reached in 1.5 hours. It then declined but continued for as long as 5 hours after irradiation. Spiegler and Norman (1970) reported that the kinetics of unscheduled DNA synthesis had two processes; a fast one, essentially completed within an hour and slow one, persisting for at least 7 hours. Brent and Wheatley (1971) found that the time dependence of repair replication was maximum immediately after irradiation and was largely completed within two hours. Clarkson and Evans (1972) reported that the rate of ^{3}H-thymidine incorporation in human lymphocytes irradiated with γ-rays was the highest during the first hour and then the rates decreased but

continued for as long as 21 hours after irradiation. The present results are in good accord with the fast processes of human lymphocytes (Spiegler and Norman, 1970) and with those of other published data. It was suggested that the initial fast reaction after ionizing radiation might involve the incorporation of only one or two nucleotides per lesions (Clarkson and Evans, 1972). From the point of view, it is tempting to conclude that the fast reaction processes might be involved in the rejoining of single strand breaks in the DNA, and that the slow processes may be associated with damage other than single strand breaks. Cleaver (1974) also suggested that ionizing radiations and alkylating agents may produce non-enzymatic breakage of DNA strands and that the repair of the breaks presumably may bypass the initial enzymatic step required for the repair of UV-induced base damage.

Ever since the unschedueld DNA synthesis and/or repair replication was reported by Rasmussen and Painter (1966), a number of investigators have been attempting to correlate this phenomenon with other cellular and subcellular recovery processes. With regard to the relationship between DNA repair synthesis and chromosome exchanges, there have been two divergent hypotheses so far proposed. The first affirmative postulation is that since DNA is the key substance in chromosome breakage and rejoining, essentially the same biological mechanisms are involved in the formation of chromosome aberration and in repair replication (Kihlman, 1971 ; Bender *et al.*, 1974). The second idea is that since the structural configuration of a chromosome is different from that of the intact DNA molecules, the induced ch omosome breaks and rejoining of chromosome aberrations are the independent ability to repair damaged DNA (Wolff and Scott, 1969 ; Scott *et al.*, 1974). The data presented in this study strongly indicate that DNA repair synthesis seems not to be related to chromosome exchanges in mammalian cells. There are several reasons why a relationship between DNA repair synthesis and chromosome aberrations does not seem to be likely. The major points are as follows; (1) Damage to DNA alone would have minimal effects of the overall chromosomal structure, whereas damage to the protein would result in disruption of the chromosome, and breaks in DNA would then be a secondary consequence of the protein damage. Protein damage would not initially involve loss of genetic information. Therefore repair could not be affected through normal protein synthetic pathways, which are active at all stages of the cell cycle. DNA damage, however, must be repaired in such a way as to preserve the genetic information. The repair of DNA, therefore, involves novel pathways such as unscheduled DNA synthesis and/or repair replication, which are distinct from normal DNA synthesis (Cleaver, 1974). (2) Root tip cells of *Vicia faba*, rat kangaroo kidney (ptKl) and fibroblast of Xeroderma pigmentosum patients, which are deficient in unscheduled DNA synthesis,

have functional chromosome rejoining processes in these cells following irradiation with X-rays and treatment with chemicals (Wolff and Scott, 1969; Shaeffer *et al.*, 1971; Wolff and Cleaver, 1973; Painter and Wolff, 1973). (3) Cycloheximide, 5'-fluorodeoxyuridine and hydroxyurea, which produce chromosome damage by preventing repair, do not inhibit unscheduled DNA synthesis (Sawada and Okada, 1970; Gautschi *et al.*, 1973).

The speculations or the results of the other studies mentioned above are strongly supported by the data presented here which show that DNA repair synthesis is not related to chromosome exchanges.

SUMMARY

Dose response and time dependence of DNA repair synthesis were investigated to determine the possible relationship between DNA repair synthesis and chromosome exchanges in γ-ray irradiated BHK-21 and KB cell lines.

DNA repair synthesis induced by γ-rays was dose dependent up to 5 kR, then leveling off occurred until 50 kR was reached. Time dependence of DNA repair synthesis was continued for up to 1~2 hours after irradiation although the initial dose responses were cell line specific. Chromosome exchanges induced by γ-rays showed different radiosensitivities in these cell lines and did not show a correlation with the DNA repair synthesis.

REFERENCES

Bender, M.A., H.G. Griggs, and J. S. Bedford, 1974. Mechanism of chromosomal aberration production. III. Chemicals and ionizing radiation. *Mutation Res.* **23** : 197—212.

Brent, T.P., and G.A. Wheatley, 1971. Repair and replication of X-irradiated HeLa cell DNA. *Int. J. Radiat. Biol.* **19** : 339—348.

Clarkson, J.M., and H.J. Evans, 1972. Unscheduled DNA synthesis in human leucocytes after exposure to UV light, *r*-rays and chemical mutagens. *Mutation Res.* **14** : 4430.

Cleaver, J.E..1974 a. Repair processes for photochemical damages in mammalian cell *In:* Advances in Radiation Biology (J.T. Lett, H. Adler, and M. Zelle eds.). Academic Press, Inc., New York **4** : 20—56.

Gautschi, J.R., B.R. Young, and F.E. Cleaver, 1973. Repair of damaged DNA in the absence of protein synthesis in mammalian cells. *Exptl. Cell. Res.* **76** : 87—94.

Hill, M., 1967. Non-S-phase incorporation of ^{3}H-thymidine into DNA of irradiated mammalian cells. *Int. J. Radiat. Biol.* **13** : 199—203.

Kang, Y.S. and S.D. Park, 1969. Studies on stage radiosensitivity and DNA synthesis of chromosomes in cultured human cells. *Radiation Res.* **37** : 371—380.

Kihlman, B.A., 1971. Molecular mechanism of chromosome breakage and rejoining.

In: Adviances in Cell and Molecular Biology (E.J. Dupraw, editor). Academic Press Inc., NewYork, **1** : 59—107.

Painter, R.B., and S. Wolff., 1973. Apparent absence of repair replication in *Vicia faba* after X-irradiation. *Mutation Res.* **19** : 133—137.

Park, S.D., 1972. X-ray induced unscheduled DNA synthesis in relation to chromosome exchange and mitotic activity in established mammalian cells. *Korean. J. Zool.* **15** : 95—100.

Rasmussen, R.E., and R.B. Painter, 1964. Evidence for repair of ultraviolet damaged deoxyribonucleic acid in cultured mammalian cells. *Nature* **203** : 1360—1362.

Richold, M., and C.F. Arlett, 1972. Repair replication and unschduled DNA synthesis in synchronous mammalian cells. *Int. J. Radiat. Biol. Biol.* **21** : 127—136.

Sawada, S., and S. Okada, 1970. Rejoining of single-strand breaks of DNA in cultured mammalian cells. *Radiation Res.* **41** : 145—162.

Scott., D., M. Fox, and B.W. Fox, 1974. The relationship between chromosomal aberrations, survival and DNA repair in tumor cell lines of differential sensitivity to X-rays and sulphur mustard. *Mutation Res.* **22** : 207—221.

Shaeffer, J., and T. Merz, 1972. Unscheduled DNA synthesis and cellular recovery in various mammalian cell lines. *Johns Hopkins Med. J. Suppl.* **1** : 174—181.

Shaeffer, J., R.A. Vincent, Jr., and T. Merz, 1971. Chromosomal rejoining in the absence of unscheduled DNA synthesis. *Mutation Res.* **11** : 256—257.

Spiegler, P., and A. Norman, 1970. Temperature dependence of unscheduled DNA synthesis in human lymphocytes. *Radiation Res.* **43** : 187—195.

Wolff, S. and J.C. Cleaver, 1973. Absence of DNA repair replication after chemical mutagen damage in *Vicia faba. Mutation Res.* **20** : 71—76.

Wolff, S., and D. Scott, 1969. Repair of radiation-induced damage to chromosomes. *Exptl. Cell Res.* **55** : 9—16.