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X-ray Induced Unscheduled DNA Synthesis in Relation to Chromosome Exchange and Mitotic Activity in Established Mammalian Cells

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哺乳動物細胞에 있어서 X-線에 의한 回復複製와 染色體交換 및 分裂活動과의 聯關性

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摘 要

回復複製(unscheduled DNA synthesis or repair replication)와 染色體交換 및 分裂活動과의 相關關係를 추구하기 위해 X-線을 處理한 4種의 哺乳動物 細胞를 材料로 回復複製 時期와 線量反應을 調査하였다.

回復複製는 調査한 4種의 細胞에서 모두 일어나고 그 率은 照射線量에 比例하며 細胞 種類에 따라 差가 난다. 그러나 回復複製가 일어나는 時期는 細胞 種類에 관계없이 X-線 照射후 2時間까지 지속되고 있다.

또 回復複製의 相對量은 細胞의 種族染色體數, 分裂活動 및 染色體交換率과 직접적인 관련이 없다. 分裂活動能力과 染色體交換은 다 線量에 比例하나 그 率은 逆比例 관계를 나타낸다.

INTRODUCTION

Unscheduled DNA synthesis, defined as the radiation-stimulated incorporation of labeled nucleotides in the DNA of non-S cells, has been a topic of much current research, since it has been equated with the repair replication and has been suggested as one of repair processes of radiation induced DNA damage (Shaeffer and Merz, 1971).

Some investigators have postulated that chromosome repair (restitution) and misrepair (exchange) occur by a process analogous to repair replication (Evans, 1966; Kihlman, 1971). Cleaver (1969), however, has reported that hydroxyurea and 5-fluorodeoxyuridine, which are supposed to enhance chromosome

damage by preventing repair, do not inhibit unscheduled DNA synthesis. Wolff and Scott (1969) have subsequently shown that rejoining of X-ray or UV-induced chromosome breakage is independent of the ability to repair DNA. Shaeffer *et al.* (1971) have also observed that rat kangaroo PtK_1 cell line which is deficient in unscheduled DNA synthesis, has shown to have a functional chromosome rejoining process. Shaeffer and Merz (1971, 1972) have recently reported that cell lines containing more chromosomes showed greater amounts of unscheduled DNA synthesis.

Thus the relation of unscheduled DNA synthesis to cell sensitivity and restitution of chromosome aberration induced by radiation remains still open to question.

The purpose of this study is to determine if any correlation exists between unscheduled DNA synthesis, modal chromosome number, chromosome exchange and mitotic activity to characterize the unscheduled DNA synthesis in mammalian cell strains.

MATERIALS AND METHODS

Materials used in this study were four established mammalian cell strains derived from normal (BHK-21 and BSC_1) and neoplastic (HeLa S_3 and KB) origin.

For the dose response experiment of unscheduled DNA synthesis, cultures were labeled with ^{3}H-thymidine (Amersham/Searle, sp. act. 27 mCi/mM) at a concentration of 5 μCi/ml for an hour. Immediately after the initiation of pulse labeling, cultures were X-irradiated with graded exposures from 0 to 10 kR (250 KVP, 15 mA and 345 R/min.). For the time dependence experiment, labeled cultures were irradiated with 2,000 R of X-rays and kept in radioactive media for 0 to 4 hours. ^{3}H-thymidine pulses were terminated by washing the cultures in cold BSS containing unlabeled thymidine. Autoradiographs were prepared by applying stripping films (Kodak AR-10) and exposed for two weeks and developed. To minimize the possibility of scoring moderately labeled S-stage cells as non-S cells, any cell with 5~25 grains was considered as unscheduled DNA synthesized cells.

For the determination of chromosome exchange and mitotic activity, cultures were irradiated from 0 to 368 R. Immediately after the irradiation, the irradiated media were changed with fresh media containing colcemid (0.06 μg/ml) and the cultures were returned to incubator and kept for two and half hours. Chromosome preparations were prepared by air drying technique described previously (Kang and Park, 1969). Chromosome exchanges and mitotic activities were scored in the same preparations.

RESULTS

Table 1 shows modal chromosome number, range of chromosome distribution and rate of spontaneous aberration.

Among four cell strains, the highest modal chromosome number is shown in BSC_1, followed by KB and HeLa S_3, and BHK-21 shows the lowest. BHK-21 represents the most stable strain, with respect of the distribution of chromosome number. The exchange type of aberration was observed in BHK-21 and BSC_1. It is assumed that the occurrence of exchange type aberration represents one of characteristics in these strains. The higher aberration rate in BSC_1 is possibly

due to the exchange type aberration. No any correlation between the chromosome number and the rate of spontaneous aberration could be found.

Table 1. Modal chromosome number, chromosome distribution and spontaneous chromosome aberration*

Cell strain	Modal chromosome number (%)	Chromosome distribution	Aberration/cell
BHK-21	44(58.7)	37- 75	0.085
BSC_1	106(11.4)	57-133	0.111
HeLa S_3**	64(42.7)	36- 92	0.081
KB	77(51.3)	43-138	0.060

*At least 100 metaphases were analyzed in each case.
**A derivative of original HeLa S_3.

The dose response of unscheduled DNA synthesis is shown in Figure 1. As shown in the figure, unscheduled DNA synthesis occurred in all strains studied. The highest values(%) at 10 kR irradiated group in BHK-21, BSC_1, HeLa S_3 and KB were 42.5, 32.9, 30.9 and 30.0 of total labeled cells, respectively. These values show almost 1/4-2/5 of all DNA synthesized cells. The relative amounts of unscheduled DNA synthesis represent that BHK-21 shows the highest (23.4), followed by HeLa S_3 (22.8) and BSC_1 (20.7), and KB shows the lowest (18.3). Dose response of unscheduled DNA synthesis seems to be dose dependent and strain-specific. Only HeLa S_3 shows a saturation after 4,000 R at the level of 30%. Other three strains show an increase as dose increases until 10 kR. Thus the amount of repair process is directly proportional to dose irradiated. However, the overall range of unscheduled DNA synthesis is not so wide in these cell strains.

Figure 2 shows the time dependence of unscheduled DNA synthesis performed at constant exposure. Although the initial rate is markedly different in different strains (BHK-21, 40.3; BSC_1, 12.7; HeLa S_3, 18.3; KB, 14.7), the unscheduled DNA synthesis is completed within 2 hours, regardless of

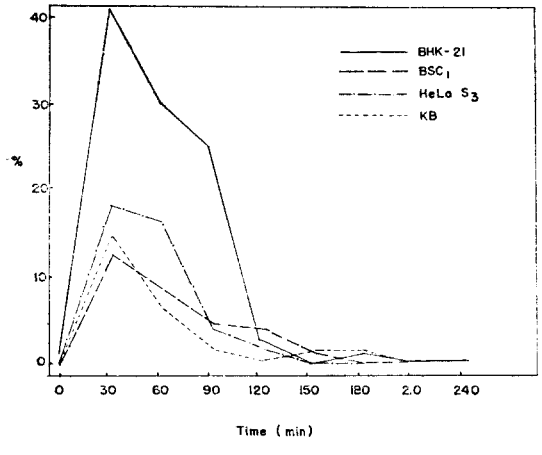

Fig. 1. Dose response of unscheduled DNA synthesis in four established mammalian cell strains irradiated with various doses of X-rays. The rate of unscheduled DNA synthesis is expressed by percentage of cells with 5-25 grains in 300 labeled cells analyzed.

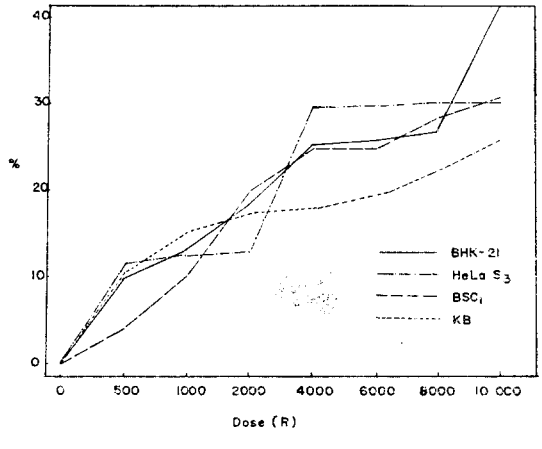

Fig. 2. Time dependence of unscheduled DNA synthesis in four established mammalian cell strains fixed at various time intervals after irradiation of 2,000 R of X-rays. Percentage represents the rate of unscheduled DNA synthesis in 300 labeled cells analyzed.

cell strains. Thus, it is assumed that though the rate of unscheduled DNA synthesis is different in different strains, the repair mechanism of damaged DNA is involved in the same enzymatic processes.

Figure 3 represents the rate of exchange type chromosome aberration. The highest rate is shown in KB, followed by HeLa S_3 and BSC_1, and BHK shows the lowest.

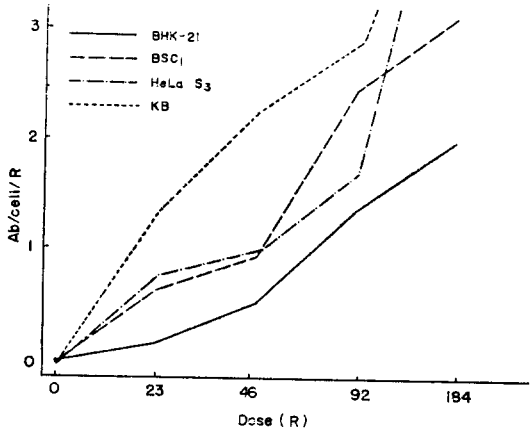

Fig. 3. Rate of chromosome exchange in four established mammalian cell strains irradiated with various doses of X-rays. Exchange rate is expressed by total chromosome exchange/cell/R in 100 metaphases.

Table 2. Relationship between unscheduled DNA synthesis, modal chromosome number, chromosome exchange, and mitotic activity

Cell strains	Total unscheduled DNA synthesis	Modal chromosome number	Total exchange rate	Relative mitotic activity
BHK-21	164.3	44	0.79	10.3
BSC_1	144.7	106	1.69	9.6
HeLa S_3	159.7	64	1.87	8.6
KB	127.9	77	3.83	7.0

These data are in good agreement with those of spontaneous aberration. The exchange rate and the relative radiosensitivity are different in different cell strains, and are directly proportional to doses irradiated.

Figure 4 shows the mitotic activity. The mitotic indices are decreased as to increases of irradiated dose. At 23 R the values were markedly decreased (1/2-1/3 times of control value), at 184 R almost same in all strains and above 368 R the mitotic indices were negligible. The relative mitotic index indicates the highest value in BHK-21 (10.3), followed by BSC_1 (9.6) and HeLa S_3 (8.6), and KB the lowest (7.0). These data are in

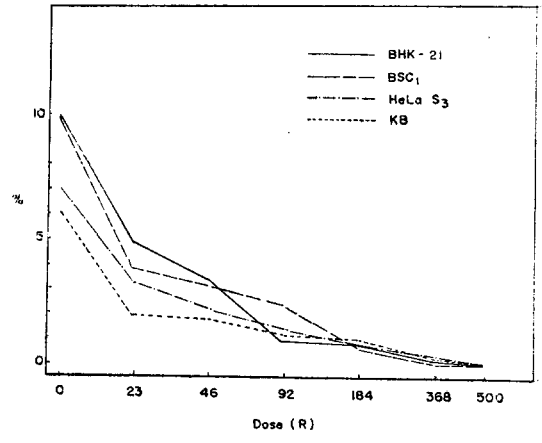

Fig. 4. Mitotic activity in four established mammalian cell strains irradiated with various doses of X-rays. Mitotic index is expressed by percentage of mitosis in 3,000 cells analyzed.

good accord with those of control.

Table 2 represents a summary of this study. From the table, it can be concluded that (1) unscheduled DNA synthesis is not directly related to chromosome number, chromosome exchange and mitotic activity of the cell, and that (2) mitotic activity is inversely related to chromosome exchanges.

DISCUSSION

Ever since the unscheduled DNA synthesis was first reported by Rassmussen and Painter (1966), a number of investigators have

attempted to shed some light on the possible relationship between this phenomenon and other recovery processes, i.e. Elkind recovery, colony forming ability and restitution of chromosome aberration.

Evans (1966) has first proposed the idea that X-ray induced chromosome aberrations are formed as a result of similar processes occurring in the DNA of chromosomes, that is, an excision of damaged DNA from two different chromosomal regions, followed by interaction and resynthesis. Kihlman and Hartley (1967, 1968), and Dubinin and Soyfer (1969) have later postulated that the rejoining of chromosome breaks is equivalent of rejoining of single stranded breaks in DNA. Spiegler and Norman (1969) have reported that the same processes are involved in the repair of DNA strand breaks and in the formation of chromosome exchange aberration. Their conclusion is primarly based on the finding that two processes of unscheduled DNA synthesis occurred likewise split dose experiments on the yield of chromosome aberration. Kihlman (1971) has still maintained this idea.

However, there are several reasons why this hypothesis does not seem likely. Among them; (1) Mitotic chromosome appear to be multistranded structures formed by coiling and folding of fiber consisting of long single DNA molecule associated with large component of protein (Kihlman, 1971). (2) Rejoining of chromosome breaks is affected by inhibitors of protein synthesis, but not affected by inhibitors of DNA synthesis (Wolff and Scott, 1969). (3) Hydroxyurea and 5-fluorodeoxyuridine which produce chromosome damage do not inhibit unscheduled DNA synthesis (Cleaver, 1969). (4) X-ray produces the same relative frequencies of exchange type aberration in DON and B14FAF of Chinese hamster cells which differ markedly in their abilities to perform unscheduled DNA synthesis (Wolff and Scott, 1969). (5) Root tip of *Vicia faba* and rat kangroo kidney cell (PtK_1), which are deficient in unscheduled DNA synthesis, represent functional chromosome rejoining processes (Shaeffer et al., 1971).

Besides, Shaeffer and Merz (1971, 1972) have recently reported that there is no apparent correlation between unscheduled DNA synthesis and radiosensitivity (cell recovery), but relationship between chromosome number and unscheduled DNA synthesis does exist.

The present results also indicate that unscheduled DNA synthesis is not necessarily related to chromosome exchange. In this respect, these data are in good agreement with those of Wolff and Scott(1969), Spiegler and Norman (1970) and Shaeffer *et al.* (1971). Moreover, no any direct correlation between unscheduled DNA synthesis and chromosome numbers could be found under the present experimental condition and materials studied. Rather, it was interesting to find that chromosome exchange and mitotic activity are both dose dependent, but their rates are inversely related. It is assumed that more severely damaged cells show less mitotic activity.

SUMMARY

Dose response and time dependence of unscheduled DNA synthesis induced by X-rays were measured to determine if any correlation exists between unscheduled DNA synth-

esis, modal chromosome number, chromosome exchange and mitotic activity in four mammalian cell strains.

Unscheduled DNA synthesis occurred in all strains studied. The rate was dose-dependent and strain-specific. Only HeLa S_3 showed a staturated dose response after 4,000 R, other cells were linearly proportional to dose increases. Time dependence of unscheduled DNA synthesis was completed within 2 hours after irradiation regardless of cell strains.

Unscheduled DNA synthesis was not directly related to modal chromosome number, total exchange rate and mitotic activity. Mitotic activity and chromosome exchange were both dose-dependent, but the rates of them were inversely related.

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