a very low affinity for its cobamide coenzyme and if, at maximum concentration of coenzyme (as after administration of 1 mg of vitamin B_{12} daily), appreciable binding of coenzyme and of substrate occur with partial restoration of catalytic function. The mechanism of such a proposed apoenzyme-coenzyme interaction is obscure but might involve interaction of enzyme subunits or an allosteric effect on a single polypeptide. The observed vitamin B_{12} dependency could also reflect abnormalities in vitamin B_{12} transport or conversion of the vitamin to its active coenzyme form, but this possibility seems much less likely.

Finally, vitamin B_{12} administration may be useful therapeutically in patients with methylmalonic aciduria, which may not be a rare inborn error, the disorder having been described in three countries in less than 1 year (*3, 4, 12*). This disorder could be detected by urinary screening techniques in newborns, and treatment with large doses of vitamin B_{12} could prevent the characteristic and potentially lethal episodes of keto-acidosis.

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5 August 1968

Frame Shift Mutations near the Beginning of the Lysozyme Gene of Bacteriophage T4

Abstract. *A pair of frame shift mutations in the lysozyme gene of bacteriophage T4 results in the substitution of a glutamyl-tyrosyl sequence for the asparagine residue that is the penultimate amino-terminal amino acid in the lysozyme of the wild-type strain. One of the mutations has been identified as the insertion of two bases, the other as the insertion of a single base.*

Earlier (*1–3*) we compared amino acid sequences of lysozyme produced by strains of phage T4 carrying pairs of frame shift mutations to that of the wild-type strain. In all cases we found a sequence of amino acids changed; our results thus confirm Crick's (*4*) theories regarding the general nature of the genetic code. In addition, we have been able to identify the mutations and to assign codons, proposed on the basis of studies in vitro (*5, 6*), to the amino acids within the regions of change.

We now describe the analysis of a strain carrying two frame shift mutations near the amino-terminal end of the lysozyme molecule. The analysis

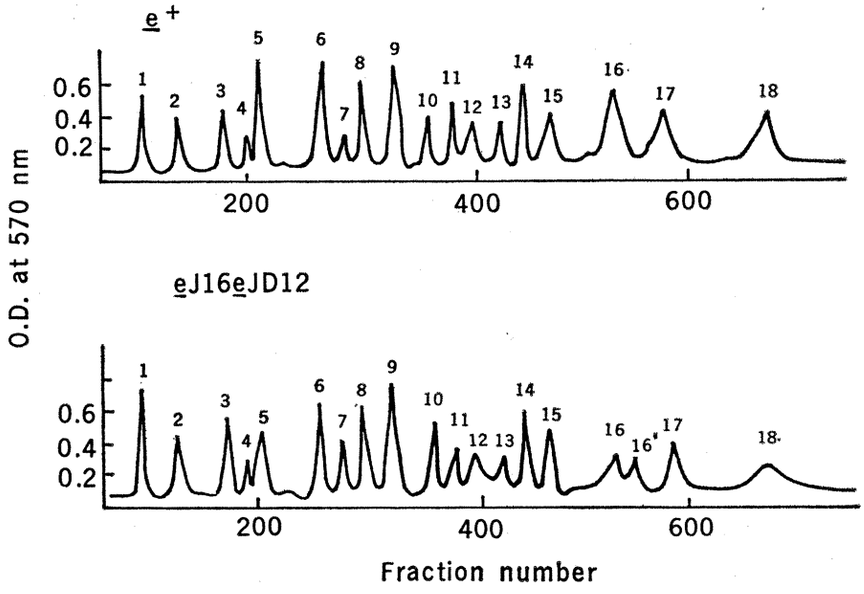


Fig. 1. Elution patterns of tryptic digests of e^+ and *e*J16*e*JD12 lysozymes. Tryptic digests of lysozyme oxidized by performic acid were applied to a Dowex-50 column (0.9 by 150 cm) and eluted by a gradient of pyridine acetate buffer of increasing *p*H and pyridine concentration by means of an eight-chamber Varigrad apparatus (*1*). Fractions of 4.0 ml were collected, and portions from alternate tubes were hydrolyzed with alkali and treated with ninhydrin; the absorbance at 570 nm was measured.

Table 1. Sequence of amino acids of peptide 16′ from *e*J16*e*JD12 lysozyme.

Method	Amino acid composition of recovered material							Amino acid sequence deduced
	Met	Glu	Tyr	Ile	Phe	Leu	Arg	
Acid hydrolysis	2.0	2.0	0.6	0.9	1.0	1.0	1.0	
Edman degradation								
Step 1	1.0	1.9	0.6	0.9	0.9	1.0	*	Met(Glu,Tyr,Ile,Phe,Glu,Met,Leu,Arg)
Step 2	0.8	1.1	0.5	0.8	0.9	1.0	*	Met-Glu(Tyr,Ile,Phe,Glu,Met,Leu,Arg)
Step 3	0.9	1.2	0	0.9	0.9	1.0	*	Met-Glu-Tyr(Ile,Phe,Glu,Met,Leu,Arg)
Step 4	0.9	1.2	0	0.1	0.8	1.0	*	Met-Glu-Tyr-Ile(Phe,Glu,Met,Leu,Arg)
Leucineaminopeptidase†	0.9	0.6	0.4	0.4	0.3			Met-Glu-Tyr-Ile-Phe(Glu,Met,Leu,Arg)
Carboxypeptidase B†							1.0	Met-Glu-Tyr-Ile-Phe(Glu,Met,Leu)Arg
Carboxypeptidase A + B†	0.1					1.1	1.0	Met-Glu-Tyr-Ile Phe-Glu-Met-Leu-Arg

* Not determined. † Molar recovery of liberated amino acid.

of this strain is relevant to the question of the possible need for a unique sequence of bases (or amino acids) near the amino-terminal end for the initiation of transcription or translation.

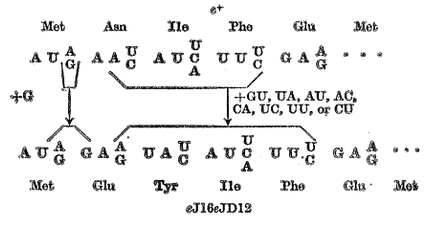
Mapping experiments demonstrate that the proflavine-induced mutation *e*J16 is located near the end of the lysozyme gene that specifies the amino-terminal end of the lysozyme molecule (*7*). Strains carrying the mutation *e*J16 produce no lysozyme activity; a pseudo-wild strain that does produce lysozyme activity was isolated from *e*J16 by a procedure already described (*3*) for isolating similar pseudo-wild strains. Among the progeny of crosses of the pseudo-wild strain to wild type, mutant phage which proved identical to *e*J16 was found. Therefore, we conclude that the pseudo-wild revertant strain must carry two mutations, *e*J16 and a new mutation which we designate as *e*JD12. This new mutation has not yet been recovered from crosses of the pseudo-wild strain to wild type. We have found (*8*), as have others (*9*), that the frequencies of the two recombinant types in crosses of closely linked frame shift mutations may be very unequal; the failure to recover the new mutation may be due to this feature.

Lysozyme produced in bacteria infected with the pseudo-wild double mutant strain *e*J16*e*JD12 was purified as described previously (*10*), and tryptic digests of this lysozyme, as well as wild-type lysozyme, were chromatographed on Dowex-50 (*1*). The only differences observed in the two chromatographic patterns (Fig. 1) are the presence of peak 16′ and the smaller size of peak 16 in the tryptic digest of the double mutant lysozyme.

Peak 16 of the e^+ digest contained three peptides which were separated by paper chromatography (with the system *n*-butanol : acetic acid : water : pyridine in the ratio 30:6:20:24). The amino acid sequences of these peptides are (*11*): ^{1}Met-Asn-Ile-Phe-Glu-Met-Leu-^{8}Arg, ^{77}Gly-Ile-Leu-^{80}Arg, ^{84}Leu-Lys-Pro-Val-Tyr-Asp-Ser-Leu-Asp-Ala-Val-^{95}Arg, (*12*) where the numbers indicate the amino acid residue, counting from the amino-terminal end of the lysozyme molecule (*11*). Peak 16 of the *e*J16*e*JD12 digest contained two peptides identical to ones from the e^+ strain (residues 77 to 80 and 84 to 95) and peak 16′ contained a new peptide derived from the amino-terminal end of the mutant lysozyme. The sequence of this new peptide, Met-Glu-Tyr-Ile-Phe-Glu-Met-Leu-Arg, was established by Edman degradation, and by leucineaminopeptidase and carboxypeptidase digestion (Table 1). The amino acid compositions of the other Dowex-50 peaks were the same for e^+ and mutant lysozymes.

These results establish that Asn, the second amino acid from the amino-terminal end of the lysozyme in the e^+ strain, is replaced by the Glu-Tyr sequence in the *e*J16*e*JD12 strain; thus an additional amino acid residue has been added in the lysozyme of the *e*J16*e*JD12 strain.

When the base triplet assignments proposed by Nirenberg *et al.* (*5*) and Khorana *et al.* (*6*) are used, our data are compatible with the following interpretation:



One of the mutations involves the addition of two bases, as found for another mutation (*2*). In this case, as in the previous one, the insertion occurs in a region of repeating base sequence.

Our results demonstrate that the wild-type sequence of amino acids, past the amino-terminal amino acid, and the sequence of bases past the first three that are translated, are not uniquely necessary for the synthesis of bacteriophage lysozyme (*13*).

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12. Abbreviations used: Met, methionine; Asn, asparagine; Ile, isoleucine; Phe, phenylalanine; Glu, glutamic acid; Leu, leucine; Arg, arginine; Lys, lysine; Pro, proline; Val, valine; Tyr, tyrosine; Asp, aspartic acid; Ser, serine; Ala, alanine; Gly, glycine; A, adenine; U, uridine; G, guanine; C, cytosine.
13. We thank D. Thomas and T. Warner for help in the preparation of lysozyme samples. Supported by a Jane Coffin Memorial Fund for Medical Research grant to Y. Okada and A. Tsugita, and by NSF grant 466GB2261 to G. Streisinger and by grant NIH GM-10982 to A. Tsugita.

24 June 1968; revised 18 September 1968