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The *fit* genes and transcription control in *Escherichia coli*

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Abstract. This article reviews the work done in the author's laboratory on the genetics and physiology of the *fit* mutants of *Escherichia coli.* Isolaton of the *fit* mutants, genetic mapping, transcription abnormalities of the *fit* mutants, the possible involvement of the *fit* gene products in transcription control and identity of the *fit*A gene as *phe*S are described.

Keywords. Transcription; RNA polymerase; *fit* genes; gene expression.

1. Introduction

Transcription is a finely regulated process in prokaryotes as well as eukaryotes. In their natural habitat, bacteria constantly face several situations of stress which threaten survival. The common stresses include nutrient limitations, stress due to changes in temperature, osmolarity and pH, DNA damage, etc. The organism has to counter these in order to survive. Entry into stationary phase and cessation of growth also demand special mechanisms to maintain survival and resumption of growth when favourable conditions are encountered. Even under normal conditions of exponential growth the organism has to exercise utmost discretion in gene expression to derive maximum advantage from the environment and avoid wasteful expenditure of resources. A large body of evidence accumulated over the years show that global regulation of gene expression, under normal as well as abnormal physiological conditions, is brought about by RNA polymerase through its interactions with DNA and other elements which might influence such interaction. Reviews on this subject are available (Ishihama 1988, 1991, 1993; Collado-Vides *et al* 1991; Matin 1991; Siegale and Kolter 1992)

2. Functional differentiation of RNA polymerase in *Escherichia coli*

RNA polymerase of *Escherichia coli* is a complex enzyme consisting of four non-identical subunits (α, β, β' and σ) in molar portions of 2:1:1:1. The complexity of the structure of RNA polymerase is a reflection of not only the complexity of its function but also the complexity of regulation of its function. The genetic loci coding for the subunits, namely *rpo*A (*α*), *rpo*B (ß), *rpo*C (β') and *rpo*D (σ) as well as the role of the individual subunits in the overall process of transcription have been well documented (reviewed by Yura and Ishihama 1979). The σ subunit (now called σ^{70}) was originally discovered (Burgess *et al* 1969) as a protein factor which could be taken off the $\alpha_2\beta\beta'$ complex (the core enzyme) by phosphocellulose chromatography with concomitant loss of the enzyme's ability to transcribe physiologically relevant templates. In recent years many more σ subunits have been discovered. One of these, called σ_{32}, encoded by the *rpo*H

gene, has been identified in *E. coli* during heat shock response (Grossman *et al* 1984). The genes expressed during heat shock are transcribed better by the core enzyme complexed with σ^{32}. Another sigma factor (σ^{54}) has been identified in nitrogen starved cells (Hirschman *et al* 1985; Hunt and Magasanik 1985). σ^{54} encoded by the *rpo*N locus, resembles eukaryotic TATA binding proteins (TBP) in that it can bind to DNA in the absence of core RNA polymerase (Buck and Cannon 1992). Sporulating *Bacillus subtilis* has several σ factors in addition to the major σ found in vegetative cells (Doi and Wang 1986; Loscik *et al* 1986). As pointed out earlier, when cells enter the stationary phase of growth cycle a set of genes are expressed, some, if not all, of which could ensure survival during the non-dividing phase (Siegele and Kolter 1992). A positive regulator of these genes has been identified to be the product of the *rpo*S (earlier called *kat*F) gene, coding for another sigma factor, σ^{38} (Mulvey and Loewen 1989). Recent evidence (reviewed by Ishihama 1993) indicates that σ^{38} might be a major sigma factor during the stationary phase, just as σ^{70} is during the exponential phase. Specific sigma factors have also been implicated in flagellar synthesis, Chemotaxis, etc. An excellent review on sigma factors is available (Helmann and Chamberlin 1988). Mutant forms of *E. coli* σ^{70} with altered promoter recognition have been described (Gradella *et al* 1989; Siegele *et al* 1989). These studies have shown that different segments of the σ^{70} polypeptide chain contact –35 and –10 regions of promoters.

Regulation by multiple sigma factors is' but one of the several ways by which gene expression is controlled. Another regulatory mechanism is through phosphorylation of RNA polymerase subunits and/or proteins interacting with RNA polymerase (for references, see Ishihama 1988). Perhaps more relevant to the present article is the interaction of the so called "accessory factors" with RNA polymerase. Experimental evidence showing the binding of extraneous proteins to RNA polymerase have come from many laboratories. Several years ago Ratner (1974) showed the binding of many proteins to RNA polymerase immobilized on agarose. In the crude state RNA polymerase exhibits physical and functional heterogeneity (Travers and Buckland 1973; Snyder 1973; Pitale and Jayaraman 1975). Many non-protein factors such as ppGpp, $tRNA_f$met and Met-$tRNA_f$met have also been shown to interact with RNA polymerase. The interchange of sigma factors coupled with the association of different accessory factors with a given holoenzyme (E σ^{70}, E σ^{32}, E σ^{x} $_{E}$ σ^{y} etc.) could be a fine mechanism of control of transcription. The accessory factors are believed to interact either with free RNA polymerase or with RNA polymerase-DNA binary complexes and activate transcription from specific promoters through protein-protein contacts. By screening mutant RNA polymerases for activation negative phenotype, Ishihama and co-workers have identified two contact sites on the enzyme. Site I covers the carboxy terminal regions of the α subunit while site II is located at the carboxy terminal region of σ^{70}. Accordingly specific accessory factors have been classified into class I and class II factors (reviewed by Ishihama 1993). It would also be rewarding to isolate mutations in the loci coding for one or more of the accessory factors and correlate transcription defects(s) *in vivo* with data from *in vitro* experiments. The main difficulty in this approach is the selection of such mutants. We have been successful in isolating a transcription mutant in which the lesion maps at a locus distinct from the loci coding for the subunits of RNA polymerase. A brief account of our work on the genetics of this locus which we have named *fit* (factor involved in transcription) is presented below.

3. Isolation of a temperature sensitive, transcription defective mutant of *E. coli*

When this work began in 1974 the map positions of only the loci which code for the β and β' subunits (*rpo*B and *rpo*C) were known. Since RNA polymerase is a vital enzyme, we reasoned that any mutation which totally inactivates it would be lethal. Therefore conditional lethal mutations, such as temperature sensitives (Ts), have to be obtained. We thought of a strategy to first pick up mutants which are likely to be transcription defective at 42°C and then actually screen them for that property. The strategy is based on the difference in the extent to which bacteriophages T4 and T7 use the host RNA polymerase for their development. While T4 utilizes the host transcription machinery throughout the developmental programme, T7 utilizes it only briefly, to transcribe a few "immediate early" genes. One of the immediate early gene products is a T7-specific RNA polymerase which is structurally very distinct from *E. coli* RNA polymerase and which is responsible for all subsequent transcriptional events during T7 development (Chamberlin and McGrath 1970; Summers and Siegel 1970). Therefore a temperature sensitive mutant in which T4 growth is sensitive to 42°C throughout the growth cycle but T7 growth 'escapes' temperature inhibition within the first few minutes of growth at the permissive temperature, could be a potential transcription mutant. With this rationale we screened approximately 200 mutants (obtained by nitorsoguanidine mutagenesis as non-growers at 42°C) for this phenotype. We found one which behaved as predicted (Jabbar and Jayaraman 1976). The mutation was named *ts*76. Figure 1 shows the escape of T7 development at 42°C after varying. times of preincubation at 30°C in a *ts*76 mutant.

4. The *ts76* mutation causes transcription abnormalities at the restrictive temperature

If the rationale outlined above is correct, the *ts*76 mutant should show transcription abnormalities at 42°C, but should be normal at 30°C. This was shown to be true (Jayaraman and Jabbar 1980). The results of our experiments could be summarized as follows:

(i) The rate and extent of gross RNA synthesis at 42°C was reduced by approximately 50% relative to that at 30°C.

(ii) When the *ts*76 mutant was pulse labelled with [^{3}H]uridine at 30°C or an hour after shift up to 42°C and the decay of such pulse-labelled RNA measured at intervals following the pulse, a very interesting result was obtained. At 30°C the pulse-labelled RNA could be distinguished into unstable species (mRNA's) and stable species (ribosomal RNA's tRNA's, etc.). The unstable species could be further distinguished into a fast decaying component and a slow decaying component. At 42°C, the stable species were totally absent while the fast decaying and slow decaying species could be observed. Figure 2 shows the results of a typical experiment.

(iii) When pulsed with [^{3}H]uridine (RNA) or [^{3}H]leucine (protein) at various times after shift-up 42°C, reduction in RNA labelling was greater in magnitude and occurred sooner than that in protein labelling. This suggested that the primary lesion at 42°C could be transcription defect, affecting translation perhaps as a

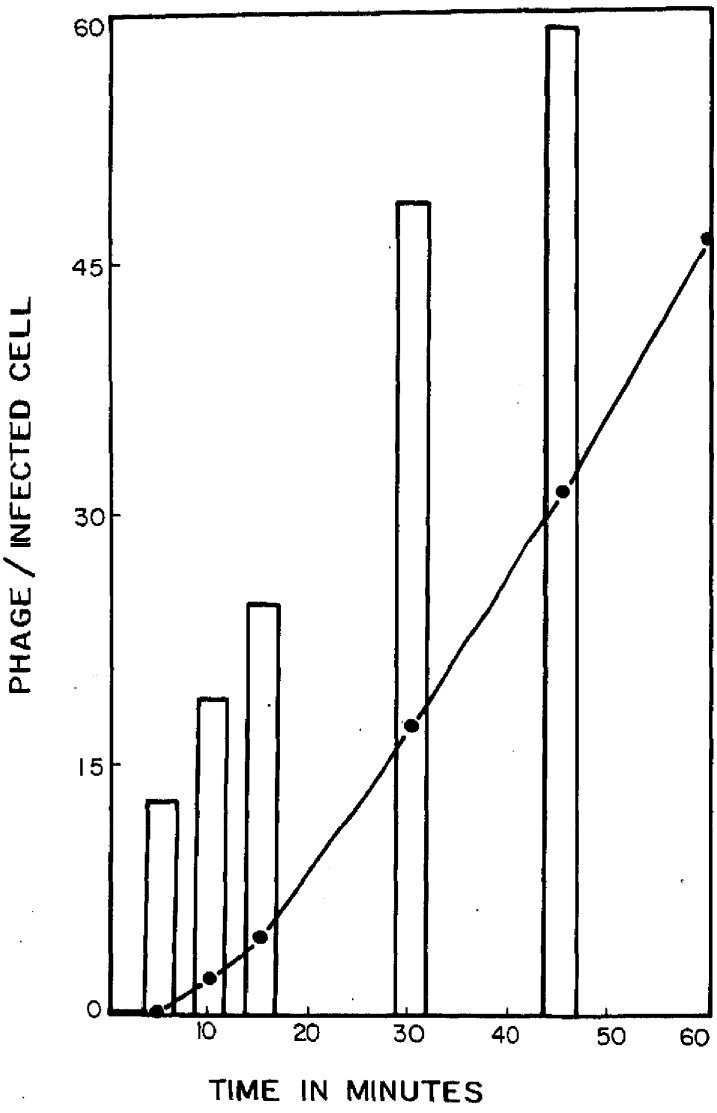

Figure 1. Kinetics of escape of T7 development from temperature inhibition in a *ts*76 (*fit*A76) mutant after preincubation at 30°C. Cells were infected with T7 at an m.o.i. of > 5 at 30°C (t_0). At the indicated times an aliquot was shifted to 42°C and another lysed with chloroform (to measure intracellular and extracellular phage present at the time of shift-up). After 90 min the tubes at 42°C were shaken with chloroform. Phage titers in all the tubes were determined. The points on the curve indicate phage titres at the time of shift-up and the bars indicate the same at 90 min. (Adapted from Jabbar and Jayaraman 1978).

consequence. Table 1 presents the data from a typical experiment.

(iv) While a small fraction of the RNA pulse labelled at 42°C sedimented in association with ribosomes as polysomes (> 70s) a substantial fraction sedimented lighter than 70s and was extremely RNase sensitive (figure 3). This was not seen with RNA pulse labelled at 30°C (data not shown).

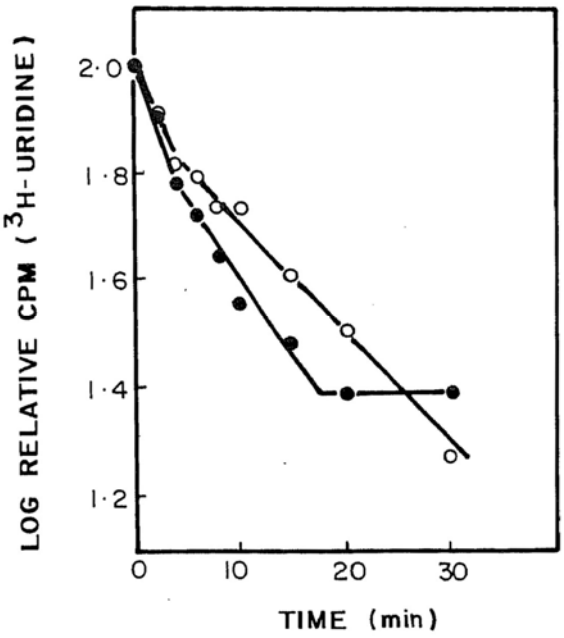

Figure 2. Kinetics of decay of pulse labelled RNA in a *ts*76 (*fit*A76) mutant. Log phase cultures were pulse labelled at 30°C or 60 min after shift-up to 42"C with [^{3}H]-uridine (sp. act. 16·5 Ci/mniol; 1 μCi/ml) for 20 s. The pulse was terminated by the addition of rifampicin and non-radioactive uridine to 100 μg/ml and 10 mM, respectively. Acid precipitable counts were measured at the indicated times after chase by conventional methods. (●) Pulse at 30°C; (O) pulse at 42°C. (Data from Sankaran 1993).

Table 1. Kinetics of inhibition of RNA and protein synthesis at 42°C in a *ts*76 (*fit*A76) mutant.

Time after shift-up (min)	Relative rates of precursor incorporation (%)	
	RNA	Protein
Pre-shift up	100	100
5	71	92
10	60	87
15	49	82
20	44	79
30	29	63
45	23	60

Precursor incorporations were determined with [^{3}H]uridine (for RNA) and [^{3}H]leucine (for protein) as counts of precursor incorporated during a 20 s pulse. The post-shift-up incorporations were normalized with respect to the pre-shift-up incorporation, taken as 100%. Data from Sankaran (1993).

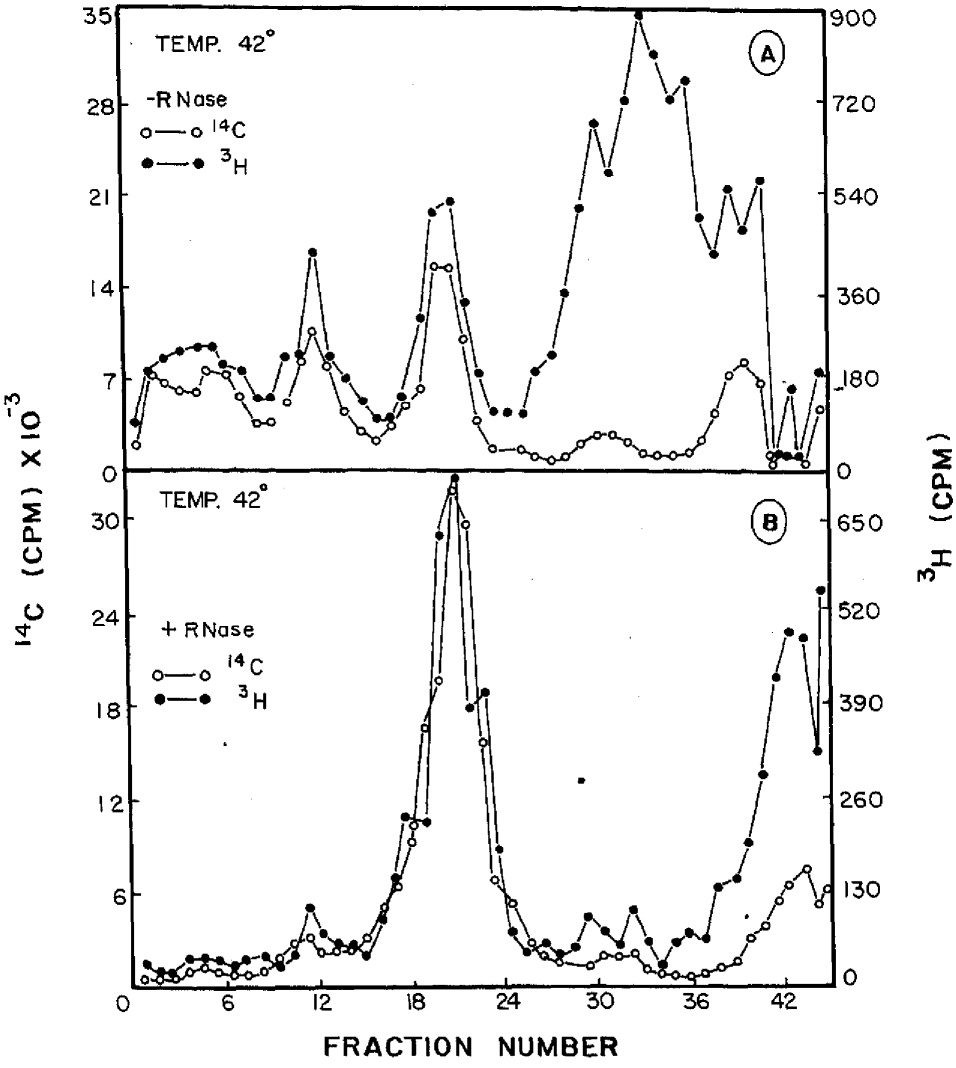

Figure .3. Sedimentation profile of RNA, pulse labelled at 42°C in a *ts*76 (*fit*A76) mutant. The cells were grown at 30°C in minimal medium containing [^{14}C]uracil (sp. act. 47 mCi/mmol; 2 μCi/ml) for 8 h (approximately 8 generations), shifted to 42°C for 1 h and pulse labelled with [^{3}H]uridine (2·3 Ci/mmol; 1 μCi/ml) for 20 s. A lysate of pulse labelled cells was centrifuged on a 15-30% sucrose gradient with and without RNase. Acid precipitable counts in fractions collected from the bottom of the gradients were measured by conventional methods. (Data from Jabbar 1979).

An important fact to emerge from these experiments was that the *ts*76 mutation does not result in total abolition of transcription, as would be expected normally, but not necessarily, of a mutation in the genes coding for the subunits of RNA polymerase subunits, but only causes a reduction. If the mutant made all the species of RNA at 42°C but at half the rate as at 30°C, one would expect it to grow slowly at 42°C (also see below). However, inhibition of growth at 42°C was total.

This implied that there could be a selective inhibition of transcription as opposed to global inhibition, leading to imbalance in RNA synthesis and consequent inhibition of growth. The *ts*76 mutation could define a locus whose product could be an accessory transcription factor essential for the expression of some but not all genes and gene groups.

5. Mapping of the *ts*76 mutation

It was necessary to know for sure whether or not the *ts*76 mutation mapped in the locus coding for any one of the subunits of RNA polymerase. Our mapping data showed that the mutation could be mapped to 37·5 min on the linkage map (Jabbar and Jayaraman 1978; also see below). None of the loci coding for the subunits of RNA polymerase maps at this position (Yura and Ishihama 1979). Therefore the *ts*76 mutation could define a gene whose product is involved in transcription but is not a subunit of the enzyme.

5. Isolation of an intragenic suppressor of the *ts*76 mutation

If the hypothesis outlined above is true, it should be possible to obtain evidence showing interaction between RNA polymerase and the product of the gene defined by the *ts*76 mutation. One way of obtaining such evidence is to isolate mutations which map in the genes coding for the subunits of RNA polymerase and which could suppress the temperature sensitivity of the *ts*76 mutation. The logic behind this classical genetic approach is that in interacting systems it is often possible to overcome the effect(s) of mutation in one of the components by a mutation in the other component(s) such that normal interaction could be restored. This has been shown in many systems. In the present case, although the compensatory (suppressor) mutation could, in principle, fall in the genes encoding any of the RNA polymerase subunits, *rpo*B was chosen mainly because it is easy to isolate *rpo*B mutants based on rifampicin resistance. Moreover, the *rpo*B gene product (β-subunit) has several Pleiotropic properties (see Dass and Jayaraman 1985a; Munavar and Jayaraman 1987). With this rationale in mind, Dass isolated a temperature-insensitive, rifampicin-resistant derivative of a *ts*76 mutant. Genetic analysis of this derivative (Dass and Jayaraman 1985a) showed that the Ts^+ phenotype of this derivative was not due to the *rpo*B mutation (named *rpo*B240) but due to a second mutation in the same gene defined by the *ts*76 mutation. The second mutation, by itself, inhibited growth and RNA synthesis at 42°C. Thus, the suppression was intragenic wherein two mutations, each conferring mutant phenotype in isolation, restore wild type phenotype in combination. At this point the locus of the *ts*76 mutation was named as *fit* (factor involved in transcription) and the mutations were designated as *fit*76 and *fit*24 (Dass and Jayaraman 1985a).

Although the *rpo*B240 mutation (which accompanied the *fit*24 mutation during the isolation of the latter) had no role in the suppression of temperature-sensitivity due to *fit*76, it did have interesting properties in a fit^+ background. A fit^+ *rpo*B240 mutant was normal with respect to growth and rifampicin resistance at 30 and 42°C in rich media such as LB. In minimal medium the mutant was rifampicin sensitive but not temperature sensitive at 42°C (Dass and Jayaraman 1985b).

Rifampicin resistance in minimal medium at 42°C could be largely restored in the presence of certain divalent cations (Mg^{2+}, Ca^{2+} and Ba^{2+}) as well as ion chelators (EDTA, EGTA and *0a*phenanthrolein). Table 2 presents a summary of the results. These observations suggested, albeit indirectly, an interaction between the *fit* gene product and RNA polymerase, especially the β-subunit, and modulation of such interaction by rifampicin, ions and chelators.

Table 2. Conditional rifampicin sensitivity and its relief in a *fit*$^+$ *rpo*B240 mutant in minimal medium.

	Relative viability (42°C/30°C) in minimal medium	
Additions	− Rif	+ Rif
None	1·0	5×10^{-4}
$MgSO_4$ (10 mM)	1·0	0·35
$MnCl_2$ (10 mM)	1·0	0·03
$CaCl_2$ (1 mM)	1·0	0·32
$BaCl_2$ (10 mM)	1·0	0·83
EDTA (0·1 mM)	1·0	0·29
EGTA (0·1 mM)	1·0	0·32
o-phenanthrolein (0·1 mM)	1·0	0·23

Relative viability was 1·0 in LB medium under all conditions listed above. Adapted from Dass and Jayaraman (1985b).

7. Isolation of an extragenic suppressor of the *fit*76 mutation

Another suppressor mutation which suppressed the temperature sensitivity of the *fit*76 mutation was isolated and characterized (Munavar and Jayaraman 1987). Genetic analysis showed that the second suppressor mutation defined a new locus, very close to *fit.* Therefore the locus of the original *fit* mutations (*fit*76 and *fit*24) was designated as *fit*A and that of the extragenic suppressor as *fit*B (Munavar and Jayaraman 1987). The available genetic data indicated that the temperature sensitivity of a *fit*A76 mutation could be suppressed intragenically by the *fit*A24 mutation or extragenically by the *fit*B mutation. It is possible that the *fit*A and *fit*B gene products function as a complex *in vivo.*

Munavar and Jayaraman (1993) measured the kinetics of decay of RNA, pulse-labelled at 30°C and 42°C in the *fit* mutants. The data are presented in table 3. It could be seen that the *fit*A24 and *fit*B mutations counter the absence of stable RNA species in the *fit*A76 mutant, pulse labelled at 42°C. Vidya and Jayaraman (1992) tentatively described two more extragenic suppressor mutations which suppressed the temperature sensitivity of the *fit*A76 mutation. The existence of one of them has not been shown unequivocally, but that of the other has been and it seems to be located near *fit*A itself.

8. Modulation of gene expression by *fit* genes

Several physiological parameters such as viability, gross RNA synthesis, induced

Table 3. Distribution of pulse labelled RNA counts into stable and unstable species in *fit* mutants.

	30°C		42°C	
Mutant	Unstable species (%)	Stable species (%)	Unstable species (%)	Stable species (%)
*fit*A$^+$ *fit*B$^+$	35	65	25	75
*fit*A76	35	65	100	0
*fit*A24	40	60	100	0
*fit*A76 *fit*A24	50	50	60	40
*fit*A76 *fit*B	35	65	50	50
*fit*B	35	65	50	50

Data from Munavar and Jayaraman (1993).

synthesis of β-galactosidase, development of phages (λ, T4 and T7) in strains bearing *fit*A76, *fit*A24, *fit*A76-*fit*A24 and *fit*A$^+$ alleles in *rpo*B$^+$ and *rpo*B240 genetic backgrounds were studied (Dass and Jayaraman 1987). The results showed that the efficiency of expression of these functions is influenced by the *fit*A and *rpo*B alleles depending upon the medium of growth and/or temperature. For instance, the *fit*A76 mutation resulted in a total inhibition of growth at 42°C while gross RNA synthesis, as measured by precursor incorporation, was down by only 50%. As pointed out earlier, if the mutation leads to partial inhibition of global transcription at 42°C one could expect a reduction in growth rate but not total inhibition of growth. A more likely possibility is that the inhibition of transcription in the *fit*A76 mutant is selective rather than global. If this were true there would be an imbalance of RNA synthesis at 42°C which could have adverse consequences. This was substantitated by the behaviour of *the fit*A76-*fit*A24 double mutant. In the*fit*A76-*fit*A24 double mutant viabilities in rich medium at 30°C and 42°C were not at all different and also the extent of gross RNA synthesis at 42°C relative to 30°C was as high as 80%. In spite of this, viability in minimal medium at 42°C was only 0·01%. Apparently the *fit*A24 mutation is not able to overcome the defect due to *fit*A76 at 42°C in minimal medium although gross RNA synthesis seems to be normal. Likewise a *fit*A76-*rpo*B240 double mutant was rifampicin resistant in rich medium at 30°C but rifampicin sensitive in minimal medium at the same temperature. The same rpoB240 mutation conferred rifampicin resistance (at 30°C) in a *fit*$^+$ or *fit*A24 background irrespective of the medium.

Recently it was shown that the *fit*B mutation conferred temperature sensitivity in rich media which lack sodium chloride (Munavar and Jayaraman 1993). The temperature sensitivity was enhanced even more if such media included glucose. Rifampicin resistant mutations, isolated in a *fit*B genetic background displayed a spectrum of phenotypes. Some were rifampicin dependent for growth at 42°C but not at 30°C; some were rifampicin sensitive in minimal media even at 30°C (like *fit*A76 *rpo*B240 strains); some were rifampicin sensitive in minimal media at 42°C (like *fit*$^+$ *rpo*B240 strains). These results strongly suggested that there is some interaction between the *fit* gene products and RNA polymerase.

Available data on the genetics and physiology of *fit* mutants has been incorporated into a general model (Munavar and Jayaraman 1993). According to this model, the *fit*A *fit*B gene products may interact with each other as well as with RNA polymerase

and modulate the efficiency of transcription. Such modulation may be mechanistically similar to that proposed by Ishihama (1988) for accessory transcription factors in general. He has suggested that promoter strength can be modulated by increasing the promoter-RNA polymerase affinity or by a more rapid isomerization of the "closed promoter complexes" to "open promoter complexes" or both. The efficiency of modulation of promoter strength may depend, among other factors, the integrity of the *fit* gene products as well as the subunits of RNA polymerase.

9. Identity of the *fit*A gene and its implications

In spite of the extensive data accumulated on the genetics and physiology of the *fit* mutants, the identity of the *fit* loci had remained equivocal. Conventional genetic mapping placed the *fit*A and *fit*B mutations in the 37·3-37·5 min region of the *E. coli* genetic map, cotransducible with the *aro*D and *pps* markers (figure 4). This region has several genes involved in translation besides other functions (see Bachmann 1990). For instance the *phe*S and *phe*T genes, coding for the α and β subunits of phenylalanyl tRNA synthetase, respectively, are obviously genes involved in translation. Besides, the gene for translation initiation factor III *(inf*C) also maps in this region. The *rpl*T locus codes for a ribosomal protein. Similarly, the *him*A gene, coding for the host integration factor, also maps here. It was quite possible that the *fit*A and *fit*B mutations could define new, unidentified genes mapping in this region or could be novel alleles of the already characterized genes located around 37·3–37·5 min. Dass and Jayaraman (1985a) had ruled out many genes as the possible loci of the *fit* mutations, based on reasoning rather than direct experiment. Even then they could not rule out *inf*C. Perhaps this was the reason for listing the fit(A) gene as a possible allele of *inf*C by Bachmann (1990). In order to resolve this ambiguity Ramalingam and coworkers (manuscript in preparation) undertook

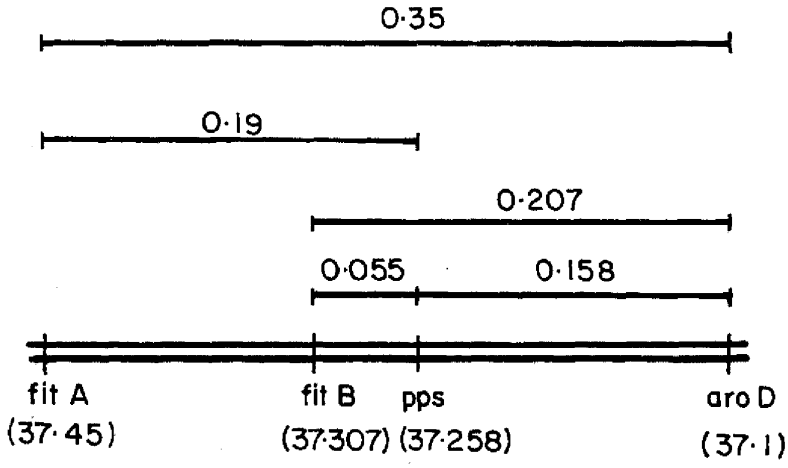

Figure 4. Partial genetic map of the *E. coli* chromosome spanning the *fit* genes. The numbers in parentheses indicate map position (in minutes) and the ones above indicate distance between markers (in minutes). (Data from Munavar and Jayaraman 1987).

cloning and molecular characterization of *fit*A and its protein product. The *fit*A gene was cloned as a 2·1 kb DNA fragment from which a 33 kDa protein was shown to be synthesized. Sequencing the 2·1 kb DNA fragment led to a surprising result, namely, the fragment contained the entire sequence of *phe*S and the initial portion of *phe*T genes. As pointed out above, the *phe*S and *phe*T loci code for the α and β a subunits of phenylalanyl tRNA synthetase, respectively. As could be expected, plasmids harbouring the 2·1 kb fragment complemented temperature sensitive mutations characterized as *fit*A mutations or *phe*S mutations, but did not complement a *fit*B mutation. These data showed that *fit*A and *phe*S genes are one and the same, thus clarifying a longstanding uncertainity on the identity of the *fit*A gene. At the same time it also raised a very basic question, namely, whether the characteristics of *fit* mutants (aberrant transcription at 42°C, postulated interaction with RNA polymerase, modulation of gene expression, etc.) documented over the years, are primary effects or secondary consequences of translational inhibition. In order to settle this question Ramalingam and coworkers (manuscript in preparation) studied the behaviour of isogenic strains harbouring temperature sensitive mutations characterised as *fit* (*fit*Al6) or *phe*S (*phe*S5) mutations. Their results showed that the characteristic abnormality of the *fit*A76 mutant, namely the absence of stable species in the RNA pulse labelled at 42°C was not manifested in the *phe*S5 mutant. Moreover RNA synthesis was inactivated at 42°C faster than protein synthesis in the *fit*A76 mutant whereas the reverse was the case in the *phe*S5 mutant. These results indicated that the two mutations lead to different phenotypic consequences although both define the same gene. Several years ago, Jabbar and Jayaraman (1978) reported that *phe*S5 and *phe*T mutants do not elicit the "T7 escape" phenotype, characteristic of *fit*A76 mutants. This has also been verified recently (Sankaran 1993). Therefore the α-subunit of phenylalanyl tRNA synthetase complex can be imagined to have two domains, one involved in transcription control and the other in charging phenylalanyl tRNA.

The role of the *phe*S (*fit*A) gene product in translation is obvious since it forms parts of the enzyme complex that activates phenylalanine for protein synthesis. However, its role as a transcription factor is not so obvious. The available genetic data suggests interaction between the *fit* gene products among themselves and also with RNA polymerase (see Munavar and Jayaraman 1993). Although the phenotype of *fit* mutants are strongly influenced by *rpo*B (rifampicin-resistant) mutations (Munavar and Jayaraman 1993) it has not been possible so far to obtain a *rif* ^{r} mutation which could suppress the temperature sensitivity of a *fitA76* mutant. Since rifampicin resistance defines only a segment of the *rpo*B gene (Jin and Gross 1988) it is possible that other regions of *rpo*B could be involved in interaction with the *fit* gene products. Similarly *rpo*A and *rpo*D could also be involved. These possibilities are currently being investigated.

Phenylalanyl tRNA synthetase of *E. coli* is unique among aminoacyl tRNA synthetases in having a complex quaternary structure (α_2 β_2), the only other enzyme of the type being glycyl tRNA synthetase (Schimmel 1987). While the significance of such quaternary structure is not known, it is believed (Schimmel 1987) that the subunits could have other functions. In the likely event of *fit*B turning out to be p*he*T (see below), it is possible that the respective gene products could function both as transcription factors as well as parts of an aminoacylating enzyme.

The genetic data of Munavar and Jayaraman (1987) indicated that *fit*B maps to

the right of *fit*A, at 37·3 min. Munavar (1991) also observed that Tn5 insertions in *fit*A were polar on *fit*B, implying that the two genes constitute an operon or are parts of one. The *phe*S and *phe*T genes are also organized into an operon, the direction of expression being *phe*S → *phe*T. Since *fit*A has turned out to be *phe*S, it is tempting to speculate that *fit*B could be *phe*T.

The elucidation of the identity of *fit*A as being the same as *phe*S has opened up several avenues for future experimentation which were hitherto not possible. It is now possible to obtain corroborative evidence *in vitro* to support the conclusions derived earlier by *in vivo* (genetic and physiological) experimentation. Such studies are in progress in our laboratory.

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