JOURNAL OF BACTERIOLOGY, May 1967, p. 1729–1731
Vol. 93, No. 5

Cotransduction with *thy* of a Gene Required for Genetic Recombination in *Escherichia coli*

PETER T. EMMERSON AND PAUL HOWARD-FLANDERS

Radiobiology Laboratories, Department of Radiology, Yale University School of Medicine, New Haven, Connecticut 06510

Received for publication 13 January 1967

AB3022 is a nitrosoguanidine-induced X-ray sensitive mutant of AB2495, a thymine-requiring derivative of *Escherichia coli* K-12. AB3022 is also recombination-deficient, as is indicated by the results of several mating experiments (Table 1). The relevant genetic characteristics of the strains used are shown in Table 2. Two-hour matings were performed by the method of E. A. Adelberg and S. N. Burns (J. Bacteriol. **79**:321, 1960). In the crosses with AB259 Hfr H and with AB2383 Hfr J2, AB3022 produces only 0.33 to 0.5% as many Pro^+ Str^R recombinants as does its parental strain, AB2495. However, AB3022 appears to conjugate normally, since there is only a twofold difference in the yields of His^+ Ilv^+ recombinants in this strain and in AB2495 in the crosses with AB2528 Hfr 313. The almost normal yield of recombinants in AB3022 is to be expected if the wild-type allele for recombination is transferred and expressed early in this cross.

TABLE 1. *Results of mating AB2495 Rec⁺ (2.2 × 10⁸/ml) and AB3022 (1.0 × 10⁸/ml) with the donor strains AB259 Hfr H, AB2383 Hfr J2, and AB2528 Hfr 313*

Donor (viable cells/ml)	Recipient	Selection	No. of recombinants per ml
AB259 Hfr H (4.7×10^7)	AB2495 *rec⁺*	Pro^+ Str^R	9.1×10^5
	AB3022 *rec-22*		2.9×10^3
AB2383 Hfr J2 (4.8×10^7)	AB2495 *rec⁺*	Pro^+ Str^R	3.4×10^6
	AB3022 *rec-22*		1.3×10^4
AB2528 Hfr 313 (6.5×10^7)	AB2495 *rec⁺*	His^+ Ilv^+	2.2×10^5
	AB3022 *rec-22*		1.1×10^5

TABLE 2. *Characteristics of the Escherichia coli K-12 strains used*

Strain	*rec*	Nutritional requirement[a]									Resistance to phage T6	Resistance to Str	Sex
		Thr	Leu	Pro	His	Thi	Arg	Thy	Ilv	Trp			
AB259	+	+	+	+	+	−	+	+	+	+	S	S	Hfr H[b]
AB2383	+	+	+	+	+		+	−	+	+	S	S	Hfr J2
AB2528	+	+	−	+	+	−	+	+	−	+	S	R	Hfr 313
AB2495	+	−	−	−	−	−	−	−	+	−	R	R	F^-
AB3022	22	−	−	−	−	−	−	−	+	−	R	R	F^-

[a] Abbreviations signify requirements for threonine, leucine, histidine, thiamine, arginine, thymine, isoleucine-valine, tryptophan; Str signifies streptomycin.

[b] The Hfr strains, obtained from E. Adelberg and G. Eggertsson, have the following marker injection sequences. AB259 Hfr H: pyrimidine, threonine, leucine; AB2383 Hfr J2: proline, leucine, arabinose; AB2528 Hfr 313: mannitol, xylose, streptomycin.

The mutation which confers recombination deficiency on AB3022 is designated *rec-22*. It seems likely that AB3022 *rec-22* is a member of a second class of Rec^- mutants that differ from those first isolated (A. J. Clark and A. D. Margulies, Proc. Natl. Acad. Sci. U.S. **53**:451,

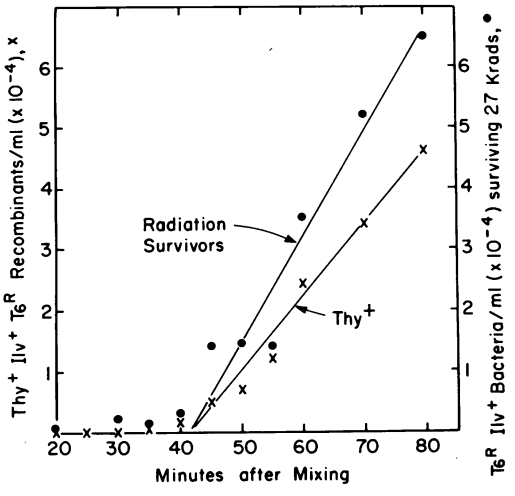

FIG. 1. *Number of Thy+ Ilv+ T6R recombinants and the number of T6R Ilv+ bacteria (per milliliter) surviving 27 krad of ionizing radiation, plotted as a function of the duration of mating of AB2528 Hfr 313 and AB3022 rec-22.*

1965; Clark et al., J. Mol. Biol. **19**:442, 1966) in ability to degrade their deoxyribonucleic acid after ultraviolet (UV) and X irradiation. This class of mutants has been discussed previously (P. Howard-Flanders and Boyce, Radiation Res. [suppl.] **6**:156, 1966).

Figure 1 shows the results of an experiment on the time of entry of genes for X-ray resistance and thymine independence. Exponentially growing cultures of AB3022 (a slow-growing strain) and AB2528 were mixed, sampled at intervals, treated with T6 phage, diluted, and plated on media selective for Thy+ Ilv+ recombinants and also on media selective for Ilv+ cells. The donor strain requires isoleucine and valine and does not grow on these plates. The plates selective for Ilv+ were incubated at 37 C for 3 hr to allow phenotypic expression, and were then irradiated with 27 krad of fast electrons from a 6-Mev linear accelerator. All selective plates were incubated for 2 to 3 days at 37 C. The thymine marker enters at approximately 40 min after the commencement of conjugation, both in this cross and in control experiments with AB2495 *rec+*. The number of radiation survivors on the plates selective for Ilv+ cells rapidly increases over the background of surviving females at roughly the same time, suggesting that the gene for X-ray sensitivity in AB3022 is close to *thy*.

Table 3 gives the results of a representative transduction experiment following the methods of Arber (Virology **11**:273, 1960) and Lennox (Virology **1**:190, 1955).

To test the transductants for X-ray sensitivity, they were grown overnight in broth and then inoculated in streaks on complete media with AB2495 and AB3022 controls. Half of each plate was irradiated with 27 krad of 6-Mev electrons. After overnight incubation, AB3022 and radiation-sensitive (X-ray^S) transductants failed to grow on the irradiated portion of the plates, or grew very sparsely, whereas AB2495 and X-ray resistant (X-ray^R) transductants grew confluently. Thy+ and Trp+ transductants were produced from AB2495 *rec+* with normal yield (7.3×10^{-6} and 3.2×10^{-6}, respectively, defined as the number of transductants per ml divided by the total number of viable cells per ml). However, although Thy+ transductants were produced in AB3022 *rec-22* with normal yield (6.8×10^{-6}) and grew in large colonies, the yield of Trp+ transductants was much lower (3.8×10^{-7}) and the colonies were small. When picked and tested, the Try+ transductants all proved to be sensitive to X-rays. Approximately 85% of 155 Thy+ transductants of AB3022 tested were X-ray^R. Seventy-seven X-ray^R and 20 X-ray^S AB3022 *thy+* transductants were tested for ability to form Pro+ Str^R recombinants with AB2383 Hfr J2.

TABLE 3. *Transduction of thy+, trp+ and the gene for X-ray resistance into AB2495 rec+ and AB3022 rec-22 by P1 phage grown on wild-type cells*

Recipient strain	Input/ml	Transductants per ml[a]			
		Thy+		Trp+	
		Total	X-ray^R	Total	X-ray^R
AB2495	1.7×10^8	1.2×10^3	Not tested	5.4×10^2	Not tested
AB3022	7.2×10^7	4.9×10^2	4.2×10^2	2.7×10^1	0

[a] The P1 phage, kindly provided by Ikeda and Tomizawa (J. Mol. Biol. **14**:85, 1965), was a virulent derivative of P1kc. It was exposed to a UV dose of 700 ergs/mm^2 before use. The multiplicity of infection, based on viability before irradiation, was approximately 1.

After 45 min of mating, all of the X-ray resistant transductants produced more than 100 times as many recombinants as did AB3022 in the same cross, and all of the X-ray sensitive transductants gave less than 1% of the number given by AB2495.

In a similar experiment to that presented in Table 3, the yields of His^+, Trp^+, Thr^+, and Thy^+ transductants in AB3022 were 3.0×10^{-7}, 3.8×10^{-7}, 7.7×10^{-7}, and 8.1×10^{-6}, respectively. Only the Thy^+ transductants gave large colonies; all other colonies were very small, even after incubation for 4 days. In the same experiment, the yields of the same transductants in AB2495 were 1.4×10^{-6}, 3.8×10^{-6}, 3.4×10^{-6}, and 4.1×10^{-6}, respectively, and colonies were large. These results show that the wild-type allele of *rec-22* promotes the formation of recombinants in transduction as well as in conjugation. However, it is notable that His^+ transductants appear to be produced at a higher frequency in AB3022 than in strains carrying *rec-13* (Hertman and S. E. Luria, J. Mol. Biol. **23:**117, 1967).

Evidence will be presented elsewhere that the *thy* with which *rec-22* is closely linked is also cotransducible with *argA*, as was reported by M. Ishibashi and Y. Hirota (J. Bacteriol. **90:** 1496, 1965). The *argA* gene controls *N*-acetylglutamate synthetase and is the *argB* of Taylor and Thoman (Genetics **50:**659, 1964).

This investigation was supported by Public Health Health Service grants GM 11014, CA 06519, and AMK 69397.