*J. Biosci.,* Vol. 12, Number 3, September 1987, pp. 229-237. © Printed in India.

# Modulation of gene expression by the product of *fit*A gene in *Escherichia coli*

S. BALACHANDRA DASS* and R. JAYARAMAN

School of Biological Sciences, Madurai Kamaraj University, Madurai 625021, India
*Present Address: Department of Microbiology, Michigan State University, East Lansing, Michigan 48824, USA

MS received 7 August 1986; revised 1 May 1987

**Abstract.** Physiological parameters such as viability, gross RNA synthesis, $\beta$-galactosidase induction, development of phages T4, T7 and $\lambda$ have been studied in temperature-sensitive *Escherichia coli* strains harbouring *fit* A76, *fit* A24 and *fit* A76*fit* A24 mutations in *rpo*B$^+$ and *rpo*B240 genetic backgrounds. The efficiently of expression of these functions is influenced by the *fit* A alleles depending upon the medium of growth and/or temperature. Strains harbouring the *rpo*B240 mutation and the *fit* A76 mutation, either alone or together with the *fit* A24 mutation, are rifampicin-sensitive even at the perfssive temperature. The results suggest possible interaction between the *fit* A gene product and RNA polymerase in *vivo.*

**Keywords.** *fit* A gene; RNA polymerase; rifampicin; transcription.

## Introduction

The selective expression of genes by RNA polymerase in *Escherichia coli* and perhaps other prokaryotes as well, depends upon the activity of a variety of elements collectively called accessory transcription factors (Doi, 1977; Ishihama *et al.,* 1983). One way of studying the interaction between these factors and RNA polymerase is to obtain and characterize mutants which are transcription-defective but in which the mutation does not affect the genes coding for the subunits of the enzyme. This approach is handicapped by the difficult in selecting for such mutants. We have developed a simple method to obtain temperature-sensitive, potential transcription mutants (Jabbar and Jayaraman, 1976). A mutant isolated by this method was shown to be transcription defective at 42°C (Jabbar, 1979; Jayaraman and Jabbar, 1980). The mutation, originally called *ts*76, was mapped (Jabbar and Jayaraman, 1978) close to the *aro*D locus which is far removed from the loci coding for the subunits of RNA polymerase. In order to obtain genetic evidence for interaction, if any, between the product of this locus and RNA polymerase, Dass and Jayaraman (1985a) isolated a rifampicin-resistant (*rif*), temperature-insensitive derivative from a strain harbouring the *ts*76 mutation. Genetic analysis (Dass and Jayaraman, 1985a) showed that the ts+ phenotype of this derivative is the result of intragenic suppersion by a second mutation in the same gene. The suppressor mutation by itself conferred temperature-sensitivity and transcription defect at 42°C. Therefore the locus was named *fit* (factor involved in transcription) and the two alleles designated as *fit* 76 and *fit* 24, respectively (Dass, 1983; Dass and Jayaraman, 1985a). More recent experiments (Munavar and J. Jayaraman, unpublished results) have shown extragenic suppression of the temperature-sensitivity of the *fit* 76 mutation by a mutation in a locus very close to *fit.* Accordingly, the locus harbouring the *fit* 76 and *fit* 24

This paper is dedicated to Proof. S. Krishnaswamy on his Sixty First Birthday.

mutations have been renamed *fit* A and the extragenic suppressor locus as *fit* B. The *rif* mutation obtained during the isolation of *fit* 24 has been named *rpo*B240 and has been shown to cause medium and temperature-dependent rifampicin sensitivity/ resistance in a *fit*$A^+$ background (Dass and Jayaraman, 1985b). In this communication we have used isogenic strains harbouring *fit* $A^+$, *fit* A76, *fit* A24, *fit* A76-*fit* A24, *rpo*$B^+$ and *rpo*B240 alleles to look for possible functional interaction between the *fit* A gene product and RNA polymerase. Our results show that the efficiency of expression of several functions depends upon the *fit* A and *rpoB* alleles.

## Materials and methods

### *Bacterialstrains*

Most of the *E. coli* strains used in this work have been described in earlier reports from this laboratory (Jabbar and Jayaraman, 1978; Dass and Jayaraman, 1985a, b). The *rpo*B240 mutation was introduced by cotransduction with *met*$A^+$ using phage P1 grown on a *met*A+ revertant of BJ240 as donor.

### *Media and viability*

Conventional LB and minimal media were used (Miller, 1972). Nutritional supplements were added at 30 $\mu$g/ml. Rifampicin, where indicated, was used at 100 $\mu$g/ml. Colony formation on appropriate plates was used as an index of viability.

### *Phage yield*

Log phase cultures in LB were infected with phage at an m.o.i of 0·1 in the presence of 10 mM sodium azide and allowed to adsorb for 15 min. The infected culture was diluted to approximately $2 \times 10^4$ cells/ml. A portion was plated for infective centres and the rest aerated at the specified temperature and the progeny phage titred according to conventional methods.

### *ß-Galactosidasesynthesis*

The protocol described by Miller (1972) was followed.

### *Gross RNA synthesis*

Log phase cultures growing in minimal medium at 30°C were shifted to 42°C for 30 min and labelled with [$^3$H]-uridine (12·7 Ci/mmol; 0·5-1 $\mu$Ci/ml). Incorporation into trichloroacetic acid precipitable material was followed at intervals. The rate of incorporation was linear upto at least 30 min. The radioactivity incorporated over 30 min was used for calculation.

## Results

The primary objective of the studies reported here was to investigate the efficiency of gene expression as a function of the *fit* A and *rpo* B alleles. We chose the following parameters. (i) Viability is a reliable index of the overall efficiently of gene expression. Even subtle changes which may not be detectable by biochemical and physico-chemical methods could be detected as changes in the viability of cells under different conditions (see also Dass and Jayaraman, 1985b). (ii) Induction of *β*-galactosidase synthesis represents the expression of a specific group of genes as opposed to overall gene expression (viability). (iii) The 3 phages used here (T4, T7 and *λ*) have intricate mechanisms of regulating their gene expression. They utilize and modify the host transcription apparatus in characteristic ways. Therefore, changes in the host transcription apparatus could influence their development. (iv) Gross RNA synthesis ($^3$H-uridine incorporation) could be used to correlate the changes in physiological activities with transcription defects, although such correlation need not necessarily be expected (Dass and Jayaraman, 1985b). These parameters were examined in isogenic strains harbouring combinations of *fit* A and *rpo*B alleles.

### *Efficiency of expression of physiological function*

To begin with we monitored the above parameters *in fit A-rpo*B$^+$ mutants (table 1). At 30°C the expression of all the functions examined was not different from the *fit* A$^+$ parent. However, drastic changes were observed at 42°C depending on mutant *fit* A alleles and the medium. For instance, the temperature-sensitivity (in LB medium) due *to fit* A76 and *fit* A24 mutations differed by 1000 fold (line 1, columns 2 and 3). The two mutations together restored fully viability in LB medium at 42°C (column 4) as reported earlier (Dass and Jayaraman, 1985a). The *fit* A76 mutant was so thermo-sensitive in minimal medium that it was difficulty to measure viability in that medium at 42°C. While the *fit* A76-*fit*A24 double mutant was fully viable in LB medium at 42°C, it was only poorly viable in minimal medium at that temperature (compare line 1, column 4 and line 2, column 4). Gross RNA synthesis at 42° C (as measured by

**Table 1.** Efficiency of expression of some functions by *fit* A mutants.

| Function | Temperature (°C) | CSH57 (*fit* A$^+$) | JAJ572 (*fit* A76) | BJ571 (*fit* A24) | BJ241 (*fit* A76-*fit* A24) |
|---|---|---|---|---|---|
| Viability in LB medium | 42/30 | 1 | $10^{-7}$ | $10^{-4}$ | 1·0 |
| Viability in minimal medium | 42/30 | 1 | — | $10^{-5}$ | $10^{-3}$ |
| Gross RNA synthesis | 42/30 | 1 | 0·45 | 0·26 | 0·79 |
| *β*-Galactosidase synthesis[a] | 42/30 | 1 | 0·10 | 0·10 | 0·10 |
| Phage yield (T4) | 42/30 | 1–2 | $10^{-4}$ | ND | 0·02 |
| Phage yield (T7) | 42/30 | 1 | $10^{-4}$ | ND | 0·10 |
| Phage yield (*λ* vir) | 42/30 | 1 | $10^{-3}$ | ND | 0·10 |

The data presented are as ratios of expression at 42°C (non permissive temperature)/expression at 30°C (perfssive temperature).

[a] *β* -Galactosidase synthesis was deterfned as described by Miller (1972).

—, Indeterminable; ND, not done.

precursor incorporation in minimal medium) did not correlate with viability. For instance the *fit* A76 mutant which is practically non-viable in minimal medium at 42°C synthesized substantial amount of RNA at that temperature (line 3, column 2) while *the fit*A24 mutant which has measurable viability at 42°C synthesized less RNA. This reinforces our earlier conclusion (Dass and Jayaraman, 1985a) that the defect in these mutants might not be solely due to amount of RNA synthesized at 42°C but due to the types and amounts of RNA synthesized at 42°C. Of the other functions examined, induction of *β*-galactosidase synthesis and phage yield were thermo-sensitive in all the mutants, the extent of sensitivity varying widely depending upon the *fit*A allele (lines 4–7). A general pattern that emerges from these results is that a *given fit* A allele affects different functions differently.

### *Effect of the rpo B*240 *mutation*

We next examined the effect of the *rpo*B240 mutation on the efficiency of expression of the representative physiological functions by the *fit* A mutants, relative to their respective *rpo*$B^+$ parents. The origin of the *rpo*B240 mutation has been described earlier (Dass and Jayaraman, 1985a). It can be seen from table 2 that the *rpo*B240

**Table 2.** Effect of the *rpo*B240 mutation on the expression of physiological functions by *fit* A mutants relative toisogenic *rpo*$B^+$ parent.

| Function | Temperature (°C) | BJ5702[a] CSH57 | BJ5721[a] JAJ572 | BJ5711[a] BJ571 | BJ240[a] BJ241 |
|---|---|---|---|---|---|
| Viability in LB medium | 30/30 | 1·0 | 1·0 | 1·0 | 1·0 |
| Viability in LB medium | 42/30 / 42/30 | 1·0 | 1·0 | 0·5 | 1·0 |
| Viability in minimal medium | 30/30 | 1·0 | 1·0 | 1·0 | 1·0 |
| Viability in minimal medium | 42/30 / 42/30 | 1·0 | — | $<10^{-3}$ | $10^{-3}$ |
| *β*-Galactosidase synthesis | 30/30 | 1·3 | 1·2 | 1·2 | 0·82 |
| *β*-Galactosidase synthesis | 42/30 / 42/30 | 0·8 | 1·5 | 1·0 | 2·6 |
| Phage yield (T4) | 30/30 | 1·6 | 1·6 | ND | 1·3 |
| Phage yield (T4) | 42/30 / 42/30 | 1·9 | 0·7 | ND | 3·0 |
| Phage yield (T7) | 30/30 | 0·4 | <0·06 | ND | 1·9 |
| Phage yield (λ vir) | 30/30 | 0·9 | 0·01 | ND | 0·01 |

For some parameters the values are given as the ratio of the respective expression indices (expression at 42°C/expression at 30°C).

[a]BJ5702, BJ5721, BJ5711 and BJ240 are *rpo*B240 derivatives of CSH57 (*fit*$A^+$), JAJ572 (*fit*A76), BJ571 (*fit*A24) and BJ241 (*fit* A76-*fit* A24), respectively.

—, Indeterminable; ND, not done.

mutation had no significant effect on the viability of the mutants in LB medium (lines 1 and 2; columns 1–4). However, the viability of *fit*A24 and *fit*A76-*fit*A24 mutants in minimal medium at 42°C was drastically reduced in the presence of the *rpo*B240 mutation (line 4; columns 3 and 4). These mutants which are moderately temperature-sensitive in minimal medium (table 1) became highly temperature-sensitive in the presence of the *rpo*B240 mutation. Apparently such potentiation of temperature-sensitivity depended upon the *fit* A allele because it did not happen in the *fit*$A^+$ *rpo*B240 strain (line 4; column 1). Parameters such as $\beta$-galactosidase synthesis and development of phage T4 at 30 and 42°C were not very much influenced by the *rpo*B240 mutation so that the ratio of the expression indices was close to unity. There was a slight (2–3 fold) stimulation in some cases (lines 5–8; columns 1–4). On the other hand the ability of the *fit*A76-*rpo*B240 mutant to support the development of phage T7 was considerably reduced even at the permissive temperature (line 9; column 2). Both the *fit*A76-*rpo*B240 and *fit* A76-*fit* A24 mutants seemed to be highly defective in supporting the development of phage $\lambda$ at 30°C (line 10; columns 2 and 4). Again this defect was not manifested in *the fit* $A^+$ *rpo*B240 background showing *that fit* A alleles are crucial. The data presented above lead to the general conclusion that the efficiency of expression of physiological functions could be modulated by combinations *of fit* A and *rpo*B alleles.

*Rifampicin-sensitivity of fitA-rpoB*240 *mutants*

The *rpo*B240 mutation conferred rifampicin resistance on all strains, irrespective of the *fit* A allele, in LB medium at 30°C. Dass and Jayaraman (1985b) reported that a *fit*$A^+$ *rpo*B240 strain became rifampicin-sensitive (but not temperature-sensitive) in minimal medium at 42°C and that the resistant phenotype could be largely restored by the addition of divalent metal ions and ion chelators. It was, therefore, of interest to see how the *fit* A-rpoB240 mutants would respond to rifampicin in LB and minimal media at 30°C and 42°C. The results are presented in table 3. The *fit*A76 *rpo*B240 mutant was rifampicin-sensitive in minimal medium even at 30°C (line 1; sub columns 3 and 7). Earlier observations of Dass and Jayaraman (1985b) reproduced here for comparison (line 4), show that the *fit* $A^+$ *rpo*B240 mutant is rifampicin-sensitive only in minimal medium at 42°C and that the degree of rifampicin-sensitivity is far less relative to the *fit* A76 *rpo*B240 mutant at 30°C. The *fit*A24 *rpo*B240 mutant, on the other hand, was rifampicin-resistant in all the media. The degree of rifampicin-sensitivity of the *fit* A76 *rpo*B240 combination was not alleviated in the presence of the *fit* A24 mutation whereas its temperature-sensitivity was totally overcome (in LB medium). Unlike in the case of the *fit*$A^+$ *rpo*B240 strain, addition of metal ions, ion chelators or aminoacids did not restore rifampicin-resistance in the *fit* A76 *rpo*B240 mutant (data not shown).

Taking viability as an index of the overall physiological integrity of the cell, table 4 presents a picture of how this parameter is influenced by *fitA* and *rpo*B alleles, medium, temperature and addition of rifampicin.

## Discussion

One of the ways of regulation of gene expression in *E. coli* is by the modulation of the specificity and amount of functional RNA polymerase molecules by loose and

**Table 3.** Rifampicin sensitivity of *fit* A-*rpo*B240 mutants in minimal and rich media at 30 and 42°C.

| | Relative viability[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Minimal medium | | Minimal medium + rifampicin | | LB medium | | LB medium + rifampicin | |
| Strain | 30°C | 42°C | 30°C | 42°C | 30°C | 42°C | 30°C | 42°C |
| BJ5721 (*fit*A76-*rpo*B240) | 1·0 | $<10^{-8}$ | $10^{-8}$ | — | 0·97 | $10^{-7}$ | 1·03 | $10^{-8}$ |
| BJ5711 (*fit*A24-*rpo*B240) | 1·0 | $<10^{-8}$ | 1·06 | $10^{-8}$ | 1·0 | $10^{-4}$ | 1·05 | $10^{-4}$ |
| BJ240 (*fit*A76-*fit*A24-*rpo*B240) | 1·0 | $5\times10^{-6}$ | $10^{-8}$ | $10^{-8}$ | 0·98 | 1·03 | 0·90 | 0·90 |
| BJ5702[b] (*fit*A$^+$-*rpo*B240) | 1·0 | 1·0 | 1·0 | $5\times10^{-4}$ | 1·0 | 1·0 | 1·0 | 0·90 |

[a] Normalized with respect to the number on minimal plates at 30°C.
[b] Reproduced from Dass and Jayaraman (1985b) for comparison.
—, Indeterminable.

**Table 4.** Summary of the effects of *fit* A-*rpo*B-rifampicin combination on viability.

| | Viability | | | |
|---|---|---|---|---|
| | Minimal medium | | LB medium | |
| Allele-ligand combination | 30°C | 42°C | 30°C | 42°C |
| *fit*A$^+$-*rpo*B$^+$ | + | + | + | + |
| *fit*A76-*rpo*B$^+$ | + | — | + | — |
| *fit*A24-*rpo*B$^+$ | + | – | + | – |
| *fit*A76-*fit*A24-*rpo*B$^+$ | + | – | + | + |
| *fit*A$^+$-*rpo*B240 | + | + | + | + |
| *fit*A76-*rpo*B240 | + | — | + | — |
| *fit*A24-*rpo*B240 | + | — | + | – |
| *fit*A76-*fit*A24-*rpo*B240 | + | — | + | + |
| *fit*A$^+$-*rpo*B240 + Rif | + | – | + | + |
| *fit*A76-*rpo*B240 + Rif | — | — | + | — |
| *fit*A24-*rpo*B240 + Rif | + | — | + | – |
| *fit*A76-*fit*A24-*rpo*B240 + Rif | — | — | + | + |

+, fully viable; –, poorly viable ($10^{-5}$– $10^{-3}$); – , non viable ($<10^{-6}$)

transient binding of accessory proteins to the enzyme (Yura and Ishihama, 1979; Ishihama *et al.,* 1980, 1983). Other modes of regulation have recently been reviewed by McClure (1985) and Reznikoff *et al.* (1985). Many accessory proteins are associated with RNA polymerase in the crude state (Travers and Buckland, 1973; Snyder, 1973; Pitale and Jayaraman, 1975). Several proteins bind to RNA polymerase immobilized on agarose (Ratner, 1974). Under gentle methods of purification

some accessory proteins copurify with the enzyme (Ishihama *et al.,* 1983). In many, if not all, cases the physiological significance of the accessory factors is obscure. Over the past few years we have been focussing our attention on the genetics and physiology of a temperature-sensitive mutant mutated in a gene whose product seemed to be involved in regulation of transcription. Our previously published data has shown that (i) the primary effect of the *fit*A76 mutation at 42°C is on transcription; (ii) the kinetics of decay of RNA pulse labelled at 42°C is different from that at 30°C; (iii) a fraction of the RNA pulse labelled at 42°C sediments in association with ribosomes as polysomes and (iv) the synthesis of *β*-galactosidase is far more sensitive to temperature than that of alkaline phosphatase (Jayaraman and Jabbar, 1980). Although there is only a partial inhibition of gross RNA synthesis ($^3$H-uridine incorporation) in the mutant at 42°C, the mutant is practically non viable at that temperature. If the mutation leads to a partial inhibition of transcription of all genes at 42°C, one would expect the mutant to be viable but perhaps grow slowly at that temperature; apparently this is not so. The alternate possibility is that there could be a selective inhibition of transcription resulting in unbalanced RNA synthesis and consequent inhibition of growth. If this were true loss of viability would be a more reliable index of transcription defect than precursor incorporation (Dass and Jayaraman, 1985a, b). Thus, our previously published data show the involvement of the *fit* A gene product in transcription. The map position of the *fit*A gene is distinctly different from those of the genes coding for the sub units of RNA polymerase (Jabbar and Jayaraman, 1978; Dass and Jayaraman, 1985a). Therefore, it is likely that the *fit* A gene product could be an accessory transcription factor involved in determining the selectivity of transcription. The present paper supports this notion. Two broad and general conclusions can be drawn from the results presented here. (i) Mutations in the *fit* A gene affect the efficiency of expression of some representative physiological functions and (ii) a particular mutation in the *rpo*B locus (*rpo*B240) in combination with *fit* A alleles causes further alterations in expression. We believe that these results tentatively suggest that the product of the *fit* A locus and RNA polymerase might interact *in vivo* and such interaction could be important in determining the selectivity of the enzyme. Perturbations in this interaction, caused by mutations *in fit* A and/or the genes coding for the subunits of the enzyme could result in aberrant transcriptt-ion, some genes being expressed optimally, some sub-optimally, or not at all, ultimately leading to cessation of growth. This idea has been discussed at length earlier (Dass and Jayaraman, 1985b). A significant point to emerge from this work is that such transcriptional aberrations manifest themselves in certain allelic combinations even at 30°C. For instance, *fit* A76 and *fit* A76*fit* A24 mutants harbouring the *rpo*B240 mutation become rifampicin sensitive in minimal medium even at 30°C. Since the *fit* A76 and the *rpo*B240 mutations confer temperature sensitivity and rifampicin resistance, respectively, one would expect a double mutant harbouring both to be rifampicin resistant at 30°C. However, this occurs only in rich media. This suggests that rifampicin resistance due to *rpo*B240 in minimal medium is dependent on *the fit* A allele. Perhaps the *fiat* A76-*rpo*B240 combination is not able to express all the genes needed for growth in minimal medium. This reinforces our earlier conclusion (Dass and Jayaraman, 1985b) that there could be (unspecified) differences between genes expressed during growth on minimal and rich media. We have shown earlier (Dass and Jayaraman, 1985b) that even in a *fit* A+ background the *rpo*B240 mutation leads to rifampicin sensitivity in minimal medium at 42°C. Apparently, the

rifampicin sensitivity in minimal medium is specific to the *fit* A76 allele since it does not occur *in fit* A24-*rpo*B240 strains.

Another significant point to come out of the present work is that the *fit*A24 mutation totally alleviates the temperature sensitivity of the *fit* A76 mutation in LB medium but only partially in minimal medium (data presented in table 1). While this alleviation in LB medium is unaffected by the additional presence of the *rpo*B240 mutation in the presence or absence, of rifampicin, it is considerably reduced in minimal medium in the absence of rifampicin and drastically reduced in minimal medium + rifampicin (data presented in table 3). The data presented herein and the ones published earlier lead to the tentative conclusion that the *fit*A gene product and RNA polymerase might interact *in vivo,* determining the selectivity of gene expression.

Some unpublished observations from our laboratory support the above notion. We have obtained a novel, temperature-insensitive 'revertant' of the *fit*A76 mutant. A fraction of the spontaneous, rifampicin-resistant mutants of this revertant, obtained at 30°C, are temperature-sensitive in LB medium myself. We hope that genetic and physiological analysis of this revertant and its *rif* derivative, currently underway, will further substantiate our hypothesis. Although we have obtained suggestive evidence for *fit*A-RNA polymerase interaction at the level of function, we are yet to demonstrate physical interaction. This would involve purification of the *fit*A gene product. Our current efforts are focussed in this direction.

## Acknowledgements

This work was supported in part by a grant from the University Grants Commission, New Delhi. One of the authors (S.B.D.) thanks the Indian Council of Medical Research and the University Grants Commission, New Delhi for the award of Fellowships.

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