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Fine-Structure Mapping of the *firA* Gene, a Locus Involved in the Phenotypic Expression of Rifampin Resistance in *Escherichia coli*

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The *firA*(Ts)*200* mutation not only eliminates the resistance to rifampin of certain genetically resistant strains, but, moreover, renders ribonucleic acid synthesis thermolabile. The *firA* gene has been mapped by P1 transduction and is located extremely close to the structural gene for deoxyribonucleic acid polymerase III at 4 min on the *Escherichia coli* linkage map.

The enteric bacterium *Escherichia coli* is normally sensitive to low levels of the antibiotic rifampin. Mutants resistant to rifampin have lesions in the *rpoB* gene (6), which codes for the β subunit of the ribonucleic acid polymerase (10). Mutations conferring rifampin resistance that map elsewhere on the bacterial chromosome have not been reported.

Mutations at other loci may reduce or eliminate the phenotypic resistance to rifampin of certain strains carrying *rif*^{r} mutations of the *rpoB* gene; these are termed *fir* mutations (R. Lathe, Ph.D. thesis, University of Brussels, Brussels, Belgium, 1976). To date, four such loci have been identified, and letters have been allocated to the first three. These are: *firA*, identified by the *firA*(Ts)*200* mutation (1); *firB*, identified by the *firB*(Ts)*290* mutation (8); and *firC*, identified by the pseudo-*rif*^{r} allele of strain RCB25 (C. Babinet, Ph.D. thesis, University of Paris, Paris, France, 1970). *recA* mutations have also been reported to give rise to the *fir* effect (11).

Importantly, the mutations *firA200* and *firB290* render ribonucleic acid synthesis thermosensitive in vivo and in vitro (1, 8), and hence the protein products of these two genes probably interact, either directly or indirectly, with the ribonucleic acid polymerase of this organism.

The *firA* gene was previously reported to be located in the *leu-proA* region of the chromosome (1). P1 transduction mapping performed by the method of Miller (7) has now enabled the order of markers in this region to be definitely established. In this work the following specific mutations were employed: *dapD2* (3), *firA200* (1), *metD279* (a new *metD* mutation isolated by the method of Cooper [4]), *popC50* (9), and *polC486* (*dnaE486;* 12). In each of 18 multiple-point crosses, an average of 175 transductants was screened, and from the data in Table 1 (R. Lathe, Ph.D. thesis) the following map order was established:

$$leu - pan - tonA - popC - dapD - \left\{ \begin{matrix} firA \\ polC \end{matrix} \right\} - metD - proA$$

To establish the relative orientations of the *firA200* and *polC486* markers, phage P1 grown upon one strain (*firA200* or *polC486*) was used to transduce a second strain (*polC486* or *firA200*) to independence of the nutritional requirement conferred by a third mutation (*popC50*) in the same region. In principle, the frequency of thermoresistant transductants (having lost both thermosensitivity markers) obtained in each cross and its reverse should establish the relative order of the two thermosensitivity markers. In all such crosses, thermoresistant transductants were found at a significant frequency (2.5 to 4%), and hence the relative order of the two markers could not be unambiguously established. Microheterogeneity and/or high negative interference might account for this result; the available data cannot discriminate, however, between these possibilities. By application of the formula of Wu (13), the maximum separation of the *polC486* and *firA200* markers can be calculated to be less than 0.1 min on the *E. coli* linkage map.

Application of the formula of Wu to the overall transduction data has allowed the distances between the markers to be calculated. The map presented in Fig. 1 has been normalized to the positions of the *leu* and *proA* markers (at 1.65 and 5.6 min, respectively) according to Bach-

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TABLE 1. *P1 transduction mapping of markers in the 4-min region of the chromosome map*

Transductional cross[a]		Marker selected	No. of transductants	Assortment of unselected markers	
				Markers	No.
P1(RL1)	× P4XSB4	pan$^+$	325	tonA firA$^+$ leu$^+$	171
pan$^+$ tonA firA200 leu	pan ton$^+$ firA$^+$ leu$^+$			tonA firA200 leu$^+$	82
				ton$^+$ firA$^+$ leu$^+$	67
				ton$^+$ firA200 leu$^+$	4
				ton$^+$ firA$^+$ leu	1
P1(RL1)	× AT980	dap$^+$	180	tonA firA$^+$	31
pan$^+$ tonA firA200 leu	dapD ton$^+$ firA$^+$			tonA firA200	55
				ton$^+$ firA$^+$	73
				ton$^+$ firA200	21
P1(P4XSB4)	× RL1	leu$^+$	496	firA200 pan$^+$	495
pan leu$^+$ firA$^+$	pan$^+$ leu firA200			firA200 pan	1
P1(RL1)	× RL51	met$^+$/metD$^+$	138	pan$^+$ firA200 tonA	9
pan$^+$ met$^+$ firA200	pan metB metD		(metD$^+$)	pan$^+$ firA200 ton$^+$	1
tonA				pan firA200 tonA	9
				pan firA200 ton$^+$	14
				pan firA$^+$ tonA	2
				pan firA$^+$ ton$^+$	103
P1(G7)	× RL51	met$^+$/metD$^+$	76	pro$^+$	71
met$^+$ proA	metB metD		(metD$^+$)	pro	5
P1(Q94)	× RL51	met$^+$/metD$^+$	129	pan$^+$ pol$^+$	9
metD$^+$ pan$^+$ polC	metB metD pan pol$^+$		(metD$^+$)	pan$^+$ polC	3
				pan pol$^+$	91
				pan polC	26
P1(594)	× RL54	pan$^+$	200	polC	173
pan$^+$ pol$^+$	polC pan			pol$^+$	27
P1(594)	× RL2	pan$^+$	250	firA$^+$	48
pan$^+$ firA$^+$	pan firA200			firA200	202
P1(RL2)	× PCMC1	pop$^+$	200	pan tonA	63
pan tonA pop$^+$	pan$^+$ ton$^+$ popC			pan ton$^+$	2
				pan$^+$ tonA	103
				pan$^+$ ton$^+$	32
P1(RL1)	× PCMC1	pop$^+$	140	firA$^+$	97
firA200 pop$^+$	firA$^+$ popC			firA200	43
P1(Q94)	× PCMC1	pop$^+$	27	pol$^+$	18
polC pop$^+$	popC pol$^+$			polC	9
P1(PCMC1)	× RL52	pan$^+$	90	popC metD$^+$	8
popC met$^+$ pan$^+$	metB metD pop$^+$ pan$^+$			pop$^+$ metD$^+$	1
				popC metD	42
				pop$^+$ metD	39
P1(PCMC1)	× RL54	pan$^+$	200	pop$^+$ pol$^+$	12
popC pol$^+$ pan$^+$	polC pan pop$^+$			pop$^+$ polC	64
				popC pol$^+$	48
				popC polC	76
P1(PCMC1)	× RL2	pan$^+$	30	popC firA$^+$	9
popC firA$^+$ pan$^+$	pop$^+$ firA200 pan			pop$^+$ firA200	15
				popC firA200	6

TABLE 1—*Continued*

Transductional cross[a]		Marker selected	No. of transductants	Assortment of unselected markers	
				Markers	No.
P1(AT980) *met*⁺ *dapD pop*⁺	× RL56 *popC metB metD dap*⁺	*pop*⁺	150	*metD*⁺ *dap*⁺	1
				metD⁺ *dapD*	16
				metD dap⁺	45
				metD dapD	88
P1(594) *pop*⁺ *firA*⁺	× RL21 *popC firA200*	*pop*⁺	100	*firA*⁺	31
				firA200	69
P1(594) *pop*⁺ *pol*⁺	× RL57 *popC polC*	*pop*⁺	100	*pol*⁺	26
				polC	74
P1(SA269T) *pan*⁺ *met*⁺ *tonA*	× RL59 *metB metD pan ton*⁺	*pan*⁺	300	*metD*⁺ *tonA*	31
				metD⁺ *ton*⁺	3
				metD tonA	162
				metD ton⁺	104

[a] P1(*A*) × *B* indicates that phage P1 grown on strain *A* was used to transduce strain *B* for the marker specified.

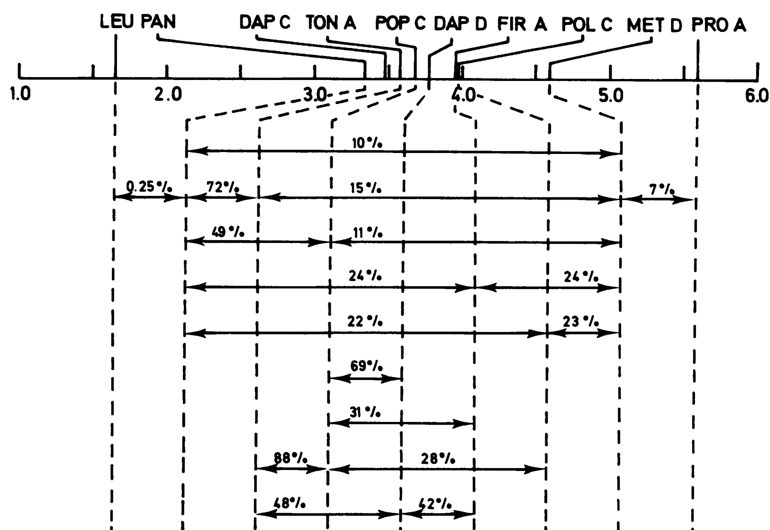

FIG. 1. *Positions of markers in the 4-min region of the chromosome map as determined by application of the formula of Wu (13) to P1 transduction data. The figures given are the overall average cotransduction frequencies (percent) between the markers indicated.*

mann et al. (2) and assumes that the length of phage P1 represents 2 min on the *E. coli* linkage map. The positioning of the *firA* gene to the left-hand (*dapD*) side of the *polC* gene is suggested by consideration of cotransduction frequencies alone and has been further confirmed by analysis of specialized transducing phages for this region (unpublished data).

It has recently been reported that the *rpsB* and *tsf* genes, coding for ribosomal protein S2 and translation elongation factor EFTs, respectively, are located in the *tonA-polC* region of the chromosome (5, 14). Biochemical and genetic evidence (to be presented elsewhere), however, suggests that the *firA200* mutation does not affect either the *rpsB* or *tsf* gene or, indeed, the proximal *polC* gene coding for deoxyribonucleic acid polymerase III. The clustering of essential functions in this small region of the chromosome raises the possibility that the expression of these genes may be coordinately controlled.

Attempts are currently in progress to identify the protein product of the *firA* gene.

During the course of this work I was a Ciba-Geigy fellow.

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