JOURNAL OF BACTERIOLOGY, May 1978, p. 677-679
0021-9193/78/0134-0677$02.00/0

Vol. 134, No. 2
Printed in U.S.A.

Mutation Affecting the Thermolability of the 50S Ribosomal Subunit in *Escherichia coli*

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Received for publication 25 October 1977

Genetic analysis of a mutation affecting the thermal response of the 50S ribosomal subunit to in vitro polyphenylalanine synthesis indicates that the gene, *rit*, is located near *metB* on the *Escherichia coli* chromosome and that the probable gene order is *metB-rit-arg-rpo*.

Recent work on ribosomal genetics has shown that: (i) the structural genes for ribosomal proteins cluster principally into two regions of the *Escherichia coli* genetic map, one near *str-spc* and the other near *rif* (6, 7, 16, 19); (ii) some exceptional genes specifying either ribosomal proteins or modifying enzymes occur outside these regions (3, 5, 13, 17, 21); (iii) three genes for rRNA occur at 83, 85, and 88 min. During an analysis of mutants with altered RNA turnover (8, 9), we found that ribosomes extracted from one particular strain, which displayed temperature-sensitive growth and longer mRNA half-life, exhibited an aberrant response to heat treatment before in vitro polyphenylalanine synthesis compared with wild type. In this paper, we report the genetic location of the mutation responsible for this effect.

McLaughlin et al. (11) have shown that preheating ribosomes to 55°C for a brief period greatly enhances their ability to bind tRNA's and synthesize protein in vitro. As expected, we found that ribosomes from our parental strain (PA3092) behaved in the same way; those of the mutant (JE15144), however, were much less responsive (Fig. 1A). Comparison of the heat inactivation curves of the ribosomes from both strains indicated that those from the mutant were more thermolabile than those from the parent (Fig. 1B) and that the thermolability resided in the 50S subunit (Fig. 1C) but not in the 30S subunit (Fig. 1D).

The location of the mutation affecting the thermolability of the 50S subunit in JE15144 was determined by two steps. The mutant strain JE15144 (F^- *argH* str^r) was first crossed with CSH47 (Hfr *sup* str^s) (12) and 20 arg^+ str^r recombinants selected and screened for in vitro protein-synthesizing activity. A total of 18 showed the wild-type response to heat treatment, and 2 showed the mutant response, suggesting that the mutation mapped in the vicinity of the *arg* marker. In the second stage, P1 transduction was used to focus on markers around the *arg* gene, using P1-sensitive derivatives of JE15144, HAK54, and HAK66 (the origins of which are described in Table 1). In the first transduction (Table 2), we selected 49 arg^+ transductants, of which the majority (30 arg^+) were recombinants for the ribosomal thermolability mutation, confirming the expectation that the mutation is located close to the *arg* marker. In the second transduction (Table 2), we selected 26 met^+ transductants, the majority of which (19 met^+) were recombinant for the ribosomal mutation, suggesting the mutation is located close to the *metB* marker. The analysis of the distribution of the unselected markers in these crosses indicated that the mutation maps within the *metB-argH* interval. We propose the name *rit* for the gene in which the ribosomal temperature sensitivity mutation occurs and take the recombination data to indicate that the gene order is *metB-rit-argH-rpo*.

Because *rit* appears to map on the *metB* side of *arg*, it is evidently not part of the *rnnB-rplA,H,J,K,L-rpo* cluster that occurs on the other side of *arg* and specifies the synthesis of some 50S ribosomal proteins, rRNA and RNA polymerase subunits (20). Among four genes known to be involved in the assembly of the 50S subunit, one *rimD* is located near *metB* (4). We consider it unlikely that *rit* and *rimD* are identical or that *rit* plays a similar role in assembly because sucrose gradient sedimentation profiles of the ribosomal subunits of JE15144 failed to reveal any maturation defects (data not shown). Comparisons of the 50S ribosomal proteins of JE15144 and PA3092 by two-dimensional gel electrophoresis also failed to reveal any obvious differences (data not shown), and, hence, we are unable at this stage to determine with certainty whether *rit* plays a structural or assembly role in the formation of the 50S ribosomal subunit.

Significantly, none of the arg^+ or met^+ recombinants from the P1 transductions showed the

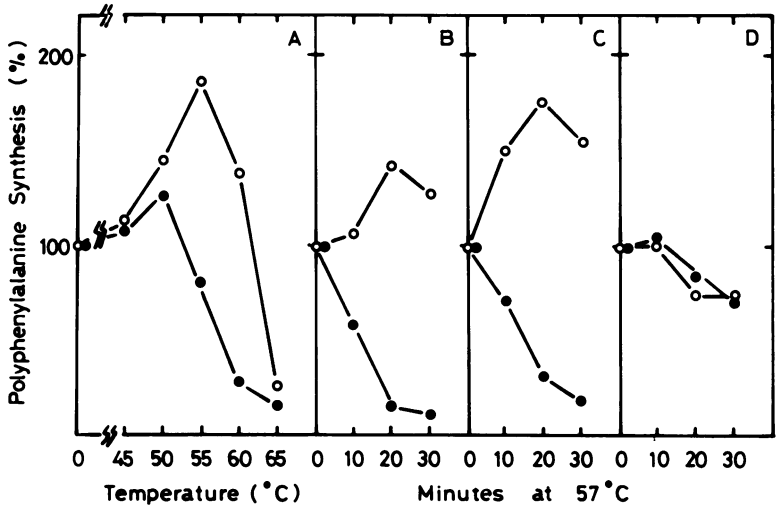

FIG. 1. *(A) Effect of preincubation temperature on 70S ribosomes with respect to in vitro polyphenylalanine synthesis. A 20-μl portion of 40 A_{260} (absorbance at 260 nm) units per ml of washed 70S ribosomes derived from PA3092 (○) and JE15144 (●), suspended in TM-2 buffer [10 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.6) 0-mM magnesium acetate], was heated for 5 min at the temperatures indicated, then chilled. Polyuridylate-directed polyphenylalanine synthesis was carried out at 35°C for 40 min in 50 μl of total reaction mixture containing 20 μl of the ribosome, 10 μl of S-100 (50 μg of protein), 40 nCi of [^{14}C]phenylalanine (112 mCi/mmol from Daiichi Pure Chemical Co., Tokyo), and other materials as described (14). The washed 70S ribosomes employed for this study were made after treatment of ribosome with 0.8 M ammonium chloride. (B) Time kinetics of heat inactivation of 70S ribosomes in the protein synthesis reaction. Portions (20 μl) of 70S ribosomes (40 A_{260} units/ml) derived from PA3092 (○) and JE15144 (●) were heated at 57°C for various times, then assayed for polyphenylalanine synthesis. The 100% levels in experiments (A) and (B) correspond to 11,977 cpm (PA3092) and 8,550 cpm (JE15144) when the background for ribosomes alone (564 for PA3092 and 641 for JE15144) is subtracted. (C) Effect of preheating 50S ribosomal subunits on protein synthesis. Portions (10 μl) of the 50S ribosomal subunit from PA3092 (○) (48 A_{260} units/ml) and JE15144 (●) (52 A_{260} units/ml) were preheated for various times at 57°C, and protein synthesis was assayed in the presence of 10 μl of the 30S ribosomal subunit from PA3092 (22 A_{260} units/ml). The 100% levels correspond to 6,408 cpm for PA3092 and 8,141 cpm for JE15144 when the activities of 50S and 30S subunit alone are subtracted. The radioactivities (counts per minute) of 50S subunit alone are 234 (PA3092) and 854 (JE15144), and those of 30S subunits alone are 427 (PA3092) and 566 (JE15144). After dialysis of washed 70S ribosome against TM-3 buffer [10 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.6)-1 mM magnesium acetate] for 5 h, 30S and 50S ribosomal subunits were separated by centrifugation in 5 to 20% sucrose gradients in TM-3 buffer at 22,000 rpm for 16 h as originally developed by Tissières et al. (18). (D) Effect of heat pretreatment of 30S ribosomal subunit upon the protein synthesis activity. Portions (10 μl) of 30S ribosomal subunits from PA3092 (○) (22 A_{260} units/ml) or JE15144 (●) (30 A_{260} units/ml) were treated for various times at 57°C, and protein synthesis was assayed in the presence of 10 μl of PA3092 50S subunit (48 A_{260} units/ml). The values (counts per minute) for 100% activity are 5,080 for PA3092 and 5,502 for JE15144 when background activity as indicated in (C) is subtracted.*

TABLE 1. *Bacterial strains used*

Strain	Genotype	Derivation
PA3092	*thr leu lacY trp his thi thy rpsL malA mtl xyl argH supE* (F^-)	Kuwano et al. (8, 9)
JE15144	As in PA3092, but *rit*[a] *ams*[b]	Kuwano et al. (8)
CSH47	*sup* (Hfr: O-*ilv*$^+$-*metB*$^+$-*leu*$^+$ ....)	Miller (12)
CSH70	*metB argE thi* (HfrC: O-*proA*$^+$-*leu*$^+$ ....)	Miller (12)
HAK54	*his thi metB rpsL malA argE* or *H*	Conjugation: CSH70 × JE15144
HAK66	As in HAK54, but *arg*$^+$	Transduction: HAK81-HAK54
W3110	*thyA* λ^- (F^-)	Bachmann (1)
HAK81	As in W3110, but *rpo*	Spontaneous rifampin resistance mutation from W3110

[a] Gene symbol for the in vitro thermolabile polyphenylalanine synthesis activity of 50S ribosomal subunit is designated *rit* (see text and Fig. 1).

[b] Gene symbol for the alteration of mRNA stability is designated *ams*.

TABLE 2. *Transduction mapping of rit in JE15144 strain*

Donor × recipient	Markers analyzed[a]				No. of colonies
	met	*rit*[b]	*arg*	*rpo*	
HAK81 (*met*$^+$ *rit*$^+$ *arg*$^+$ *rpo*) × HAK54 (*met rit arg rpo*$^+$)	0	1	1	0	16
	0	0	1	0	5
	1	1	1	0	10
	1	0	1	0	2
	0	1	1	1	3
	0	0	1	1	10
	1	1	1	1	1
	1	0	1	1	2
Total					49
	1	1	0	0	13
	1	0	0	0	5
	1	1	1	0	5
	1	0	1	0	1
	1	1	1	1	1
	1	0	1	1	1
Total					26

[a] The symbols 1 and 0 show donor and recipient phenotype, respectively.

[b] The *rit*$^+$ and *rit* transductants show the wild (PA3092) type and the mutant (JE15144) type of heat inactivation kinetics in the in vitro polyphenylalanine synthesis after pretreatment of washed ribosomes at 57°C for 5, 10, 15 and 30 min (see Fig. 1b). The ribosome fraction was prepared from each recombinant strain cultured at middle-log phase in 200 ml of enriched L broth medium (10).

temperature-sensitive growth and altered mRNA stability displayed by JE15144. It seems likely, therefore, that the *rit* mutation is not responsible for these characteristics and that they derive from secondary mutations fortuitously present in this mutant.

We thank greatly G. Wilson (University of California at Berkeley) for critically reading the manuscript and also T. Horiuchi (Kyushu University) and Y. Takeda (University of California at Berkeley) for fruitful comments and discussion. We give our sincere thanks to H. Endo for his encouragement.

This work was supported by a grant-in-aid from the Ministry of Education, Science and Culture, Japan.

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