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# Aberrant transcription in *fit* mutants of *Escherichia coli* and its alleviation by suppressor mutations

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**Abstract.** Earlier work from this laboratory had identified, mapped and characterised an intragenic suppressor (*fitA*24) as well as an extragenic suppressor (*fit*B) for the temperature-sensitive transcription defective mutation *fit*A76 in *Escherichia coli* In this communication we report the results of experiments on RNA synthesis and decay of pulse labelled RNA in strains harbouring *fit* A76, *fit*B, *fit*A24, *fit*A76-*fit*A24, *fit*A76-*fit*B mutation(s) as well as in the isogenic *fit*A$^+$ *fit*B$^+$ strain. Taken together with earlier results, this indicates that the *fit*A and *fit*B gene products could be involved in the expression of some classes of genes including genes coding for ribosomal proteins. The implications of these results for the *in vivo* control of transcription in *Escherichia coli* are discussed.

**Keywords.** *fit*A; *fit*B; RNA polymerase; transcription; transcription control.

## 1. Introduction

Control of gene expression in prokaryotes is, by and large, regulated at the level of transcription. This is a diligently controlled process, wherein several molecules regulate gene expression by acting in concert with either RNA polymerase or DNA or both. These molecules are collectively referred to as accessory transcription factors (Ishihama 1988). Among such factors, those that interact with DNA (repressors, CAP, etc.,) are relatively better understood than those which interact with RNA polymerase. One way by which the interaction between accessory transcription factors and RNA polymerase can be studied is by isolating and characterizing mutations that impair the transcription process *per se* but do not affect genes coding for the subunits of RNA polymerase. The difficulty in this approach lies in the isolation of such mutations. A simple strategy to isolate temperature sensitive, potentially transcription defective mutations was reported by Jabbar and Jayaraman (1976). A Ts mutation (named *ts*76) isolated by this method was shown to cause defects in transcription at 42° C, but did not map in the genes coding for RNA polymerase subunits (Jabbar and Jayaraman 1978; Jabbar 1979; Jayarman and Jabbar 1980). The map position of this mutation (37·5 min) suggested that this locus might define a gene for an accessory transcription factor. Subsequently Dass and Jayaraman (1985a) isolated an intragenic suppressor (named *ts*24) that suppressed the Ts phenotype of *ts*76 but by itself conferred temperature sensitivity for growth; it was transcription defective at 42° C. The defect in these mutants could have been the inhibition of transcription of some but not all genes (Dass and Jayaraman 1985a). Dass and Jayaraman (1985a) named this locus

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*fit* (*f*actor involved in transcription) and the *ts*76 and *ts*24 mutations were named *fit*76 and *fit*24 respectively. A *rif* mutation (named *rpo*B240) that accompanied the *fit*24 mutation has been shown to cause medium- and temperature-dependent rifampicin sensitivity/resistance in $fit^{+}$, background (Dass and Jayaraman 1985b). Combinations of $fit^{+}$, *fit*76, *fit*24, *fit*76-*fit*24 mutations in $rpoB^{+}$ and *rpo*B240 genetic backgrounds have been found to affect the expression of different gene groups differently (Dass and Jayaraman 1987). They suggested that the *fit* factor could bind to the *ß*-subunit of RNA polymerase and thus function as an accessory transcription factor. During the course of our attempts to see whether the Ts phenotype (due to the *fit*76 mutation) could be extragenically suppressed, we found that a mutation mapping at 37·3 min, very close to the *fit* locus, could achieve this. Accordingly the locus for *fit*76 and *fit*24 mutations was renamed *fit* A and that of the extragenic suppressor as *fit*B (Munavar and Jayaraman 1987). Some of our preliminary results (Munavar 1991) suggested that the two *fit* genes could form, or be part of, a single operon. The phenotypes of strains harbouring various combinations of *fit* and *rpo*B alleles suggested possible interaction between the two *fit* gene products with each other as well as with RNA polymerase to exert Fit activity. Based on this, a model was proposed for interaction between the two *fit* gene products *in vivo* (Munavar 1991). This aspect will be dealt with in detail in a subsequent report. Recent studies of Vidya and Jayaraman (1992) have identified one more suppressor of the *fit*A 76 mutation, again mapping close to the *fit* AB loci. Whether the *fit* mutations define discrete genetic loci or are novel alleles of already known ones in the region is unclear. Dass and Jayaraman (1985a) have argued in favour of the former possibility. However, the current genetic map of *E. coli* (Bachmann 1990) lists *fit* as a *possible* allele of *inf*C. This question can be settled unambiguously only after cloning and sequencing the *"fit"* genes. Such studies are in progress and will be published elsewhere. Until the question is resolved we will stick to the term *fit* for the sake of continuity.

In the present communication we report the results of experiments carried out to determine the nature and amounts of RNA species synthesised in various *fit* mutants. Our results indicate that the *fit* gene products could be involved in the transcription of only some classes of genes, possibly including gene(s) coding for ribosomal protein(s). The implications of these results are discussed in relation to control of transcription by Fit factors *in vivo.*

## 2. Materials and methods

### 2.1 *Bacterial strains*

The *E. coli* strains used in this work are listed in table 1. The genetic nomenclature is according to Demerec *et al* (1966) as well as Bachmann (1990).

### 2.2 *Media and chemicals*

Conventional LB and minimal media were used (Miller 1972). Nutritional supplements were added at 30 $\mu$g/ml. Rifampicin was from Sigma Chemical

**Table 1** List of strains used/referred in this study

| Strain | Relevant genotype | Source/construction |
|---|---|---|
| CSH57 | F⁻ *his trp pur*E *leu met*A *ilv rps*L *arg*G | CSH collection |
| JAJ572 | F⁻ *his fit*A76 *trp pur*E *leu met*A *rps*L *arg*G | *fit*A76 derivative of CSH57 Dass and Jayaraman (1985a) |
| HMJ01 | F⁻ *his fit*A76 *fit*B *nal trp pur*E *leu met*A *rps*L *arg*G | Munavar and Jayaraman (1987) |
| BJ241 | F⁻ *his fit*A76 *fit*A24 *trp pur*E *leu rps*L *arg*G | Dass (1983) |
| BJ571 | F⁻ *his fit*A24 *pur*E *leu met*A *rps*L *arg*G | Dass (1983) |
| HMJ03 | F⁻ *his fit*B *trp pur*E *leu met*A *rps*L *arg*G *zdi 276::Tn10 nal* | This work (same as HMJ01 but *fit*A76⁺ and has *zdi*276::Tn*10*) |
| HMJ10 | Same as CSH57 but *pur*E⁺ | This work (*pur*E⁺ transductant of CSH57) |
| HMJ07 | Same as JAJ572 but *pur*E⁺ | This work (*pur*E⁺ transductant of JAJ572) |
| HMJ08 | Same as HMJ01 but *pur*E⁺ | This work (*pur* E⁺ transductant of HMJ01) |
| HMJ09 | Same as HMJ03 but *pur*E⁺ | This work (*pur* E⁺ transductant of HMJ03) |
| JMM1 | Same as BJ571 but *pur*E⁺ | This work (*pur* E⁺ transductant of BJ571) |
| JMM4 | Same as JAJ572 but has *fit*A24 | This work |
| JMM5 | Same as JMM4 but *pur*E⁺ | This work (*pur* E⁺ transductant of JMM4) |

Company, USA while all the other chemicals were from local sources. [$^3$H] uridine was from Bhabha Atomic Research Centre, Bombay. The ready made scintillation flui (INSTA-GEL) was from Packard Instrument Company Inc., USA.

### 2.3 *Measurement of [$^3$H] uridine incorporation*

Cells were grown to mid-log phase at 30° C with shaking in glucose minimal medium and divided into two equal portions. One portion was shaken at 30°C and the other at 42°C for lh. After this pre-incubation, [$^3$H] uridine (sp. Activity 165 mCi/mmol: 0·5 $\mu$ Ci/ml) was added to each culture and shaken at the respective temperatures. Two-tenth ml samples were withdrawn at 5 min intervals, added to 1 ml of ice cold 10% TCA and kept chilled. The precipitate was collected on Whatman glass micro fibre filters, washed 5 times with 5% TCA containing 50$\mu$g/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%. Incorporation was found to be linear with time. For quantitative comparison the cpm at 30 min was taken into consideration.

### 2.4 *Pulse-labelling of RNA and decay of pulse-labelled RNA*

Cells were grown to mid-log 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, 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 preincubation, RNA was pulse-labelled with [$^3$H] uridine (16,500 mCi/mmol; 0·5 $\mu$Ci/ml) for 20 s. The pulse was terminated by adding rifampicin and non radioactive uridine, to 100 $\mu$g/ml and 10 mM, respectively. Two-tenth ml aliquots were removed at different time intervals, added to 1·0 ml of ice cold 10% TCA, processed and counted as above.

For effective labelling with [$^3$H ] uridine we found that the strain should be *pur*$^+$*;* otherwise the adenine present in the minimal medium can inhibit labelling. Therefore all the strains used herein were converted to *pur*$^+$ by P1 transduction before use.

## 3. Results

### 3.1 *Gross RNA synthesis in fit mutants*

Previous results of Jayaraman and Jabbar (1980) and Dass and Jayaraman (1985a) indicated that RNA synthesis is reduced in *fit* A76, *fit* A24 and fit A76-fit A24 mutants at 42°C. In order to measure RNA synthesis in the *fit*B mutant and in the extragenically suppressed *fit*A76-*fit*B strain we studied the extent of [$^3$H] uridine incorporation in these strains. The data are presented in table 2. It can be seen that in the *fit*A76-*fit*B and *fit*B strains gross RNA synthesis goes down by approximately 25%, indicating a marginal transcription defect in both.

**Table 2.** [$^3$H] uridine incorporation *in fit* strains.

| Strain (genotype) | Relative incorporation of [$^3$H] uridine* 30° C | 42° C | Source/ref |
|---|---|---|---|
| HMJ10 (*fit* A$^+$ *fit* B$^+$) | 100 | 115 | This work |
| HMJ07 (*fit* A76) | 100 | 50 | This work |
| HMJ08 (*fit* A76-*fit* B) | 100 | 75 | This work |
| HMJ09 (*fit* B) | 100 | 75 | This work |
| BJ571 (*fit* A24) | 100 | 25 | Dass and Jayaraman (1985a, 1987) |
| BJ241 (*fit* A76-*fit* A24) | 100 | 79 | Dass and Jayaraman (1985a, 1987) |

*Maximum counts/min incorporated at 30°C was taken as 100% and the incorporation at 42°C was normalised with respect to it. In all cases the maximum counts/min incorporated was found to be of the order of $10^3$ or more.

However, we found that gross RNA synthesis as measured by [$^3$H] uridine incorporation did not correlate with the viability of the strains at 42° C. For instance, the *fit* A76 mutant, which is virtually non viable at 42° C in minimal medium (relative colony forming units at 42°C/30°C being $10^{-8}$ or lower),

synthesized a substantial amount of RNA (50%) while the *fit* A24 mutant, which has measureable viability (relative colony forming units approximately $10^{-4}$), synthesized only 25% RNA (Dass and Jayaraman 1985a, 1987). Similarly, although the extragenically suppressed strain (*fit*A76-*fit*B) and the strain harbouring only the suppressor (*fit*$A^+$-*fit*B) were comparable in terms of [$^3$H]uridine incorporation, they showed drastically different growth patterns in minimal medium (unpublished). This is true also of the *fit* A76-*fit* A24 and *fit* A76-*fit* B strains: the extent of RNA synthesis is approximately the same, whereas the viability is very different at 42°C in minimal medium (Munavar and Jayaraman 1987). Thus the transcription defect in these mutants is not confined merely to the amount of RNA synthesized at 42°C but could also encompass the types of RNA synthesized (see below).

### 3.2 *Kinetics of decay of pulse-labelled RNA in fit mutants*

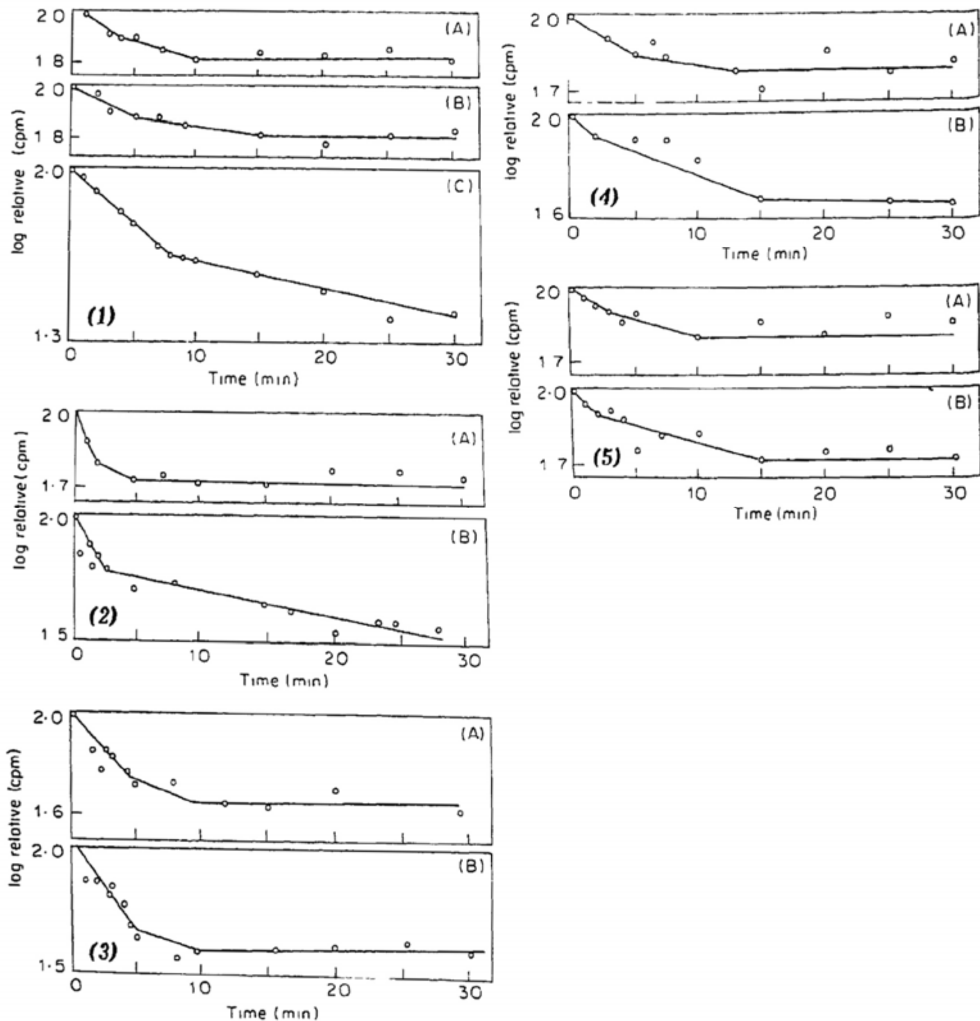
One way of analysing the types of RNA synthesized in the *fit* mutants is to measure the proportion of stable RNA (rRNAs and tRNAs) and unstable RNA (mRNAs) synthesized at 42°C. This could be done by pulse-labelling RNA and measuring the proportion of stable versus unstable species by following the kinetics of decay of pulse-labelled counts. Jayaraman and Jabbar (1980) found that the RNA synthesized at 42°C in *the fit* A76 mutant could not be differentiated into stable or unstable species whereas the RNA synthesized at 30°C could be so distinguished. They observed that the RNA pulse labelled at 42°C in the *fit* A76 mutant decayed with a very long half life, of the order of approximately 20 min. Doing similar experiments in other *fit* mutants would reveal the types of RNA synthesized by them and thus throw light upon the nature of involvement of the *fit* gene products *in vivo.* Therefore we carried out such experiments *in fit* A76, *fit* A24, *fit* A76-*fit* A24, *fit* A76-*fit*B, *fit*B and *fit*$A^+$-*fit*$B^+$ strains. Initially when we examined the kinetics of decay of pulse-labelled RNA in the wild type strain at 30°C, we found a triphasic pattern of decay consisting of a fast, a slow and a stable component (figure 1a). A triphasic pattern of decay was observed at 42°C also (data not shown).

The same triphasic pattern was observed in the *fit* A76 mutant at 30°C. But at 42° C the decay was biphasic consisting of only the fast and slow decaying species (figure 1 b, c). The stable species was completely missing at 42° C. It should be noted that the differentiation of unstable species into fast and slow decaying components was not emphasized earlier by Jayaraman and Jabbar (1980) either *in fit* $A^+$ or in *fit* A76 strains. Further, they had reported that in the *fit* A76 mutant at 42°C, the RNA decays monophasically with a longer half life whereas more careful measurements have now shown that it has two components (fast and slow) with different half lives.

As can be seen from figure 2, the pattern of decay of RNA pulse-labelled at 30°C in the *fit* A24 mutant is triphasic, whereas at 42°C, it is identical to what was observed with the *fit*A76 strain, the stable species being absent.

In the extra- and intragenically suppressed strains the decay pattern was triphasic with all three components present at 30°C and 42°C (figures 3 and 4). The same was also the case with the *fit* B strain (figure 5).

The above results suggest that the lesion in the *fit* mutants could be anamolous RNA synthesis at 42° C. The amount of stable versus unstable RNA (both fast and




**Figures1-5.** Kinetics of decay of pulse-labelled RNA. (**1**) Wild type strain HMJ10 at 30°C (**A**), *fit* A76 mutant (HM J07) at 30° C (**B**) and *fit* A76 mutant at 42° C (**C**). (**2**) *fit* A24 strain (JMM1) at 30° C (**A**) and 42° C (**B**). (**3**) Intragenically suppressed *fit* A76-*fit* A24 strain (JMM5) at 30° C (**A**) and 42° (**B**). (**4**) Extragenically suppressed *fit* A76*fit* B strain (HMJ08) at 30° (**A**) and 42° C (**B**). (**5**) *fit* B strain (HMJ09) at 30° C (**A**) and 42° C (**B**). Each point in the graph is the average of three different experiments. Experimental details are given under §2. Initial counts/min were always of the order of $10^3$ or more.

slow decaying) observed in various *fit* mutants are summarized in table 3. As can be seen, the *fit* A76 and *fit* A24 mutants suffer mainly in the extent of stable RNA. In these mutants stable RNAs are hardly detectable at 42°C. In both intragenically and extragenically suppressed strains, and also in the strain bearing only an extragenic suppressor, stable RNAs are detectable.

**Table 3.** Amount of pulse-labelled RNA detectable as stable and unstable species in *fit* mutants at 30° C and 42° C.

| | % RNA at 30° C | | % RNA at 42° C | |
|---|---|---|---|---|
| Strain (genotype) | Unstable species | Stable species | Unstble species | Stable species |
| HMJ10 (*fit* A⁺ -B⁺) | 35 | 65 | 25 | 75 |
| HMJ07 (*fit* A76) | 35 | 65 | 100 | 0 |
| JMM1 (*fit* A24) | 40 | 60 | 100 | 0 |
| JMM5 (*fit* A76-*fit* A24) | 50 | 50 | 60 | 40 |
| HMJ08 (*fit* A76-*fit* B) | 35 | 65 | 50 | 50 |
| HMJ09 (*fit* B) | 35 | 65 | 50 | 50 |

## 4. Discussion

One of the ways in which regulation of transcription is brought about is by regulation of promoter selectivity, in turn achieved by means of several mechanisms such as the association of RNA polymerase with different sigma factors, structural modification of the enzyme due to phosphorylation and ADP ribosylation, interaction with various transcription factors etc., (reviewed by Yura and Ishihama 1979; Ishihama 1988). Many proteins have been found to be associated with RNA polymerase in the crude state (Snyder 1973; Travers and Buckland 1973; Pitale and Jayaraman 1975). Ratner (1974) reported that several proteins bind to RNA polymerase immobilised on agarose. Many proteinaceous factors copurify with RNA polymerase under mild conditions of purification (Ishihama *et al* 1983). In most cases the physiological significance of these accessory transcription factors is obscure. Only recently several lines of direct evidence have come up, implicating RNA polymerase-associated factors in regulation of transcription during phase transition in growth and response to stresses such as heat shock and nutrient limitation (reviewed by Ishihama 1988). Our laboratory has been working on the genetics and physiological aspects of temperature sensitive, transcription defective mutants (see § 1 for references). The present results show that the products of the *fit* loci could function as accessory transcription factor(s). *fit* A76 and *fit* A24 mutations result in aberrant transcription at 42° C when present alone but normal transcription in combination. Similarly the *fit* B mutation corrects the aberrant transcription *in fit* A76 mutant at 42° C but has no apparent effect by itself. In both the *fit* A76 and *fit* A24 mutants stable RNA species are hardly detectable at 42° C.

What could be the reason for the reduction in the level of stable RNAs in the *fit* mutants? Could it be due to lack of synthesis in *fit* A76 and *fit* A24 mutants? Or could it be due to the lack of protection of the synthesized stable RNAs because of the absence of ribosomal proteins which normally protect them from decay? Previous observations of Jayaraman and Jabbar (1980) favoured the latter possibility. They reported that in a *fit* A76 mutant, when RNA was pulse-labelled at 42° C, only a small fraction of the label sedimented in association with ribosomes as polysomes (heavier than 70s). A substantial fraction of the pulse-labelled RNA sedimented below 70s and was sensitive to RNase. This was not observed when

RNA was pulse-labelled at 30°C. It is likely that the RNase sensitive RNA could be rRNA, underprotected because of nonavailability of ribosomal proteins. If this were true, the RNA pulse-labelled at 42°C in the *fit* A76 mutant would be protected from decay at 30°C due to the resumption of the synthesis of ribosomal proteins. This was indeed found to be true. Moreover they also found that when RNA pulse-labelled at 30°C was allowed to decay at 42°C, it decayed normally, indicating that temperature *per se* is not responsible for the abnormal decay of RNA in a *fit* A76 mutant. Considered together these observations indicate that there could be a defect in the transcription of genes coding for ribosomal proteins in *the fit* A76 mutant at 42°C. The extragenically and intragenically suppressed strains might overcome this defect and allow apparently normal RNA synthesis at 42° C.

It is important to stress two points. Although the intragenic (*fit* A24) and extragenic (*fit* B) suppressors are seen to restore "normal" RNA synthesis at 42°C as measured by labelling experiments, the phenotypes of the *fit* A76 *fit* A24 and *fit* A76 *fit* B strains are very different; while the former is still temperature sensitive in minimal medium the latter is not (Munavar and Jayaraman 1987). This implies that there could be subtle transcription defects in the *fit* A 6-*fit* A24 mutant, not detectable by experiments of the kind reported here but detectable by measurements of viability. Another point we wish to emphasize is that our results do not say anything about the transcription of tRNA genes in the *fit* mutants at 42°C. All the pulse labelled counts (at least more than 95%) at 42° C decayed away in *fit* A76 and *fit* A24 mutants (see table 3). Whether this is due to lack of synthesis of any tRNA at 42°C or the insensitivity of the experiments to detect them is difficult to distinguish. Additional experiments are necessary to clarify this point.

Based on our results, it is tenable to conclude that the *fit* factors function as accessory transcription factors and regulate the expression of only certain classes of genes which might include the genes coding for ribosomal proteins. According to the model proposed by Ishihama (1988) core RNA polymerase with a given sigma subunit ($E\sigma_\chi$) and transcription factor (designated TF) might transcribe a group of genes; the same $E\sigma_\chi$ with another TF might express some other group of genes. It is likely that the *fit* factors function as one such TF. Our genetic data indicate a strong interaction between the *ß*-subunit of RNA polymerase and *fit* factors (see § 1). Therefore it is possible that the *fit* factors may be copurified along with RNA polymerase and their activity can be checked *in vitro* using relevant *fit*-dependent genes as templates. These studies are currently underway.

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