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RIBOSOMAL ASSEMBLY DEFECTIVITY IN A SET OF AMBER MUTANTS OF ESCHERICHIA COLI

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Summary

Five amber mutations affecting essential genes of Escherichia coli have been isolated. The procedure relies on P1-mediated localized mutagenesis(1) and on the use of a recipient strain carrying a strong but instable suppressor gene and a particular thermoinducible λ prophage which kills suppressor hosts at 42°C (2). All five mutations map close to the spcA gene, in a region which codes essentially for ribosomal proteins. Strains harboring the mutations were studied biochemically ; all five exhibit defective ribosomal assembly upon loss of suppression.

Introduction

Several independent approaches have shown that many genes coding for ribosomal proteins are clustered in the aroE-strA region of the chromosome (72 minutes on the genetic map). Genetic and biochemical studies relying on the use of λ transducing phages carrying ribosome genes clearly demonstrated that ribosomal protein genes, in this cluster, are organized in four transcriptional units (3). Understanding of the functional organization of ribosomal protein genes and of the mechanisms which modulate their expression might be facilitated by the existence of polar amber mutations. With this objective in mind, we have recently developped a general method to obtain amber mutations in Escherichia coli essential genes (2). It relies on P1-mediated localized mutagenesis (1) and on the fact that the recipient carries an efficient but genetically unstable suppressor and a particular thermoinducible λ prophage which kills suppressor hosts at 42°C. Exposure of these bacteria to the non-permissive temperature yields frequent suppressor-free derivatives while none will be found if the strain carries an amber mutation in an essential gene. In the same report, we analysed three amber mutations leading to ribosomal assembly defectivity. The present communication extends our investigations to five other amber mutations in the aroE-spcA region. All five have been mapped with respect to the spcA gene (coding for ribosomal protein S5) and introduced into a thermosensitive suppressor recipient to monitor ribosomal assembly upon loss of suppression.

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Table 1 Bacterial strains

Designation	Chromosomal markers	Origin
RH2569(K12S)	prototroph	Appleyard, R.
RH3134	ara_{am}, $lac125_{am}$, $galU_{am}$, galE, tsx_{am}, spcA, aroE353	Delcuve *et al* (2)
RH3149	RH3134(Ø80sus2suIII)(λN7N53029cI857)	obtained by lysogenisation for each phage
RH3187	ara_{am}, $lac125_{am}$, $galU_{am}$, galE, trp_{am}, tsx_{am} suIIIA81* (thermolabile suppressor tRNA)	Delcuve, G., Ph.D. thesis
RH3184	RH3187 aroE353, spcA	Delcuve, G. *et al* (2)
RH3801	RH3187 aroE353, eryA	Denicourt, D., unpublished data
RH3804	RH3187 spcA am3804	this work
RH3829	RH3187 am3829	
RH3830	RH3187 am3830	
RH3198	RH3187 am3198	
RH3199	RH3187 am3199	

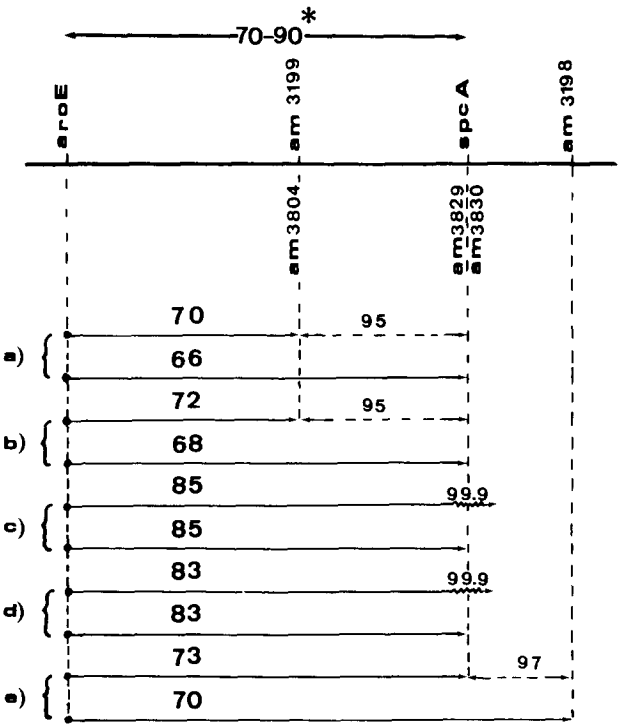

Figure 1 - Mapping of amber mutations
Figures on the map refer to % cotransduction ; small size numbers refer to deduced values. * cotransduction frequency aroE-spcA as compiled from the litterature (5). Selection was for aroE$^+$ in all cases.
(a) 325 transductants (b) 401 transductants (c) 261 transductants
(d) 210 transductants (e) 233 transductants.

Material and Methods

a) Escherichia coli strains Table 1

b) Phages : λN7N53029cI857 ; Ø80su2suIII (2)

c) P1 transduction procedures were described previously (4)

d) P1 mutagenesis with hydroxylamine follows the procedure of Hong and Ames (1)

e) In vivo ribosomal assembly : as described in (2), with a minor modification. Labelling of cells was performed for 90 instead of 30 minutes.

Results

Phage P1 grown on strain RH2569 was mutagenised with hydroxylamine (1). The rate of survival was 0.5 %. After concentration, the mutagenised stock was used to transduce the aroE-spcA region into strain RH3149, lysogen for Ø80sus2suIII and λN7N53029cI857. After selection for aroE$^+$ colonies at 32°C, we screened for complete thermosensitivity. Roughly 1% of the transductants are unable to grow at 42°C. Five mutants have been purified and analysed. The mutations they carry have been mapped with respect to spcA. As seen in Figure 1, cotransduction frequencies range

Table 2 P1-mediated transduction of amber mutations in various recipients (selection for $aroE^+$)

Mutation	Recipient		
	RH3149 (suIII)	RH3134 (su^-)	RH3184 (suIIIA81*)
am3198	+	-	+
am3199	+	-	+
am3804	+	-	+ *
am3829	+	-	+
am3830	+	-	+

+, survival of the recipient after transduction ; -, no survival

* in the case of am3804, which was initially obtained in a spc^R transductant, we used the recipient RH3801, which is $spcA^+$ in order to obtain mapping data

Table 3 Ribosomal assembly defectivity in amber mutants

Strain	at 30°C	at 42°C	Remark
RH3198	reduction of 50S content	nearly no 50S	no detectable 50S precursors
RH3829	-	reduction of 50S content	no detectable 50S precursors
RH3804	-	nearly no 50S	no detectable 50S precursors
RH3199	reduction of 30S content	no 30S	no detectable 30S precursors
RH3830	-	no 30S	accumulation of incomplete particles (S value around 16S)

from 95 to 99.9%. In two instances, am3829 and am3830, we were unable to separate the mutations from the spcA marker.

The five mutants harbor an amber mutation ; this was shown by transduction of the aroE-spcA region into a recipient strain, RH3134, which does not carry a suppressor. Selection was made for $aroE^+$, in all cases, it was impossible to recover thermosensitive transductants (Table 2).

In order to carry out biochemical analysis of these mutations, we transduced them into a recipient carrying a thermosensitive suppressor ($suIIIA81^*$) ; the resulting strains became thermosensitive, further indicating the amber nature of the mutations (Table 2). The strains carrying the thermosensitive suppressor and the mutations am3804, am3198, am3199, am3829 and am3830 are referred to as RH3804, RH3198, RH3199, RH3829 and RH3830 respectively.

Exposure of the mutants to high temperature will inactivate the suppressor tRNA. As a consequence, genes carrying amber mutations will not be expressed and their products, if they are indeed ribosomal proteins, will be lacking during in vivo ribosomal assembly. This situation will lead to the production of immature or aberrant particles. We have checked ribosomal assembly in vivo by comparing sedimentation profiles of cell extracts from parent and mutant strains labelled respectively with [^{3}H] or [^{14}C]uracil at 42°C. Figure 2 illustrates the results of such experiments. Strain RH3198, at the non-permissive temperature, does not assemble 50S particles correctly. On the other hand, strain RH3199 is defective in 30S assembly.

Data for all five amber mutants are compiled in Table 3 ; it should be noted that even at 30°C, strains RH3198 and RH3199 present already a partial defect in ribosomal assembly. This observation is consistent with the fact that the suIII thermosensitive mutation at 30°C has a relatively low efficiency of suppression (~ 28% efficiency : Delcuve, G., unpublished data).

Conclusion

In this report, we describe some properties of five new amber mutants of Escherichia coli. The mutations have been mapped close to the spcA gene which codes for ribosomal protein S5. Although the identification of the altered components has yet to be performed, it is highly probable that the five amber mutations affect genes coding for ribosomal proteins. In support of this hypothesis, we have found that ribosomal assembly in vivo is impaired upon loss of suppression. Amber mutations in ribosomal protein genes are potentially useful in the study of the expression of ribosomal

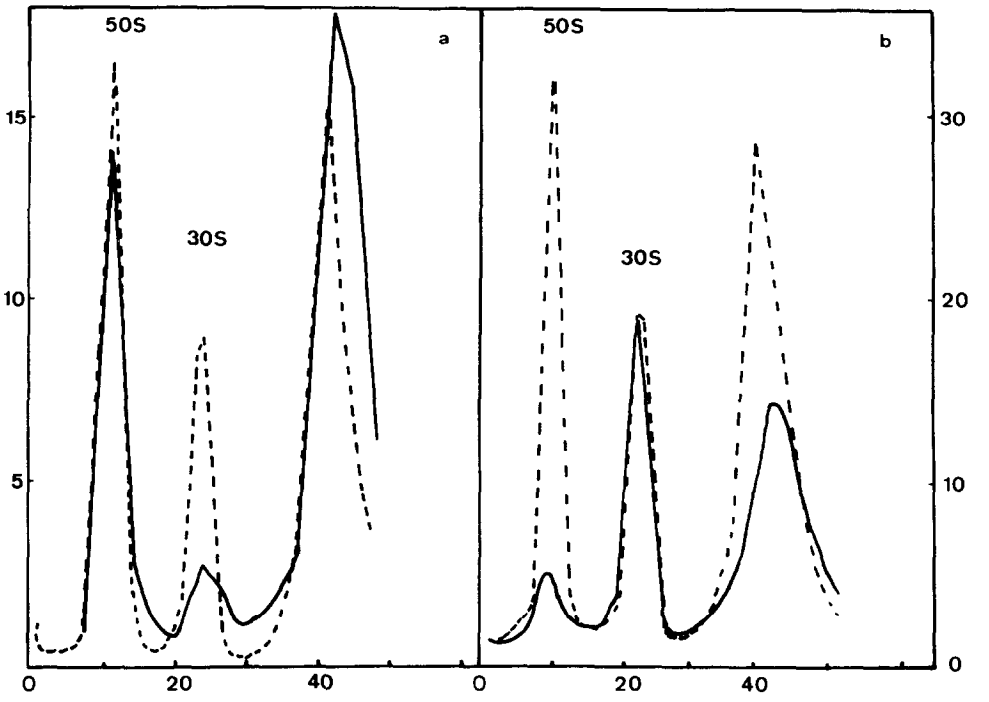

Figure 2
Sucrose gradient sedimentation analysis of extracts from cells incubated at 42°C in the presence of radioactive uracil. Abscissa indicate the number of fractions from bottom of the gradient. Ordinates are in cts/min x 10^3.
Left scale is for [^{14}C] (parent strain) and right scale for [^{3}H] (mutants).
(a) RH3187 [^{14}C] – – – – × RH3199 [^{3}H] ————
(b) RH3187 [^{14}C] – – – – × RH3198 [^{3}H] ————

transcriptional units. It is indeed well known that amber mutations in classical operons (e.g. lac, trp) may be polar, thus reducing not only the expression of the gene they affect but also the expression of distal genes. We are presently investigating such effects in the amber mutants described in this paper.

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