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Induction of invertant males in *E. Coli* K12*

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1. INTRODUCTION

When the *E. coli* K12 Hfr H male strain (Hayes, 1953) is submitted to increasing doses of X-rays (Marcovich, 1961) or α-particles (Wood & Marcovich, 1964), a diminution of transmission of its markers to the recipient cell is observed. The rate of diminution is directly related to the distance of the markers from the origin of injection of the chromosome. This effect is attributed to lesions distributed randomly on the chromosome, which prevent the transfer of the markers located behind them with respect to the origin of transfer (Wood & Marcovich, 1964). It has been shown that transfer lesions and lethal events are independent; thus killed males (cells not able to make colonies) can still transmit their genetic determinants to females, while viable males may nevertheless carry transfer lesions (Marcovich, 1961). Ultra-violet light (Jacob & Wollman, 1955; Joset & Wood, 1965) and ^{32}P disintegrations (Fuerst *et al.*, 1956; Krisch, 1965) produce the same type of damage.

The same study has been made using another Hfr strain, Ra 1, (see Fig. 1 for its mode of chromosome transfer), which differs from Hfr H both by its original source and its mode of chromosome transfer. It has been found that Ra 1 does not yield the same simple relationship as Hfr H between the dose of radiation and the inactivation of transfer as a function of the length of chromosome separating the selected marker from the origin of transfer (Joset *et al.*, 1964). After irradiation, the diminution of transmission of some of its distal markers is smaller than that of the proximal ones: the gradient curves, instead of showing regularly increasing slopes with increasing doses, as is the case with Hfr H, are broken, showing a relative increase of transmission of some distal markers, as compared to proximal ones. The distal markers affected by this effect are *Arg*, and to a lesser extent *Thr*, *Leu* and *Try*. Interrupted mating experiments have explained these results by showing that radiations actually modify the times of entry of the markers: thus *Arg*, originally transferred at 78 min., now appears in the recombinants after only 7 min. of mating. Similar modifications were observed for the other markers. These results were best explained by assuming that radiations induce Ra 1 to transfer its genetic material from a new origin, situated at approximately 7 min. from *Arg*, and in the reverse direction. The induced modified Hfr has been called 'invertant'.

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These results led us to examine several other male strains from various sources. The ability to produce invertants has been found in several cases.

2. MATERIALS AND METHODS

Strains—Males. The males used form the three groups listed below, according to their sources. The Ra group includes Hfr's isolated after ultra-violet irradiation of F+ W1485. All B strains are spontaneous Hfr's arising from F+ W1655 and were

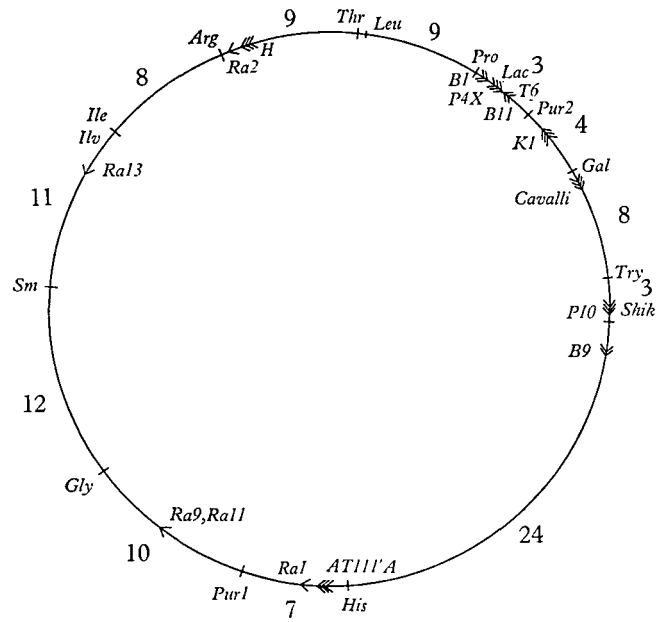

Fig. 1. Characterization of the Hfr's used. The *E. coli* chromosome is represented as a circular unit. The markers studied in this paper have been placed according to their relative distances. The arrows indicate the origin and direction of transfer of the various males:

males of the Ra group

males of the B group

males of other sources

The figures indicate the length of chromosome in minutes of transfer (Jacob & Wollman, 1961).

Symbols—*Thr:* Threonine; *Leu:* Leucine; *Pro:* Proline; *Lac:* Lactose; *T6:* resistance to phage T6; *Gal:* Galactose; *Try:* Tryptophane; *Shik:* Shikimic acid; *His:* Histidine; *Pur:* Purine; *Gly:* Glycine; *Sm:* resistance/sensitivity to Streptomycin; *Ile:* Isoleucine-valine; *Arg:* Arginine.

kindly given to us by Dr Broda. The other Hfr's are all from independent and different sources. Fig. 1 gives the order of chromosome transfer of each male and explains the symbols used.

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Ra 1	B1 *met*$^-$	H
Ra 2	B9 *met*$^-$	P10
Ra 9	B11 *met*$^-$	K1 *Thr*$^-$
Ra 11		P4X-6 Pur_2^- *met*$^-$ *Sm*^{r}
Ra 13		P4X-8 *met*$^-$
		Cavalli
		AT111A

It should be noted that all the Hfr's of the Ra group are unstable, and revert to the F$^+$ form with a high frequency. Hfr's must be frequently re-isolated.

Females. Only the selective markers used in this work are listed:

PA 309	*Thr*$^-$, *Leu*$^-$, *Gal*$^-$, *Try*$^-$, *His*$^-$, *Sm*^{r}, *Arg*$^-$
PA 330-1	*Thr*$^-$, *Leu*$^-$, *Pro*$^-$, *His*$^-$, Pur_1^-, *Sm*^{r}, *Arg*$^-$
PA 3344	*Thr*$^-$, *Leu*$^-$, *Pro*$^-$, *Try*$^-$, Pur_1^-, *Sm*^{r}, *Ile*$^-$, *Arg*$^-$
PA 309-1	*Thr*$^-$, *Leu*$^-$, *Gal*$^-$, *Try*$^-$, *His*$^-$, *Sm*^{r}, *Ile*$^-$, *Arg*$^-$
PA 360	*Thr*$^-$, *Leu*$^-$, *His*$^-$, *Gly*$^-$, *Sm*^{r}, *Arg*$^-$

*Preparation of Hfr's from F$^+$ W*1485: The ultra-violet irradiated F$^+$ population was grown in broth for 3 hours, then plated. Replicas of the surviving colonies on selective plates spread with 10^9 females per plate were examined after 20 hours of incubation at 37°C. (Jacob & Wollman, 1956).

Media: Difco Tryptone broth has been used to grow the cultures. Synthetic medium (Vogel & Bonner, 1956), supplemented with the appropriate growth factors and antibiotic, was used for recombinant selection.

Table 1. *Mating conditions for the different males studied*

Males	Females	Selected markers	Female selection marker	Duration of mating in min.
H	PA 309	*Thr*(8)*Leu*(8·5)*Try*(32)*His*(59)*Arg*(107)	*Sm* (88)	90
Ra 1	PA 309	*His*(5)*Try*(32)*Leu*(55)*Thr*(56)*Arg*(65)	*Sm* (84)	90
Ra 2	PA 309	*Thr*(8)*Leu*(8·5)*Try*(32)*His*(59)*Arg*(107)	*Sm* (88)	90
Ra 9	Pa 330-1	Pur_1(5)*His*(12)*Pro*(54)*Leu*(63)*Arg*(72)	*Sm* (91)	90
Ra 11	PA 3344	Pur_1(5)*Try*(39)*Pro*(54)*Leu*(63)*Arg*(72) *Ile*(80)	*Sm* (91)	90
Ra 13	PA 309-1	*Ile*(5)*Arg*(13)*Leu*(22)*Try*(46)*His*(73)	*Sm* (102)	90
B1	PA 309	*Leu*(8)*Thr*(9)*Arg*(18)*His*(66)*Try*(93)	*Sm* (37)	90–100
B9	PA 309	*Try*(10)*Leu*(33)*Thr*(34)*Arg*(43)*His*(91)	*Sm* (62)	90–100
B11	PA 309	*Try*(13)*His*(40)*Arg*(88)*Thr*(97)*Leu*(98)	*Sm* (69)	90–100
K1	PA 309	*Gal*(4)*Try*(12)*His*(39)*Arg*(87)*Leu*(97)	*Thr* (96)	100
P10	PA 309	*Try*(3)*Leu*(26)*Arg*(36)*His*(84)	*Sm* (55)	90
P4X-8	PA 3344	*Pro*(1)*Leu*(8)*Thr*(9)*Arg*(18)*Ile*(26) Pur_1(59)*Try*(93)	*Sm* (37)	90
P4X-6	PA 360	*Leu*(9)*Arg*(19)*Gly*(50)*His*(67)	Pur_2 (106)	90
Cavalli	PA 330-1	*Pro*(7)*Leu*(15)*Thr*(16)*Arg*(25)	*Sm* (44)	45
AT111A	PA 309	*His*(5)*Try*(32)*Leu*(55)*Thr*(56)*Arg*(65)	*Sm* (84)	90

The figures represent entry times in minutes, as given by Jacob & Wollman (1961).

Mating conditions: A mixture of exponentially growing cultures of male and female cells, in a ratio of 1/10, was gently agitated at 37°C. The duration of the mating was 90–100 min., except in the case of Hfr Cavalli, for which it was 45 min. These lengths of time are chosen according to the times of entry of the selected markers. The numbers of recombinants were scored after 48 hours of growth on selective media. Table 1 indicates, for each male studied, the exact conditions of mating and counter-selection.

Radiations: The following types of radiation have been used: X-rays (37·5 kV), γ-rays (from a ^{60}Co source), α-particles (from a ^{210}Po source), ultra-violet light (254 mμ), and decay of incorporated ^{32}P. The cells have been irradiated either in liquid suspension (broth for X- and γ-rays and synthetic medium for ultra-violet light) or on Millipore filters (α-particles). All irradiations were carried out at 0°C., the duration being from less than a minute up to about 15 min. with γ-rays and α-particles. The medium and conditions described by Fuerst *et al.* (1956) have been used for ^{32}P incorporation and suicide.

Note: The males induced by irradiation of an Hfr to transfer their chromosome in a new way have been called 'invertants', no matter what their actual mode of transfer is.

3. RESULTS

The results will be presented by grouping the strains according to the F$^+$'s from which they are derived.

Figures summarizing the behaviour of the various strains studied are given in Table 2. Doses allowing survival of the male strains of approximately 10^{-3} have been chosen. No difference was found when various types of radiation were used on the same strain. The double arrows indicate the normal order of transfer of the males. The figures given for each of the selected markers represent degrees of inactivation of transmission of the markers to the females. They correspond to the ratios of the number of recombinants after irradiation to that without irradiation, for each marker. This permits a direct comparison of the degrees of inactivation of the various markers as a function of their chromosomal locations, by normalizing the inactivation of transmissions of the various markers.

Thus it can be seen that the degree of diminution of transmission of the markers of Hfr H, given as a comparison, increases proportionally to their distances from the origin of transfer.

(1) *Males of the Ra group*: Four Hfr's isolated from F$^+$ W1485, Ra 2, Ra 9, Ra 11 and Ra 13, have been studied, besides Ra 1 (Joset *et al.*, 1964) (see Fig. 1 for their modes of chromosome transfer). None of these strains yield the same pattern of inactivation as Hfr H. Though their modes of transfer are different, they all show the smallest inactivation for the transmission of the *Arg* marker. One exception, Hfr Ra 9, gives the smallest inactivation for *Pur*$_1$, *Arg* coming next. As a whole, two directions of increasing inactivation can be found: one, from the origin towards the end of the chromosome, has the same orientation as the transfer; the other starts at *Arg* and continues through the *Thr–Pro–Try* side.

Table 2. *Effects of irradiation on the transmission to females of the markers of several male strains*

Hfr	*Thr*	*Leu*	*Pro*	*Gal*	*Try*	*His*	*Pur*$_1$	*Ile*	*Arg*	Radiations
H	←80	70			10	1			0·4◀	X, α, γ, u.v., ^{32}P
Ra 1	1·5	0·7			0·2	0·5→▶			←○ 10	X, α, u.v., ^{32}P
Ra 2	←5	2·5			0·35	0·5			←○ 70 ◀	X
Ra 9		0·1	0·05			0·15	20→▶		←○ 3	X
Ra 11	10		2		1		7→▶	2·5	←○ 80	X
Ra 13		4			0·2	5		◀←10	←○ 40	X, α
K1		15		○→◀←7	1	0·09			0·3	X
P10		0·4			30→▶					X
P4X-8	50	30	60→▶←○		30		4	10	40	X
Cavalli	40		50	→▶					30	α
AT111A		0·7			3	100 →▶			0·1	X, γ, ^{32}P*
B1		150	→▶		0·1	1			1·5	γ
B9		1			79→▶					γ
B11	80		○→◀←		1·5	0·6			20	γ

* D. Schwartz, personal communication.

The figures represent the frequencies of transmission, multiplied by 10^2, of the markers after a dose giving 10^{-3} surviving males, normalized to the transmission of the same markers without irradiation. The markers are ordered as on the chromosome. The normal mode of transfer, together with the position of the F factor is indicated for each strain (→▶), as well as the induced origin and direction of injection (←○). The data given for strains Ra 11, Ra 13, AT111A, and B11 are obtained from the corresponding curves of Figs. 2 (Ra 11) and 3 (Ra 13, AT111A, and B11).

A typical experiment is given for Ra 11 (Fig. 2) after X-ray irradiation. Fig. 2A gives the absolute numbers of recombinants as a function of dose. The male survival curve is indicated. Fig. 2B, deduced from the former by normalizing the number of recombinants to the total input males, gives the gradient curves. The distortion of the curves is greater in the *Arg* region, and then decreases for the *Thr*, *Pro* and *Try* markers. The transfer of *Ile* is not affected by this modification.

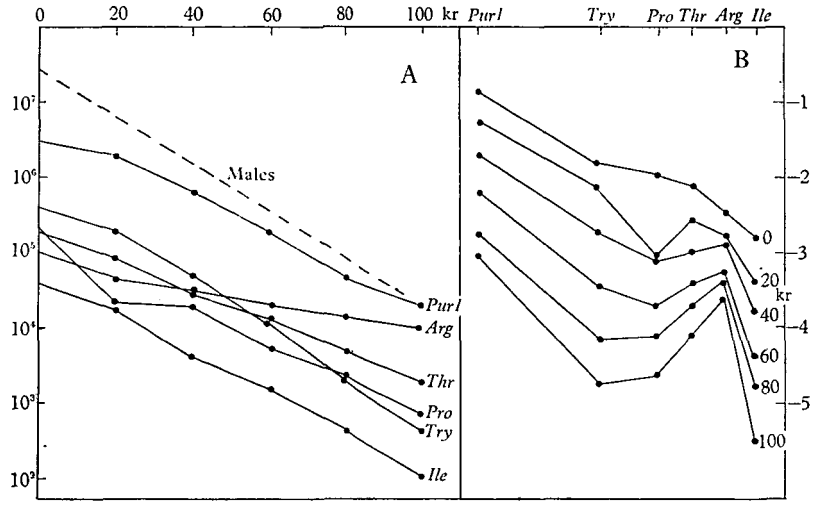

Fig. 2. Effect of X-ray irradiation on the transmission of Ra 11 markers. Ra 11 grown in broth to 3.10^8 cells/ml., is irradiated and immediately mated for 90 min. with PA 3344. The recombinants are scored after 48 hours of growth at 37°C. on selective media. Part A: absolute values of males and recombinants as a function of dose. Part B: variation of the gradient of transfer as a function of dose. The gradients are obtained by plotting the ratio of the number of recombinants to the total input males as a function of distance of markers from the origin.

Figure 3A shows the modification of the gradient of transmission of Ra 13 as a function of dose: the *Ile* transmission decreases rapidly while that of *Arg* is only slightly diminished, the other markers disappearing afterwards according to their distance from the origin.

(2) *Hfr's derived from* F^+ W1655: Among three that we have studied (see Fig. 1 for characterization of the transfer order), two, B1 and B9, behave as Hfr H, in that their markers give a direct proportionality between sensitivity of transmission and distance from the origin (Table 2). With the third one, B11, two directions of increasing inactivation are observed: one corresponding to the order of transfer, and one corresponding to the reverse order (Table 2 and Fig. 3B).

(3) *Hfr's from various sources*: The five strains chosen transfer their genetic material each in a different way (see Fig. 1).

Three of them, P10, AT111A, Cavalli, give results identical to those obtained with Hfr H: the rate of inactivation of transfer of a given marker is proportional to its distance from the origin of transfer (Table 2). Fig. 3C illustrates the results obtained

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with AT111A: the regular increase of the slope of the gradient reflects the increase of the inactivation of transfer of the selected markers as a function of both dose and distance from the origin.

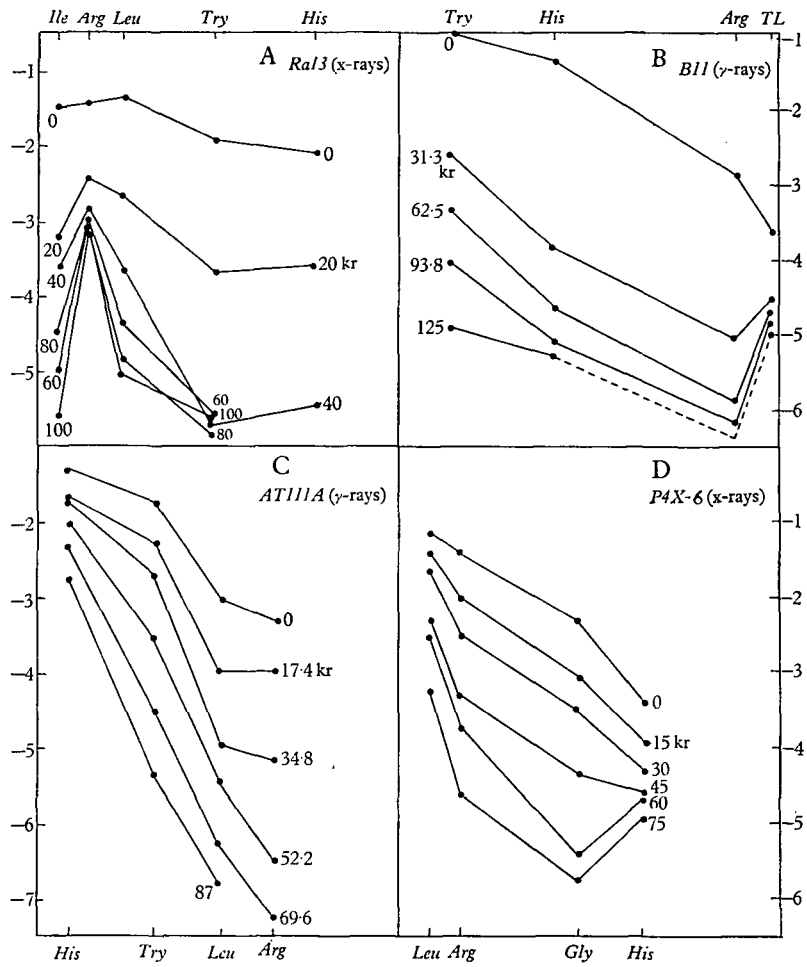

Fig. 3. Variation of the gradient of transmission after irradiation. The males are irradiated and mated as in Fig. 2. The gradients are obtained as the ratios of the number of recombinants to the total input Hfr's plotted as a function of distance of markers from the origin. The survival of the males, not shown, is approximately 10^{-3} at 100 kr.

The two other strains, P4X and K1, on the contrary, behave differently when irradiated (Table 2). The results obtained with P4X are given in Fig. 3D; a relative increase in the frequency of transmission of the distal markers studied, *His* and *Gly* is observed. Other experiments using different markers nearer to the end of the chromosome have shown that they too show a smaller degree of diminution.

The same behaviour is observed for K1, in which the transmissions of both proximal and distal markers are less inactivated.

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4. DISCUSSION

It is interesting to compare the present results with those previously obtained with Ra 1 (Joset *et al.*, 1964), which led to the conclusion that this Hfr is induced by radiations to transfer its chromosome in a new sequence. The strains studied here fall in two classes. One gives a direct proportionality between distance from the origin of transfer and rate of inactivation of transmission of the markers. The same model of inactivation can be applied to these strains as to Hfr H (Marcovich, 1961; Wood & Marcovich, 1964). The second class includes a number of strains for which no simple model of inactivation can be given. However, several facts strongly recall the results obtained with Ra 1: the appearance, in the same strain, of two gradients of inactivation; the capacity of all types of radiation used to produce this effect; and the instability of the phenomenon (preliminary experiments have shown that the modified gradient of transmission disappears as soon as the irradiated population has undergone one division).

Thus, though more experimental work is necessary to obtain definite proof, it is quite probable that these strains too are induced to transmit their chromosome in a new sequence.

This sequence can be approximately determined from the gradient curves or from the data of Table 2. The two gradients of inactivation generally found reflect the normal and the induced orders of transfer, supposing that both normal and induced strains follow a pattern of inactivation similar to that of Hfr H. The location of the induced origins of transfer can be grossly determined: the new proximal marker should show the least inactivation of transmission, that of the following markers increasing with their distances from this origin. These approximate origins are shown in Table 2.

The study of the inheritance of unselected male markers by the recombinants confirms this conclusion (unpublished results).

The progressive modification of the gradient curves with increasing doses (Figs. 2 and 3) indicates that the proportion of 'invertant' males among the original population increases with dose. Though no figure can at the moment be given for the efficiency of induction, an idea of its size can be obtained by comparing the results found with Ra 11 and AT111A; these two strains inject almost identically, but only Ra 11 is induced to transfer in another direction. At a survival of 10^{-3}, 90% of the Ra 11 cells which should transfer Pur_1 first are lost, while the number of *Arg* recombinants is increased by a factor of 10^3, as compared to that in AT111A.

The Ra group is remarkable for the fact that all the males of this series seem to give rise to the same invertant, no matter what their own mode of transfer is.

On the contrary, no correlation has been found in the other strains between the kind of invertant produced and the source of the males or their mode of injection. However, is should be noted that each male always gives rise to the same invertant.

SUMMARY

A number of Hfr strains have been studied as to the action of radiations on the transmission of their genetic material to female cells. They form two classes, one

in which irradiation produces only lesions that prevent transfer, and one in which new modes of chromosome transfer seem to be induced. One group of strains, derived from a common F^+ male, appears to give rise to the same induced mode of transfer, while no correlation could be found in the other Hfr's studied.

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