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Chromosomal Regulation of Sexual Expression in *Escherichia coli*

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We report a genetic analysis of a recessive chromosomal mutation of *Escherichia coli* K-12 that is responsible for masking the sexual expression of an F factor that it carries. We call this new bacterial gene, which is closely linked to *thr*, *fex*, for F expression.

The capacity of Hfr or F^+ *Escherichia coli* cells to act as donors in conjugation depends upon specific properties determined by the F or R sex factors. Thus, for any strains to mate, at least one of the pair must be F^+ (5). Recently, it was brought to our attention that certain supposedly female strains of *E. coli* K-12 were capable of mating, although with a much delayed appearance of progeny (T. Anderson and J. Ou, personal communication). Upon further investigation of this female-female mating, we discovered that one of the strains, OU3091, contains a chromosomal mutation responsible for "masking" the expression of an F factor which it carries. Therefore, though scored as female by the criterion of resistance to male-specific phage (7), OU3091 is actually a repressed male, capable of undergoing classical conjugation and recombination but in a limited way. We propose to call this new bacterial gene, which is closely linked to the genes for threonine biosynthesis at 0 min on the revised map (3), *fex*, for F expression.

The crosses performed to analyze the results of Ou and Anderson were (i) OU3091 × OU41112 ("F^-" *leu-6* × F^- *metA28*); (ii) OU3091 × MX554 ("F^-" *leu-6* × F^- *metB*); (iii) OU3091 × D30 ("F^-" *leu-6* × HfrC *metB*), and (iv) OU41112 × K17 (F^- *metA28* × F *leu-6*). The full genotypes and origins of these strains are listed in Table 1. In all instances, after mixing the cultures and plating, we selected Met^+ Leu^+ recombinants on minimal plates supplemented with arginine, threonine, histidine, tryptophan, and thiamine. The first two crosses yielded recombinants only after 5 days of incubation at 37°C, whereas the third cross yielded recombinants after 2 days. The last cross yielded no recombinants at all. Few revertant colonies were found on control plates of each unmixed parent. The recombinant frequency for cross 3 was about 10^{-3} for each Hfr cell. No real frequency could be determined for crosses 1 and 2 since colonies grew in areas of considerable background on the plates, characteristic of plate mating and probably accounting for the delayed appearance. Twenty recombinants were picked from each cross and purified by two streakings on the selection plates. These colonies grew up in 2 days. The unselected markers were determined both by replica plating (6) and by growth in liquid culture. The results are summarized in Tables 2-4.

We predicted that if two females were to conjugate and their genomes recombine, the recombinants should show no bias toward the markers of either parent. Our results show that this is not the case. In the first two crosses, it appears that OU3091 is acting as a polar donor rather than as a symmetrical recipient. However, in the cross with the Hfr, the OU3091 markers predominate in the recombinants and the D30 markers appear with the expected gradient of transmission (14).

We also compared the ability of the parental strains and the recombinants to plate the female-specific phage T7 and the male-specific phage f2. It is necessary to use sensitivity to both male and female phages to define sexuality since there exist F factors that are fi^+, i.e., fertility inhibited, and strains that have sex factor mutations affecting phage sensitivity and/or gene transfer (11, 13). The plating results are also shown in Tables 2 to 4.

The development of phage T7 is inhibited in cells containing the F factor due to the expression of the F *pif* genes (9). The F^- strains MX554 and OU41112 plated T7 with the same efficiency. The uniform plaques were about 1 cm in diameter. However, OU3091 plated with a 10-fold lower efficiency, and the plaques were small and irregular. D30 plated T7 at an efficiency of less than 10^{-4}. A T7 plaque that was picked from the OU3091 lawn replated with the same low efficiency and plaque morphology on that strain. This rules out the influence of a restriction-modification system of the type specified by some fi^+

TABLE 1. *Bacterial strains used*

Strain no.	Mating type	Genotype/phenotype	Derivation or source
OU3091	F^+ (repressed)	*thr-1 leu-6 thi-1 lacY malA* λ^r *xyl-7 ara-13 mtl-2 tonA2 gal-6 (gal-b) his-1 argH1 trp nalA str-9 fex*	T. Anderson and J. Ou *nalA* mutant derived from PA309. Source of F and *fex* unknown
OU41112	F^-	*metA28 gal arg tsx thi-1 lac xyl*	T. Anderson and J. Ou
MX554	F^-	*metB leu*(Am) *lacZ*(Am) *galK*(Am) *galE trp*(Am) *rpsL supD43*	M. Oeschger, unpublished
K17	F^-	*lacY1 thr-1 leu-6 thi-1 tonA21 supE44 str* λ^-	C600 *str* (2)
D30	Hfr	HfrCavalli P02A *metB1 pyrD*(Am) *supD rel-1 tonA22* $T2^r$	S. Brenner
AB313	Hfr	HfrAB313 P012 *thr-1 leu-6 thi-1 sup-49 lacZ4 str-8*	A. L. Taylor and E. A. Adelberg (10)
KLF1/AB2463	F′	F′101/*thr-1 leuB6 thi-1 argE3 his-4 proA2 recA13 lacY1 galK2 mtl-1 xyl-5 ara-14 strA31 tsx-33* λ^- *supE44*	K. B. Low (7)

TABLE 2. *Analysis of OU3091 × OU41112 cross*

Frequency	Genotype[a]							T7[b]	f2[c]
	thr	*tonA*	*tsx*	*trp*	*his*	*nalA*	*str*		
10	x	x	x	x	x	x	x	o	*
4	x	x	x	x	x	x	x	x	
1	x	x	x	x	x	x	o	o	*
1	x	x	o	x	x	x	x	o	*
1	o	x	x	x	x	x	o	x	
1	o	x	x	x	x	x	o	o	
1	o	x	x	x	o	o	o	o	
1	o	x	x	o	o	o	o	o	

[a] Selection for Met^+ Leu^+ recombinants. In all tables, o refers to the OU3091 genotype and x refers to the genotype of the other parent, i.e., OU41112. The markers are listed in the order of their appearance on the *E. coli* map, starting with *thr* at 0 min and proceeding clockwise to *str* at 64 min.

[b] Those classes of recombinants that plate phage T7 as the OU3091 parent are denoted by o. Those that plate the phage as the other parent are denoted by x (see text).

[c] Those classes of recombinants that are f2 sensitive are denoted by an asterisk.

TABLE 3. *Analysis of OU3091 × MX554 cross*[a]

Frequency	Genotype							T7	f2
	thr	*tonA*	*tsx*	*trp*	*his*	*nalA*	*arg*		
4	x	x	x	x	x	x	o	x	
3	o	x	x	x	x	x	x	x	
2	x	x	x	x	x	x	o	o	*
2	x	x	x	x	x	o	o	o	
2	o	x	x	x	x	x	x	o	*
1	x	x	x	x	x	x	x	x	
1	x	x	x	x	x	x	o	o	
1	x	x	x	o	o	o	o	o	*
1	x	o	o	x	x	x	o	o	*
1	o	x	x	x	x	x	o	o	
1	o	o	x	x	x	o	o	o	
1	o	o	x	o	o	o	o	o	

[a] See Table 2, footnotes *a-c*.

TABLE 4. *Analysis of OU3091 × D30 cross*[a]

Frequency	Genotype							T7	f2
	thr	*lac*	*trp*	*his*	*nalA*	*str*	*arg*		
8	x	x	o	o	o	o	o	x	*
4	x	x	o	o	o	o	o	o	*
2	x	o	o	o	o	x	o	x	*
2	x	o	o	o	o	o	o	x	*
1	x	x	o	o	o	o	x	o	*
1	o	x	o	o	o	o	o	x	
1	o	x	o	o	o	o	o	o	
1	o	o	o	o	o	o	o	o	

[a] See Table 2, footnotes *a-c*.

and fi^- R plasmids as an explanation for the aberrant plating properties (12). This result indicates partial expression of the *pif* genes of an F factor. However, many kinds of host mutations, e.g., receptor mutations, could also effect a similar phenotype. Similar results were obtained with the female-specific phage ϕII (data not shown).

Infection by the male-specific phage f2 is dependent upon proteins encoded within the F transfer operon (1). Phage f2 does not plate on OU3091, OU41112, MX554, or K17; it does plate on the Hfr strain D30. A large number of the recombinants were sensitive to f2 (Tables 2 to 4), and this property was infectious, i.e., these cells could transfer f2 sensitivity to other strains at a high frequency (data not shown). It was noteworthy that so many of the progeny of the Hfr cross (85%) were sensitive to f2. In a classical Hfr cross, only those recipients that receive the entire Hfr chromosome become sensitive to male-specific phages; this is a rare event, occur-

ring only under optimal conditions (4). In addition, we noted that most of the recombinants that failed to plate f2 were threonine auxotrophs, a property inherited from the OU3091 parent. The male-specific phage, f1, plated as did f2 (data not shown).

Thus we conclude that OU3091 contains a sex factor, one that is in a repressed state. At a very low frequency, repression is relieved, the chromosome is mobilized, and classical conjugation and recombination ensue. This would account for the prolonged incubation period (5 days) that was necessary to obtain recombinants. When this sex factor was moved to new genetic backgrounds, as would be the case with many of the recombinants, the factor expressed.

The high correlation between f2 resistance and threonine auxotrophy in the recombinants leads us to postulate that a locus (*fex*, for F expression) responsible for the expression of the F factor is linked to threonine. The linkage of *thr* and *fex* has been confirmed by P1 transduction experiments.

When OU3091 was transduced to Thr^+ with P1 grown on normal Hfr, F^+, or F^- Thr^+ strains, over 80% of the transductants also plated f2. *fex* is closer to *thr* than to *leu*, since transduction of OU3091 to Leu^+ gave only 5% f2 sensitivity. As expected, the recombinants from crosses 1 and 2 that were thr^- and f2 resistant but which plated T7 poorly could readily be transduced to f2 sensitivity. These cotransduction experiments place the OU3091 *fex* gene in the region of 0 min on the *E. coli* map. Further support for the position of *fex* comes from transducing *fex* into another strain. AB313 was transduced to Thr^+ with P1 grown on an OU3091 Thr^+ revertant. More than 85% of the transductants lost the ability to plate f2. In no instance did the OU3091 *fex* marker corevert with the threonine marker. The results of these experiments verify that the *fex* gene is capable of turning on and off expression of the F factor, in both its autonomous and integrated forms.

It would seem that the F transfer operon or some of its cistrons is the target of regulation by the *fex* gene. However, the expression of other genes associated with the same F agent is not affected, e.g., a galactose or lactose gene on an F′ (data not shown).

The *fex* gene in OU3091 is recessive. Merodiploids of the threonine-leucine region were formed by mating a cured OU3091 derivative with KLF1/AB2463. The presence of the F′101 *fex* gene led to full F expression. Therefore, we suggest that *fex* codes for a positive regulator of the expression of F which probably acts at the level of transcription. The manner in which the *tra* operon of the F factor is controlled by *fex* is under study.

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