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Genetic evidence for interaction between *fitA*, *fitB* and *rpoB* gene products and its implication in transcription control in *Escherichia coli*

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Abstract. The *fitB* mutation (Fit, factor involved in transcription) in *E. coli* was earlier identified as an extragenic suppressor of the *fitA76* mutation, which confers a temperature-sensitive transcription defect. Here we show that the *fitB* mutation by itself confers a temperature-sensitive phenotype depending on the presence or absence of NaCl or glucose, or both, in the medium. The *fitB* mutation suppresses the temperature-sensitive phenotype due to the *fitA24* mutation also. However, suppression of *fitA24* by *fitB* is restricted to rich medium, unlike suppression in the *fitA76 fitB* combination where it is independent of the medium. The strain harbouring *fitA76*, *fitA24* and *fitB* mutations shows the extragenically suppressed (as in *fitA76 fitB*) phenotype. Several *rif* (*rpoB*) alleles isolated in a *fitB* genetic background affect growth of the *fitB* mutant, depending on the medium of growth, temperature, and presence or absence of rifampicin. We propose a model for interaction between *fitA* and *fitB* gene products and involvement of the *fit* genes in transcription control *in vivo*.

Keywords. *fitA*; *fitB*; *rpoB*; transcription; accessory transcription factors.

1. Introduction

A vital feature of transcription regulation is its selectivity. A number of elements help in this process by acting in concert with RNA polymerase or DNA or both. These elements are collectively referred to as accessory transcription factors (Yura and Ishihama 1979; Ishihama 1988). Our laboratory has been working on the genetic and physiological aspects of temperature-sensitive transcription-defective mutants of *Escherichia coli*. The first such mutation identified (originally named *ts76*) was mapped to 37·5 min on the *E. coli* chromosome (Jabbar and Jayaraman 1978). The fact that this mutation did not map in the genes coding for the subunits of RNA polymerase suggested that it might define the gene for an accessory transcription factor. Physiological studies revealed that the transcription defect in this mutant might be due to a failure to transcribe some classes of genes at 42°C (Jabbar and Jayaraman 1978; Jabbar 1979; Jayaraman and Jabbar 1980). Subsequent isolation of an intragenic suppressor (originally named *ts24*), which suppressed the Ts phenotype due to the *ts76* mutation under certain conditions, but by itself conferred a Ts phenotype and a transcription defect at 42°C, supported the above notion. Hence this locus was named *fit* (factor involved in transcription) and the *ts76* and *ts24* mutations were redesignated *fit76* and *fit24* respectively (Dass and Jayaraman 1985a). A *rif* mutation (*rpoB240*) that accompanied the *fit24* mutation was shown to cause medium-dependent and temperature-dependent rifampicin sensitivity in a fit^+ background (Dass and

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Jayaraman 1985b). Differential expression of gene groups in strains harbouring various *fit* alleles in combination with *rpoB240* or *rpoB*$^+$ suggested an interaction between the *fit* factor and the β-subunit of RNA polymerase (Dass and Jayaraman 1985b, 1987). Further studies on the suppression of the Ts phenotype due to the *fit76* mutation led to identification of an extragenic suppressor (Munavar and Jayaraman 1987). Consequently the locus of *fit76* and *fit24* mutations was renamed *fitA* and that of the extragenic suppressor *fitB*. The *fitB* mutation also mapped very close to *fitA*, and the order of markers in the region was deduced to be *fitA76-fitA24-fitB-pps-aroD* (Munavar and Jayaraman 1987). Some of our results (Munavar 1991) indicated that *fitA* and *fitB* might constitute an operon by themselves or be part of one, the direction of transcription being *fitA* → *fitB*. Results of experiments on RNA synthesis and decay of pulse-labelled RNA in various *fit* mutants, together with the earlier observations of Jayaraman and Jabbar (1980), revealed that the Fit factors regulate expression of only certain classes of genes and that these might include genes coding for ribosomal proteins (Munavar *et al.* 1993). Recent studies have tentatively identified another suppressor of *fitA76* (Vidya and Jayaraman 1992).

Whether the *fit* mutations define discrete genes or are novel alleles of previously identified loci in the region had hitherto remained unsettled (Dass and Jayaraman 1985a; Munavar *et al.* 1993). Recent work of Sandhya Ramalingam in our laboratory (manuscript in preparation) on cloning and sequencing of the *fitA* gene as well as on the ability of the cloned DNA to complement temperature-sensitivity mutations previously characterized as *fitA76* or *pheS5* has shown that *fitA* and *pheS* genes are one and the same. The *pheS* gene product functions as the α-subunit of phenyl-alanyl tRNA synthetase; the β-subunit is encoded by the *pheT* gene. The *pheS* and *pheT* genes define an operon whose direction of transcription is *pheS* → *pheT* (Plumbridge and Springer 1980). Earlier results of Jabbar and Jayaraman (1978) showed that temperature-sensitive mutants in *pheS* and *pheT* did not show the phenotype characteristic of *fitA76*, that is escape of phage T7 development at 42°C following a brief preincubation at 30°C (Jabbar and Jayaraman 1976). More recent experiments in our laboratory have shown that the same temperature-sensitive *pheS* mutant (*pheS5*) also does not show transcription abnormalities characteristic of *fitA76* (Sudha Sankaran, M. Hussain Munavar and R. Jayaraman, to be published). It is possible that the product of *pheS* acts as an accessory transcription factor also, a function rendered defective by *fitA76* and *fitA24* mutations. The juxtaposition of *fitA* and *fitB* as well as the direction of their transcription (see above) suggest that *fitB* could be *pheT*. (We shall continue to use the designation *fit* in this as well as in future reports to describe transcription defects due to mutations in these loci.)

In this communication we report the suppression potential and phenotype of the *fitB* mutation as well as genetic evidence to support the notion that the two *fit* gene products interact with each other and with RNA polymerase to exert Fit activity.

2. Materials and methods

2.1 *Bacterial strains*

The *E. coli* strains used in this study are listed in table 1.

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

Strain	Relevant genotype	Source/construction/ reference
CSH57	F⁻ *argG his trp ilvA metA leu purE rpsL*	CSH collection
JAJ572	F⁻ *argG his trp metA leu purE rpsL fitA76*	*fitA76* derivative of CSH57 (Dass and Jayaraman 1985a)
HMJ01	F⁻ *argG his trp metA leu purE rpsL fitA76 fitB* Nal^r	Ts$^+$ Nal^r derivative of JAJ572 (Munavar and Jayaraman 1987)
BJW72	F⁻ *thr leu pro zdi276*::Tn*10 his rpsL argE*	B. J. Bachmann
HMJ03	F⁻ *argG his trp metA leu purE rpsL fitB zdi276*::Tn*10* Nal^r	This work, P1/BJW72 × HMJ01
BJ507	F⁻ *fitA24 aroD pyrD his edd pfkA? rpsL*	Dass and Jayaraman (1985a)
BJ241	F⁻ *argG his trp metA leu purE rpsL fitA76 fitA24*	Dass 1983
JAM1	Same as HMJ01; has *pps*::Tn*10*	This work
BW6165	Hfr *argE*::Tn*10*	B.J. Bachmann
JAM7	Same as HMJ03; has *rpoB7*	This work
JAM42	Same as HMJ03; has *rpoB42*	This work
JAM55	Same as HMJ03; has *rpoB55*	This work
JAM7A	Same as BW6165; has *rpoB7* and *argE*$^+$	This work
JAM42A	Same as BW6165; has *rpoB42* and *argE*$^+$	This work
JAM55A	Same as BW6165; has *rpoB55* and *argE*$^+$	This work
KL159	F⁻ *his aroD proA recA*	B.J. Bachmann
KLF48/ KL159	F⁻ KL159/F′ 148 *his*$^+$ *fitA*$^+$ *fitB*$^+$ *aroD*$^+$	B.J. Bachmann

2.2 *Methods*

All the genetic techniques employed were conventional ones as described in Miller (1972). In transduction experiments, AroD$^+$ were selected on minimal medium lacking tryptophan, phenylalanine, tyrosine and shikimic acid; Tet^r on LB plates containing 20 μg ml^{-1} tetracycline; and Arg$^+$ and Met$^+$ on minimal medium lacking arginine and methionine respectively.

2.3 *Media*

LB and minimal medium (MM) were as given in Miller (1972). The salt-minus medium consisted of beef extract (0·15%), yeast extract (0·3%) and peptone (0·6%).

3. Results

3.1 *Phenotype of fitB mutants*

An unanswered question in our previous report (Munavar and Jayaraman 1987) was the phenotype of the *fitB* mutant. Our earlier results indicated that the *fitB* mutation

did not confer a Ts phenotype, at least in rich medium. This conclusion was based on the following observation. Since *aroD* is closer to *fitB* than to *fitA*, in a transductional cross *fitA*$^+$ *fitB*$^+$ *aroD* (recipient) × *fitA76 fitB aroD*$^+$ (donor) a large fraction of the AroD$^+$ transductants should have become *fitA*$^+$ *fitB aroD*$^+$. If the *fitB* mutation by itself conferred a Ts phenotype, a substantial fraction of the transductants should have been Ts. But this was not the case (Munavar and Jayaraman 1987). Therefore it was concluded that either the *fitB* mutation by itself does not confer a Ts phenotype (at least in LB medium, in which the Ts/Ts$^+$ phenotypes were scored) or the *fitA*$^+$ *fitB* mutants are nonviable in minimal medium, in which the AroD$^+$ transductants were selected. To resolve this question we used the strain BJW72, which is *fitA*$^+$ *fitB*$^+$ and has a Tn*10* insertion near 38 min, that is to the left of *fitA*. When phage P1 propagated on BJW72 is used to transduce *tet*^{r} to a *fitA fitB* recipient, a fraction of the transductants may be expected to become *fitA*$^+$ *fitB*. Hence we used strain HMJ01 as recipient. The Tet^r transductants, selected at 30°C, were screened for temperature sensitivity on minimal and LB media. If a fraction of them turned out to be Ts, they could be *fitA*$^+$ *fitB*. Data presented in table 2 for cross 1 show that 9% of the Tet^r transductants were Ts (poor growth) on minimal medium whereas all were Ts$^+$ on rich medium. Since *fitA* and the Tn*10* insertion of BJW72 are cotransduced (table 2, cross 2), the former cross (cross 1) should include some *fitA*$^+$ *fitB* transductants. Thus the colonies that showed poor growth on minimal medium at 42°C should be (*fitA*$^+$) *fitB*. A strain bearing the *fitB* mutation alone was designated HMJ03.

The Ts phenotype of HMJ03 (*fitB*) on minimal medium with glucose as carbon source prompted us to check its growth with another carbon source, such as glycerol. While we expected poorer growth with glycerol than with glucose this was not the case; the *fitB* mutant grew better on glycerol. Interestingly, growth of the *fitB* mutant was affected at 42°C by the presence of glucose even when glycerol was provided. Moreover, addition of glucose inhibited growth of the *fitB* mutant at 42°C even in LB medium. We found that the effect of glucose was more pronounced if the medium was richer (with beef extract, yeast extract and peptone, but devoid of NaCl) than the conventional LB medium (yeast extract, peptone and NaCl). The phenotypes of three *fit* mutants under various growth conditions are summarized in table 3. It is clear from the table that the *fitA76* mutant is completely temperature-sensitive under all the conditions tested. However the *fitA76 fitB* strain was Ts$^+$ under all conditions but one, in which simultaneous absence of sodium chloride and presence of glucose affected its growth at 42°C. Thus the *fitB* mutation suppresses the Ts phenotype of *fitA76* in all media except one lacking sodium chloride and containing glucose,

Table 2. Phenotype of the *fitB* mutation in minimal medium.

Cross no.	Donor	Recipient	Selected character	Unselected character	Cotransduction frequency (%)
1	BJW72 (*fitA*$^+$ *fitB*$^+$; has Tn*10* near 38 min)	HMJ01 (*fitA76 fitB*)	Tet^r	Ts on LB Ts on MM*	0 (0/274) 9 (26/274)
2	BJW72	JAJ572 (*fitA76*)	Tet^r	Ts$^+$ on LB	27 (44/164)

* Poor growth

Table 3. Influence of glucose and sodium chloride on the growth of *fitB*, *fitA76* and *fitA76 fitB* strains.

Medium of growth	Growth of HMJ03 (*fitA*$^+$ *fitB*)		HMJ01 (*fitA76 fitB*)		JAJ572 (*fitA76*)	
	30°C	42°C	30°C	42°C	30°C	42°C
MM + Glu	+	±	+	+	+	−
MM + Gly	+	+	+	+	+	−
MM + Glu + Gly	+	±	+	+	+	−
LB	+	+	+	+	+	−
LB + Glu	+	±	+	+	+	−
Salt-minus medium	+	−	+	+	+	−
Salt-minus medium + Glu	+	−	+	±	+	−

See Materials and methods for description of the media.
Glu, Glucose; Gly, glycerol
+, Normal growth; −, no growth; ±, poor growth

wherein the suppression is only partial. Once the phenotype of the *fitB* mutation was identified, we reexamined the cotransduction of *fitB* and *aroD* by scoring for *fitB* on appropriate media (see above) and found it to be 73%, which is consistent with our earlier report on the suppression of the Ts phenotype of *fitA76* by *fitB* (Munavar and Jayaraman 1987).

The *fitB* mutation was found to be recessive as determined by complementation using strain KLF48/KL159, which has F'148 carrying wild-type alleles of genes lying between *his* and *aroD* (except *cheB* and *cheC*). This implies that the *fitB* mutation defines a structural gene, as was the case with *fitA76* and *fitA24* mutations.

3.2 *The fitB mutation can suppress the Ts phenotype due to fitA24 also*

To ascertain the effect of the *fitB* mutation on a *fitA24* strain the following experiment was done. P1 phage propagated on HMJ01 (*fitA76 fitB aroD*$^+$) was used to transduce *aroD*$^+$ to BJ507 (*fitA24 fitB*$^+$ *aroD*). In this cross AroD$^+$ transductants arise as a result of two crossover events, one to the right and the other to left of the *aroD* locus. Depending on the site of the second crossover, the genotype, and hence the phenotype, of the transductants will vary. Figure 1A illustrates such a cross and table 4 presents the predicted genotypes and phenotypes of the AroD$^+$ transductants. A crossover occurring between *fitA24* and *fitB* would yield transductants with the genotype *fitA76*$^+$ *fitA24 fitB aroD*$^+$. The Ts/Ts$^+$ phenotype of this class of transductants would reveal the effect of the *fitB* mutation on the *fitA24* mutation. We had reported earlier that in a similar cross, using different partners, approximately 54% of the AroD$^+$ transductants arose as a result of crossovers between *fitB* and *fitA24* (Munavar and Jayaraman 1987). A similar frequency could be expected here too. Table 5 presents the results of an experiment in which *aroD*$^+$ was transduced into BJ507 using P1/HMJ01 and the transductants grouped as indicated. A substantial fraction (67%) of the AroD$^+$ transductants with *fit* genotype *fitA24 fitB* were temperature insensitive on rich medium and showed poor growth on minimal medium. This shows that the *fitB* mutation suppresses the Ts phenotype due to the *fitA24* mutation also, but only

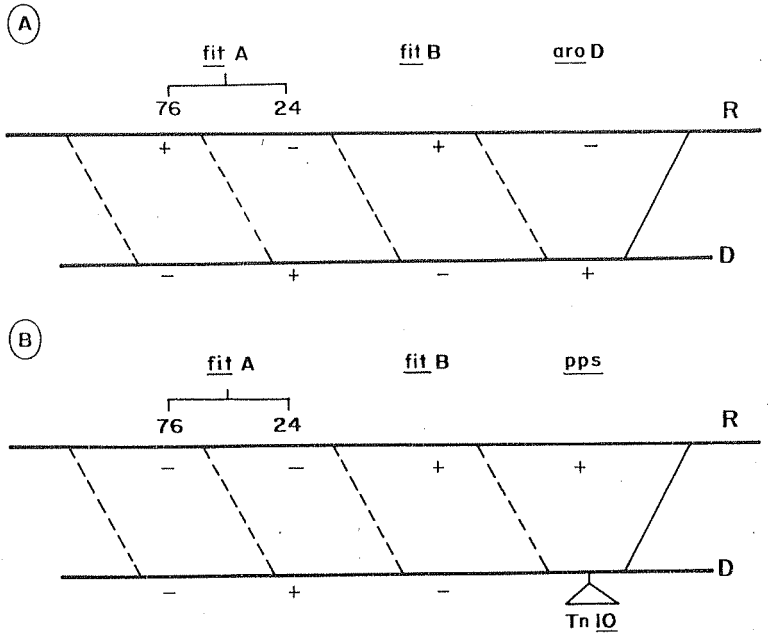

Figure 1. Schematic illustration of the transductional crosses: A, *fitA76 fitB aroD*$^+$ (D) × *fitA24 fitB*$^+$ *aroD* (R); B, *fitA76 fitB pps*::Tn*10* (D) × *fitA76 fitA24 fitB*$^+$ (R). The first crossover is shown as a solid line and the second crossover as broken lines. R, Recipient; D, donor. The figure is not drawn to scale.

Table 4. Predicted genotypes and phenotypes of the AroD$^+$ transductants in the transductional cross illustrated in figure 1A: *fitA76 fitB aroD*$^+$ (D) × *fitA24 fitB*$^+$ *aroD* (R).

Interval of the second crossover	Genotype of AroD$^+$ transductants	Predicted phenotype of AroD$^+$ transductants
aroD–fitB	*fitA76*$^+$ *fitA24 fitB*$^+$ *aroD*$^+$	Ts$^\pm$ on LB, MM (and salt-minus medium) (parental type but aroD$^+$)
fitB–fitA24	*fitA76*$^+$ *fitA24 fitB aroD*$^+$	?
fitA24–fitA76	*fitA76*$^+$ *fitA24*$^+$ *fitB aroD*$^+$	Ts on salt-minus medium and Ts$^\pm$ on MM with glucose
Beyond *fitA76*	*fitA76 fitA24*$^+$ *fitB aroD*$^+$	Ts$^+$ on MM, LB and salt-minus medium

D, Donor; R, recipient

Table 5. Results of the transductional cross illustrated in figure 1A: HMJ01 (*fitA76 fitB aroD*$^+$) (D) × BJ507 (*fitA24 fitB*$^+$ *aroD*) (R); selected marker AroD$^+$.

		Phenotype of AroD$^+$ tranductants at 42°C				
Interval of the second crossover	Genotype of AroD$^+$ transductants	LB	LB + Glu	Salt-minus medium	MM (Glu)	Frequency (%)
aroD–fitB	*fitA76*$^+$ *fitA24 fitB*$^+$ *aroD*$^+$	±	±	±	±	19 (18/97)
fitB–fitA24	*fitA76*$^+$ *fitA24 fitB aroD*$^+$	+	+	+	±	67 (65/97)
fitA24–fitA76	*fitA76*$^+$ *fitA24*$^+$ *fitB aroD*$^+$	+	±	−	±	2 (2/97)
Beyond *fitA76*	*fitA76 fitA24*$^+$ *fitB aroD*$^+$	+	+	+	+	12 (12/97)

+, Normal growth; ±, poor growth; −, no growth

in rich medium (LB or LB + glu or salt-minus medium). In minimal medium the suppression is only partial, whereas suppression of *fitA76* by *fitB* is independent of the medium (except in salt-minus medium with glucose wherein the suppression is partial).

3.3 *Suppression of temperature sensitivity due to fitA76 by fitB is dominant over suppression by fitA24*

Strains harbouring both *fitA76* and *fitA24* mutations grow well only on rich medium at 42°C (Dass and Jayaraman 1987). On the other hand strains harbouring *fitA76* and *fitB* mutations grow well at 42°C on rich as well as minimal media (Munavar and Jayaraman 1987). To ascertain the phenotype of strains harbouring all three mutations the following experiment was done. Phage P1 propagated on JAM1 (*fitA76 fitB pps*::Tn*10*) was used to transduce *pps*::Tn*10* into BJ241 (*fitA76 fitA24 fitB*$^+$) and Tet^r transductants were selected at 30°C. This cross is schematically illustrated in figure 1B. Since cotransduction of *pps* and *fitB* is more than 90% (Munavar and Jayaraman 1987), a majority of the Tet^r transductants in this cross would have acquired the genotype *fitA76 fitA24 fitB*. The other class of recombinants in the cross would be *fitA76 fitA24*$^+$ *fitB* irrespective of whether the second crossover occurred between *fitA76* and *fitA24* or beyond *fitA76* to its left. When the Tet^r transductants obtained in this cross were screened for growth almost all the colonies tested (393/400) grew normally on rich and minimal media at 42°C. This means that a strain in which the extragenic suppressor *fitB* is present with *fitA76* shows the extragenically suppressed phenotype irrespective of the presence of the intragenic suppressor *fitA24*. Hence we suggest that the extragenic suppression of *fitA76* by *fitB* is dominant over the intragenic suppression by *fitA24*. It is interesting to note that different *fit* combinations give different phenotypes with respect to growth on minimal medium at 42°C. For example, a *fitA76 fitA24*$^+$ *fitB* strain is Ts$^+$ on minimal medium, a *fitA76*$^+$ *fitA24 fitB* strain is Ts$^\pm$, and a *fitA76 fitA24 fitB* strain is Ts$^+$. These differences are incorporated into the model we propose (see Discussion).

3.4 *Influence of rpoB mutations on growth properties of a fitB strain*

The possible interaction of the *fitA* gene product with the β-subunit of RNA polymerase is well documented (see Introduction). To see whether the *fitB* gene product also interacts with RNA polymerase, the following experiment was done. The approach was to see whether *rif* mutations could suppress the *fitB* phenotype. HMJ03 (*fitB*) was plated on LB + rifampicin (50 μg ml^{-1}) plates at 30°C to select for spontaneous Rif^r mutants. Surprisingly, spontaneous Rif^r mutants arose at a very low frequency (approximately 10^{-9}) in the *fitB* background compared to the usual frequency of approximately 10^{-7} in a *fitB*$^+$ background (data for shown). Sixtyeight Rif^r mutants obtained from HMJ03 were checked for growth on various media at 30°C and 42°C. They displayed a spectrum of phenotypes at 42°C. Some were rifampicin-dependent for growth; some were resistant to high concentrations of rifampicin; some were rifampicin-sensitive in glycerol minimal medium only at 42°C; some were rifampicin-sensitive on glycerol minimal medium even at 30°C; some were rifampicin-sensitive on LB medium at 42°C, but resistant in minimal medium irrespective of the carbon source or temperature. Table 6 gives the phenotypes of a few representative Rif^r

Table 6. Growth pattern of some spontaneous Rif^r isolates of a *fitB* strain (HMJ03).

	Growth on plates													
	30°C							42°C						
Colony no.	LB	LB Rif 50	LB Rif 100	MM Glu	MM Glu Rif 50	MM Gly	MM Gly Rif 50	LB	LB Rif 50	LB Rif 100	MM Glu	MM Glu Rif 50	MM Gly	MM Gly Rif 50
7	+	+	+	+	+	+	+	(−	+	+)	±	±	+	+
16	+	+	+	+	+	+	+	(−	±	+)	±	±	+	+
21	+	+	+	+	+	(+	±)	+	+	+	±	±	(+	±)
23	+	+	+	+	+	+	+	(−	±	−	−	−)	+	+
27	+	+	+	+	+	+	+	(−	+	+)	±	±	(+	−)
29	+	+	+	+	+	(+	±	±	+	+)	±	±	(+	−)
31	+	+	+	+	+	+	+	+	+	+	(−	−)	+	+
35	+	+	+	+	+	(+	±)	+	+	+	±	±	(+	−)
24,34, 42,43, 44	+	+	+	+	+	+	+	+	+	+	±	±	(+	−)
55	+	+	+	(+	−)	+	+	(±	+	−	−	−)	+	+
60,63	+	+	+	+	+	+	+	(−	±	−)	±	±	+	+
64	+	+	+	+	+	(+	−)	+	+	+	±	±	(+	−)
66	+	+	+	(+	−	+	−	±	−	−	−	−	+	−)

+, Normal growth; ±, partial growth; −, no growth
Rif, Rifampicin (used at 50 or 100 $\mu g\,ml^{-1}$); MM, minimal medium; Glu, glucose; Gly, glycerol
In each case the phenotypic change due to *rif* mutation is indicated within parentheses.

isolates. We chose colonies 7, 42 and 55 for further analysis and designated them JAM7, JAM42 and JAM55 respectively. We first confirmed that these *rif* mutations do map in the *rpoB* locus by their cotransduction with *argE3* using a recipient that carried *argE*::Tn*10* (BW6165). Accordingly the *rif* mutations were designated *rpoB7*, *rpoB42* and *rpoB55* respectively. The ArgE$^+$ Rif^r transductants of BW6165 harbouring these *rpoB* mutations have been designated JAM7A (has *rpoB7*), JAM42A (has *rpoB42*) and JAM55A (has *rpoB55*). To ascertain the effect of these three selected *rif* alleles in other *fit* genetic backgrounds, P1 phage propagated on JAM7A, JAM42A and JAM55A was used to transduce DNA into JAJ572 (*fitA76*), HMJ01 (*fitA76 fitB*) and CSH57 (*fitA*$^+$ *fitB*$^+$), all of which are *metA*, and MetA$^+$ transductants were scored for rifampicin resistance. Ten different Rif^r colonies from each batch were tested under conditions listed in table 6. None of the three *rpoB* alleles had any effect (besides rifampicin resistance) in other *fit* backgrounds (data not shown). Thus the *rpoB* alleles tested above seem to influence the phenotype of only the *fitB* mutant.

4. Discussion

We have shown that the *fitB* mutation by itself confers a unique phenotype, namely total temperature sensitivity on salt-minus medium with or without glucose and partial temperature sensitivity (poor growth) on LB and minimal medium containing glucose. This phenotype is not observed with either the *fitA76* or the *fitA24* mutation. Since the

fitB$^+$ allele is dominant over *fitB*, the *fitB* locus probably defines a structural gene, as was the case with *fitA*.

The isolation of an extragenic suppressor (*fitB*) for the *fitA76* mutation, and the reduction in suppression by F′ bearing *fitA76/24 fitB*$^+$ or *fitA*$^+$ *fitB*$^+$ strongly suggested that the products of these two genes might interact with each other and function as a complex *in vivo* (Munavar and Jayaraman 1987). It is of interest that the *fitB* mutation could suppress the Ts phenotype due to *fitA24* also and that the extragenic suppression (of *fitA76* by *fitB*) is dominant over the intragenic suppression (of *fitA76* by *fitA24*). These observations suggest that the mutant *fitB* product could interact with mutant *fitA* product altered because of either *fitA76* or *fitA24*, or both. The fact that the phenotypes of strains harbouring *fitA76*, *fitA24*, *fitB*, *fitA76 fitA24*, *fitA76 fitB*, *fitA24 fitB*, *fitA76 fitA24 fitB*, and *fitA*$^+$ *fitB*$^+$ differ implies that in each case the efficiency of interaction is different, leading to differences in functional efficiency of the complex, which is reflected in the pattern of growth. On the basis of this hypothesis we propose a model for interaction between the *fitA* and *fitB* gene products *in vivo* (figure 2). We propose that the conformation of the FitA–FitB complex, which, in turn, is determined by the individual conformations of FitA and FitB products, is crucial to its activity as an accessory transcription factor. The *fitA76* mutation is postulated to confer such a conformation at 42°C that interaction

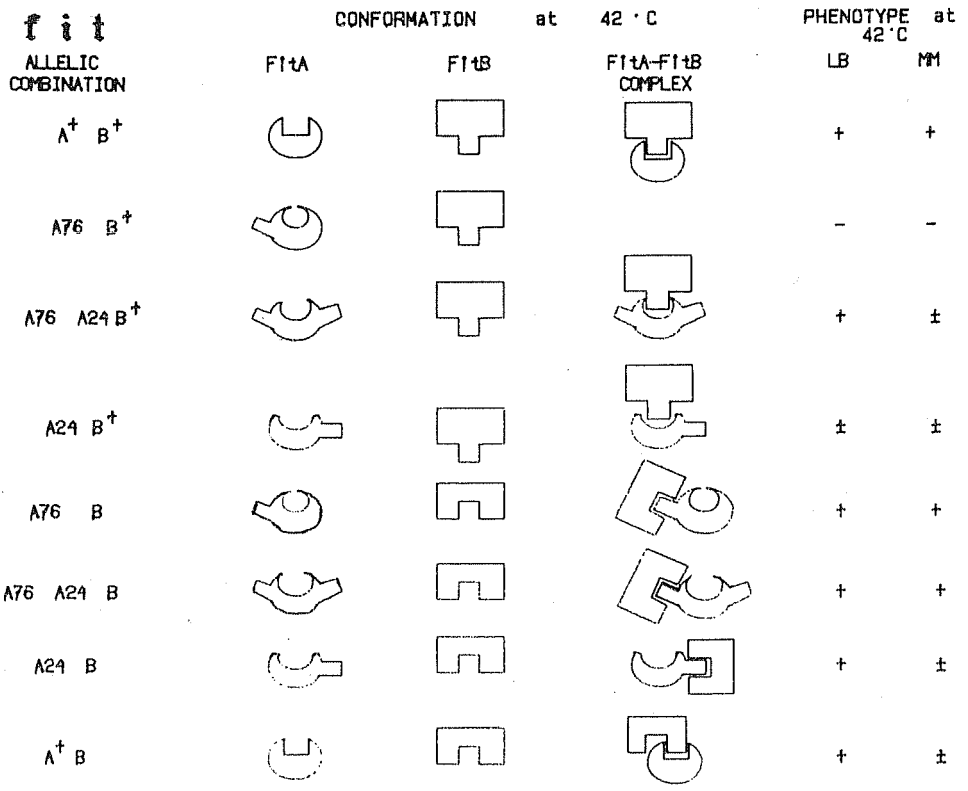

Figure 2. Proposed model for interaction of *fitA* and *fitB* gene products *in vivo*. The cartoons are intended merely to serve as aids in following the argument of the model and are not illustrations of the hypothesized complexes.

with the normal *fitB* gene product does not occur or is unstable. This would lead to loss of expression of Fit-dependent genes at 42°C and hence cessation of growth. The *fitB* mutation is proposed to allow or stabilize the interaction between *fitA76* and *fitB* gene products at 42°C such that phenotypic normalcy is restored. Other *fit* alleles, alone or in combination, function with varying efficiencies. It should be noted that, with the exception of *fitA76*, in all the cases where temperature sensitivity is manifested, it is only partial temperature sensitivity. This means that in such cases there is some level of function, which may not however be sufficient for healthy growth.

The spectrum of phenotypes of Rif^r isolates from a *fitB* mutant reported here is convincing genetic evidence for possible interaction between RNA polymerase and the *fitB* gene product, in association with that of *fitA*. Different *rif* (*rpoB*) alleles may specify different RNA polymerase conformations. It is known that mutations leading to Rif^r alter the conformation of RNA polymerase (for references see Dass and Jayaraman 1985b). Thus on one hand we can have RNA polymerase molecules of different conformations and on the other FitA–FitB complexes of different conformations. Combinations of these would generate RNA polymerase–FitA–FitB complexes of varying specificities or functional efficiencies or both. Superimposed on this are effects brought about by ligands (rifampicin, ions, chelators, etc.), temperature and growth medium.

Conformational changes in RNA polymerase accompanying rifampicin binding have been proposed to be of importance for its activity (Krakow *et al.* 1976; Nakamura and Yura 1976; Doi 1977). There is evidence to suggest that rifampicin may bind to RNA polymerase from Rif^r mutants also, since some Rif^r mutants are sensitive to derivatives of the antibiotic (for references see Dass and Jayaraman 1985b). By isolating and sequencing 42 Rif^r mutants, which constituted 17 alleles with mutations affecting 14 different amino-acid residues of the β-subunit of RNA polymerase, Jin and Gross (1988) have suggested that as many as four regions of the β-subunit protein are crucial for rifampicin sensitivity/resistance. Of these, one is near the N-terminus and three are in the middle of the protein. They cooperate to form the rifampicin binding domain, which requires enzyme assembly to achieve the native conformation. On the basis of the above, it is possible to explain the various phenotypes of the *rif* mutations we have reported here. For example: The strain JAM7 (*fitB rpoB7*) is rifampicin-dependent for growth at 42°C in LB medium but not in minimal medium. Thus the (FitA$^+$) FitB RpoB7 RNA polymerase complex might require rifampicin to assume a conformation necessary to express LB-medium-specific genes but not minimal-medium-specific genes at 42°C. While the *fitB rpoB7* strain requires rifampicin for growth at 42°C in LB medium the *fitB*$^+$ *rpoB7* strain does not. Thus at 42°C the (FitA$^+$) FitB RpoB7 RNA polymerase complexed with rifampicin may be conformationally and functionally equivalent to the (FitA$^+$) FitB$^+$ RpoB7 RNA polymerase without rifampicin. Our results are consistent with the earlier reports of Dass and Jayaraman (1985b, 1987), who reported that the efficiency of expression of various functions can be modulated by combinations of *fitA* and *rpoB* alleles and such modulation could be further influenced by temperature, medium, rifampicin, ions and chelators.

Selectivity of gene expression depends on the interplay of the conformational states of the genome as well as of RNA polymerase complexed with various accessory transcription factors. For example, the extent of supercoiling of different regions of the chromosome may partially determine selectivity of transcription (Smith 1981),

and many promoters have been reported to be sensitive to DNA supercoiling *in vitro* (Wood and Lebowitz 1984; Borowiec and Gralla 1987; Dixon *et al.* 1988). The extent of supercoiling can be affected by parameters such as temperature (Goldstein and Drlica 1984), growth phase (Dorman *et al.* 1988), and nutritional status or medium of growth (Worcel and Burgi 1972; Balke and Gralla 1987). By examining the regulation of several genes that respond to osmotic and anaerobic stress, Ni Bhriain *et al.* (1989) have suggested that there is a class of "stress-regulated" genes, the regulation of which is brought about by a common mechanism, namely change of supercoiling in response to different environmental signals. Attenuation in rich medium and expression in minimal medium of amino acid biosynthetic operons are dependent on structural features (Kolter and Yanofsky 1982). Likewise, the cAMP–CRP complex recognizes distinct sequences in several catabolite-sensitive operons (Rosenberg and Court 1979). Operons that are stringently regulated under amino-acid starvation have many signals that set them apart from others (Travers 1988). In addition, selectivity of gene expression could also involve specific conformations of RNA polymerase complexed with sigma factors or accessory transcription factors or ligands, or particular combinations of them. Evidence for this stems from the association of many proteins with RNA polymerase in the crude state (Snyder 1973; Travers and Buckland 1973; Pitale and Jayaraman 1975). Several proteins bind to RNA polymerase immobilized on agarose (Ratner 1974). Under mild conditions of purification, many proteinaceous factors copurify with RNA polymerase (Ishihama *et al.* 1983). According to the model proposed by Ishihama (1988), heterogeneity of RNA polymerase, brought about by the association of sigma factors/accessory transcription factors/ligands, is a major factor in selectivity of gene expression. It is possible that a fraction of RNA polymerase molecules could be complexed with the Fit factors *in vivo*. The phenotypes of the various *fit* and *rpoB* mutants described here and in our earlier reports could be the result of failure to form such complexes or of altered stability of the complexes.

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