The Effect of Mitomycin C on the Recombination in *Escherichia coli K-12**

Jacob *et al.* (1958) reported that when ultraviolet (UV)-irradiated *Hfr* were mated with normal F^- cells, the frequency of the simultaneous integration of the linked markers into the recombinant chromosome was considerably reduced, though the rate of transfer of all the markers was the same. It has been reported that the antibiotic mitomycin C has similar effects to UV light on bacteria and phages (Shiba *et al.*, 1958; Otsuji *et al.*, 1959; Iijima *et al.*, 1959; Otsuji *et al.*, 1960; and Iijima 1961, 1962). The present paper shows that mitomycin C also has the same effect as UV light on bacterial recombination.

E. coli K-12 Hfr L-118 ($Lp^- B_1^-$) was used as a donor and *W678* ($F^-TL^-T_1^R$ $Lac^- Mb^S Gal^- Lp^- B_1^-$) was used as a recipient. This *Hfr* transfers chromosomal fragments of the O-TL-Gal region into F^- cells at high frequency. Donor cells in the logarithmic phase of growth in nutrient broth were harvested by centrifugation and resuspended in a minimum medium supplemented with 1

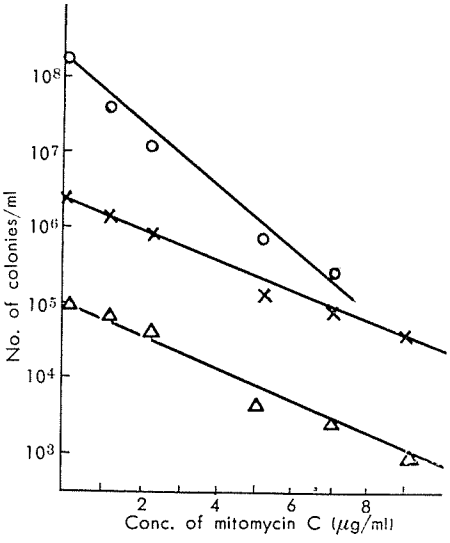

Fig. 1. The Effect of Treatment of *Hfr* Cells with Mitomycin C on the Recombination frequency: *Hfr L-118* × *W678*

-○- the number of colony formers of *Hfr L-118*; -×- the number of TL^+Sm^R recombinants; -△- the number of Lac^+Sm^R recombinants.

* This study has been reported at the 32nd Meeting of the Genetic Society of Japan held in Fukuoka in October, 1960 (Yuki and Iijima)

mg/ml of aspartate and were treated with various concentrations of mitomycin C (1-9 μg/ml) for 15 minutes at 37°C. As shown in Fig. 1, the number of colony forming cells decreased exponentially with increase in the concentration of mitomycin. Deoxyribonucleic acid (DNA) synthesis in *Hfr* cells was completely inhibited by 5 μg/ml and 7 μg/ml of mitomycin, but at a concentration of 1 μg/ml, DNA synthesis took place though at a lower rate than in the control. After washing with the minimum medium, these mitomycin-treated *Hfr* cells were mated with non-treated F^- cells for 60 minutes at 37°C and TL+ Sm^R recombinants and Lac+Sm^R recombinats were selected. The number of recombinants also decreased exponentially according to the concentration of mitomycin, it decreased less than the viability of the *Hfr* cells (Fig. 1). This seems to suggest that some of the mitomycin-inactivated *Hfr* cells still have the ability to donate their chromosomes which can then play a normal role in recombinational events in the recipient cells. Another possible explanation is that mitomycin treatment of *Hfr* cells enhances the recombination rate.

As can be seen in Fig. 1, the number of TL+Sm^R recombinants decreased at the same rate as the Lac+Sm^R recombinants. This indicates that the rate of transfer of the distal markers does not decrease so much as that of the proximal ones. In other words, the chromosomes which were transfered from mitomycin-treated *Hfr* to female cells were not broken down by the action of mitomycin. However, it was found that the genetic constitution of TL+Sm^R recombinants was affected by treatment with mitomycin (Table 1). In the control cross, among

Table 1. The Genetic Constitution of TL+Sm^R Recombinants Derived from the Cross: Mitomycin-treated Hfr L-118 × W678

TL	T_1	Lac	Mb	0	1	3	7 μg/ml.
1	1	1	1	14 (13.3%)	5 (5.0%)	3 (2.9%)	1 (1.0%)
1	1	1	0	13 (12.4)	10 (10.0)	7 (6.7)	3 (2.9)
1	1	0	0	43 (41.0)	44 (44.0)	33 (31.4)	38 (36.2)
1	0	0	0	31 (29.5)	41 (41.0)	61 (58.1)	63 (60.0)
1	0	1	1	3 (2.9)	0 (0)	1 (1.0)	0 (0)
1	0	1	0	1 (1.0)	0 (0)	0 (0)	0 (0)
	Total			105	100	105	105

1 : Marker of Hfr L-118
0 : Marker of W 678

the TL+Sm^R recombinants examined, the frequency of TL+T_1^SLac+Mb^R was 13.3%; TL+T_1^S Lac+Mb^S, 12.4%; TL+T_1^S Lac−Mb^S, 41.0% and TL+T_1^R Lac−Mb^S, 29.5%. But, in the cross in which *Hfr* cells treated with 7 μg/ml of mitomycin were mated with normal F^- cells, the frequency of TL+T_1^S Lac+Mb^R recombinants was 1.0%; TL+T_1^S Lac+Mb^S, 2.9%; TL+T_1^S Lac−Mb^S, 36.2%

and $TL^+T_1^R$ Lac^-Mb^S, 60.0%. This result clearly shows that the rate of simultaneous integration of unselected markers of *Hfr* with selective markers is reduced by the action of mitomycin C.

The same results were obtained using another kind of *Hfr*, Cavalli's *Hfr*, which transfers O-Lac-LT segments of its chromosome to the recipients at high frequency.

In order to compare the effect of UV irradiation with that of miomycin C, UV-irradiated *Hfr L-118* cells were mated with normal F^- cells, and similar results were obtained.

The above results clearly show that mitomycin C has similar effects as UV light on the genetic recombination. Since during bacterial conjugation, cytoplasmic components of *Hfr* cells scarcely migrate into recipient cells, it can be concluded that the chromosomes of mitomycin-treated *Hfr* transfered to the female cells suffer some damages which loosen the linkage of the genetic markers in the recombinational events. However the mechanism of genetic recombination is still obscure.

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