52. *Sister Chromatid Exchanges in the Uridine requiring Chinese Hamster Cell Line, UR-216, cultured in the Uridine Rich or Starved Medium**

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It is generally recognized that the sister chromatid exchange (SCE) is induced by the treatment with several chemicals giving damage or modification directly to chromosome DNA (Latt *et al.* 1981). According to an experiment that the SCE to be induced by BUdR was suppressed by the administration of deoxycytidine, Davidson *et al.* (1980), however, suggested that the SCE can also be induced by the metabolic perturbation. On the induction of SCE by the administration of an excess amount of deoxyribonucleosides, Suzuki and Yosida (1983) explained that the SCE induced by such a treatment could be due to the metabolic perturbation.

Metabolic perturbation, however, can induce in auxotrophic variant cells by cultivation under the starved condition of the requiring nutrients. As a variant of the Chinese hamster V-79 cells, a uridine requiring cell line, UR-216, has been established by Kusano *et al.* (1976). Condition of the metabolic perturbation can easily be induced in this variant cell line by culture with the uridine starved medium. The present paper deals with an examination of frequency of SCE in the UR-216 cells cultured in the uridine rich or starved medium to know whether, or not, the SCE can really be induced by metabolic perturbation.

Material and methods. A uridine requiring variant, UR-216, established by Kusano *et al.* (1976) from Chinese hamster V-79 cells was used in the present study. The UR-216 cell were maintained *in vitro* by the use of medium F12 with the supplement of 25 μ M uridine, 25 μ M thymidine and 10% fetal calf serum by the routine procedure at 37°C. The cells were cultured in the glass flasks of 10 cm in diameter at a concentration of 2×10^5 cells. One day after

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the routine culture, the flask was washed with PBS, and then several concentrations of uridine and BUdR were added to 5 cc of F12 medium with 10% FCS. The cells were harvested at 30 hr after 50 μ M uridine was added and 36 hr after 2.5 μ M uridine.

The chromosome examination was made one hour after treatment with 0.05 μ g/ml of colcemid, and the SCE was counted from slides stained by FPG method (Kanda and Kato, 1979).

Results and discussion. Modal chromosome number of the uridine requiring UR-216 cells of the Chinese hamster was 45 which were categorized to be the pseudo-tetraploidy, although that in the parental V-79 line was the pseudo-diploidy. The frequency of SCE in the UR-216 cells cultured in the uridine rich medium (50 μ g)

Table I. SCE of UR-216 cells cultured in uridine rich medium

Concentration (μ M) of		No. of cells counted	SCE/cell
Uridine	BUdR		Means $\pm$ S. E.
50	1	25	23.1 $\pm$ 2.1
50	5	25	24.3 $\pm$ 1.4
50	10	25	24.7 $\pm$ 1.6
50	100	25	28.8 $\pm$ 1.3

ranged from 23.1 $\pm$ 2.1 to 28.8 $\pm$ 1.3 at the concentrations of 1 to 100 μ M of BUdR (Table I and Fig. 1). In the case of a uridine starved condition (2.5 μ M), however, the frequency of SCE in the UR-216 cells increased to 46.0 $\pm$ 3.7 and 46.6 $\pm$ 3.7 at 1 and 5 μ M of BUdR, respectively. The number of SCE in the uridine starved medium was about twice that of the cells cultured at the uridine rich condition (50 μ M) (Table II and Fig. 2).

Table II. SCE of UR-216 cells cultured in uridine starved medium

Concentration (μ M) of		No. of cells counted	SCE/cell
Uridine	BUdR		Means $\pm$ S. E.
2.5	1	25	46.0 $\pm$ 3.7
2.5	5	25	46.6 $\pm$ 2.7
50.0	1	25	21.2 $\pm$ 6.5
50.0	5	25	23.9 $\pm$ 7.3

Based on the above two experiments, a higher frequency of SCE in the uridine starved medium seemed not to be resulted from an increase of incorporation of BUdR into the chromosome DNA, but due to the effect of metabolic perturbation. Interesting is the fact that SCE in UR-216 cells cultured in the uridine starved medium showed a nearly normal frequency by the administration of 10 μ M of

deoxycytidine (dC) in the culture medium (Table III). This result suggested strongly that the DNA synthesis could be proceeded by a salvage pathway by utilizing of dC.

Figs. 1-2. Sister chromatid exchanges (SCE) in uridine requiring UR-216 cells of the Chinese hamster. 1: SCE in uridine rich medium (50 μ M). 2: SCE in uridine starved medium. Arrows indicate SCEs in one chromosome as an instance.

Table III. Effect of dC of SCE of UR-216 cells cultured in uridine starved medium

Concentration (μ M) of			No. of cells counted	SCE/cell Means $\pm$ S. E.
Uridine	BUdR	dC		
50	1	0	25	17.2 $\pm$ 2.1
50	1	10	25	21.9 $\pm$ 1.8
1	1	0	25	39.0 $\pm$ 3.1
1	1	10	25	26.9 $\pm$ 1.6

According to Latt *et al.* (1981) most chemicals which can induce SCE give damages directly to DNA. On the other hand, some chemicals such as methotrexate (Banarjee and Benedict, 1979), 5-fluorodeoxyuridine (Sono and Sakaguchi 1980; Suzuki *et al.* 1982) hydroxyurea (Ishii and Bender, 1980); and an excess amount of deoxyribonucleosides (Suzuki and Yosida, 1983) giving no direct effect to DNA can also induce SCE. Davidson *et al.* (1980) explained the latter phenomenon as due to the metabolic perturbation. In the present experiment, in which the auxotrophic variant cells were used, SCEs were induced by culture in a nutrition starvation medium. Based on these studies it may be concluded that SCE would be in-

duced not only by direct effect to chromosome DNA, but indirect effect such as metabolic perturbation. It has been remained unknown at present what mechanism concerns to the induction of SCE by the metabolic perturbation.

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