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Allele-specific suppression of the temperature sensitivity of *fitA/fitB* mutants of *Escherichia coli* by a new mutation (*fitC4*): isolation, characterization and its implications in transcription control

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The temperature sensitive transcription defective mutant of *Escherichia coli* originally called *fitA76* has been shown to harbour two missense mutations namely *pheS5* and *fit95*. In order to obtain a suppressor of *fitA76,* possibly mapping in *rpoD* locus, a Ts^+ derivative (JV4) was isolated from a *fitA76* mutant. It was found that JV4 neither harbours the lesions present in the original *fitA76* nor a suppressor that maps in or near *rpoD*. We show that JV4 harbours a modified form of *fitA76* (designated *fit*A76*) together with its suppressor. The results presented here indicate that the *fit95* lesion is intact in the *fitA76** mutant and the modification should be at the position of *pheS5*. Based on the cotransduction of the suppressor mutation and/or its wild type allele with *pps*, *aroD* and *zdj-3124*::Tn*10 kan* we have mapped its location to 39·01 min on the *E. coli* chromosome. We tentatively designate the locus defined by this new extragenic suppressor as *fit*C and the suppressor allele as *fitC4*. While *fitC4* could suppress the Ts phenotype of *fitA76** present in JV4, it fails to suppress the Ts phenotype of the original *fitA76* mutant (harbouring *pheS5* and *fit95*). Also *fitC4* could suppress the Ts phenotype of a strain harbouring only *pheS5*. Interestingly, the *fitC4* Ts phenotype could also be suppressed by *fit95*. The pattern of decay of pulse labelled RNA in the strains harbouring *fitC4* and the *fitA76** resembles that of the original *fitA76* mutant implying a transcription defect similar to that of *fitA76* in both these mutants. The implications of these findings with special reference to transcription control by Fit factors *in vivo* are discussed.

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1. Introduction

Regulation of gene expression in prokaryotes occurs mostly at the level of transcription. This involves use of multiple sigma factors, antisigma factors and several macromolecular elements collectively called "accessory transcription factors" (Yura and Ishihama 1979; Ishihama 1988, 1993, 2000). These factors interact either with DNA or RNA polymerase or both and confer selectivity on the process and thus regulate gene expression. Our laboratory has been studying the control of transcription by accessory transcription factors in *Escherichia coli* for a long time. Our earlier work in this area has been reviewed (Jayaraman 1994). Specifically, two genes have been identified designated *fitA* and *fitB*, mapping close to each other at 38.7 min. The products of these genes are believed to interact with each other

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as well with RNA polymerase and control the expression of few classes of genes (which might include some of the genes coding ribosomal proteins). A tentative model has been proposed to explain the interaction between FitA and FitB products with each other as well as with RNA polymerase. These conclusions stem from the initial isolation and characterization of a temperature sensitive transcription defective mutant (*fitA76*) and two of its suppressors (*fitA24* and *fitB*) and modulation of growth properties of these mutants by four *rpoB* mutations (*rpoB240, rpoB7, rpoB55* and *rpoB42*) in an allele specific manner (Jabbar and Jayarman 1976, 1978; Jayaraman and Jabbar 1980; Dass and Jayaraman 1985a,b, 1987; Munavar and Jayaraman 1987; Munavar *et al* 1993). Polar effect of Tn*5* insertions in *fitA* on *fit*B expression suggested that *fitA* and *fitB* could form an operon by themselves or be components of one, the direction of expression being *fitA→fitB* (Munavar 1991). Initially, it was believed that the *fitA* and *fitB* mutations defined hitherto unidentified genes and the lesions could be single base change in the respective genes.

Molecular characterization of a recombinant plasmid clone harbouring 2.1 kb fragment from wild type *E. coli* which complemented the Ts phenotype of the *fitA76* mutant and the corresponding chromosomal region of *fitA76* mutant revealed that *fitA76* harbours two missense mutations: a $G_{293}\rightarrow A_{293}$ transition in *pheS* locus (which codes for alpha subunit of phenylalanyl tRNA synthetase) and the other named *fit95* (possibly located in the *pheT* locus which codes for the beta subunit of the phenylalanyl tRNA synthetase). The presence of the same G→A transition at position of 293 of *pheS* gene in the temperature sensitive translation defective *pheS* mutant namely *pheS5* (Kast *et al* 1992) and absence of transcriptional abnormalities characteristic of *fitA76* in either *pheS5* or *fit95* mutants justify the need for both mutations to elicit the phenotype characteristic of the *fitA76* mutant (Ramalingam *et al* 1999; Sudha *et al* 2001; B Praveen Kamalakar and M H Muanvar, unpublished results). These results and the similarity in the organization and expression of *fitA* and *fitB* genes *vis-à-vis pheS* and *pheT* genes (Springer *et al* 1982) led to the proposal that *fitA* and *fitB* genes could be same as *pheS* and *pheT* and that the subunits of phenylalanyl tRNA synthetase could also function as selective transcription factors interacting perhaps with β subunit of RNA polymerase (Ramalingam *et al* 1999; Sudha *et al* 2001).

In this investigation, in an effort to know whether the selective transcription regulation by Fit factors stems from their interaction with the σ subunit of RNAP, we sought for mutation(s) in *rpoD* capable of suppressing the *fitA76* Ts phenotype. Starting from a *fitA76* mutant, we isolated a Ts^+ derivative (JV4). This report describes the genetic and physiological characterization of this derivative.

2. Materials and methods

2.1 *Media and chemicals*

M9 minimal media and LB medium were prepared according to Miller (1972, 1992). Antibiotics were used at the following concentrations: kanamycin 30 μg/ml; tetracycline 10 μg/ml; rifampicin 50 μg/ml. Rifampicin was obtained from Sigma Chemical Company, USA while all other antibiotics and other chemicals were from local sources. [^{3}H] uridine was from Bhabha Atomic Research Centre, Mumbai. The ready-made scintillation fluid (INSTA-GEL) was from Packard Instrument Company Inc., USA.

2.2 *Bacterial strains, and bacteriophages*

The *E. coli* strains used in this study are listed in table 1. The P1 phage (P1 *vir*) used in this investigation is from our laboratory collection.

2.3 *Methods*

All genetic techniques were according to Miller (1972, 1992).

2.4 *Curing of Tn*10

This was done according to the method of Maloy and Nunn (1981). Fresh overnight cultures to be cured of Tn*10* were sub cultured into fresh LB medium and allowed to grow till midlog phase. One ml of the cells were centrifuged down and resuspended in the same volume of saline. Approximately 10^5 – 10^6 cells were plated on Bochner's medium and incubated at 37°C. The colonies which appeared were segregated twice on Bochner's medium and checked for the Tet^s phenotype.

2.5 *Pulse labelling of RNA and decay of pulse-labelled RNA*

Cells were grown to midlog phase at 30°C with shaking in glucose minimal medium, centrifuged, resuspended in the original volume of 0.01 M Tris-HCl, pH 8 containing 1 mM disodium EDTA and aerated at 30°C for 10 min. The Tris EDTA treatment was given to allow rapid permeation of rifampicin that is used to arrest RNA synthesis after pulse labelling. The cells were again centrifuged down, resuspended in the original volume of pre-warmed growth medium, divided into two halves and shaken at 30°C and 42°C for 1 h. After pre-incubation, RNA was pulse labelled with [^{3}H] uridine (16,500 mCi/mmol; 0.5 μCi/ml) for 20 s. The pulse was terminated by adding rifampicin (100 μg/ml) and non-radioactive uridine (10 mM). After this 0.2 ml aliquots

Table 1. List of *E. coli* strains used in this study.

Strain	Relavent genotype	Source/Reference
CSH57	F⁻ *arg his trp ilv metA leu purE rpsL*	Laboratory collection
AB1157	F⁻ *hisG4Δ (gpt-pro) leuB6 thr1 argE3 rpsL31*	Laboratory collection
JAJ572	F⁻ *fitA76* (*pheS5 fit95*) derivative of CSH57	Jabbar and Jayaraman 1978
JV4/Tn*10*	A Tet^r Ts$^+$ derivative of JAJ572	This study
HfrC K10	HfrC wild type	Berlyn, CGSC, USA
K10 Tet^R	*pps*::Tn*10* derivative of HfrC	This study
CAG18578(K34)	*zdj3124*::Tn*10kan* derivative MG1655	Berlyn, CGSC, USA
RT500	F⁻ *his pps aroD pyrD edd pfkA*? *rpsL*	Laboratory collection
JV4	Tet^s derivative of JV4/Tn*10*	This study
JV41	*pps*::Tn*10 fitA*$^+$ *fitB*$^+$Ts (*fitC4*) derivative of JV4	This study
JV43	*zdj 3124*::Tn*10kan fitC4 fitA*$^+$ *fitB*$^+$ *pps*::Tn*10* derivative of JV4	This study
JV57	*aroD pps*::Tn*10* derivative of CSH57	This study
JVS5	*pps*::Tn*10 pheS5* derivative of CSH57	This study
JV *fitA*76*	*aroD*$^+$ Ts (*fitA76* fitC*$^+$) derivative of JV57	This study
PMJ*fitA*76*	*zdj 3124*::Tn*10kan fitC*$^+$ *fitA76** derivative of JV4	This study
PMJ02	*pps*::Tn*10 fitA76* fitC*$^+$ derivative of HfrC K10	This study
PMJ50	*pps*::Tn*10* derivative of PMJ *fitA76**	This study
SMJ01	Same as K10 but has *pps*::Tn*10 fitA76* (*pheS5-fit*95)	Sudha *et al* 2001
NP37	Same as HfrC K10 but *pheS5*	Bachmann, CGSC, USA
SMJ02	Same as HfrCK10 but has *pps*::Tn*10 pheS5*	Sudha *et al* 2001
PKM01	Same as HfrCK10 but has *pps*:: Tn*10 fitC4*	This study
PKM01 *pps*$^+$	Same as PKM01 but *pps*$^+$ Tet^S	This study
PKM57	*pps*::Tn*10 fit*C4 *fitA*$^+$ *fitB*$^+$derivative of CSH57	This study
PKM57 *pps*$^+$	Same as PKM57 but *pps*$^+$ Tet^S	This study
PMJ95	Same as AB1157 but has *fit95 pps*::Tn*10 rpoB201*	This study

were removed at different time intervals, added to 0.5 ml of ice cold 10% TCA, and kept chilled. The precipitate was collected on Whatman glass microfibre filters, washed 5 times with 5% TCA containing 50 μg/ml of non-radioactive uridine and once with 95% ethanol and air dried. The dried filters were counted in a liquid scintillation counter at an efficiency of 65%.

3. Results

3.1 *Isolation of a temperature insensitive derivative from a* fitA76 *mutant*

When the work reported in this paper was initiated it was neither known nor suspected that the *fitA76* mutant could harbour two mutations (*pheS5* and *fit95*), although its transcription defects at 42°C were well documented (Jayaraman and Jabbar 1980; Dass and Jayaraman 1985 a,b; Munavar *et al* 1993). We thought it would be of interest to see if a mutation in the *rpoD* gene (coding for the σ^{70} subunit) could suppress the Ts phenotype of the *fitA76* mutant. Therefore, a derivative of *E. coli* C600 (*rpoD*$^+$) having a Tn*10* insertion close to *rpoD* was mutagenized with MNNG and grown overnight in LB. Phage P1 propogated on the mutagenized culture was used to transduce Tn*10* (Tet^r) to a *fitA76* mutant (JAJ572). The transduced cells were plated on LB-Tet medium and incubated at 30°C until the Tet^r transductants appeared as tiny colonies. Four plates containing approximately 2000 tiny Tet^r transductants in total were shifted to 42°C and incubated at that temperature for 24 h more. Of the four colonies, which grew bigger in size (see table 2, cross 1), one that grew well at 42°C in both LB and minimal medium was designated as JV4/Tn*10* and used for further studies.

3.2 *The suppressor mutation is not located in the* rpoD *region in JV4/Tn*10

In order to verify whether the Ts phenotype of the *fitA76* is suppressed by mutation(s) in or near the *rpoD* locus, the Tn*10* (linked to *rpoD*) from JV4/Tn*10* was transduced again into a *fitA76* recipient and the Tet^r transductants were screened for Ts$^+$ phenotype. None of the 230 Tet^r transductants checked was Ts$^+$ implying the absence of a suppressor mutation in or near *rpoD* (cross 2, table 2). It is possible that while selecting for Ts$^+$ derivatives we might have picked up either a true revertant or a colony harbouring a suppressor elsewhere on the chromosome. This is reminiscent of the observations of Dass and Jayaraman (1985a) who isolated *fitA24* as an intragenic suppressor of *fitA76* while attempting to isolate suppressor mutation(s) in *rpoB*. A similar

Table 2. Isolation of a Ts^+ derivative (JV4) from a *fitA76* mutant and demonstration of the presence of *fitA76* in it.

Donor	Recipient	Selected marker/ character	Unselected phenotype	Cotransduction (%)
P1/ C600(has Tn*10* near *rpoD*$^+$) MNNG mutagenized	JAJ572(*fitA76*) (Ts)	Tet^r	Ts^+	0.2 (4/2000)
P1/ JV4/ Tn*10* (a Ts^+ colony from cross 1)	JAJ572(*fitA76*)	Tet^r	Ts^+	< 0.40 (0/230)
P1/ JAJ572(*fitA76*)	RT500(*aroD*)	*aroD*$^+$	Ts	45.00 (46/103)
P1/ JV4/ Tn*10*	RT500(*aroD*)	*aroD*$^+$	Ts	40.00 (137/340)
P1/ JV4 (Tet^s derivative of JV4/ Tn*10*)	RT500(*aroD*)	*aroD*$^+$	Ts	43.00 (71/165)

Ts, temperature sensitivity; Ts^+, temperature insensitivity.

observation was made by Munavar and Jayaraman (1987), who isolated *fitB* as an extragenic suppressor of *fitA76* while trying to isolate suppressor mutation(s) in *gyrA*. Therefore, we looked for the retention of the *fitA76* lesion in JV4/Tn*10*.

3.3 *The* fitA76 *lesion is intact in JV4/Tn*10 *and the suppressor mutation maps close to* fitA76 *to its left*

The *fitA76* lesion cotransduces approximately 50% and 60% with *aroD* and *pps,* respectively (Dass and Jayaraman 1985a; see also cross 3, table 2). When P1 propagated on JV4/Tn*10* was used as the donor to transduce the *aroD*$^+$ allele into RT500 (the same recipient used in cross 3), 40% of the *aroD*$^+$ transductants became temperature sensitive (cross 4, table 2). This result showed the presence of a Ts lesion, possibly *fitA76*, in JV4/Tn*10* near *aroD* and also implied that the suppressor mutation could lie elsewhere on the chromosome. Before proceeding to know the position of the suppressor mutation it was necessary to cure JV4/Tn*10* of its Tn*10* because such a Tet^s derivative would allow mapping of the suppressor mutation by replacing it with its wild type allele along with linked Tn*10* or Tn*5* insertions from the Carol Gross collection (Nicholas *et al* 1998). The curing was done as described under §2. The cured (Tet^s) derivative was designated JV4. The curing of Tn*10* had no obvious effect on the phenotype of JV4 because it grew as well at 42°C as did JV4/Tn*10*. Also, when P1 propagated on JV4 was used to transduce the *aroD*$^+$ marker into RT500, 40% of the *aroD*$^+$ transductants became temperature sensitive, as expected (see table 2, crosses 4 and 5).

The previously isolated both intra and extragenic suppressors of *fitA76* Ts, namely, *fitA24* and *fitB* by themselves conferred a Ts phenotype. In order to know if this will be true in the present instance also the effect of introduction of the *fitA*$^+$ allele into JV4 was studied. P1 propagated on HfrC *pps::*Tn*10* (*fitA*$^+$) was used to transduce *fitA*$^+$ *via* the linked *pps::*Tn*10* into JV4 and the Tet^r transductants obtained were screened for growth at 42°C on LB medium. Since both the donor and recipient in this cross are Ts^+ (the former being *fitA*$^+$ is Ts^+ and the latter being *fitA76* harbouring a suppressor is phenotypically Ts^+) replacement of *fitA76* by *fitA*$^+$ allele would not be expected to give temperature sensitive transductants in this cross, unless the suppressor mutation by itself confers temperature sensitivity in a *fitA*$^+$ background. The fraction of temperature sensitive colonies (if obtained) among Tet^r transductants should equal the cotransduction between *pps* and *fitA* (~ 60%), provided the locus of the suppressor and *fitA* are not linked. The *fitA-pps* cotransduction frequency using *pps::*Tn*10 fitA*$^+$ as donor and an authentic *fitA76* as recipient was observed to be approximately 66% (table 3, cross 1). However, when same donor P1 was used to transduce *pps*::Tn*10* linked *fitA*$^+$ to JV4 (Tet^S) only 34% of the Tet^r transductants became temperature sensitive (table 3, cross 2). This showed that the suppressor mutation is located in the vicinity of the *fitA*, possibly to its left (see below) and confers temperature sensitivity in *fitA*$^+$ background. It could be linked to *fitA* since its cotransduction frequency with *pps* is only 34% as against the expected cotransduction frequency of 60%, if unlinked. This cross is schematically illustrated in figure 1A.

In order to verify these conclusions, we randomly picked up a temperature sensitive Tet^r transductant from the above cross and designated the same as JV41 with a presumptive genotype *sup-fitA*$^+$*-pps::*Tn*10 aroD*$^+$. When P1 propagated on JV41 was used to transduce the *aroD*$^+$ marker into an *aroD fitA*$^+$ recipient (RT500), 21% of the *aroD*$^+$ transductants became Ts (cross 3, table 3). When the same P1 lysate was used to transduce *pps*::Tn*10* in to the *pps*$^+$*fit*$^+$ strain CSH57, 40% of the Tet^r transductants became Ts (table 3, cross 4; figure 1B,C). The *aroD*-suppressor and *pps*-suppressor cotransduction frequencies (21% and 40% respectively) place the suppressor mutation approximately

Table 3. Location of the suppressor of *fitA76* near *fitA* in JV4.

Donor	Recipient	Selected marker/ character	Unselected phenotype	Cotransduction (%)
P1/HfrC *fitA*$^+$ *pps*:: Tn*10*	JAJ572 (*fitA76*)	Tet^r (*pps*::Tn*10*)	Ts$^+$	66.00 (71/107)
P1/HfrC *fitA*$^+$ *pps*:: Tn*10*	JV4 Tet^s (derivative of JV4/Tn*10*)	Tet^r (*pps*::Tn*10*)	Ts	34.00 (77/230)
P1/JV41(a *pps*:: Tn*10* Ts transductant of JV4 from cross 2)	RT500 *(aroD fitA*$^+$)	*aroD*$^+$	Ts	21.0 (19/91)
P1/JV41	CSH57 (*fit*$^+$ *pps*$^+$)	Tet^r (*pps*::Tn*10*)	Ts	40.0 (53/134)

Ts, temperature sensitivity; Ts$^+$, temperature insensitivity.

0.83 and 0.61 min to the left of *aroD* and *pps* respectively. It is known that *fitA* is located 0.35 min to the left of *aroD* and 0.2 min to the left of *pps* (reviewed by Jayaraman 1994). Therefore, the suppressor should be located to the left of *aroD*, *pps* and *fitA*, approximately at 39.01 min on the *E. coli* linkage map (see below for details), leading to the following order of markers in the Ts$^+$ derivative JV4 or JV4/Tn*10*: suppressor- *fitA76 – pps – aroD* counterclockwise on the *E. coli* genetic map (Berlyn 1998). However, it was shown earlier (see table 2) that JV4 harbours a Ts mutation, which cotransduces 40% with *aroD* possibly *fitA76* itself (for the sake of clarity this Ts lesion will be called *fitA76* until we show below that it is indeed a modified form of *fitA*76). After the position and phenotype of the suppressor were known it can be retrospectively inferred that the Ts transductants obtained in cross 5 table 2 should be the result of an event leading to the inheritance of only *fitA76* (excluding the suppressor allele) along with selected marker (*aroD*$^+$) as shown in the figure 1D.

3.4 *Unmasking the latent temperature sensitivity of JV4 by the introduction of the wild type allele of the suppressor*

The data presented so far shows that the relevant genotype of JV4 is: *sup*$^-$*– fitA76 – pps*$^+$ *– aroD*$^+$. It was also shown that replacement of *fitA76* by *fitA*$^+$ allele in JV4 also results in temperature sensitivity (see above). Therefore replacement of the mutant suppressor allele by the wild type allele in JV4 should also result in Ts phenotype. For this purpose the choice of the donor is very crucial. The donor should have a Tn*10*/Tn*5* insertion located within transducible limits to the left of suppressor but beyond transducible limits from *fitA*$^+$. Such a donor could be used to co-transduce only the locus of the suppressor along with the Tn*10*/Tn*5* insertion and exclude the cotransduction of *fitA*$^+$. Testing five different strains carrying Tn*10::kan* insertions located near the *fit* region, we found one (*zdj-3124::*Tn*10 kan*) which met the criterion. Therefore we chose CAG18578 bearing the above insertion to transduce the wild type allele of the suppressor into JV4. In a transductional cross: P1/CAG18578 (donor) x JV4 (recipient), ~ 4% of the kan^r transductants (6/161) were temperature sensitive. The cotransduction frequency places the suppressor well away from *zdj-3124::*Tn*10 kan* to its right. The order of markers in the Ts transductants obtained in the above cross is inferred to be *zdj-3124::*Tn*10 kan*-suppressor$^+$ *fitA76 – pps*$^+$ *– aroD*$^+$.

3.5 *The map position of the suppressor mutation in relation to other* fit *mutations and its implications*

The map positions of *fitA* and *fitB* alleles relative to their neighbours, especially *aroD* and *pps*, have been worked out very well (reviewed by Jayaraman 1994). Now it is known that *fitA* is the same as *pheS* and *fitB* could possibly be *pheT* (Ramalingam *et al* 1999; B Praveen Kamalakar, M H Munavar and R Jayarman, unpublished results) *fitA* and *fitB* map at 38.7 and 38.6 min respectively, on the *E. coli* chromosome. The *aroD*-suppressor and *pps*-suppressor distances (0.83 and 0.61 min, respectively; see above) place the suppressor locus at 39.01–39.03 min. Also the wild type suppressor allele cotransduces with *zdj3124::*Tn*10 kan* around ~ 4% (see above) and the mutant allele cotransduces with same insertion around 12% (see below). The average cotransduction (8.0%) places the suppressor 1.3 min away from the *zdj3124::*Tn*10 kan* insertion, at 39.01 min. The map position of the suppressor mutation indicates that it could be an extragenic suppressor. The Ts phenotype of the suppressor mutation implies that it defines an essential function. We have tentatively designated the locus defined by the suppressor mutation as *fitC* and the mutation will henceforth be referred to as *fitC4*. Figure 2 summarizes the position of *fitC* in relation to *fitA* and *fitB*, on the linkage map.

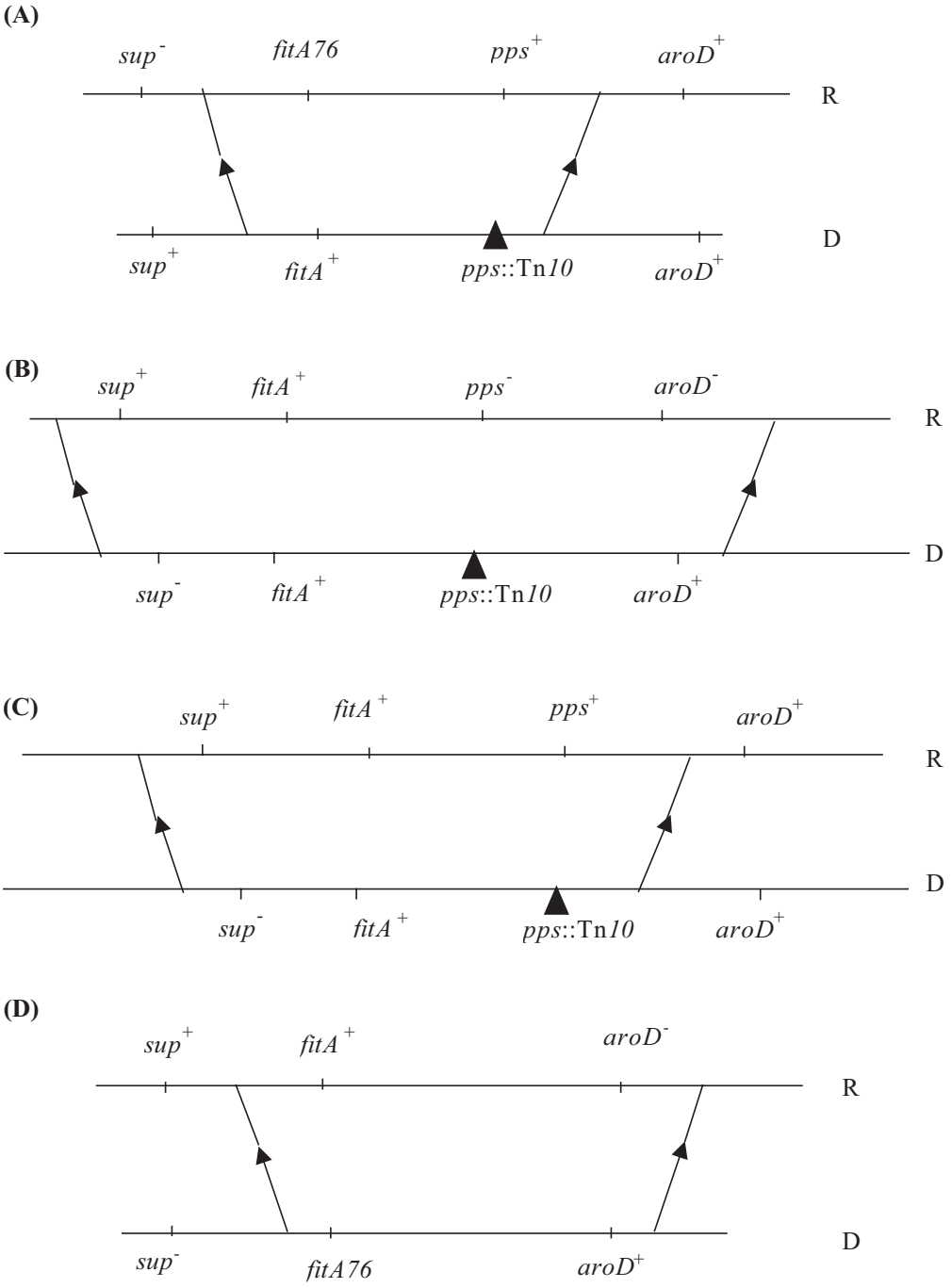

Figure 1. Schematic illustration of the transductional cross described in cross 2, table 3 (**A**), cross 3, table 3 (**B**), cross 4, table 3 (**C**) and cross 5, table 2 (**D**). (A) HfrC *sup*$^+$*fitA*$^+$*pps*::Tn*10* (D) x JV4 *sup*$^-$ *fitA76 pps*$^+$ (R). The two cross over events needed to generate the transductants of the type *sup*$^-$ *fitA*$^+$*pps*::Tn*10*, one to the right of the selected marker (*pps*::Tn*10*) and the other to its left between *sup*$^+$ and *fitA*$^+$ are shown. **(B)** JV41(*sup*$^-$ *fitA*$^+$ *pps*:: Tn*10 aroD*$^+$) (D) x RT500 (*sup*$^+$ *fitA*$^+$ *pps-aroD*$^-$) (R). The two cross over events needed to generate the transductants of the type *sup*$^-$ *fitA*$^+$ *aroD*$^+$ one to the right of the selected marker (*aroD*$^+$) and the other to its left, beyond *sup*$^-$ are also shown. **(C)** JV41(*sup*$^-$ *fitA*$^+$ *pps*:: Tn*10 aroD*$^+$) (D) x CSH57 (*sup*$^+$ *fitA*$^+$ *pps*$^+$*aroD*$^+$) (R). The two cross over events needed to generate the transductants of the type *sup*$^-$ *fitA*$^+$ *pps::*Tn*10* one to the right of the selected marker (*pps::*Tn*10*) and the other to its left, beyond *sup*$^-$ are also shown. **(D)** JV4 *sup*$^-$ *fitA*76 *aroD*$^+$ (D) x RT500 *sup*$^+$*fitA*$^+$*aroD* (R). The two cross over events needed to generate the transductants of the type *sup*$^+$*fitA76 aroD*$^+$ one to the right of the selected marker (*aroD*$^+$) and the other to it's left between *sup*$^-$ and *fitA76* are also shown (figure not drawn to scale). R, Recipient; D, donor

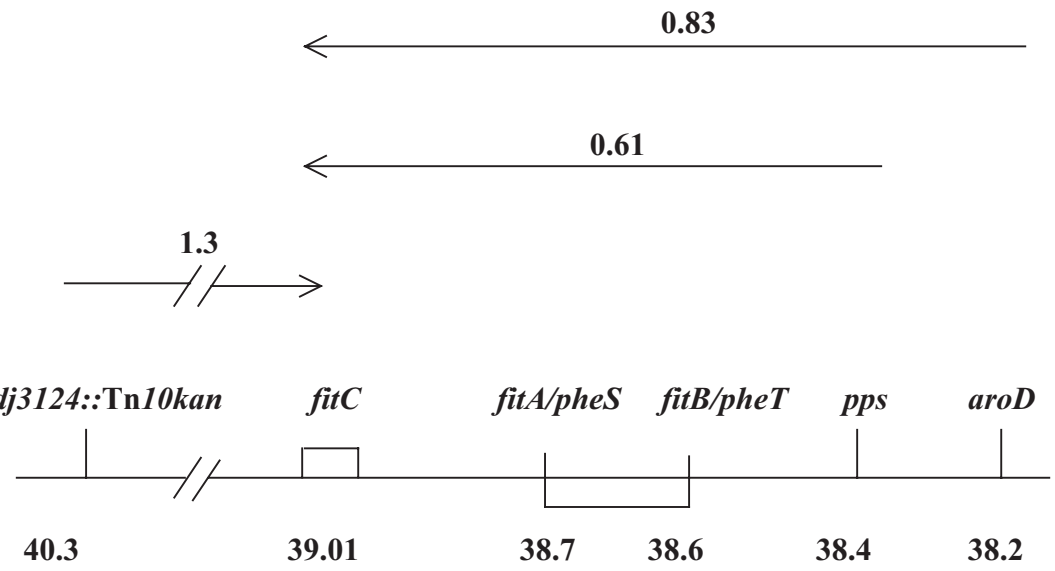

Figure 2. The map postion of *fitC* (in minutes) calculated from co-transduction frequencies using Wu's formula (Wu 1966). The position given for *zdj3124*::Tn*10kan* is from Nicholas *et al* (1998). The positions of *fitA* and *fitB* are from our previous publications (reviewed by Jayaraman 1994) and *pps aroD* positions are from Berlyn (1998).

3.6 *Differential suppression of the Ts phenotype of* fitA76 (pheS5 fit95) *and* pheS5 *mutants by the* fitC4 *mutation*

It was shown above that introduction of *fitC*$^+$ allele into JV4 renders the latter Ts. The reciprocal experiment, namely, introduction of the *fitC4* allele into *fitA76* would be expected to render the latter Ts$^+$ (reconstruction of JV4). Since *fitA76* is now known to harbour two lesions *pheS5* and *fit95* (Ramalingam *et al* 1999) it would be interesting to look into the effect(s) of the *fitC4* mutation on *pheS5* and if possible with *fit95* also. These experiments required a strain from which *fitC4* could be mobilized into desired recipients. Such a strain of genotype *zdj3124*::Tn*10kan fitC4 fitA*$^+$ *fitB*$^+$ *pps*::Tn*10* was constructed as follows. JV4 was transduced with P1 grown on a *fitA*$^+$*B*$^+$*C*$^+$*pps*::Tn*10* and the Tet^R transductants screened for temperature sensitivity to select the transductants that retained *fitC4* but received *fitA*$^+$*B*$^+$ (*fitC4 fitA*$^+$*B*$^+$ *pps*::Tn*10*). *zdj 3124*:: Tn*10kan* was then introduced and Ts colonies (which retained *fitC4*) among the kan^R transductants were picked up. The final construct, *zdj*3124::Tn*10kan fitC4 fitA*$^+$ *fitB*$^+$ *pps*::Tn*10* was named JV43. When P1/JV43 was used to transduce a *fitA76* recipient (JAJ572) none (out of 160) of the kan^r transductants were Ts$^+$ (table 4, cross 1) whereas one would expect approximately 4% to have become Ts$^+$ (see § 3.5). However, 12% of the kan^r transductants obtained using a *pheS5* strain as recipient were Ts$^+$ (table 4, cross 2). The inability of *fitC4* to suppress the *fitA76* lesion was totally unexpected since *fitC4* was isolated as a suppressor of a *fitA76* strain. Moreover a Ts lesion was shown to be present in JV4 at a site expected of *fitA76* (see table 2, crosses 3–5). In order to clarify the discrepancy the ability of *fitC4* to suppress the Ts lesion present at the position of *fitA76* in JV4 was tested. As shown in table 4, crosses 3 and 4, *fitC4* does suppress the same at the same frequency as it suppresses *pheS5* (12%). The above data shows that the lesions present in original *fitA76* and that present in the position of *fitA76* in JV4 are not the same. The Ts phenotype due to former could not be suppressed by the *fitC4* mutation whereas the latter (henceforth referred to *fitA76**) could be.

3.7 *Reconstruction of JV4* (fitC4 fitA76*) *in another genetic background*

As could be seen from the above the JV4 was isolated as a Ts$^+$ derivative of JAJ572 (a CSH57 derivative; see table 1) during an attempt to isolate suppressor mutations(s) in *rpoD*. Genetic characterization of JV4 reported above clearly indicate that it neither harbours a suppressor of *fitA76* in/near *rpoD*, nor harbours the original *fitA76* lesion. Instead, it is shown to harbour a modified form of *fitA*76 (*fitA*76*) together with its suppressor *fitC4*. Since most observations made during the characterization of JV4 were unexpected, it was imperative to move these two mutations (*fitC4* and *fitA76**) into a totally new genetic background such as HfrC, and reconfirm that suppression occurs independent of genetic background. Since JV4 is a Ts$^+$

Table 4. Transductional crosses illustrating the differential suppression of *fitA76* and *pheS5* Ts mutants by *fitC4*.

Donor	Recipient	Selected marker/ character	Unselected phenotype	Cotransduction (%)
P1/JV43(*zdj 3124*::Tn*10 kan fitC4 fitA*$^+$ *B*$^+$ *pps*::Tn*10*)	JAJ572 (*fitA76*)	Kan^r	Ts$^+$	< 0.6 (0/160)
P1/JV43(*zdj 3124*::Tn*10 kan fitC4 fitA*$^+$*B*$^+$ *pps*::Tn*10*)	JV5S (*pheS5*)	Kan^r	Ts$^+$	12 (10/85)
P1/JV4 (*fitC4*$^-$ *fitA76* ? *aroD*$^+$)	JV57 (*fitC*$^+$ *fitA*$^+$*B*$^+$ *pps*::Tn*10 aroD*)	*aroD*$^+$	Ts	40 (43/106)
P1/JV43(*zdj3124*::Tn*10 kan fitC4 fitA*$^+$*B*$^+$ *pps*::Tn*10*)	an *aroD*$^+$ -Ts transductant obtained in cross 3 (JV *fitA76**)	Kan^r	Ts$^+$	12 (15/125)

Ts$^+$/Ts refers growth or no growth on LB plates at 42°C.

Table 5. Transductional crosses involved in the reconstruction of *fitA76* fitC4* (Ts$^+$) in HfrC and CSH57 backgrounds.

Donor	Recipient	Selected marker/ character	Unselected phenotype	Cotransduction (%)
CAG18578 (*zdj 3124*::Tn*10 kan fitC*$^+$ *fitA*$^+$ *fitB*$^+$)	JV4 (*fitA76** and *fitC4*)	Kan^R	Ts	3 (8/250)[a]
HfrC K10 (*fitC*$^+$*fitA*$^+$*fitB*$^+$ *pps*::Tn*10*)	PMJ *fitA76** (kan^r *fitC*$^+$, *fitA76** from cross 1)	Tet^R	Ts	16 (14/85)[b]
PMJ50(*pps*::Tn*10 fitA76** *fitC*$^+$kan^r from cross 2)	HfrC K10	Tet^R	Ts	50 (44/88)[c]
PMJ02 (*fitC*$^+$*fitA76**Ts *pps*::Tn*10* from cross 3)	PKM01 *pps*$^+$ (HfrC *fitC4* Ts *pps*$^+$ Tet^S)	Tet^R	Ts$^+$	~ 1(3/316)
PMJ02 (*fitC*$^+$*fitA76**Ts *pps*::Tn*10* from cross 3)	PKM57 *pps*$^+$ (CSH57*fitC4* Ts *pps*$^+$ Tet^S)	Tet^R	Ts$^+$	~ 1 (2/208)

[a] Ts transductant of this cross (kan^r *fitC*$^+$ *fitA76**) was named PMJ*fitA76**.
[b] Ts transductant of this cross (*fitC*$^+$ *fitA76** *pps*::Tn*10*) was named PMJ50.
[c] A Ts Transductant of this cross was named PMJ02.

derivative of CSH57, the same experiment can also be done in CSH57. These were done as follows.

P1 prorogated on the strain K34 (*zdj3124*::Tn*10kan*; figure 2) was used to transduce the Kan^R marker into JV4. The Kan^R transductants that received *fitC*$^+$ but retained *fitA76** would be Ts. It was observed that ~ 3% (8/250) of the Kan^R transductants exhibited Ts phenotype as could be expected of the cotransduction between *zdj3124*::Tn*10kan*^{R} and *fitC4* (see above and also cross 1, table 5). One of the Ts colonies (PMJ*fitA76**) was purified and was used as a recipient to transduce *pps*::Tn*10* using P1/HfrC *fitC*$^+$*A*$^+$*B*$^+$ *pps*::Tn*10* (K10 Tet^R, table 1). In this cross any transductant receiving only *pps*:: Tn*10* but not *fitA*$^+$ will continue to have *fitA76** thus would be Ts. As can be seen from the table 5 cross 2, 14 out of 85 Tet^R colonies retained the *fitA76** allele and were Ts at 42°C. One of the resultant Ts colonies was named PMJ50 (see table 5). When P1/PMJ50 was used to mobilize the *fitA76** allele into wild type HfrCK10 with linked *pps*::Tn*10*, 50% of the Tet^R transductants were Ts at 42°C as was expected (table 5 cross 3). One Ts (PMJ02), one Ts$^+$ colony was purified and used for further studies.

First in order to make sure that the *fitC4* confers a Ts phenotype regardless of genetic background, P1/JV41 [*pps*::Tn*10 fitB*$^+$ *fit*A$^+$ *fitC4* (Ts) derivative of JV4] was used to transduce *fitC4* Ts with linked *pps*::Tn*10* into HfrC, AB1157 and CSH57. In all the three genetic backgrounds around 40% of Tet^R colonies became Ts (data not shown) as expected of the cotransduction between *fitC4* and *pps*::Tn*10*. This confirms that the Ts phenotype of *fitC4* is

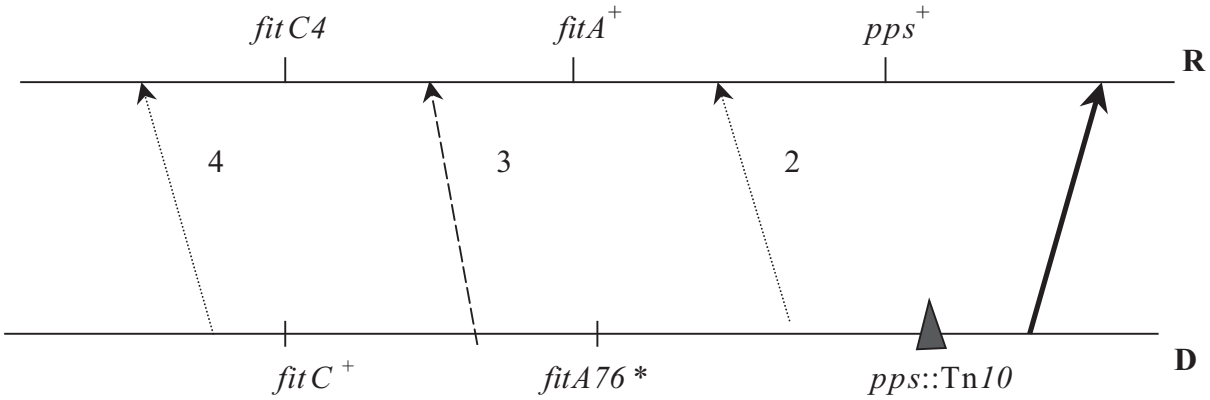

Figure 3. Schematic illustration of the transductional cross involved in the reconstruction of *fitA76* fitC4* and hence Ts^+ strain (similar to JV4) using relevant donor and recipient strains, see crosses 4 and 5 of table 5. The relevant genotype of both the recipient stains (PKM01*pps*$^+$ and PKM57 *pps*$^+$) are *pps*$^+$ *fitA*$^+$ *fitC4* and genotype of the donor strain is *pps*::Tn*10 fitA76* fitC*$^+$ (PMJ02). To generate the Ts^+ (*fitC4 fitA76**) transductant, one crossover (1) should occur to the right of *pps*::Tn*10* locus (bold line) and the other second cross over should occur in between *fitC*$^+$ and *fitA76** (3) (figure not drawn to scale). For other details see text.

not grossly affected by genetic background although there is some minor variation (B Praveen Kamalakar and M H Munavar; unpublished results). We chose one *pps***::**Tn*10 fitC4* Ts derivative of HfrC (named PKM01) and one *pps***::**Tn*10 fitC4* derivative of CSH57 (named PKM57) and intended to transduce *fitA76** into both to make sure that in both cases the resultant *fitC4 fitA76** transductants become Ts^+ phenotype. However, the presence of *pps*::Tn*10* in PKM01 and PKM57 rendered them unsuitable for use as recipients to introduce *fitA76** along with *pps*::Tn*10*. Therefore it was imperative to eliminate *pps*::Tn*10* from PKM01 and PKM57. This was done by transducing them to *pps*$^+$ by P1 propogated on a wild type (*pps*$^+$*fitA*$^+$*fitB*$^+$*fitC*$^+$) strain. Among the *pps*$^+$ (lactate$^+$) transductants from both the crosses, one that retained *fitC4* and hence Ts were saved. To the *pps*$^+$*fitC4* derivatives of PKM01 (PKM01 *pps*$^+$) and PKM57 (PKM57 *pps*$^+$), we introduced *fitA76** with linked *pps***::**Tn*10* using P1 made on PMJ02. Such a cross is schematically illustrated in figure 3. As could be seen from the figure in such crosses, transductants which received only *pps***::**Tn*10* (1:2 crossovers) will exhibit Ts phenotype due to *fitC4* mutation. Also the transductants which received both *fitA76** and *fitC*$^+$ with *pps***::**Tn*10* will also become Ts due to *fitA76** (1:4 crossovers). However, the transductants which receive only *fitA76** with *pps***::**Tn*10* (1:3 crossovers) will have the genotype *fitC4 fitA76** and thus should exhibit Ts^+ phenotype due to suppression of each others' Ts phenotype. But a fraction of such colonies will be low because the second crossover should occur between *fitC*$^+$ and *fitA76** (figure 3). As was expected, about 1–2% of the Tet^R transductants in both crosses became Ts^+ (see table 5). These results confirm that the introduction of *fitC*4 and *fitA*76* in any genetic background confers Ts^+ phenotype.

3.8 fit95 *is intact in JV4*

Considering the fact that *fitA76* has two mutations (*pheS*5 and *fit*95; Ramalingam *et al* 1999), we speculated that the *fitA76** in JV4 might have arisen due to a change either at the *pheS5* position or at the *fit95* position. Initially we wanted to check whether *fit95* is intact in JV4. It is known that *fit95* when present alone confers a Ts phenotype on rich media devoid of NaCl (referred as – salt Ts phenotype, Ramalingam *et al* 1999). In the *fitA76* mutant the order of markers is *pheS5-fit95-pps-aroD*, counter-clockwise on the *E. coli* chromosome. The *fit95* and *pheS5* could be separated at a frequency of approximately 1–2% in transductional crosses (Ramalingam *et al* 1999 see also cross 1, table 6). If JV4 harbours intact *fit95* we could expect that in a cross P1/JV4 X *aroD fitA*$^+$ recipient like JV57, 1–2% of the *aroD*$^+$ transductants should be *fitA*$^+$ *fit95-aroD*$^+$ and hence Ts on – salt media. As can be seen from the data presented in table 6, cross 2, approximately 2% of the *aroD*$^+$ transductants were Ts on LB-NaCl media. These results confirm the presence of the *fit95* mutation in JV4. Therefore, the modified form of *fitA76* (*fitA*76*) present in JV4 might have acquired a change at the position of *pheS5*. Sequence analysis has confirmed this conclusion (also see §4).

3.9 *Evidence that* fit95 *could also suppress the* fitC4 *Ts phenotype*

When we transduced a *fitA76* (*pheS5 fit95*) recipient for Kan^R using P1 grown on *zdj3124***::**Tn*10kan fitC4* strain (JV43) none of the Kan^R transductants became Ts^+ (see table 4 cross 1; this cross is also shown as cross 1 in table 7 for the sake of clarity). However, in a reciprocal cross (in

Table 6. Transductional crosses showing the presence of the *fit95* mutation in the Ts$^+$ derivative JV4.

Donor	Recipient	Selected marker/ character	Transductants exhibiting the –salt Ts[a] (*fit95*) phenotype (%)
P1/HfrC *fitA76* (*pheS5 fit95 pps*::Tn*10*)	AB1157 (*pps*$^+$ *fit*$^+$)	Tet^r	1 (10/735)
P1/JV4 *fitC4fitA76* aroD*$^+$	JV57 (*aroD pps*:: Tn*10 fit*$^+$)	*aroD*$^+$	2 (6/285)

[a] –Salt Ts refers to temperature sensitivity on rich media devoid of sodium chloride at 42°C.
For cross 1 data reproduced from Ramalingam *et al* (1999).

Table 7. Evidence for suppression of *fitC4* Ts phenotype by *fit95*.

Donor	Recipient	Selected marker/ character	Unselected phenotype	Cotransduction (%)
P1/JV43 *(zdj 3124::*Tn*10 kan fitC4 fitA*$^+$ *fitB*$^+$ *pps::*Tn*10*)	JAJ572 *fitA76* (*pheS5-fit95*)	Kan^R	Ts$^+$	< 0.6 (0/160)
P1/JAJ572 (*fitA76 pps*$^+$)	JV43(*zdj 3124*:: Tn*10kan fitC4 fitA*$^+$ *fitB*$^+$ *pps::*Tn*10*)	*pps*$^+$	Ts$^+$	5 (10/202)
P1/JAJ572 (*fitA76 pps*$^+$)	RT500(*pps*$^-$)	*pps*$^+$	Ts	70 (148/211)

which donor and recipient of cross 1 were interchanged) 5% of the *pps*$^+$ transductants became Ts$^+$ (see table 7, cross 2). These results are unexpected due to the following reasons. If the *fitA76* Ts phenotype could be suppressed by the *fitC4* mutation, then 60–70% of the *pps*$^+$ transductants in cross should have become Ts$^+$ because it is known that the *fitA76* cotransduces with *pps* around 60–70% (Jabbar and Jayaraman 1978; Dass and Jayaraman 1985a; see also cross 3 of table 7). On the other hand if the *fitA76* Ts phenotype could not be suppressed by the *fitC4* mutation then none of the transductants should have become Ts$^+$. Therefore, 5% Ts$^+$ transductants obtained in cross 2 could be explained by postulating that the *fit95* mutation present in the *fitA76* (between *pheS5* and *pps*), could suppress the *fitC4* Ts phenotype. Figure 4 represents schematic illustration of the transductional cross 2 of table 7. The predicted genotypes and phenotypes of various possible classes of *pps*$^+$ transductants are shown in table 8. As can be seen from table 8 all the *pps*$^+$ transductants that arise due to second crossovers in all possible intervals other than between *fit95* and *pheS5* should be temperature sensitive. However, a second crossover event between *fit95* and *pheS5* would yield a class of transductants with genotype *fitC4*$^-$ *pheS*$^+$ *fit95* whose phenotype could not be predicted. Also the frequency of generation of such transductants would be much low (see above). Therefore, the 5% Ts$^+$ transductants obtained in the above cross could be of the type *fitC4 pheS*$^+$ *fit95 pps*$^+$ only. These results not only reconfirm that the original *fitA76* mutant indeed harbours a second mutation, *fit95*, in addition to the *pheS5* but also clearly indicates that this mutation suppresses the Ts phenotype due to *fitC4*.

We have reconfirmed that *fit95* indeed suppresses *fitC4* Ts phenotype by transducing *fit95* along with linked *pps*::Tn*10* using P1 made on a *fit95 pps*::Tn*10* strain (PMJ95) into the recipient *pps*$^+$ *rpoB201*, a derivative of PKM01(harbouring *fitC4*). As was expected the transductants which received both *fitC4* and *fit95* mutations exhibited Ts$^+$ phenotype regardless of media (data not shown) confirming the above results. In this cross both donor and recipient strains carry the *rpoB201* mutation known to stabilize the *fit95* – salt Ts phenotype (Ramalingam *et al* 1999; Rukmani 1996).

3.10 *Kinetics of decay of pulse labelled RNA in strains bearing* fitC4 *and the modified form of* fitA76 (fitA76*): *Evidence that both mutants behave like the original* fitA76 *mutant*

Upon a temperature shift from 30°C to 42°C in isogenic *fitA76* and *pheS5* mutants, it was observed that RNA synthesis was inhibited earlier and faster than protein synthesis in *fitA76* mutant and the reverse was seen in the *pheS5* mutant. This strongly suggested that the *fitA76* mutant is primarily transcription defective and *pheS5* mutant is primarily translation defective (Ramalingam *et al* 1999). Kinetics of decay of pulse labelled RNA in the *pheS5* and *fitA76* mutants showed the following. In the *pheS5* mutant, the pattern of decay of RNA pulse labelled at 30°C and 42°C was found to be triphasic (similar to that found with the wild type strain) consisting of fast decaying and slow

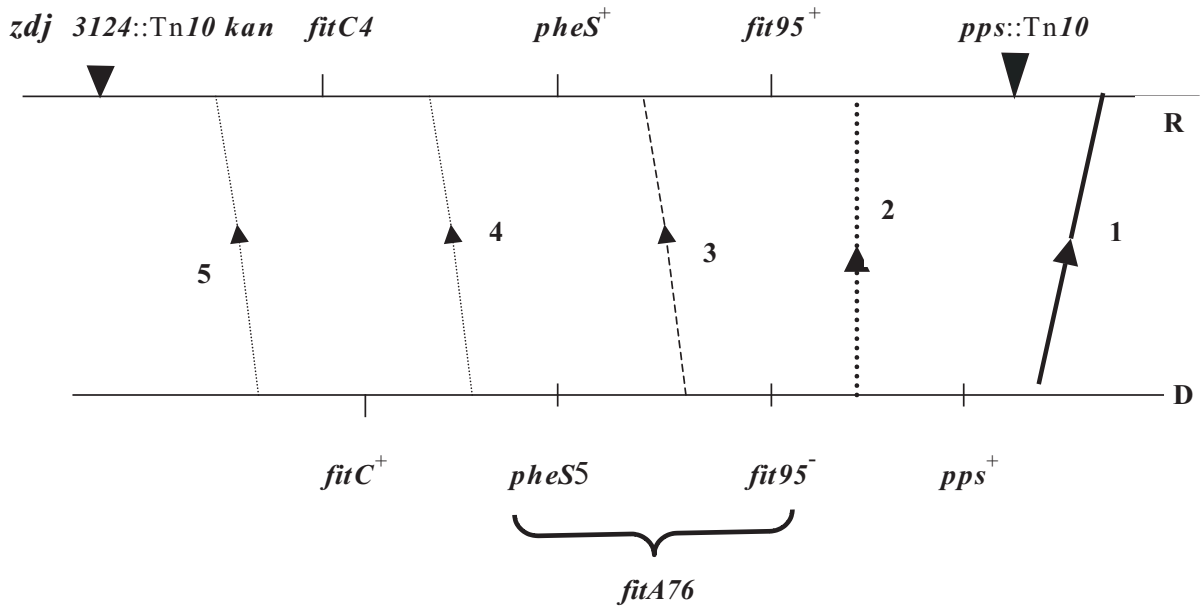

Figure 4. Schematic illustraion of the transductional cross 2 shown in table 7. JAJ572 (*fitC*$^+$ *pheS5 fit95*$^-$ *pps*$^+$) (D); X JV43 (*zdj3124*::Tn*10 kan fitC4 pheS*$^+$ *fit95*$^+$ *pps*::Tn*10*) (R). The first cross over is shown as a solid line and the second crossover as broken lines. It should be noted that only the second cross over occurring between *pheS5* and *fit95*$^-$ (1:3) would yield transductants of the type *fitC4 pheS*$^+$ *fit95*$^-$. All the other second cross overs will yield only Ts transductants (see text and table 8).

Table 8. Predicted genotypes of the various classes of *pps*$^+$ transductants in the transductional cross-illustrated in figure 4. JAJ572 (*fitC*$^+$ *pheS5-fit95* (*fitA76*) *pps*$^+$ (donor); X JV43 (*zdj 3124::*Tn*10 kan fitC4 pheS*$^+$ *fit95*$^+$ *pps*::Tn*10* (recipient).

Interval of the second cross over	Genotype of the *pps*$^+$ transductants	Predicted phenotype of the *pps*$^+$ transductants
pps$^+$*-fit95*$^-$ (2)	*fitC4 pheS5*$^+$ *fit95*$^+$ *pps*$^+$	Ts[a]
fit95-pheS5 (3)	*fitC4 pheS5*$^+$ *fit95*$^-$ *pps*$^+$	?
pheS5-fitC$^+$ (4)	*fitC4 pheS5 fit95*$^-$ (*fitA76*) *pps*$^+$	Ts[b]
Beyond *fitC*$^+$ to its left (5)	*fitC*$^+$ *pheS5 fit95*$^-$ *pps*$^+$	Ts[c]

The numbers shown in brackets refer to the second cross over intervals.
[a] Ts because these transductants are same as the recipient (*fitC4*) but *pps*$^+$.
[b] Ts because *fitC4* mutation cannot suppress the Ts phenotype of the original *fitA76* (*pheS5-fit95*) mutant.
[c] Ts because these transductants are same as *fitA76* but *pps*$^+$.

decaying species (representing mRNAs) and stable species (representing rRNAs and tRNAs) implying that all the three species of RNAs are made in this mutant at both temperatures. In the *fitA76* mutant RNA pulse labelled at 30°C decayed triphasically like that observed in a wild type strain. However, RNA pulse labelled at 42°C decayed biphasically comprising of only fast and slow decaying species (Sudha *et al* 2001); the stable species could not be detected at all in the *fitA76* mutant at 42°C. This together with earlier results of Jayaraman and Jabbar (1980) indicated that the absence of detectable stable RNA species in the *fitA76* mutant at 42°C could be due to lack of protection of nascent stable RNA at 42°C which in turn could be due to lack of expression (transcription) of genes coding for (at least some) ribosomal proteins required to stabilize nascent ribosomal RNA from decay. Based on this, it was postulated that Fit factors function as selective transcription factors regulating the expression of few classes of genes; the genes coding for ribosomal proteins could be among them (Jayaraman and Jabbar 1980; Munavar *et al* 1993; Ramalingam *et al* 1999; Sudha *et al* 2001). Thus absence of detectable stable RNA synthesized at 42°C is one of the characteristic properties of the *fitA76* mutant.

In order to know whether *fitC4* mutant and also the strain bearing the modified form of *fitA76* (*fitA76**) behave like the original *fitA76* mutant or like the *pheS5* mutant, we studied the

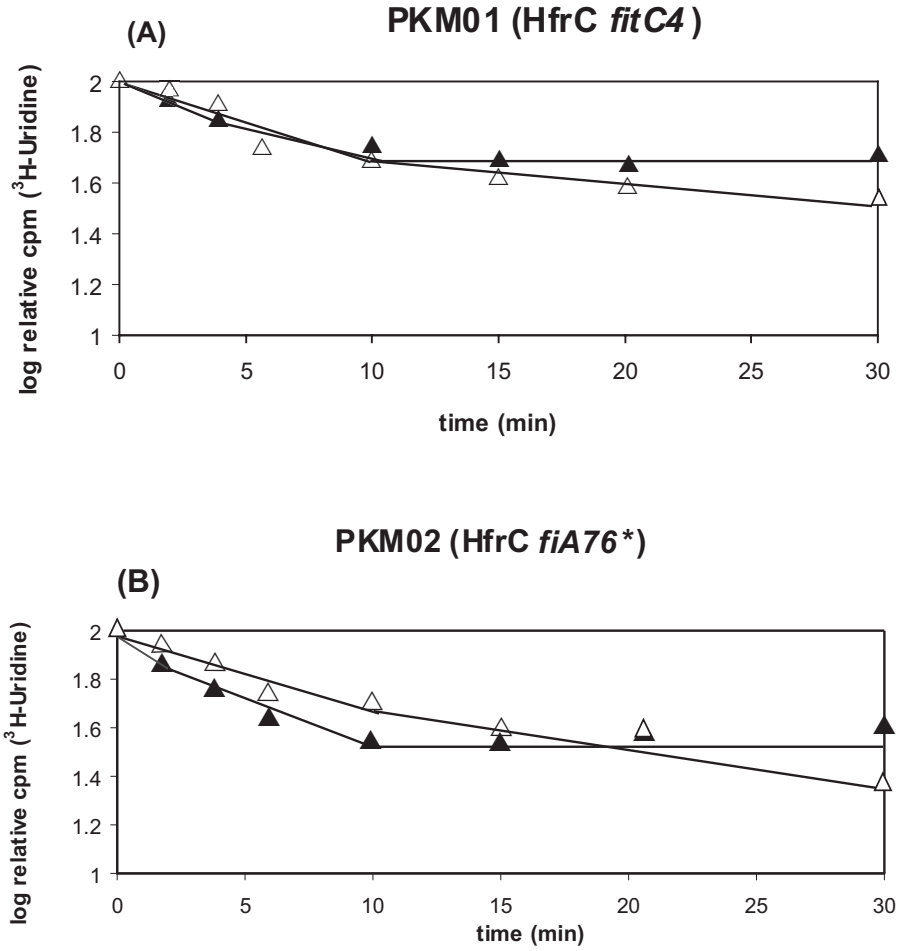

Figure 5. **(A)** Kinetics of decay of pulse-labelled RNA in HfrC *fitC4* mutant (PKM01) at 30°C(▲) and 42°C (△). The initial CPM is taken as 100% and the values at other time points are normalized with respect to it. Each point in the graph indicates an average of three experiments. See text for other details. **(B)** Kinetics of decay of pulse-labelled RNA in HfrC *fitA76** mutant (PKM02) at 30°C(▲) and 42°C (△). The initial CPM is taken as 100% and the values at other time points are normalized with respect to it. Each point in the graph indicates an average of three experiments. See text for other details.

kinetics of decay of pulse-labelled RNA in both. The relevant strains [PKM01 and PKM02 (PKM02 is genotypically same as PMJ02)] were grown in M9 medium and divided into two portions. One was kept at 30°C and the other was shifted to 42°C and incubated for 1 h. The two mutants at both temperatures were pulse-labelled for 20 s with ^{3}H-uridine and pattern of decay was followed (see § 2.3 for details). We found the pattern of decay of RNA pulse-labelled at 42°C to be biphasic, and triphasic when pulse-labelled at 30°C. In both the mutants stable RNA species could not be detected when pulse-labeled at 42°C as the case with original *fitA76* (*pheS5 fit95*) mutant (see figure 5). For comparison the patterns of decay of pulse-labelled RNA in the original *fitA76* and *pheS5* mutants are also given (figure 6).

4. Discussion

Our earlier work provided genetic, physiological and biochemical evidence for the involvement of the *fitA* and *fitB* gene products in selective regulation of transcription in *E. coli*. It was originally believed that *fitA* and *fitB* were unidentified genes and their products interact with each other as well as with RNAP and regulate the expression of few classes of genes (Jabbar and Jayaraman 1978; Dass and Jayaraman 1985a,b, 1987; Munavar and Jayaramn 1987; Jayaraman 1994). It has been shown recently (Ramalingam *et al* 1999) that *fitA* is same as *pheS*, coding for the α subunit of phenylalanyl-tRNA synthetase (PheRS). There is suggestive evidence (genetic mapping, polarity of transposon

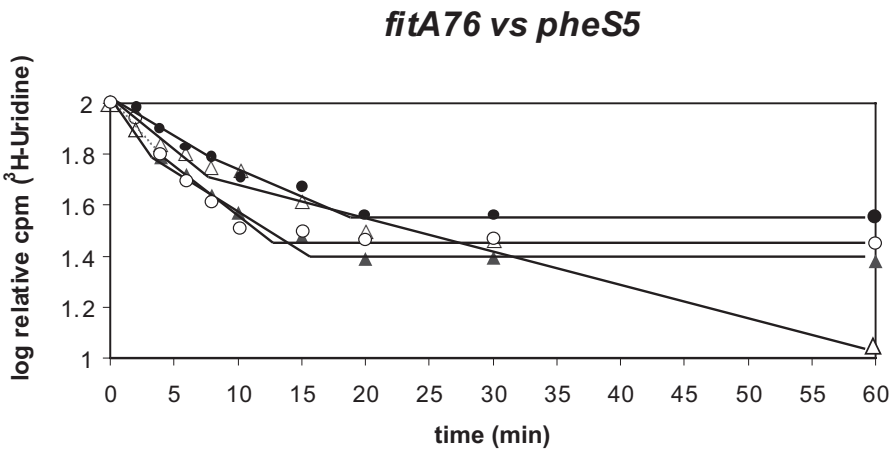

Figure 6. Kinetics of decay of pulse-labelled RNA in *fitA76* and *pheS5* mutants at 30°C (▲, ●) and 42°C (△, ○) (adapted from Sudha *et al* 2001).

insertions in *fitA* on *fitB* expression and complementation with Kohara phages) to show that *fitB* could be *pheT,* coding for the β subunit of PheRS (Munavar 1991; B Praveen Kamalakar and M H Munavar, unpublished results). Taken together it was proposed that *fit* function is a second to function associated with phenylalanine-tRNA synthetase, the subunits of which also function as selective transcription factors possibly by interacting with the β subunit of RNA polymerase (Ramalingam *et al* 1999; Sudha *et al* 2001).

In the present investigation, started at a time when the molecular details of the lesions present in the *fitA76* mutant were not known, we attempted to isolate a suppressor of *fitA76* mapping in *rpoD*. Starting from the *fitA76* mutant we isolated a Ts^+ derivative (JV4). We have shown here that JV4 neither harbours a suppressor of *fitA76* mapping in/near *rpoD* nor is the original *fitA76* lesion (*pheS5 fit95*) is intact in JV4. It is shown here that JV4 harbours a modified form of *fitA76* (referred to as *fitA76**) together with its suppressor. The suppressor mutation by itself confers a Ts phenotype and maps around 39.01 min. The map position of the suppressor clearly indicates that it is extragenic. We have tentatively designated this locus as *fitC* and the mutation as *fitC4*. While *fit*C4 could suppress the Ts phenotype due to *fitA76**, *pheS5* and *fit95* mutants, it failed to suppress the Ts phenotype of original *fitA76* (*pheS5-fit95*) mutant. The presence of the *fit95* mutation in JV4 implies that some modification could have occurred at the position of *pheS5* to generate *fitA76**. This postulate has been verified to be true. The new *pheS* mutation present in JV4 (named *pheS4*) has been cloned, sequenced and shown to be a G_{293}–C_{293} transversion (S Vidya, B Praveen Kamalakar, M H Munavar and R Jayaraman, unpublished results).

Extensive molecular analysis revealed that *pheS4* could suppress *fitA76* when present on a multicopy plasmid (S Vidya, B Praveen Kamalakar, M H Munavar and R Jayaraman, unpublished results). The inability of *fitC4* to suppress the Ts phenotype of the transcription defective *fitA76* mutant but its ability to do so in a translation defective *pheS5* mutant raises the question whehter *fitC4* is primarily transcription defective (like *fitA76*) or translation defective (like *pheS5*). The same question may be extended to *fitA76** also. Results on the kinetics of decay of pulse labelled RNA in isogenic *fitC4* and *fitA76** mutants reported herein clearly indicates that both the mutations behave like the original *fitA76* mutant. Moreover, the phenotype characteristic of *fitA76* mutant *viz* the escape of phage T7 growth at 42°C after a brief period of incubation of infected cells at 30°C (Jabbar and Jayaraman 1976, 1978), has been found to be true of both *fitC4* and *fitA76** mutants (Saini 1997). Based on the above we propose that both *fitC4* and *fitA76** mutants are primarily transcription defective. Measurement of gross RNA synthesis at 30°C and 42°C and the pattern of inhibition of RNA and protein synthesis after a shift to 42°C would help to know the actual extent of transcription defect in these mutants. It would be worthwhile to know how the *fitC4* mutation, which confers a primary defect in transcription, suppresses the phenotype of translation defective *pheS5* mutant. Similarly it would be interesting to study the effect of *fitC4* mutation in *fitA24* and *fitB* genetic backgrounds. Such studies are currently underway.

Although, based on map position we might postulate that the *fitC4* mutation defines a new unidentified locus (*fitC*), it remains to be clarified whether this is true or *fitC4* is an allele of a known gene present at that position. However, the suppression properties of the *fitC4* mutation imply that the

product coded by it directly or indirectly interacts with the *fitA/pheS* and/or *fitB/pheT* products. How far would such interaction influences the transcription specificity of FitAB complex? Does the *fitC* gene product by itself interact with subunits of RNA polymerase? We believe that molecular cloning of *fitC* gene, identifying the *fitC4* lesion and isolation of suppressor(s) of *fit*C4 mapping in the locus/loci coding for subunit(s) of RNAP would help elucidate the actual mode of functioning of *fitC* product. These are some facets of our current efforts.

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