CHROMOSOMAL LOCATION OF A FUSIDIC ACID RESISTANT MARKER IN ESCHERICHIA COLI.

Nobuo Tanaka, Genji Kawano, and Tadatoshi Kinoshita
Institute of Applied Microbiology, University of Tokyo, Tokyo.

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Summary: A mutation in Escherichia coli to resistance to fusidic acid, a steroidal antibiotic, alters G factor. Fusidic acid resistance (fus) is located at minute 64, about 0.2 min. from the strA gene, and may lie between the strA and malA loci.

This communication reports the mapping of a new locus (fus), affecting G factor in Escherichia coli K-12.

Fusidic acid has been demonstrated to inhibit protein synthesis by preventing the ribosome-dependent activity of G factor: i.e. GTPase reaction and translocation of peptidyl-tRNA on the ribosome (1-3). In fusidic acid resistant mutants, the resistance is associated with G factor but not with ribosomes (2). It has been also shown that fusidic acid binds with G factor and a fusidic acid-G factor-GDP-ribosome complex is formed in a molar ratio of 1:1:1:1. Less binding of the antibiotic is observed when fusidic acid resistant G factor is used (4,5).

Several fusidic acid resistant mutants were recovered from the culture of E. coli K-12 strain AB312 treated with N-methyl-N'-nitro-N-nitrosoguanidine by the method of Adelberg et al. (6). One of them was used in most of the experiments. As shown in Table 1, it was demonstrated that fusidic acid resistance (Fus^r) is associated with G factor in this mutant by the procedure as described previously (2). G factor from the resistant mutant showed Km value of 3.5×10^{-4}M for GTP in the GTPase reaction, whereas Km of G factor from the sensitive parent strain was 7.0

Table 1. Effect of fusidic acid on GTPase reaction catalyzed by combination of G factor and ribosomes, obtained from antibiotic-sensitive or -resistant E. coli K-12.

Ribosomes	G factor	GTP hydrolyzed (mμmoles) - Fus	GTP hydrolyzed (mμmoles) + Fus	% Inhibition
S	S	14.3	2.28	84
S	R	4.65	3.98	14
R	S	13.8	2.05	85
R	R	4.72	3.83	19

Fus: fusidic acid 20 μg/ml. S: E. coli K-12 strain AB312 (Fus-sensitive). R: the Fus-resistant mutant.

x 10^{-5}M. Another Fus^r mutant was obtained from strain HfrC by the same procedure.

For mating the Hfr culture in exponential growth was added to 9 volumes of the F^- culture and incubated at 37° for 60 or 120 min. Transduction was performed following the method of Lennox (7), using P1 phage (8). L broth, L agar and soft agar (7) were used in mating, transduction and growing cells to prepare P1 lysates. For selection of Str^r recombinants or transductants, the cells were spread on penassay agar; and after 3 hours of incubation at 37°, soft agar containing 100 μg/ml of streptomycin was poured over it. Media for selection of the other recombinants or transductants were eosin-methylene blue media: EMB-lactose medium and minimal medium (EMS) supplemented with sugar 10 mg/ml, amino acids 10 μg/ml each, and/or thiamine 1 μg/ml. Fusidic acid was added to EMB-lactose medium at the concentration of 800 μg/ml, supplemented with 2 mM EDTA, which increased the sensitivity of E. coli to the antibiotic and did not significantly affect viability of the organism.

Mapping by conjugation and transduction was used to test the linkage of fus to known markers. The Fus^r mutant (met, fus) from HfrC was mated with F^- strain JE1011 (thy, thr, leu, his, trp, xyl, ara, lac, mtl, gal, strA); and Thr-Leu^+Str^r, Lac^+Str^r and Xyl^+Str^r

Table 2. Linkage of *fus* in conjugation.

(a) Recombination frequencies in HfrC x JE1011 cross.

Phenotype selected	% of markers of donor										No. scored
	Trp$^+$	His$^+$	Str$^+$	Xyl$^+$	Mtl$^+$	Met$^-$	Thr-Leu$^+$	Ara$^+$	Lac$^+$	Fus^r	
Thr-Leu$^+$Str^r	9	8	0	7	6	9	100	97	64	3	160
Lac$^+$Str^r	29	5	0	10	7	10	92	91	100	1	164
Xyl$^+$Str^r	12	7	0	100	82	15	83	85	70	0	160

Donor: Fus^r mutant from HfrC (*met*, *fus*).
Recipient: JE1011 (F$^-$; *thy*, *thr*, *leu*, *his*, *trp*, *xyl*, *ara*, *lac*, *mtl*, *gal*, *strA*). Mating was interrupted at 120 min.

(b) Recombination frequencies of *fus*.

Cross	Phenotype selected	Unselected marker of donor Fus^r (%)	No. scored
AB312 x W3110	Str^r Thr-Leu$^+$	95	197
AB312 x W4183	ArgG$^+$Thr-Leu$^+$	91	637

Donor: Fus^r mutant from AB312 (Hfr; *thr*, *leu*, *thi*, *lac*, *strA*, *fus*).
Recipients: W3110 (F$^-$) and W4183 (F$^-$; *argG*, *strA*).
Mating was interrupted at 60 min.

recombinants were selected and scored for Fus^r. Only a few percent of recombinants selected for Str^r were Fus^r (Table 2-a). The results suggested that *fus* may situate near the *strA* gene. The Fus^r mutant (*thr*, *leu*, *strA*, *fus*) from Hfr strain AB312 was mated with F$^-$ strain W3110. Fus^r was observed in 95% of Str^r recombinants (Table 2-b), in harmony with the results of the previous cross (Table 2-a). The same donor was mated with F$^-$ strain W4183 (*argG*, *strA*); and 91% of recombinants selected for ArgG$^+$ were Fus^r (Table 2-b). The results indicated that *fus* may be near the *argG* locus.

Transduction experiments by P1 phage were performed between the Fus^r donor strain AB312 (*strA*, *fus*) and recipient strain HfrC, AB 1450 (*malA*) or W4183 (*argG*). It was found that 17% of MalA$^+$ transductants of AB1450 and <0.2% of the ArgG$^+$ ones of W4183 inherited the donor Fus^r marker. The *fus* and *strA* were cotransduced at a frequency of 86 to 90% in the transductant clones of HfrC (Table 3).

Table 3. Linkage of *fus* in transduction by P1 phage.

Recipient	Selected transductants	Transductants per 10^8 P1 ϕ	No. scored	% of transductants that scored as Str^r	% of transductants that scored as Fus^r
HfrC	Fus^r	54	395	86	
	Str^r	20	359		90
AB1450	MalA$^+$	12	134		17
W4183	ArgG$^+$	152	682		0

P1 donor: Fus^r mutant (*thr*, *leu*, *thi*, *lac*, *strA*, *fus*) from AB312. Recipients: HfrC (*met*), AB1450 (*ilv*, *arg*, *met*, *his*, *thi*, *xyl*, *gal*, *malA*, *lac*, *tsx*, *strA*) and W4183 (*argG*, *strA*).

The results indicated that the *fus* gene is located at minute 64.2, about 0.2 min. from *strA*.

It is of interest that G factor mutation (*fus*) is near the *strA* gene, where ribosomal mutations lie in a cluster between the *argG* and *malA* loci (9). The present genetic study indicates that G factor is a member of, or closely related to, the ribosomal protein, although it is present in the supernatant fraction of cell extracts as well as in the unwashed ribosomes.

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