Proc. Natl. Acad. Sci. USA
Vol. 75, No. 9, pp. 4470–4473, September 1978
Genetics

Mutants of *Escherichia coli* altered in both genes coding for the elongation factor Tu

[protein biosynthesis/mocimycin (= kirromycin)-resistant *tufA* gene product/nonfunctional *tufB* gene product/amber and temperature-sensitive *tufB* mutants/genetic characterization]

JOS A. M. VAN DE KLUNDERT*, PETER H. VAN DER MEIDE*, PIETER VAN DE PUTTE†, AND LEENDERT BOSCH*

Departments of *Biochemistry and †Molecular Genetics, State University, P.O. Box 9505, 2300 RA Leiden, The Netherlands

Communicated by Severo Ochoa, July, 7, 1978

ABSTRACT **Genetic analysis of a mutant of *Escherichia coli* resistant to the antibiotic mocimycin is presented. This resistance is due to alterations in both *tuf* genes coding for the elongation factor Tu. Mocimycin resistance is recessive. Bacteria carrying only one *tuf* gene from the resistant mutant are still mocimycin sensitive. If the mutant gene is the *tufA* gene, the sensitive cells can be made resistant through inactivation of the *tufB* gene by insertion of the bacteriophage Mu genome. Conditional mocimycin-resistant mutants can also be isolated when the *tufB* gene is altered by an amber or a temperature-sensitive mutation. When only the *tufB* allele from the original mocimycin-resistant mutant is present, inactivation of the wild-type *tufA* gene fails to give viable mocimycin-resistant progeny. We conclude that the *tufA* mutant allele codes for a functional mocimycin-resistant EF-Tu, whereas the mutant *tufB* gene does not code for a functional product.**

In *Escherichia coli* the protein synthesis elongation factor (EF) Tu plays a role in several cellular processes. Besides its well-documented role in protein synthesis (for a review see ref. 1), EF Tu is also involved in bacteriophage Qβ RNA replication (2) and the EF Tu·EF Ts complex has been reported to stimulate the synthesis of ribosomal RNA *in vitro* (3). EF Tu is present in the cell in large amounts (4), both in the cytoplasm and associated with the cell membrane (5). To improve our understanding of the function of this protein it would be useful to have available well-characterized mutants.

The isolation of a temperature-sensitive mutant of *E. coli* with an altered EF Tu was previously reported from this laboratory (6). However, multiple mutations caused by the use of nitrosoguanidine as mutagen greatly hindered the genetic analysis of this mutant.

In 1975 Young and Neidhardt‡ isolated a temperature-sensitive mutant of *E. coli* partially resistant to the EF Tu inhibitor L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK). This mutation apparently affects EF Tu. More recently, Pedersen *et al.* (7) showed that the mutant strain HAK 88 carrying a temperature-sensitive EF Ts (8) also carries an altered EF Tu. The isoelectric point of this mutant protein is shifted approximately 0.1 pH unit in the acidic direction, apparently without any functional consequences. The desirability of having well-characterized EF Tu mutants increased through the discovery by Jaskunas *et al.* (9) of two genes on the *E. coli* chromosome coding for EF Tu, *tufA* at 72 min and *tufB* at 88 min of the recalibrated *E. coli* linkage map (10).

The specific selection of EF Tu mutants was made possible by the isolation of a new class of antibiotics that affect the function of EF Tu in protein synthesis, namely mocimycin (11), kirromycin (12), and antibiotic X5108 (13). These antibiotics are structurally related, but a pyridone-*N*-methyl group present in antibiotic X5108 is absent from the other two compounds (14). Their mode of action has been elucidated by Parmeggiani and coworkers (15–17), who showed that these antibiotics prevent the release of EF Tu from the ribosome, thus blocking further protein synthesis.

By selecting for mocimycin resistance, we isolated an EF Tu mutant (LBE 2012; ref. 18) harboring mutations in at least two genes, which were located at 72 and 88 min on the *E. coli* chromosome (18).

In this paper we show that cells carrying only one mutant *tuf* gene are mocimycin sensitive. If the mutant allele is *tufA*, the cells can be made mocimycin resistant by inactivating the *tufB* genes. However, if the mutant allele is *tufB*, inactivation of the *tufA* gene is lethal.

MATERIALS AND METHODS

Bacteria and Phages. Bacterial strains used in this investigation are listed in Table 1 (for nomenclature see ref. 10). Bacteriophages P1*kc*, Mu, and Mu*Bam* were obtained from the stock of the Laboratory for Molecular Genetics, Leiden. Mocimycin Na salt was provided by R. Beukers (Gist Brocades N.V., Research and Development, Delft, The Netherlands); ethyl methanesulfonate ($MeSO_2OEt$) was purchased from Eastman; fusidic acid Na salt from Leo Pharmaceutical Products, Denmark; rifampicin from Boehringer Mannheim; streptomycin from Rhône-Poulenc; culture media from Difco Laboratories; chemicals from Merck.

Media. LC medium contained, per liter of deionized water: tryptone, 10 g; yeast extract, 5 g; NaCl, 8 g. It was supplemented with Tris, 0.12 g; glucose, 2 g; $CaCl_2 \cdot 2H_2O$, 0.37 g; $MgSO_4 \cdot 7H_2O$, 1 g; and thymine, 0.02 g. VB medium contained, per liter of deionized water: $MgSO_4 \cdot 7H_2O$, 0.2 g; citric acid, 2 g; K_2HPO_4, 10 g; and $Na(NH_4)HPO_4 \cdot 4H_2O$, 3.5 g. It was supplemented as required with glucose, amino acids, vitamins, etc. Agar plates contained 1.5% or 1.8% agar in LC medium or VB medium, respectively. Soft agar overlays contained 0.6% agar.

Isolation of Antibiotic-Resistant Mutants. Mutants were isolated on VB medium agar plates containing fusidic acid Na salt, 1.000 μg/ml; rifampicin, 75 μg/ml; or streptomycin, 100 μg/ml. All plates contained EDTA (2 mM) to prevent the occurrence of antibiotic resistance as a result of a diminished antibiotic uptake.

P1 transduction, $MeSO_2OEt$ mutagenesis, isolation of Mu lysogens, and isolation of nonsense suppressor mutants were done as described by Miller (19). The isolation of strain LBE 2012 has been described previously (18).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: EF, elongation factor; $MeSO_2OEt$, ethyl methanesulfonate.

‡ Young, F. S. & Neidhardt, F. C. (1975) *Abstracts of the Annual Meeting of the American Society for Microbiology, 75th Annual Meeting, New York*, 161.

Table 1. *E. coli* K-12 strains used or obtained during this study

Strain	Parent strain	Mutagen or transducing agent	Selected phenotype*	Sex	Genotype		Phenotype*	Source†
LBE 1001	‡			F^-			Moc^s	a
LBE 2012	LBE 1001	$MeSO_2OEt$	Moc^r	F^-	*xyl*	*tufA tufB*	Moc^r	b
LBE 2013	LBE 2012	Spontaneous	Str^r	F^-	*xyl, rpsL*	*tufA tufB*	Moc^r	c
LBE 2014	LBE 2012	Spontaneous	Rif^r	F^-	*xyl, rpoB*	*tufA tufB*	Moc^r	c
LBE 2015	LBE 2012	Spontaneous	Fus^r	F^-	*xyl, fus*	*tufA tufB*	Moc^r	c
KMBL 1164	‡			F^-	*supE, thi,* Δpro–$lac_{X\text{-}111}$		Moc^s	
KA 40‡				F^+	*cys-am, gal-am, his, mal, lam*		Moc^s	d
LBE 2026	KMBL 1164	P1(2015)	Fus^r	F^-	*supE, thi,* Δpro–$lac_{X\text{-}111}$, *fus*	*tufA*	Moc^s	c
LBE 2027	KA 40	P1(2015)	Fus^r	F^+	*cys-am, gal-am, his, mal, lam, fus*	*tufA*	Moc^s	c
LBE 2030	KA 40	P1(2014)	Rif^r	F^+	*cys-am, gal-am, his, mal, lam, rpoB*	*tufB*	Moc^s	c
LBE 2032	KMBL 1164	P1(2014)	Rif^r	F^-	*supE, thi,* Δpro–$lac_{X\text{-}111}$, *rpoB*	*tufB*	Moc^s	c
LBE 2034	LBE 2027	$MeSO_2OEt$	Moc^r	F^+	*cys-am, gal-am, his, mal, lam, fus*	*tufA tufBam53*	Moc^r	c
LBE 2035	LBE 2027	$MeSO_2OEt$	Moc^r	F^+	*cys-am, gal-am, his, mal, lam, fus*	*tufA tufBam60*	Moc^r	c
LBE 2036	LBE 2034	Spontaneous	Su^+	F^+	*cys-am, gal-am, his, mal, lam, fus, sup*	*tufA tufBam53*	Moc^s	c
LBE 2039	LBE 2035	Spontaneous	Su^+	F^+	*cys-am, gal-am, his, mal, lam, fus, sup*	*tufA tufBam60*	Moc^s	c
LBE 2040	LBE 1001	Spontaneous	Fus^r	F^-	*fus*		Moc^s	c
LBE 2041	LBE 1001	Spontaneous	Rif^r	F^-	*rpoB*		Moc^s	c
LBE 2042	LBE 2026	Mu	Moc^r	F^-	*supE, thi,* Δpro–$lac_{X\text{-}111}$, *fus*	*tufA tufB*::(*Mu*)	Moc^r	c
LBE 2043	LBE 2927	Mu	Moc^r	F^+	*cys-am, gal-am, his, mal, lam, fus*	*tufA tufB*::(*Mu*)	Moc^r	c
LBE 2044	LBE 2042	Spontaneous	Rif^r	F^+	*cys-am, gal-am, his, mal, lam, fus, rpoB*	*tufA tufB*::(*Mu*)	Moc^r	c
LBE 2045	LBE 2043	Spontaneous	Rif^r	F^+	*cys-am, gal-am, his, mal, lam, fus, rpoB*	*tufA tufB*::(*Mu*)	Moc^r	c
LBE 2046	LBE 2036	Spontaneous	Rif^r	F^+	*cys-am, gal-am, his, mal, lam, fus, sup, rpoB*	*tufA tufBam53*	Moc^s	c
LBE 2047	LBE 2039	Spontaneous	Rif^r	F^+	*cys-am, gal-am, his, mal, lam, fus, sup, rpoB*	*tufA tufBam60*	Moc^s	c

The genetic nomenclature is that used by Bachmann *et al.* (10).
* Phenotype symbols: ^{r}, resistant; ^{s}, sensitive; Moc, mocimycin; Str, streptomycin; Fus, fusidic acid; Rif, rifampicin; Su^+, amber suppressor.
† a, LBE 1001 = W1485, nonsuppressor; b, see ref. 19; c, this paper; d, Devoret 63, no. 112.
‡ These strains were obtained from the stock of the Laboratory of Molecular Genetics, State University, Leiden.

Selection of Antibiotic Resistant Transductants. After P1 transduction the cells were harvested by centrifugation, washed once with physiological saline, and resuspended in saline at a concentration of about $2 \cdot 10^9$ cells per ml. A 0.1-ml sample of this suspension was spread over the surface of a VB medium agar plate without the antibiotic and incubated for 2.5–3 hr at 37°C to allow expression of the resistance. After this preincubation the agar layer was carefully placed on top of a second agar plate containing the appropriate concentration of the antibiotic. Incubation was continued for 16–40 hr at 37°C.

RESULTS

Mocimycin resistance is caused by mutations in both the *tufA* and the *tufB* gene. Previously (18) we described the isolation of a mocimycin-resistant mutant of *E. coli* with an altered EF Tu due to at least two mutations on the *E. coli* chromosome, located at 72 and 88 min. Moreover, the mocimycin-resistant phenotype of the mutant strain (LBE 2012) was abolished by introducing either the wild-type *tufA* or the wild-type *tufB* allele. In Table 2 the cotransduction frequencies of the mutations causing the mocimycin-resistant phenotype with *rpsL*, *metA*, *fus*, and *rpoB* are summarized.

The results of the transduction experiments suggest that both *tuf* genes are altered in the mocimycin-resistant mutant. Furthermore, the results show that mocimycin resistance is recessive and that the mocimycin resistance phenotype is expressed only if both altered *tuf* genes are present.

In order to exclude the possibility that more mutations than the two already found are required for mocimycin resistance, we showed that resistance can be transferred from the mutant to wild-type strains in two successive P1 transductions. Two strains were derived from the mocimycin-resistant LBE 2012, one carrying a spontaneous fusidic acid resistance (LBE 2015: *tufA, tufB, fus*), the other a spontaneous rifampicin resistance (LBE 2014: *tufA, tufB, rpoB*). Bacteria of the strain LBE 1001 were infected with a P1 lysate prepared on strain LBE 2015 and fusidic acid-resistant transductants were isolated. Some of these transductants would have acquired the *tufA* mutation because *tufA* and *fus* are cotransducible. Ten of these transductants, still mocimycin-sensitive and irrespective of whether they had acquired the *tufA* mutation, were subjected to a second P1 transduction using the same P1 (LBE 2015) lysate. Selection for transfer of the *tufB* gene could now be done directly by picking mocimycin-resistant colonies. Nine of the 10 strains yielded mocimycin-resistant transductants, thus indicating that the frequency of cotransduction of *tufA* and *fus* in the first

Table 2. Frequency of cotransduction of the *tuf* genes with known markers on the *E. coli* chromosome

tuf gene	Selected gene	Frequency of cotransduction	Colonies tested
tufA	*rpsL*	0.89	230
	fus	0.96	200
tufB	*metA*	0.36	200
	rpoB	0.82	217

The markers indicated in the second column were transferred into the mocimycin-resistant strain LBE 2012 by P1 transduction. The transductants were subsequently tested for mocimycin resistance. Loss of mocimycin resistance was considered as a proof for the cotransduction of the *tuf* gene.

transduction was about 0.9 and that the mocimycin resistance phenotype could be transferred in two successive transductions. In the control experiment without P1, however, the number of spontaneous mocimycin-resistant colonies was equal to the number of mocimycin-resistant transductants. This means that the frequency of spontaneous mutations in the *tufB* gene is of the same order of magnitude as the frequency of transduction. To avoid this problem we did not select directly for mocimycin-resistant colonies in the second transduction experiment but determined the cotransduction of mocimycin resistance (*tufB*) with rifampicin resistance. It appeared that 116 out of the 142 rifampicin-resistant transductants were mocimycin resistant, whereas in the control without P1 all the spontaneous rifampicin-resistant mutants were still mocimycin sensitive.

The experiment was also carried out in the reversed order. In the first transduction, strain LBE 1001 was infected with a P1 (LBE 2014: *tufA*, *tufB*, *rpoB*) lysate and 15 rifampicin-resistant transductants were isolated, some of which must be *tufB*. These transductant strains were infected once more with a P1 (LBE 2014) lysate and a selection was made for mocimycin-resistant transductants (*tufA* transfer). In 13 out of the 15 rifampicin-resistant transductant strains tested, the mocimycin resistance phenotype could be obtained after the second transduction. In this case the frequency of appearance of spontaneous mocimycin-resistant mutants was very low (*ca* 10^{-8}). The explanation for this is that the nature of the mutation in *tufA* is different from that in *tufB* in the mutant strain LBE 2015. This will be discussed.

Nature of the Mutations in the *tufA* and *tufB* Genes in Strain LBE 2012. Having established the double mutant character of strain LBE 2012, we explored the nature of the mutations in the *tufA* and the *tufB* gene of the mocimycin-resistant mutant. In principle, three different genotypes may give rise to a resistant phenotype as shown in Table 3. In the first case both genes code for a functional mocimycin-resistant EF-Tu. In the second and third cases, only one gene actually codes for a functional mocimycin-resistant EF Tu, whereas the second gene does not code for an active product. The nature of both mutations was examined by transferring them separately into different strains so that one wild-type and one mutant *tuf* gene would be present. As predicted, the resultant strains were mocimycin sensitive. Subsequently, the wild-type gene was inactivated in different ways and the consequence of this inactivation was studied.

Insertion of Bacteriophage Mu. We attempted to inactivate the remaining wild-type gene by the insertion of the bacteriophage Mu. This temperate phage can integrate randomly in the host chromosome and abolish the normal phenotypic expression of the host genes located at, or adjacent to, the chromosomal sites of integration (20). Mocimycin-resistant cells lysogenic for phage Mu were isolated from the mocimycin-sensitive strains LBE 2026 and LBE 2027. Both of these parental strains carried a mutant, mocimycin-resistant *tufA* and a wild-type, mocimycin-sensitive *tufB* gene. Evidence that mocimycin resistance resulted from the integration of the Mu prophage into, or close to, the wild-type *tufB* gene was provided by the observation that transduction of the *tufB* gene to the parental strain was always accompanied by transfer of the Mu prophage. Apparently the *tufA* gene in the original mocimycin-resistant mutant codes for a mocimycin-resistant EF Tu, and expression of the *tufB* gene is not required for growth. When the Mu prophage was inserted into the mocimycin-sensitive strains LBE 2030 and LBE 2032, carrying a *tufB* gene from the mocimycin-resistant mutant and a wild-type (mocimycin-sensitive) *tufA* gene, no mocimycin-resistant Mu lysogens were detected. In this case, insertion of the Mu prophage seems to eliminate an essential gene. Because no vital genes are located distal to the *tufA* gene (21), it appears that inactivation of this gene is lethal in cells carrying the mutant *tufB* gene.

Table 3. Possible genotypes causing a mocimycin resistance phenotype

Case	Genotype *tufA*	Genotype *tufB*
I	R	R
II	R	–
III	–	R

R, codes for a functional mocimycin-resistant EF Tu; –, does not code for a functional EF Tu.

Nonsense Mutations in the *tufB* Gene. The fact that mocimycin-sensitive cells carrying the *tufA* gene from the mocimycin resistant mutant become mocimycin resistant upon inactivation of the wild-type *tufB* gene was used to construct conditionally mocimycin-resistant mutants in which the *tufB* gene is altered by an amber mutation. The selection of these mutants was based on the elimination of mocimycin resistance by introduction of a nonsense suppressor.

Strain LBE 2027, used for this experiment, carries two suppressible amber mutations (*cys-am* and *gal-am*), besides a mutant *tufA* and a wild-type *tufB* gene. When plated on a medium selective for the simultaneous reversion of both amber mutations, only those cells that have acquired a nonsense suppressor gene will be able to grow. The strain was mutagenized with $MeSO_2OEt$, and 64 mocimycin-resistant mutants were isolated. Descendants of each strain carrying a nonsense suppressor were isolated. The presence of the suppressor mutation was verified by the ability of these strains to propagate a Mu*Bam* phage. In two of the 64 mocimycin-resistant mutants isolated, the introduction of the nonsense suppressor was accompanied by the loss of mocimycin resistance, indicating that the *tuf* mutation present was suppressible. Because in both cases the frequency of cotransduction of the nonsense mutation with *rpoB* was consistent with the data reported in Table 2, it is likely that the mutation is in the *tufB* gene. Isolation of the *tufBam* gene product will allow testing this possibility. No suppressible mocimycin-resistant mutants could be isolated from strain LBE 2030, which carries a wild-type (mocimycin-sensitive) *tufA* and a mutant *tufB* gene. This illustrates once more the incompatibility of the mutant *tufB* gene with a nonfunctional *tufA* gene product.

Temperature-Sensitive Mutants. We have also isolated a mutant in which mocimycin resistance is temperature dependent. The mocimycin-sensitive strain LBE 2026, which carries the *tufA* gene from the mocimycin-resistant mutant and a wild-type *tufB* gene, was used to isolate mocimycin-resistant mutants at 39°C. Four hundred mutants were isolated and tested for mocimycin resistance at 32°C. One of them appeared to be mocimycin sensitive at this temperature. Presumably, this mutant carries a *tufB* gene coding for a protein that functions at 32°C but not at 39°C. We have not yet succeeded in isolating a conditional mocimycin-resistant mutant from a strain carrying the *tufB* gene from the original mocimycin-resistant mutant and a wild-type *tufA* gene.

Phenotypic Lag after P1 Transduction of the Two Mutant *tuf* Genes. Another indication of the different character of the mutations in the *tufA* and *tufB* genes is the time lapse required for phenotypic expression after transduction of a *tuf* gene from the mocimycin-resistant mutant to a cell carrying the other mutant *tuf* gene. When the mutant *tufA* gene is transduced into a cell carrying the mutant *tufB* gene, the phenotypic lag is 2–3 hr. However, when the mutant *tufB* gene is transduced into a cell carrying the mutant *tufA* gene, there is either no phenotypic lag or a very short one.

DISCUSSION

Recently Jaskunas *et al.* (9) reported that each of two transducing λ phages (λ d*rif*^{d}18 and λ d*fus*-3) derived from two different chromosomal regions (72 and 88 min) carries a host gene coding for a protein identified physicochemically and immunologically as EF Tu. One may ask whether each gene normally codes for a functional EF Tu, and how their expression is regulated.

In this and the earlier study (18), we have shown that mocimycin resistance, which is due to an alteration in the target protein EF Tu, can only be achieved by a double mutation, affecting genes mapping at 72 and 88 min on the *E. coli* chromosome. This implies that the parental strain (LBE 1001) has two genes that code for an active mocimycin-sensitive EF Tu. The fact that mocimycin resistance can be transferred by two successive transductions with phage P1 would seem to rule out the existence of a third gene on the *E. coli* chromosome coding for a functional EF Tu. The results obtained by phage Mu insertion and the isolation of nonsense and missense mutations in the *tufB* gene clearly demonstrate that the mutant *tufA* gene codes for a functional mocimycin-resistant EF Tu, whereas the mutant *tufB* gene apparently fails to produce a functional product. These conclusions are further substantiated by the frequency with which mocimycin-resistant mutants are obtained when one of the mutant genes is already present in the cell (0.15×10^{-5} and 10^{-8} for the mutant *tufA* and *tufB* genes, respectively). This may be explained by the fact that, in general, inactivation of a protein does not demand as much from the mutational event as the transition of a protein from antibiotic sensitivity to resistance. In well-documented cases, such as streptomycin resistance (22), antibiotic resistance is achieved only by very specific single amino acid substitutions. Additional support for the proposed character of the mocimycin-resistant mutant is offered by the difference in the phenotypic lag caused by the two mutant genes. It is well known that, after mutation or phage P1 transduction, recessive antibiotic resistance is not expressed until the parental chromosomes have segregated and the cellular pool of sensitive protein is depleted and replenished by resistant protein (23). Particularly in the case of EF Tu, in which the target protein is present in large amounts, the phenotypic lag can be expected to last for several generations. The observed difference in phenotypic lag between the two mutant *tuf* genes can be explained by assuming that, when the *tufA* gene is initially present, the resistant protein is already present in significant amounts before the mutant *tufB* gene is introduced, whereas in the other case the appearance of resistance depends wholly on *de novo* protein synthesis.

A fact emerging from this study is that the *tufB* gene product is dispensable for bacterial growth, because there is only a slight effect on the growth rate when this protein is missing. This again raises questions concerning the regulation and the functioning of the two *tuf* genes. The mutant strains isolated in the course of this study are likely to prove very useful in providing some answers to these questions.

The possibility of isolating Mu insertions in the *tufB* gene already answers one question. Apparently no vital genes are inactivated by this insertion. Thus, if the *tufB* gene is in the same operon as genes coding for ribosomal proteins (24), it must be located at the promoter-distal end of the operon. The amber mutants of the *tufB* gene will make the isolation of the *tufA* gene product possible. At present all EF Tu preparations must be considered to consist of a mixture of the *tufA* and the *tufB* gene products. Moreover, the amber products are in themselves of great interest in EF Tu research. Whereas the only EF Tu fragments now available for structural and functional studies lack the NH_2-terminal region of the protein (25, 26), the amber mutation products lack the COOH-terminal portion.

The temperature-sensitive mutant may offer the possibility of constructing bacterial strains that could be used to examine the pleiotropic effects of the elimination of EF Tu on the various cellular processes in which EF Tu is involved.

The kirromycin-resistant mutant isolated by Fischer *et al.* (27, 28) shows phenotypic similarity to our mutant, but genetic analyses suggest that here the *tufB* gene codes for a resistant EF Tu while the *tufA* gene is inactivated.

We thank Dr. R. Beukers, Gist Brocades N. V., Delft, The Netherlands, for the generous gift of the mocimycin; Marianne Groothuis for her technical advice; and Dr. B. W. Glickman for his help in the preparation of the manuscript. This research was supported by the Netherlands Foundation for Chemical Research (SON), which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO).

1. Miller, D. L. & Weissbach, H. (1977) in *Molecular Mechanisms of Protein Biosynthesis*, eds. Weissbach, H. & Pestka, S. (Academic, New York), pp. 334–349.
2. Blumenthal, T., Landers, T. A. & Weber, K. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1313–1317.
3. Travers, A. (1973) *Nature (London)* **244,** 15–18.
4. Furano, A. V. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4780–4784.
5. Jacobson, G. R. & Rosenbusch, J. P. (1976) *Nature (London)* **261,** 23–26.
6. Lupker, J. H., Verschoor, G. J., De Rooij, F. W. M. & Bosch, L. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 460–463.
7. Pedersen, S., Blumenthal, R. M., Reeh, S., Parker, J., Lemaux, P., Laursen, R. A., Nagarkatti, S. & Friesen, J. D. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1698–1701.
8. Kuwano, M., Endo, H. & Yamamoto, M. (1972) *J. Bacteriol.* **112,** 1150–1156.
9. Jaskunas, S. R., Lindahl, L , Nomura, M. & Burgess, R. R. (1975) *Nature (London)* **257,** 458–462.
10. Bachmann, B. J., Low, K. B. & Taylor, A. L. (1976) *Bacteriol. Rev.* **40,** 116–167.
11. Vos, C. (1972) *Chem. Abstr.* **77,** 32742.
12. Wolf, H. & Zähner, H. (1972) *Arch. Mikrobiol.* **83,** 147–154.
13. Maehr, H., Blount, J. F., Evans, R. H., Leach, M., Westley, J. W., Williams, T. H., Stempel, A. & Büchi, G. (1972) *Helv. Chim. Acta* **55,** 3051–3054.
14. Vos, C. & Verwiel, P. E. J. (1973) *Tetrahedron Lett.* **52,** 5173–5176.
15. Wolf, H., Chinali, G. & Parmeggiani, A. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4910–4914.
16. Chinali, G., Wolf, H. & Parmeggiani, A. (1977) *Eur. J. Biochem.* **75,** 55–65.
17. Wolf, H., Chinali, G. & Parmeggiani, A. (1977) *Eur. J. Biochem.* **75,** 67–75.
18. Van de Klundert, J. A. M., Den Turk, E., Borman, A. H., Van der Meide, P. H. & Bosch, L. (1977) *FEBS Lett.* **81,** 303–307.
19. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
20. Taylor, A. L. (1963) *Proc. Natl. Acad. Sci. USA* **50,** 1043–1051.
21. Nomura, M. (1976) *Cell* **9,** 633–644.
22. Funatsu, G. & Wittmann, H. G. (1972) *J. Mol. Biol.* **68,** 547–550.
23. Carlberg, D. M. (1977) *Essentials of Bacterial and Viral Genetics* (Thomas, Springfield, IL), p. 120.
24. Bendiak, D. S., Parker, J. & Friesen, J. D. (1977) *J. Bacteriol.* **129,** 536–539.
25. Arai, K.-I., Nakamura, S., Arai, T., Kawakita, M. & Kaziro, Y. (1976) *J. Biochem.* **79,** 69–83.
26. Jacobson, G. R. & Rosenbusch, J. P. (1976) *Biochemistry* **15,** 5105–5110.
27. Fischer, E. (1976) Dissertation (Universität Tübingen, Tübingen, West Germany).
28. Fischer, E., Wolf, H., Hantke, K. & Parmeggiani, A. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4341–4345.