A SUPPRESSOR IN *ESCHERICHIA COLI* N4316 WHICH SUPPRESSES T4 NONSENSE AND FRAMESHIFT MUTATIONS

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ABSTRACT

A SUPPRESSOR IN ESCHERICHIA COLI N4316 WHICH SUPPRESSES T4 NONSENSE AND FRAMESHIFT MUTATIONS

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Escherichia coli strain N4316 carries a temperature-dependent suppressor gene which can suppress bacteriophage T4 lysozyme UAA and UGA nonsense mutants at 36 C but not at 31 C. In this thesis, I have further characterized this suppressor strain.

I have assayed forty-five T4 mutants on N4316. N4316 is able to suppress four UAG mutants, six UAA mutants, five UGA mutants and one frameshift mutant. Missense and deletion mutants are not suppressible. Mutations affecting the lysozyme, rII, DNA polymerase, head component and tail fibre genes of T4 phage are suppressible indicating that N4316 does not suppress by providing an alternative pathway for the suppressible mutations. Temperature-dependent suppression by N4316 is demonstrated by measuring lysozyme activity in cells infected with suppressible lysozyme mutants.

The suppression pattern of N4316 was compared with those of other nonsense suppressors, and found to be

different. This indicates that the amino acid inserted by N4316 in response to a nonsense codon is different from those inserted by the other suppressors. Further evidence for this comes from heat inactivation studies of wildtype T4 and a suppressible strain that is mutated in a tail fibre gene. The rate of heat inactivation of the mutant phage grown on N4316 is different from that of phage grown on a known suppressor indicating that the phage particles are different when grown on the two strains. A model for a suppression mechanism involving tRNA modification has been proposed.

DEDICATED TO

MY

GRANDPARENTS

AND

PARENTS

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
MATERIALS AND METHODS	
Bacterial strains	10
Bacteriophage strains	10
Media	
(1) AB medium	10
(2) Tryptone medium	15
(3) Citrate agar	15
(4) Bray's Scintillation fluid	15
Bacteriophage assay	15
Plating assay for lysozyme mutants of T4	16
Lysozyme assay	
(1)Preparation of lysozyme substrates	17
(2)Procedure for lysozyme assay	18
Preparation of phage lysate from a single plaque	19
Thermostability test of lysates prepared from different hosts	20
RESULTS	
Suppression pattern of N4316	21
Lysozyme assay	27
Thermostability test of lysates prepared from different hosts	32
Suppression of the T4 nonsense mutants with various other suppressor strains of *E. coli*............	37
DISCUSSION	43

TABLE OF CONTENTS (continued)

	Page
REFERENCES	55
APPENDIX	60

LIST OF FIGURES

		Page
Figure 1.	Heat inactivation rates of various lysates at 60 C	34-35
2.	Possible mechanism of frameshift suppression by an under-modified tRNA recognizing AAAA	52
3.	Possible mechanism of frameshift suppression by an under-modified tRNA recognizing nonsense codon	53

LIST OF TABLES

		Page
Table 1.	List of Escherichia coli strains	11
2.	List of bacteriophage T4 strains	12-14
3.	Efficiencies of suppression by N4316 on various strains of T4 mutants	22-25
4.	Lysozyme assay	30
5.	F values for the test of equality of slopes between each pair of regression lines	36
6.	List of Escherichia coli suppressor strains	38
7.	Suppression of the T4 nonsense mutants with various tRNA suppressor strains of E. coli	39-40
8.	Amino acid and nucleotide sequence of T4 lysozyme containing the J44 mutation	50

INTRODUCTION

This thesis involves further characterization of a suppressor in *Escherichia coli* N4316 that can suppress bacteriophage T4 lysozyme UAA and UGA nonsense mutants at 36 C but not at 31 C (Phillips, Schlessinger and Apirion, 1969(a)).

Suppression is the phenomenon whereby a secondary mutation dominates a primary mutation so that the wildtype phenotype is restored. In this situation, the secondary mutation is called a suppressor.

There are two main categories of suppression (Hartman and Roth, 1973): (1) intragenic suppression, and (2) intergenic suppression.

Suppression in which the secondary mutation occurs in the same cistron as the primary mutation is called intragenic. This is not to be confused with reversion to wildtype, for although the wildtype phenotype has been restored, the original mutation still exists in the cistron. It would be more appropriate to call it a 'pseudo-wildtype'. An example of intragenic suppression is a double frameshift mutation in the rIIB cistron (Crick *et al.*, 1961).

Deletion or addition of one base pair in the DNA sequence is called a frameshift mutation. Since the genetic code is read by triplets, an addition or deletion of one base will distort the reading frame. rII frameshift mutants

cannot produce plaques on *E. coli* K derivatives because without the rII gene product, they fail to grow. For example, the T4 phage mutant FC0 has an addition of one base pair in the rII B cistron. Mutants such as this one which lack an active rII gene product cannot grow in *E. coli* K strains. The FC0 mutation can be suppressed by crossing with a second mutant FC1 which has a deletion of one base near by in the same cistron. Double mutants from this cross are able to grow on *E. coli* K strains indicating that the displaced reading frame is restored (Crick *et al*., 1961).

In intergenic suppressions, the secondary mutation affects a different cistron from that of the primary mutation. Intergenic suppression can be further divided into two classes: (a) functional suppressors and (b) informational suppressors (Hawthorne and Leupold, 1974).

Functional suppressors restore the mutant phenotype to that of the parents by offering an alternative pathway to bypass the block caused by the primary mutation. An example is found in *Saccharomyces*. An isoleucine-requiring strain cannot utilize threonine as substrate in place of isoleucine. However, in the presence of a suppressor gene, the isoleucine-requiring strain can use threonine in the absence of isoleucine. This suppressor gene segregated independently from the isoleucine alleles. The suppressor was shown to provide an alternative pathway from threonine to isoleucine (Mortimer and Hawthorne, 1969; Kakar, 1963).

Informational suppressors act at the level of

transcription or translation to partially or completely restore activity to the protein whose activity was altered by the primary mutation. Suppression of nonsense mutations by tRNA suppressors is an example of informational suppression (Gorini, 1970).

As early as 1961, it has been proved that nucleotide sequences are read by triplets (Crick et al., 1961). All except three of the sixty-four triplet codons code for amino acids. The other three codons are UAG (amber), UAA (ochre), and UGA (opal). These nonsense codons signal termination for the translational processes and normally there are no aminoacyl-tRNA's which can recognize the nonsense codons.

A point mutation in many amino acid coding triplets can result in a nonsense codon. This will lead to premature termination and therefore an inactive protein. Such nonsense mutations can be suppressed when a second mutation occurs in a tRNA gene and that gene produces an altered tRNA which can recognize a nonsense codon and read it as "sense" and would suppress the mutation (Hartman and Roth, 1973). One example is the suppressor supD in E. coli (Bachmann and Low, 1980) which suppresses UAG mutations because the serine-tRNA gene is mutated. The altered serine-tRNA can base pair with the UAG nonsense codon and inserts the amino acid serine in that position. If the tertiary structure of the suppressed protein is not seriously affected by the substituted serine, the protein will be functional, and a wildtype or pseudo-wildtype could be restored.

This thesis involves the study of a peculiar suppression by an E. coli mutant, strain N4316. This strain was isolated by Phillips, Schlessinger and Apirion (1969(b)) during the process of isolating ribosomal mutants. The parent of this mutant strain is E. coli D10. E. coli D10 was mutagenized by treatment with N-methyl-N'-nitro-N-nitrosoquanidine and mutants which could not form functional ribosomes at 43 C and so could not grow at 43 C were selected. N4316 is one of these mutants.

In addition to being unable to grow at 43 C, N4316 was later shown to be a suppressor strain (Phillips, Schlessinger and Apirion, 1969(a)). N4316 suppressed two UAA and two UGA nonsense mutants in the lysozyme gene of bacteriophage T4 but did not suppress any of the four UAG mutants tested (Phillips, Schlessinger and Apirion, 1969(a)). In E. coli, UAA suppressors usually suppress UAG as well while UGA and UAG suppressors are specific (Steege and Soll, 1979). N4316 is the only known suppressor which can suppress UAA and UGA nonsense mutants. One major concern of this thesis is to further determine the suppression pattern of N4316. A total of forty-five mutants of phage T4 have been tested to see whether suppression by N4316 is specific for UAA and UGA mutations. This study shows that the suppression by N4316 is not specific for UAA and UGA mutations since four UAG mutants and one frameshift mutant were suppressible by N4316.

Phillips, Schlessinger and Apirion (1969(a)) only

tested the suppression of N4316 on lysozyme mutants. The mutation in N4316 might be a functional suppressor if it provides an alternative pathway for cell lysis in which lysozyme is no longer needed. In this case, all lysozyme mutants should be suppressible by N4316. However, not all lysozyme mutants are suppressible by N4316 therefore this suppressor is not a functional suppressor. The suppressor in N4316 is probably an informational suppressor since this is the only other alternative.

Suppression by N4316 is temperature dependent; it can suppress only at 36 C but not at 31 C. There are only a few suppressor strains which exhibit temperature dependent suppression. They were selected from known tRNA suppressors. They include mutants of _supD_, _supC_ and _supF_ (Smith _et al_., 1970; Gallucci, Pacchetti and Zangrossi, 1970; Nagata and Horiuchi, 1973; Oeschger and Woods, 1976). All these suppressors function at low temperature (31 C) but not at high temperature (42 C) because the altered tRNA is more labile to heat and therefore fail to suppress at the high temperature. N4316 is the only known suppressor to behave in the reverse fashion. This might indicate a different mechanism of suppression.

Since N4316 cannot grow at 43 C, there is probably a thermolabile component of the mutation. _In vitro_ protein synthesis using natural mRNA such as R17 RNA is inhibited at 43 C and polypeptides accumulate on ribosome _in vitro_ suggesting a defect in termination process of protein

synthesis (Phillips, Schlessinger and Apirion, 1969(a)). A protein factor from wildtype E. coli which can restore protein synthesis in N4316 at 43 C was isolated by two groups of workers. It is called "Z" factor by Phillips (1971) and "Rescue factor" by Ganoza et al. (1973). This non-ribosomal factor is required for release of the polypeptide chain from the ribosome during protein synthesis. Therefore it was postulated that protein synthesis in N4316 is defective because the Z factor is thermolabile at 43 C. Without this factor, the synthesized proteins are not released and so further synthesis is blocked (Phillips, Schlessinger and Apirion, 1969(a)). This might also explain the temperature-dependent suppression property of N4316. At 31 C, the Z factor is stable and active therefore protein synthesis and polypeptide chain termination occurs naturally. This also includes the premature chain termination caused by nonsense mutations. Therefore suppression is not observed at 31 C. However, at 36 C the Z factor might be partially inactive so that not all termination signals are obeyed and therefore permitting some suppression of the nonsense mutants. At 43 C, the Z factor becomes totally inactive and none of the synthesized proteins are released from the ribosomes. As a result, the strain cannot survive (Phillips, Schlessinger and Apirion, 1969(a); Phillips, 1971). However, there is no evidence of partially defective Z factor in N4316 at 36 C and it is not yet clear whether the mutation affecting

protein synthesis is in fact the suppressor mutation.

In bacteriophage genetics, efficiency of plating (E.O.P.) (Ellis and Delbruck, 1939) has been widely used as a quantitative measure of suppression. In the agar layer method of plating (Hershey, Kalmanson and Bronfenbrenner, 1943), the number of plaque forming units (P.F.U.) represents the relative survival rate of the phage on the particular host. If a lysate of a phage mutant is titred on a suppressor bacterial strain which can suppress the mutation, the phage particle will have a chance to survive, multiply and form a plaque forming unit. Therefore comparing the E.O.P. on various hosts with different suppressors, will indicate the relative efficiency of the suppressors. Although this method is an indirect way to estimate the efficiency of suppression, it has the advantage of some quantitative comparison in terms of survival rate and is relatively easy to do. This is particularly helpful in cases where some gene products might not be measurable biochemically.

One also wishes to study the immediate events of a suppression. In the case of informational suppressors, one would like to know how well the mutation is recognized by the suppressor and how functional is the gene product after the suppression. Therefore in my work, I have also attempted to measure the efficiency of suppression by measuring the lysozyme activities of some suppressible lysozyme mutants. Measuring the activity of a restored

protein is a more direct way of evaluating the efficiency of suppression. The obtained efficiencies of suppression were also compared with those from the efficiencies of plating.

T4 strain opc105 is an UGA mutant. The mutation occurs in gene 34 which codes for a subunit of the tail fibre. This mutant will grow happily on E. coli strain CAJ64 because CAJ64 is a suppressor strain with mutated tryptophan tRNA. This tRNA will read the UGA signal as a tryptophan codon and substitute the amino acid tryptophan in place of it. As shown in this study, strain opc105 is suppressible by N4316. Although N4316 might not be a tRNA suppressor, in order to form a viable phage particle, the suppressor of N4316 must have substituted an accepted amino acid in response to the termination codon so that the tail fibre could be completed. I want to compare the phage particles of opc105 made on CAJ64 to that made on N4316 by the heat inactivation experiment. Heat can inactivate phage particles. The rate of inactivation follows first order kinetics (Adams, 1959). Heat inactivation of phage is due to the denaturation of the phage structural proteins (head proteins and tail fibres). The amino acid sequences of these proteins dictate the tertiary structures which in turn dictate the heat inactivation rate. If the substituted amino acid is not tryptophan and if the amino acid affects the tertiary structure and therefore the heat stability of the tail fibre protein, one would expect to see a difference in rates of heat inactivation for the opc105 phage grown on

N4316 and on CAJ64.

MATERIALS AND METHODS

Bacterial strains

All the bacterial strains used are *E. coli* derivatives. Their genotypes and sources are listed in Table 1. Stocks were kept at -20 C in glycerol or in stab vials (Miller, 1972). Strains were maintained as streaks on AB agar plates (see "Media"). In the case of strain N4316, a fresh plate was streaked every week because the cells on the old plates did not grow well when subcultured into liquid medium.

Bacteriophage strains

Forty-five T4 bacteriophage mutants were used in this study. They include eight UAA (ochre) mutants, seventeen UAG (amber) mutants and nine UGA mutants. In addition, there were six missense mutants, four frameshift mutants and one deletion mutant. Their affected genes and sources are listed in Table 2.

Media

(1) AB medium (Apirion, 1966) - This nutrient medium consists of 10 grams of Difco nutrient broth and 10 grams of vitamin-free casamino acids in one litre of distilled water. AB bottom agar contains 15 grams of Difco agar per litre of AB medium. Each agar plate contains approximately 25 ml of AB bottom agar. AB soft agar contains 6.5 grams of Difco

TABLE 1. List of *Escherichia coli* strains

Strain[a]	Genotype[b]	Source	Reference
AB2834	*aroE353 mal-352 tsx-352* λ^R,λ^- *supE42*	CGSC[c]	Pittard and Wallace(1966)
B		S.Champe	
C		E.Newman	
CA165	*supB lacI lacZ*	M.C.Ganoza	Hirsch (1971)
CA265	*trp-49 relA1 lacZ125 supF66*	CGSC	Brenner and Beckwith(1965)
CAJ64	*lac supUGA*	J.Friesen	Sambrook *et al*.(1967)
CR63	*supD60* F^+ λ^s	M.C.Ganoza	Bachmann and Low(1980)
D10	*metB thy rna*	M.C.Ganoza	Gesteland(1966)
K223	F' *lac supUGA*	T.Blumenthal	Beremand and Blumenthal (1979)
N4316	*metB thy rna sts-1 Sut*$^-$	J.L.Phillips	Phillips *et al*.(1969a,b)
RG11	*his rpsL* (*chlD-pgl*) λ^s	R.Grant	
[illegible]11R1d	*phoA6 relA1 tonA22* $T2^R$ *supG46*	CGSC	Galluci and Garen(1966)

a All strains except B and C are *E. coli* K12 derivatives.
b Symbols are from Bachman and Low
c CGSC-Coli Genetic Stock Center, Yale University (B.J. Bachmann, Curator).

TABLE 2. List of bacteriophage T4 strains

Strain	Affected gene	Affected gene product	Source*	Reference
T4	Wildtype			
UAG mutants				
JC1916	e	lysozyme	II	Person and Osborn (1968)
M103	e	lysozyme	IV	Stresinger et al.(1961)
M91	e	lysozyme	IV	Ibid.
M41	e	lysozyme	IV	Ibid.
HB118	rII	membrane protein	I	Benzer and Champe(1961)
SM10	rII	membrane protein	III	Drake and McGuire (1967)
UV200	rII	membrane protein	III	Benzer and Champe(1961)
JC1913	15	tail component	II	Person and Osborn (1968)
JC1914	15	tail component	II	Edgar and Lielausis (1964)
JC1915	15	tail component	II	Person and Osborn (1968)
E18	18	tail sheath subunit	V	
JC1917	23	head component	II	Edgar and Lielausis (1964)
E605	30	DNA ligase		
JC1918	34	tail fibre subunit	II	Person and Osborn (1968)
N58	34	tail fibre subunit	V	
JC1912	43	DNA polymerase	II	Edgar and Lielausis (1964)
M69	63	tail fibre attachment	V	Stresinger et al.(1961)

TABLE 2. List of bacteriophage T4 strains (continued)

Strain	Affected gene	Affected gene product	Source*	Reference
<u>UAA mutants</u>				
L3	e	lysozyme	IV	Yahata, Ocada and Tsugita (1970)
JC1921	e	lysozyme	II	<u>Ibid</u>.
JC1922	e	lysozyme	II	<u>Ibid</u>.
JC1924	e	lysozyme	II	<u>Ibid</u>.
L5	e	lysozyme	II	<u>Ibid</u>.
JC1926	?	?	II	Person and Osborn (1968)
N21	rII	membrane protein	I	Benzer and Champe (1961)
SM10	rII	menbrane protein	III	Drake and McGuire (1967)
<u>UGA mutants</u>				
JC1927	e	lysozyme	II	
JC1928	e	lysozyme	II	
X655	rII	membrane protein	I	Benzer and Champe (1961)
UV200	rII	membrane protein	III	<u>Ibid</u>.
SM10	rII	membrane protein	III	Drake and McGuire (1967)
opc6	17	head completion protein	V	Wilson and Kells (1972)
opc100	23	head component	V	<u>Ibid</u>.
opc105	34	tail fibre	V	<u>Ibid</u>.
opc23	37	tail fibre	V	<u>Ibid</u>.

TABLE 2. List of bacteriophage T4 strains (continued)

Strain	Affected gene	Affected gene product	Source*	Reference
Frame-shift mutants				
FC1	rII	membrane protein	I	Crick *et al.*(1961)
FC0	rII	membrane protein	I	*Ibid.*
J44	e	lysozyme	IV	Terzaghi et al.(1966)
J42	e	lysozyme	IV	*Ibid.*
Deletion mutant				
221	rII	membrane protein	I	Benzer (1959)
Mis-sense mutants				
PS75	rII	membrane protein	III	
SM25	rII	membrane protein	III	Drake and McGuire (1967)
T10	rII	membrane protein	III	
AP129	rII	membrane protein	I	Benzer and Champe (1961)
N74	rII	membrane protein	I	*Ibid.*
r607	rII	membrane protein	I	*Ibid.*

*Sources of strains:

I. S.P.Champe, Rutgers University.

II. A.J.Clark, Department of Molecular Biology, University of California, Berkeley.

III. J.W.Drake, National Institute of Environmental Health Sciences, North Carolina.

IV. J.Owen, Institute of Molecular Biology, University of Oregon.

V. W.Wood, University of Colorado.

agar per litre of AB medium. AB soft agar was stocked in 150 ml bottles and melted in a boiling bath prior to assay. (In the latter part of this study, regular casamino acids was used instead of the vitamin-free casamino acids. There was no noticeable difference.)

(2) Tryptone medium (Emrich, 1968) consists of ten grams of tryptone (Difco) and five grams of NaCl in one litre of distilled water. All media were autoclaved before use.

(3) Citrate agar (Okada <u>et</u> <u>al</u>., 1966) - Tryptone medium was used in preparing citrate agar. For bottom agar, eleven grams of Noble agar(Difco) were added to one litre of tryptone medium. Seven grams of Noble agar were used for preparing soft agar. After the agar has been autoclaved, 50 ml of 1.0M tris-HCl (pH 8.0) and 10 ml of 25% sodium citrate:2 H_2O were added to each litre of the medium.

(4) Bray's Scintillation fluid (Bray, 1960) consists of 60 grams of Naphthalene, 4 grams of scintanalyzed PPO (2,5-Diphenyl-oxazole), 0.2 gram of Dimethyl-POPOP (2,2'-p-phenylenebis (4-methyl-5-phenyl)-oxazole) (Fisher Scintillar), 100 ml of 100% methanol and 20 ml of ethylene glycol. p-Dioxane was added to make up the total volume of one litre. The fluid was stocked in a light-proof bottle.

Bacteriophage assay

The method of plating was used to titre phage lysates as well as to measure the efficiencies of suppression.

Plates containing AB bottom agar were used. To obtain optimal plaque formation, the AB plates were no older than one day. All bacterial cultures were grown in AB medium. 0.1ml of the overnight culture was added to a sterile 250ml Erlenmeyer flask containing 10ml of AB medium. The flask was aerated by shaking at 31 C(or 37 C) until mid-log phase. 0.1ml of the appropriate culture and 0.1ml of the phage diluted in AB medium were mixed in 2.5ml of melted AB soft agar kept at 45 C. This was poured immediately onto an AB plate. After a few minutes to allow the soft agar layer to solidify, the plates were incubated at 31 C or 36 C overnight.

Plating assay for lysozyme mutants of T4

Some T4 mutants cannot produce lysozyme because of a frameshift mutation in the lysozyme gene (eg. J42, J44). These mutants do not form plaques on common _E. coli_ strains. In order to assay the titre of these lysozyme mutants, a method was used in which lysozyme was supplemented in the soft agar during plating (Okada _et al._, 1966). The general procedure is the same as described above except citrate bottom and top agar were used in place of AB agar. In addition, a lysozyme solution was prepared prior to plating; 5mg of egg white lysozyme (Sigma) was dissolved in one ml of 0.05M tris-HCL (pH 8.0). 0.01ml of this lysozyme solution, 0.1ml of plating bacteria (_E. coli_ D10) and 0.1 ml of diluted phage were mixed into 2.5ml of citrate

top agar. The mixture was poured onto a citrate agar plate and was incubated as required. As a control, 0.1ml of the lysozyme solution and 0.1ml of the plating bacteria were plated in the absence of phage. This plate did not have any plaque formation. The reversion rate could be estimated by plating 0.1ml of the bacterial culture and 0.1ml of phage in the absence of lysozyme.

Lysozyme assay

A. Preparation of lysozyme substrates (Herrlich and Schweiger, 1974)

An aliquot (0.1ml) of an overnight culture of _E. coli_ C was added to a 250ml flask containing 10ml of tryptone medium and 0.1ml ^{3}H-Diaminopimelic acid (^{3}H-Diaminopimelic acid was obtained from Amersham, the radioactivity was about 10uCi/ml, the specific activity is 1Ci/mmol). The flask was aerated by shaking and was grown overnight at 31 C. Since diaminopimelic acid is a characteristic component of the _E. coli_ cell wall, cells from the overnight culture have ^{3}H-labelled cell walls. These cells were fixed into filter papers to be used as substrates. Aliquots (0.1ml) of the culture were added to 2.3cm Whatman 3MM filter paper discs. The discs were dropped into a beaker containing 250ml of cold 10% TCA (trichloroacetic acid). After four hours of soaking in 10% TCA, they were washed twice in 200ml of 5% cold TCA for 10 minutes. Then they were washed in 200ml of

95% ethanol and ether mixture (volume ratio is 1:1) for another 10 minutes. The discs were then transferred into a beaker of boiling 0.1M ammonium acetate for 10 minutes. They were then washed twice with cold 0.1M ammonium acetate and were stored in 0.1M ammonium acetate at 4 C. They were stable for at least a month.

B. Procedure for lysozyme assay

Using overnight culture as inoculum, bacterial cells were grown in AB medium to an O.D. 560 of 0.4, with a Bausch and Lomb Spectronic 21 spectrophotometer. Then the cells were concentrated by centrifuging for ten minutes at 1,000g and resuspended in 1/10 of the original volume. To the concentrated cell suspension, $MgSO_4$ was added to a concentration of 0.01M. Tryptophan was also added to give a final concentration of 20µg/ml. The cells were infected by the T4 mutant phages with a multiplicity of infection of three. They were allowed to stand for ten minutes for phage absorption. Samples were aerated by bubbling with air. They were incubated at a 37 C water bath for three hours to allow suppression to take place. After three hours, all tubes were kept in ice and aeration was stopped. Each sample was sonicated for one minute to release the lysozyme in the cells (using Sonic 300 dismembrator by Fisher, intermediate tip,35%). After sonication, all samples were kept in ice prior to lysozyme assay. 50µl of the sonicate was added to a vial containing one treated disc prepared as

above. The vial also contain 0.45ml of 0.1M ammonium acetate. The vials were incubated at 37 C for 12 hours. After the incubation, 0.2ml of the incubation mixture was added to 5ml of Bray's scintillation fluid in a scintillation vial. The vials were counted for ^{3}H for one minute using a LKB scintillation counter.

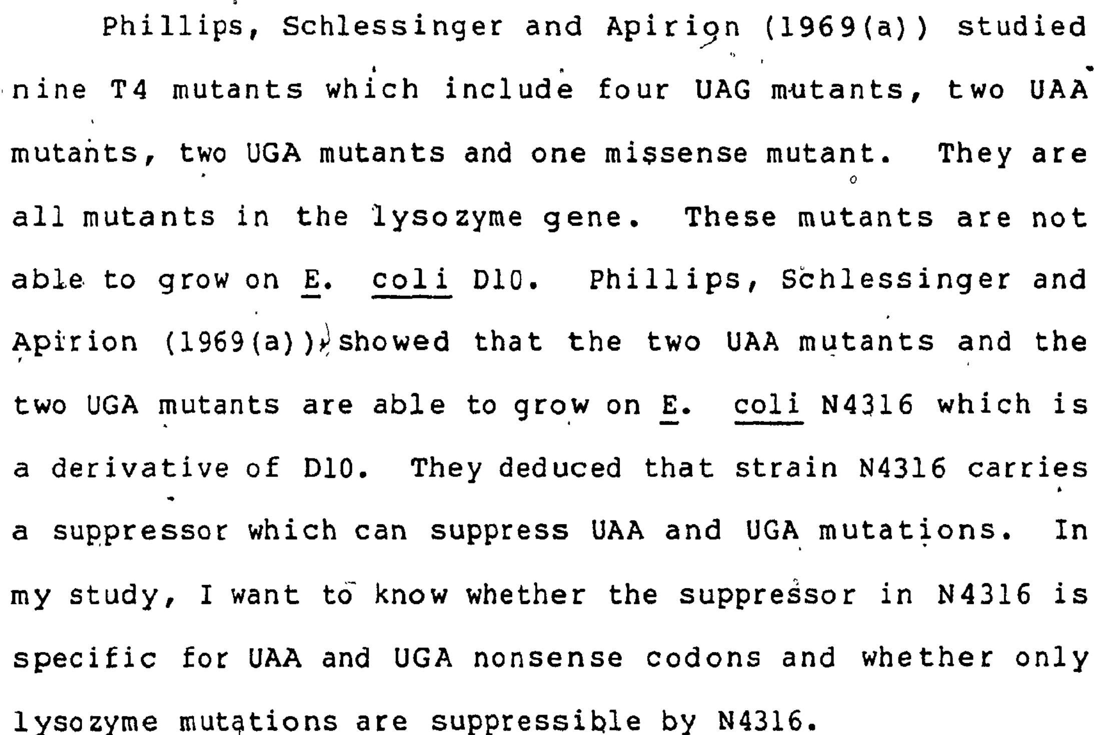
Preparation of phage lysate from a single plaque

Upon receipt of all the mutant phages from various sources, they were plated on the bacterial strain which they can grow on as described above. Plaques were picked and each was transferred to one ml of AB medium. They were plated on E. coli D10 to test for revertants. Only samples containing very few revertants were plated on the host strain so that plates were confluently lysed. About 0.5ml of chloroform was spread on each plate to disinfect the plate. After the plate appeared to be free of chloroform, three ml of AB medium was then added to the plate to extract the phage. The plates were allowed to sit for four to five hours with ocassional agitation. Using propippete, the medium was then transferred into small tube with screw cap. A few drops of chloroform was added to the tube. After the tubes had been shaken vigorously for ten seconds, they were stored in the cold. The lysates were titred as described previously. Wildtype T4 lysate was prepared in a similar manner. This method could usually give around 10^{11} PFU/ml of lysate.

Thermostability test of lysates prepared from different hosts

The UGA mutant opc105 was shown to be suppressed by N4316 with high efficiency at 36 C. The usual bacterial host strain for UGA mutants is CAJ64. Plate lysates of opc105 were prepared by using N4316 and CAJ64 as hosts.These lysates were named opc105/N and opc105/C respectively. Wildtype T4 plate lysates were also prepared by using D10, N4316 and CAJ64 as the hosts and the lysates were named T4/D, T4/N and T4/C respectively.These lysates were diluted 10,000 fold using sterile saline (0.9% NaCl). They were then incubated in a 60 C water bath. Samples were taken at zero time and five minute intervals to measure the number of surviving phages. The rates of heat inactivation of the five lysates were compared.

RESULTS



Suppression pattern of N4316

Phillips, Schlessinger and Apirion (1969(a)) studied nine T4 mutants which include four UAG mutants, two UAA mutants, two UGA mutants and one missense mutant. They are all mutants in the lysozyme gene. These mutants are not able to grow on *E. coli* D10. Phillips, Schlessinger and Apirion (1969(a)) showed that the two UAA mutants and the two UGA mutants are able to grow on *E. coli* N4316 which is a derivative of D10. They deduced that strain N4316 carries a suppressor which can suppress UAA and UGA mutations. In my study, I want to know whether the suppressor in N4316 is specific for UAA and UGA nonsense codons and whether only lysozyme mutations are suppressible by N4316.

Forty-five T4 mutants were assayed on D10, N4316 and their permissive hosts (the permissive host for a T4 mutant is the bacterial strain that the mutant can grow on). The permissive hosts for UAG, UAA, UGA, rII and lysozyme mutants are CR63, CA165, CAJ64, B and D10+lysozyme respectively). The efficiencies of plating of these strains were determined(Table 3). The efficiency of plating (E.O.P.) is the ratio of the number of plaque forming units (PFU) on D10 (or N4316) to the number of PFU on the permissive host. Since the suppression by N4316 is temperature dependent, the E.O.P. of various strains was assayed at both temperatures (31 C and 36 C). The results are shown in Table 3. As

TABLE 3. Efficiencies of suppression by N4316 on various strains of T4 mutants

(a) UAG mutants

Phage strain	Affected gene	Efficiency of plating*			
		D10		N4316	
		31 C	36 C	31 C	36 C
Suppressible					
JC1916	e	1.5x10^{-7}	7.9x10^{-6}	1.4x10^{-7}	6.4x10^{-4}
M103	e	3.0x10^{-6}	3.2x10^{-6}	2.0x10^{-3}	1.4x10^{-1}
HB118	rII	2.8x10^{-8}	8.2x10^{-8}	4.1x10^{-6}	4.7x10^{-2}
JC1912	43	3.7x10^{-8}	3.5x10^{-8}	2.8x10^{-5}	1.9x10^{-5}
Non-suppressible					
M91	e	1.7x10^{-5}	2.3x10^{-4}	3.3x10^{-5}	9.7x10^{-4}
M41	e	1.2x10^{-6}	7.3x10^{-5}	1.0x10^{-6}	6.0x10^{-5}
SM10	rII	3.6x10^{-7}	9.9x10^{-7}	8.9x10^{-8}	9.9x10^{-7}
UV200	rII	2.3x10^{-7}	2.2x10^{-7}	1.1x10^{-7}	1.1x10^{-7}
JC1913	15	6.1x10^{-7}	5.4x10^{-7}	6.1x10^{-7}	5.1x10^{-7}
JC1914	15	5.0x10^{-9}	6.3x10^{-9}	4.3x10^{-9}	9.1x10^{-9}
JC1915	1[illegible]	4.3x10^{-7}	4.4x10^{-7}	5.3x10^{-7}	4.9x10^{-7}
E18	18	1.6x10^{-6}	1.4x10^{-6}	1.5x10^{-6}	1.7x10^{-7}
JC1917	23	2.7x10^{-7}	2.6x10^{-7}	2.7x10^{-7}	4.2x10^{-7}
E605	30	2.1x10^{-4}	5.4x10^{-5}	1.4x10^{-4}	3.7x10^{-4}
JC1918	34	4.8x10^{-7}	5.2x10^{-7}	5.8x10^{-7}	7.4x10^{-7}
N58	34	1.7x10^{-7}	1.4x10^{-7}	1.6x10^{-7}	1.6x10^{-7}
M69	63	3.7x10^{-5}	3.3x10^{-5}	4.3x10^{-5}	6.5x10^{-5}

TABLE 3. Efficiencies of suppression by N4316 on various strains of T4 mutants (continued)

(b) UAA mutants

Phage strain	Affected gene	Efficiency of plating*			
		D10		N4316	
		31 C	36 C	31 C	36 C
Suppressible					
L3	e	5.6×10^{-6}	3.4×10^{-4}	1.6×10^{-4}	1.9
JC1921	e	3.3×10^{-5}	6.3×10^{-5}	4.2×10^{-5}	3.1×10^{-2}
JC1922	e	6.8×10^{-6}	1.8×10^{-4}	4.3×10^{-6}	5.6×10^{-1}
JC1924	e	3.1×10^{-5}	9.6×10^{-5}	6.0×10^{-5}	1.4×10^{-1}
L5	e	2.8×10^{-5}	2.8×10^{-5}	5.6×10^{-5}	2.9×10^{-1}
JC1926	?	5.9×10^{-5}	1.1×10^{-4}	2.2×10^{-4}	7.6×10^{-1}
Non-suppressible					
N21	rII	1.0×10^{-6}	4.4×10^{-7}	4.8×10^{-7}	3.0×10^{-6}
SM10	rII	1.0×10^{-7}	1.0×10^{-7}	1.0×10^{-7}	1.0×10^{-7}

(c) UGA mutants

Phage strain	Affected gene	Efficiency of plating*			
		D10		N4316	
		31 C	36 C	31 C	36 C
Suppressible					
JC1927	e	3.8×10^{-6}	1.3×10^{-5}	6.9×10^{-6}	1.8×10^{-2}
JC1928	e	2.1×10^{-4}	2.3×10^{-4}	3.6×10^{-4}	5.2×10^{-2}
X655	rII	1.5×10^{-5}	2.2×10^{-5}	2.6×10^{-5}	8.8×10^{-3}
opc100	23	6.4×10^{-6}	6.5×10^{-6}	7.1×10^{-6}	2.1
opc105	34	5.4×10^{-7}	6.8×10^{-7}	4.3×10^{-7}	1.6
Non-suppressible					
UV200	rII	1.5×10^{-4}	1.0×10^{-6}	1.0×10^{-6}	2.0×10^{-6}
SM10	rII	2.0×10^{-6}	1.5×10^{-6}	7.9×10^{-7}	2.9×10^{-6}
opc6	17	3.0×10^{-8}	4.0×10^{-8}	1.0×10^{-8}	5.0×10^{-8}
opc23	37	1.2×10^{-5}	1.3×10^{-5}	1.4×10^{-5}	1.5×10^{-5}

TABLE 3. Efficiencies of suppression by N4316 on various strains of T4 mutants (continued)

(d) Frame-shift mutants

Phage strain	Affected gene.	Efficiency of plating*			
		D10		N4316	
		31 C	36 C	31 C	36 C
Suppressible					
J44 (+1)	e	$2.1x10^{-3}$	$3.7x10^{-2}$	$8.5x10^{-3}$	14.3
Non-suppressible					
FC1 (-1)	rII	$2.2x10^{-6}$	$2.5x10^{-6}$	$6.5x10^{-6}$	$7.6x10^{-6}$
FC0 (+1)	rII	$1.6x10^{-6}$	$1.6x10^{-6}$	$1.6x10^{-6}$	$1.6x10^{-6}$
J42 (-1)	e	$1.3x10^{-3}$	$1.3x10^{-3}$	$1.8x10^{-2}$	$7.5x10^{-2}$

(e) Deletion mutant

Phage strain	Affected gene	Efficiency of plating*			
		D10		N4316	
		31 C	36 C	31 C	36 C
Non-suppressible					
221	rII	$1.7x10^{-9}$	$2.1x10^{-9}$	$1.7x10^{-9}$	$2.1x10^{-9}$

TABLE 3. Efficiencies of suppression by N4316 on various strains of T4 mutants (continued)

(f) Mis-sense mutants

Phage strain	Affected gene	Efficiency of plating*			
		D10		N4316	
		31 C	36 C	31 C	36 C
Non-suppressible					
PS75	rII	$3.7x10^{-8}$	$5.0x10^{-8}$	$3.7x10^{-8}$	$5.0x10^{-8}$
SM25	rII	$8.9x10^{-8}$	$6.4x10^{-8}$	$1.6x10^{-7}$	$1.1x10^{-7}$
T10	rII	$2.5x10^{-7}$	$2.5x10^{-7}$	$2.5x10^{-7}$	$2.5x10^{-7}$
AP129	rII	$5.6x10^{-9}$	$4.6x10^{-7}$	$6.8x10^{-11}$	$3.5x10^{-10}$
N74	rII	$3.8x10^{-8}$	$2.6x10^{-8}$	$3.8x10^{-8}$	$4.1x10^{-9}$
r607	rII	$3.3x10^{-7}$	$5.0x10^{-7}$	$2.1x10^{-7}$	$5.1x10^{-7}$

*Efficiency of suppression was represented by the efficiency of plating (E.O.P.) which is the ratio of the number of plaque forming units on N4316 (or D10) to that on the permissive host. The permissive host for UAG mutants is CR63; for UAA mutants is CA165; for UGA mutants is CAJ64. The permissive host for the rII mutants is E. coli B. Strains J42 and J44 were assayed on D10 with lysozyme supplemented.

The data are the averages of four to five assays and the variations are not significant.

expected, the E.O.P. values of all the mutants on strain D10 were very low because D10 is the non-permissive host, and only wildtype revertants can form plaques on D10. If the E.O.P. on N4316 is approximately the same as on D10, the mutant is not suppressible, while if the E.O.P. on N4316 is at least 100 fold greater than that on D10, the mutant is suppressible.

Seventeen strains of UAG mutants were assayed (Table 3(a)). Of these, four strains were suppressed by N4316. Two of the lysozyme mutants (JC1916, M103) and one rII mutant (HB118) were suppressed by N4316 at 36 C. The E.O.P. for JC1916 on N4316 at 31 C was essentially the same as the reversion rate on D10. However for strains M103 and HB118, their E.O.P. on N4316 at 31 C is considerably higher than the reversion rate. Strain JC1912 was found to be suppressible by N4316 but the suppression did not seem to be temperature-dependent. The E.O.P. of JC1912 on N4316 at both 31 C and 36 C were about 1,000 fold higher than the reversion rate and therefore indicates suppression.

As shown in Table 3(b), eight UAA mutants were tested on N4316. Contrary to the original conclusion of Phillips, Schlessinger and Apirion(1969(a)), not all UAA mutants were suppressible. Of the eight strains tested, two rII mutants (N21, SM10) showed no signs of suppression on N4316. The other six strains were suppressible with variable efficiencies of plating. The suppression was clearly temperature-dependent. A "gradient" of suppression

efficiencies was observed since the E.O.P. for the strains varied from 3.1×10^{-2} (JC1921) to 1.9 (L3). L3 grew particularly well on N4316 at 36 C because it formed twice as many plaques on N4316 than on the regular host CA165.

The effciencies of suppression for UGA mutants are shown in Table 3(c). Nine UGA mutants were tested. Of these, two lysozyme mutants, one rII mutant, one head protein mutant and one tail fibre mutant were suppressible by N4316. The suppression of these strains was temperature-dependent. A gradient of suppression was also observed. The E.O.P. of the suppressible mutants varied from 8.8×10^{-3} (X655) to 2.1 (opc105). Mutants opc100 and opc105 were extremely well suppressed by N4316.

Four frameshift mutants were tested (Table 3(d)). Two of them are mutants carrying a deletion of one base and the other two are mutants carrying an addition of one base. The lysozyme mutant J44 (addition of one base) was suppressed extremely well by N4316. This finding is most surprising. From this result, N4316 is the first known suppressor strain to be able to suppress all three nonsense mutations as well as a frameshift mutation. This might indicate that the suppressor of N4316 involves a defect in the translational machinery which could cause general mis-reading. However, the six missense mutants and one deletion mutant tested were not suppressible by N4316 (Table 3 (e,f)).

Lysozyme Assay

Some lysozyme mutants were suppressible by N4316 in the plating assays. In this experiment, I have attempted to measure the eifficiency of suppression by measuring the lysozyme activity in extracts from infected cells. Lysozyme was assayed by its ability to solublize ^{3}H from E. coli cells grown in ^{3}H-Diaminopimelic acid. This compound is incorporated specifically into cell walls. The labelled cells were fixed to filter discs (see method) and when the disc is treated with lysozyme, some of the tritium is released from the disc into the incubation buffer. The amount of label released is proportional to the amount of lysozyme added (Herrlich and Schweiger, 1974). This is also true under the present experimental conditions. Incubation of a disc with 50µl of a sonicate of wildtype T4-infected D10 results in the release of 100,000cpm. Five lysozyme mutants suppressible in the plating assay were tested in this assay to see if these mutants made more lysozyme on N4316 at 36 C. In this experiment, four aeration tubes were set up for each mutant. The protocol used was as follows: Tube 1-(phage + permissive host at 36 C). Tube 2-(phage + N4316 at 31 C). Tube 3-(phage + N4316 at 36 C). Tube 4-(phage + D10 at 36 C). Tube 4 is a control because only wildtype T4 can produce lysozyme on D10. Lysozyme activity of any mutant on D10 is caused by the wildtype revertants or background activity of the extract. The efficiencies of suppression of the lysozyme mutants by N4316 were expressed

as the percentage lysozyme activity of the T4 wildtype. The efficiencies obtained (Table 4) were probably overestimates of the actual efficiencies of suppression since the amount of radioactivity released by the T4 wildtype was very near the maximum radioactivity releaseable from the filter discs. Howvever, the main purpose of this experiment is to demonstrate suppression of the lysozyme mutants on N4316 at 36 C and therefore the relative efficiency of suppression is more important.

As shown in Table 4, at least three mutants (M103, L3 and JC1927) were clearly suppressible by N4316 at 36 C because the percentage lysozyme activity of these strains on N4316 was much higher at 36 C than at 31 C. For comparison, the efficiencies of plating (from Table 3) for these strains were also shown in brackets in Table 4. For M103, the restored lysozyme activity by N4316 at 31 C was higher (37%) than the lysozyme activity on D10 (23%). The E.O.P. of this mutant is also high at 31 C. This might indicate that suppression by N4316 is not strictly temperature-dependent. For JC1916, the lysozyme activities restored by N4316 at 31 C and 36 C were about the same but both were higher than the activity on D10.

Both M103 and JC1916 were UAG mutants. The permissive host used for UAG mutants was _E. coli_ CR63. CR63 is an tRNA suppressor which inserts serine in place of a UAG codon. This assay indicated that the efficiency of this suppressor was high (100% activity of the wildtype for M103

TABLE 4. Lysozyme assay

Strain	Mutant type	% enzyme activity of wild-type*			
		Permissive+ host at 36 C	N4316 31 C	N4316 36 C	D10 36 C
T4	Wild-type	100			
M103	UAG	100	37 (2.0×10^{-3})	59 (1.4×10^{-1})	23 (3.2×10^{-6})
JC1916	UAG	63	24 (1.4×10^{-7})	25 (6.4×10^{-4})	17 (7.9×10^{-6})
JC1922	UAA	4	3 (4.3×10^{-6})	4 (5.6×10^{-1})	2 (1.8×10^{-4})
L3	UAA	14	3 (1.6×10^{-4})	25 (1.9)	5 (3.4×10^{-4})
JC1927	UGA	28	28 (6.9×10^{-6})	43 (1.8×10^{-2})	33 (1.3×10^{-5})

*The corresponding efficiencies of plating of various strains (taken from Table 3) are shown in brackets.

+Permissive hosts used for T4 wildtype, UAG, UAA and UGA mutants are *E. coli* D10, CR63, CA165 and CAJ64 respectively.

and 63% activity of the wildtype for JC1916). This is in agreement with other reports that UAG suppressors are very efficient (Brenner, Stretton and Kaplan, 1965).

The lysozyme activities in extracts of N4316 infected by JC1922 are relatively low at both temperatures and the difference is probably not significant. For mutant L3, it was clearly suppressible by N4316 at 36 C because lysozyme activity of L3 on N4316 is 25% of wildtype as versus 3% at 31 C. More lysozyme was produced by L3 grown on N4316 than on CA165. This indicates that for L3, the efficiency of suppression by N4316 is higher than the efficiency of suppression by CA165. This agrees with the E.O.P. data. The permissive host used for UAA mutants was E. coli CA165 with a glutamine-tRNA suppressor which inserts glutamine in place of the UAA nonsense codons. In this assay, the enzyme activities of JC1922 and L3 on CA165 were low although for both mutants, the UAA mutation occurred in a triplet originally coding for glutamine residue in the lysozyme gene. This could be due to either a weak suppressor tRNA recognition process or the suppression had been limited by the experimental conditions. Efficiencies of UAA suppressors are generally low (Brenner and Beckwith, 1965).

The only UGA mutant tested was JC1927. It was suppressed by N4316 since the lysozyme activity of this strain on N4316 is much higher at 36 C than at 31 C. The enzyme activity of JC1927 on the permissive host CAJ64 was very low when compared to the enzyme activity on D10. One

possible explanation is that suppression of JC1927 by the permissive host produced very unstable lysozyme which had failed to show any activity in this particular assay but were sufficient to form plaque forming units after overnight incubation. The efficiency of UGA suppressors is thought to be low (Kao and McClain, 1977).

Thermostability test of lysates prepared from different hosts

Recall that opc105 is an UGA mutant. The UGA mutation occurs in the tail fibre gene. In D10,opc105 phage particles lack tail fibre and therefore no infective phages are made. However, E. coli strain CAJ64 suppresses this mutation by substituting tryptophan in place of the UGA codon thereby completing the tail fibre protein. Since N4316 can also suppress the mutation in opc105, it must have substituted an acceptable amino acid in place of the UGA codon in the tail fibre gene. If the substituted amino acid is not tryptophan and if the substitution results in a more (or less) stable tertiary structure of the tail fibre protein, one would expect that the stability of the phage particles would be different.

Five lysates were prepared for this experiment. They include two opc105 lysates, opc105/C and opc105/N, which were prepared by growing on E. coli CAJ64 and N4316 respectively and three T4 wildtype lysates, T4/N, T4/C and T4/D, were prepared from E. coli N4316, CAJ64 and D10

respectively. The survival rates of these lysates at 60 C were measured. As in the heat denaturation of protein, the rate of heat inactivation of phage particles follows first order kinetics (Adams, 1949). To transform a first order sigmodal curve into a straight line, phage survival is plotted as log N/N_0 against time where N is the number of phages left at time t of incubation and N_0 is the number of phages present at time zero. The results are shown in Figure 1. Each line represents the rate of heat inactivation of a lysate as described in the legend. Each data point represents the average of four to five assays. The slopes were obtained by linear regression of the data points.

The test for equality of slopes was adapted from Sokal and Rohlf (1969) . To compare between each pair of regression lines, Fs values were obtained by using the formula as shown in Appendix 1.

The Fs values for each pair of the regression lines are shown in Table 5. Since $F_{0.001}[1,22] = 14.40$, it means that any F value greater than 14.40 will indicate that the slopes are significantly different at the 0.001 level. At this level of significance, the rates of heat inactivation for T4/C, T4/D and opc105/C are the same.

The T4 wildtype lysates were used as controls. However, the rate for T4/N was significantly different from those of T4/C and T4/D. One might attribute such difference to be caused by N4316. There was also a difference between

FIGURE 1. Heat inactivation rates of various lysates at 60 C

		M*	C*
▲——▲——▲	opc105/N	-0.0085	-0.954
●——●——●	T4/N	-0.0138	-0.991
△——△——△	opc105/C	-0.0251	-0.990
○——○——○	T4/C	-0.0248	-0.995
⊙——⊙——⊙	T4/D	-0.0288	-0.989

* M = slope of regression line
C = correlation coefficient

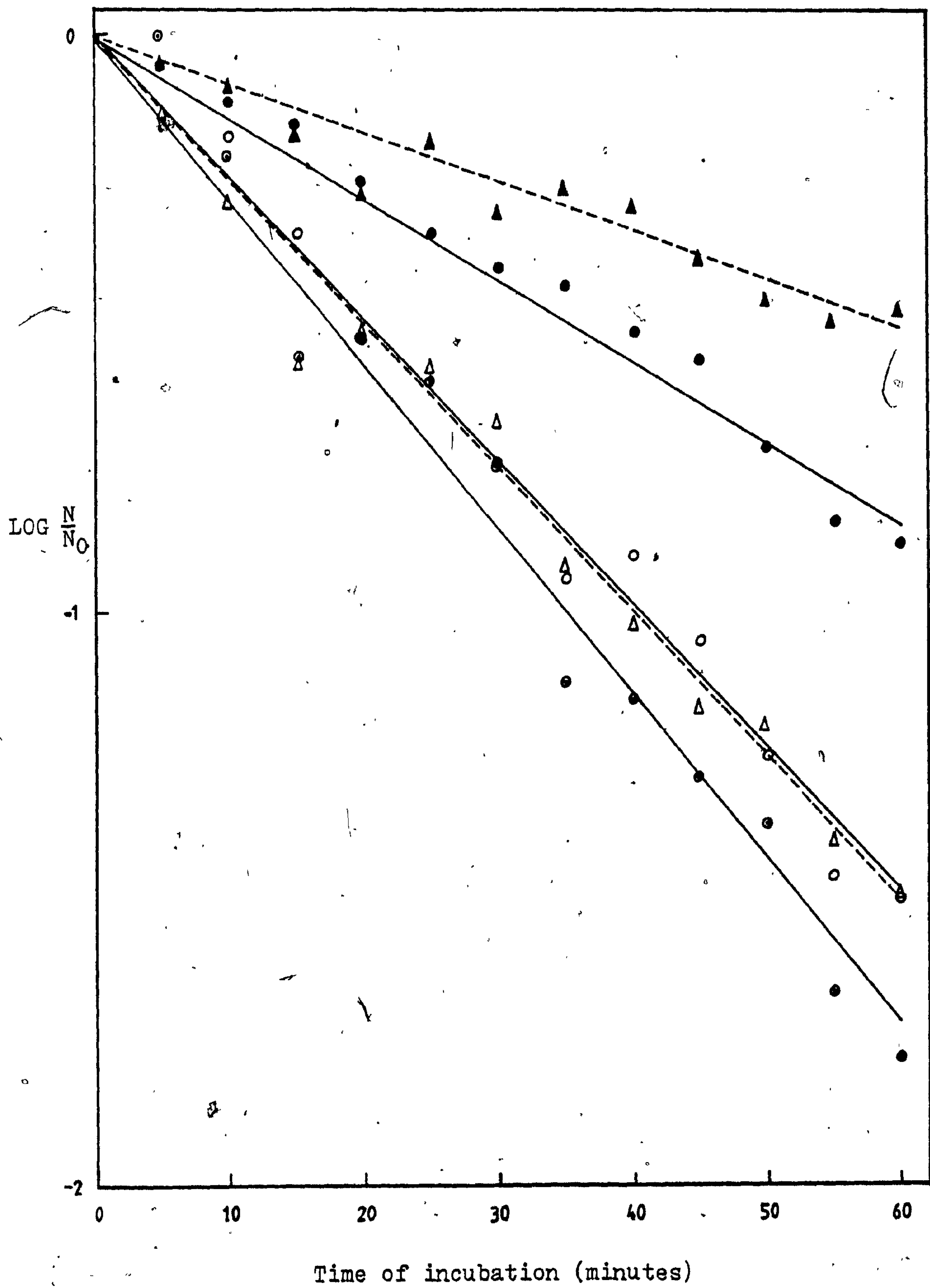

0
-1
-2
LOG $\frac{N}{N_0}$
0
10
20
30
40
50
60
Time of incubation (minutes)

TABLE 5. F values for the test of equality of slopes between each pair of regression lines

Pairs of regression lines		F_s *
opc105/N	opc105/C	181.63
opc105/N	T4/N	60.87
opc105/N	T4/C	308.42
opc105/N	T4/D	227.30
T4/N	T4/C	122.77
T4/N	T4/D	112.13
T4/N	opc105/C	63.35
T4/C	T4/D	7.91
T4/C	opc105/C	1.59
T4/D	opc105/C	12.82

* Since $F_{.001}(1,22)=14.40$, any F value greater than 14.40 indicates that the slopes are significantly different at the 0.001 level.

opc105/C and opc105/N, and between opc105/N and T4/N. The rate of heat inactivation of opc105/C is the same as that of T4/C and T4/D, indicating that the tryptophan present in the tail fibre of the opc105/C particles as a result of suppression of the UGA mutation has no effect on the heat stability of the phage particle. Thus, if N4316 suppresses UGA mutations by inserting a tryptophan, the heat inactivation of opc105/N particles should be the same as the heat inactivation of the T4/N particles. This was not observed and so N4316 does not insert tryptophan for a UGA codon.

Suppression of the T4 nonsense mutants with various other suppressor strains of _E. coli_.

The best characterized suppressors are the tRNA suppressors. They have mutated aminoacyl-tRNA's which can recognize the nonsense codons and therefore suppress the mutations. In this experiment, the efficiencies of plating of all the T4 nonsense mutants on several tRNA suppressor strains of _E. coli_ (Table 6) were assayed at 36 C. The suppression pattern of each tRNA suppressor was compared to the suppression pattern of N4316. If N4316 suppresses a nonsense mutation by substituting the same amino acid as the known tRNA suppressor strain does, one would expect the suppression patterns of N4316 and that tRNA suppressor strain to be the same. The results are summarized in Table 7. "-" indicates that the E.O.P. of the mutant on the

TABLE 6. List of Escherichia coli suppressor strains

Strain	Suppressor gene	Amino acid substituted	Suppressible nonsense codon
CA165	supB	glutamine	UAA,UAG
CAJ64	supUGA	tryptophan	UGA
CR63	supD	serine	UAG
U11R1d	supG	lysine	UAA,UAG
AB2834	supE	glutamine	UAG
CA265	supF	tyrosine	UAG
K223	supUGA	tryptophan	UGA

TABLE 7. Suppression of the T4 nonsense mutants with various tRNA suppressor strains of E. coli*

(a) UAG

T4 mutant	Suppressor strain / amino acid substituted				
	N4316	CR63 serine	U11R1d lysine	AB2834 glutamine	CA265 tyrosine
JC1916	+	+++	-	++	+++
M103	+	++	+++	++	+++
M91	-	+++	-	-	++
M41	-	+++	-	-	++
HB118	++	+++	-	++	+++
SM10	-	+++	-	++	+++
UV200	-	+++	-	+	+++
JC1913	-	+++	-	++	-
JC1914	-	+++	-	++	+++
JC1915	-	+++	-	++	++
E18	-	+++	-	-	+++
JC1917	-	+++	-	++	++
E605	-	+++	-	++	+++
JC1918	-	+++	-	-	++
N58	-	+++	-	++	++
JC1912	+	+++	+	++	++
M69	-	+++	-	++	-

TABLE 7. Suppression of the T4 nonsense muatnts with various tRNA suppressor strains of *E. coli** (continued)

(b) UAA

T4 mutant	Suppressor strain / amino acid substituted		
	N4316	CA165 glutamine	U11R1d lysine
L3	+++	+++	-
JC1921	++	+++	+++
JC1922	++	++	++
JC1924	++	+++	++
JC1926	++	++	++
L5	++	++	++
N21	-	++	-
SM10	-	-	-

(c) UGA

T4 mutant	Suppressor strain / amino acid substituted		
	N4316	CAJ64 tryptophan	K223 tryptophan
JC1927	+	+++	+++
JC1928	+	++	++
X655	+	++	+
UV200	-	++	++
SM10	-	+++	+++
opc6	-	+++	+++
opc100	+++	+++	+++
opc105	+++	+++	+++
opc23	-	+++	+++

* All assays were done at 36°C. The relative efficiency for a suppressor is expressed in "+" or "-" signs. "+++" means the highest efficiency of suppression. "-" indicates the strain is not suppressible.

suppressor strain is similar to the E.O.P. on D10. "+", "++" and "+++" indicate that a mutant was suppressed and whether the E.O.P. was moderate, high or very high. It was found that the suppression pattern of N4316 did not resemble the suppression pattern of any of the tRNA suppressor strains.

All the UAG mutants were suppressible by CR63 which inserts serine. This was expected because most mutants were characterized as UAG mutants by their ability to grow on the UAG suppressor strain CR63. Suppressor strain U11R1d which inserts lysine suppressed only M103 and JC1912. AB2834 which inserts glutamine suppressed all strains except M91, M41, E18 and JC1918. CA265 which inserts tyrosine suppressed all strains except JC1913 and M69. N4316 can suppress only JC1916, M103, HB118 and JC1912 . The suppression pattern of N4316 did not resemble any of the UAG suppressor strains studied and so it probably does not insert serine, lysine, glutamine or tyrosine in response to the UAG codon.

For the UAA mutants, N4316 suppressed all but N21 and SM10. CA165 suppressed all but SM10. U11R1d suppressed all except L3, N21 and SM10. This suggests that N4316 is not substituting glutamine or lysine in place of the UAA codon because the suppression pattern of N4316 did not resemble the two UAA suppressor strains in this experiment.

For the UGA mutants, N4316 can suppress five out of the nine strains tested. UV200, SM10, opc6 and opc23 were not

suppressible by N4316. CAJ64 and K223 suppressed all the UGA mutants and they shared the same suppression pattern because they carry the same suppressor gene. The result suggests that N4316 is not substituting tryptophan in place of the UGA codon. This conclusion is consistent with the results from the heat inactivation experiment which also indicates that N4316 did not substitute tryptophan in place of the UGA codon in the tail fibre gene of mutant opc105.

In general, no two suppressors shared the same pattern of suppression. The E.O.P. varied from strain to strain. The suppression pattern of N4316 did not resemble any of the other suppressor's. This might suggest that if N4316 suppresses nonsense mutation by substituting an amino acid, the amino acid might not be serine, lysine, glutamine, tyrosine nor tryptophan. However, it is also possible that N4316 is substituting a different amino acid in place of a different nonsense codon. It this is true, N4316 might not be substituting the amino acids glutamine and lysine in place of UAA, might not be substituting the amino acids glutamine, serine, lysine and tyrosine in place of UAG, might not be substituting the amino acid tryptophan in place of UGA.

DISCUSSION

One major theme of this thesis is to study the suppression pattern of the mutant strain N4316. In the present study, N4316 is able to suppress all three nonsense codons plus a frameshift mutation. This finding is contrary to the original conclusion that the suppression by N4316 is specific for UAA and UGA mutations (Phillips, Schlessinger and Apirion, 1969(a)). The pattern of suppression by N4316 has provided us with clues for elucidating the suppression mechanism.

From the suppression pattern of N4316, it is highly probable that the suppressor mutation in N4316 is not a functional suppressor which provides the suppressible mutants with an alternative pathway to bypass the mutation. The reasons are, first, not all lysozyme mutants are suppressible by N4316. If N4316 suppresses by providing an alternative mechanism for lysis in which lysozyme is no longer required, one would expect that at least all lysozyme nonsense mutants should be suppressible by N4316. Furthermore, if the defect in lysozyme production has been bypassed by N4316, one would not expect variations in the level of suppression of the lysozyme mutants. Secondly, lysozyme mutants are not the only type of mutants suppressible by N4316. Other suppressible mutations affect rII, DNA polymerase, head component and tail fibre genes of T4 phage. Therefore, if N4316 is not a functional

suppressor strain, the only other suppression mechanism for N4316 is an informational suppressor acting at the transcriptional or translational level.

Suppression by N4316 did not appear to be an all-or-none phenomenon. Not all mutations in each category of nonsense mutants are suppressible by N4316. From the plating experiments, N4316 was able to suppress 6 out of 8 strains of UAA mutants tested; 4 out of 17 strains of UAG mutants tested; and 5 out of 9 strains of UGA mutants tested. Furthermore, different efficiencies of suppression were observed. If suppression by N4316 is informational, this observed variation in the efficiencies of suppression might reflect two things. First, the nonsense codon may be recognized at a different rate by the suppressor depending on where the nonsense codon is located. The neighbouring base sequence might influence the recognition process (Akaboshi, Inouye and Tsugita, 1976; Fluck, Salser and Epstein, 1977). Secondly, the stability and the activity of the gene product produced after suppression depends on what amino acid is inserted in response to the nonsense codon. If the amino acid inserted by the suppressor results in a protein that is less active or less stable than the wildtype protein, the efficiency will be low even if the nonsense codon is translated efficiently. If the amino acid inserted by the suppressor is the same amino acid found in the wildtype protein, then the efficiency of suppression will depend only on how much of the protein is present, and this

in turn will depend on the efficiency of translation of the nonsense codon.

The suppression pattern of N4316 was compared to various other tRNA suppressor strains. The amino acids substituted by these suppressors are already known (Table 6). As shown in Table 7, the suppression pattern of N4316 did not resemble any of the other suppressor strains. This indicates that those amino acids substituted by the tRNA suppressors are not the same as those substituted by N4316. The results from the heat inactivation experiment support the conclusion that tryptophan is not substituted in place of the UGA codon in the opc105/N tail fibre protein.

N4316 is the first _E. coli_ suppressor strain known to suppress all three nonsense codons and one frameshift mutation. In _E. coli_, the only known suppressor which can suppress all three nonsense codons is a ribosomal mutation called _ram_ (Rosset and Gorini, 1969). _ram_ stands for "ribosomal ambiquity". The mutation alters the 30S ribosomal subunit protein S4 (Zimmermann _et al._, 1971) and this causes a reduction of the coding fidelity of the ribosome in accepting and rejecting aminoacyl-tRNA during the process of translation thus _ram_ mutation suppresses nonsense mutations by enhancing ribosomal misreading.

Suppressors capable of suppressing all three nonsense codons have also been found in yeast. They are called "omnipotent" suppressors (Hawthorne and Leupold, 1974). These suppressors had been well characterized genetically in

Saccharomyces. They all fall into two genetic loci: Sup35 (chromosome IV) and Sup45 (chromosome II). These are weak and recessive suppressors for all three nonsense codons. There is strong evidence suggesting that these are ribosomal mutations. N4316 can suppress all three nonsense mutations and one frameshift mutation suggesting that the mutation might affect the ribosomes and enhances general misreading. If this suppression results from misreading during translation, the level of misreading of the nonsense codons must be quite high since some mutants are very efficiently suppressed. A high level of errors during translation would probably interfere with cell growth and survival. Therefore, the mutation in N4316 might not be ribosomal.

Phillips, Schlessinger and Apirion(1969(a)) thought that N4316 was specific for UAA and UGA mutations only and proposed that the mutation in N4316 might affect the protein synthesis termination factor RF2. This factor is required for termination at UAA and UGA codons. Since N4316 can also suppress UAG mutants and a frameshift mutant, an alteration in RF2 alone could not be responsible for the suppression.

Most of the well characterized suppressor strains of E. coli have a mutation in the anticodon region of a tRNA gene. If this is true of N4316, then the question arises whether such an altered aminoacyl-tRNA could recognize all three nonsense codons. The Wobble Hypothesis (Crick, 1966) proposes that the third base of the codon can basepair with other nucleotides in addition to the standard complementary

base. This has been supported by the finding that all UAA suppressors in _E. coli_ known so far can also suppress UAG mutants (Hartman and Roth, 1973). The anticodon UUA on the altered tRNA can recognize both UAA and UAG because basepairing between U and A; and between U and G are permissible. It is possible that the middle base of the anticodon could also "wobble". An altered tRNA with the anticodon UUA would then be able to recognize all three nonsense codons, since U can basepair with A or G. However, there is no evidence for such mispairing and so it seems unlikely that a single mutation in a tRNA gene could result in a tRNA which can recognize all three nonsense codons.

There could be several tRNA species altered by the mutation in N4316 such that different tRNA's are involved in suppression of three nonsense codons and the frameshift mutation. In fact, the most likely suppression mechanism of N4316 might involve tRNA modification. The only other known suppressor which can suppress frameshift as well as nonsense mutations is the _supK_ in _Salmonella typhimurium_ reported by Atkins and Ryce (1974). This suppressor can suppress an UGA mutant and a +1 frameshift mutation in the histidine operon. The _supK_ involves a defect in a tRNA methylase gene. Some tRNA species are undermethylated and therefore can recognize both UGA and a frameshift mutation. At least two tRNA species (alanine-tRNA and serine-tRNA) had been identified as possible substrates for the _supK_ tRNA methylase (Pope, Brown and Reeves, 1978). The under-methylated nucleoside on

the anticodons of the two tRNA's might be responsible for recognizing the UGA and the frameshift mutation. Similarly, the suppressor mutation of N4316 might affect tRNA modification.

Ribolini and Baylor (1975) reported a "multi-nonsense" suppressor in bacteriophage T4D. This suppressor psu$^+$SB can suppress all three nonsense mutations with varying degrees of efficiency. Even more interesting, this suppressor is also temperature sensitive; it can only suppress at 36 C but not at 42 C. The suppression also appeared to be informational as it can suppress nonsense mutations in several different genes such as lysozyme, tail fibre and rII genes.Although the exact suppression mechanism of this "multi-nonsense" suppressor is still not clear, it had been suggested that psu$^+$SB might involve a tRNA modifying enzyme. N4316 is also a "multi-nonsense" suppressor strain which can suppress all three nonsense mutations and in addition, it can also suppress a frameshift mutation. It is likely that the suppressor mutation in N4316 involve tRNA modification.

If the suppression property of N4316 involves an enzyme for tRNA modification, there are two possible explanations for the temperature-dependence of the suppression. First, at 36 C the mutated modifying enzyme might be inactive so that the tRNA's would not be modified. The undermodified tRNA's are capable of suppressing nonsense and frameshift mutations. At 31 C, the modifying enzyme functions normally and with normal tRNA's, N4316 does not suppress. Secondly,

the mutated modifying enzyme is inactive at all temperatures but the structures of the undermodified tRNA's at 31 C and at 36 C might be different such that the ones at 36 C can suppress nonsense and frameshift mutations.

In this study, the frameshift mutant J44 was found to be suppressible by N4316. Fortunately, this lysozyme mutant J44 had been well studied(Terzaghi et al., 1966) and therefore some postulates of the mechanisms of frameshift suppression by N4316 are possible. The lysozyme mutants of the bacteriophage T4 are useful for studying suppression because the T4 lysozyme protein has been purified and well studied. The lysozyme of wildtype T4 contains 164 amino acid residues (Tsuigita and Inouye, 1968). The corresponding mRNA sequence could be deduced from the polypeptide map. The suppressible frameshift mutant J44 has an addition of one guanine (G) in the 41st triplet coding for the amino acid alanine (Table 8)(Terzaghi et al., 1966). Due to the addition of one base the reading frame has been shifted. Note particularly that the shift in the reading frame generates a UAA codon at the 43rd triplet and results in premature termination of translation.

The first possible way of suppressing the frameshift mutation by N4316 is comparable to that reported in Salmonella. In Salmonella, there are two types of intergenic frameshift suppressors (Riddle and Roth, 1972). The first type (SufA, SufB and SufC) suppresses those frameshift mutations which were caused by an addition of one

TABLE 8. Amino acid and nucleotide sequence of T4 lysozyme containing the J44 mutation

Position	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
Amino acid residue+	Leu	Asn	Ala	Ala	Lys	Ser	Glu	Leu	Asp	Lys	Ala	Ile	Gly	Arg	Asn
Nucleotide sequence*	CUU	AAY	GCX	GCX	AAZ	AGY	GAZ	UUZ	UGY	AAZ	GCX	AUV	GGX	WGX	AAY
J44 mutation			+G GGCX												
Shifted reading frame	CUU	AAY	GGC	XGC	XAA	ZAG	YGA	ZUU	ZUG	YAA	ZGC	XAU	VGG	XWG	XAA
Possible nonsense codon					UAA		UGA			UAA				UAG	UAA
Possible repeated base					AAA	AA									

*V = U,C or A
W = A or C
X = U,C,A or G
Y = U or C
Z = A or G

+ Terzaghi *et al.*(1966)

cytosine in a repeated sequence of cytosines. Normally, the codon CCC is recognized by proline-tRNA's with the anticodon GGG. For SufA and SufB, the normal proline-tRNA is altered (Riddle and Roth, 1972). The anticodon of these mutant tRNA's contains four bases GGGG which can recognize the codon CCCC. Since these mutant tRNA's read four bases instead of three, they can suppress the frameshift mutation with the addition of one C adjacent to CCC.

Frameshift suppressors SufD, SufE and SufF suppress GGGG rather than CCCC. SufD and SufE are glycine-tRNA mutations (Riddle and Roth, 1972; Riddle and Carbon, 1973). The suppressor SufD produces an altered glycine-tRNA with the anticodon CCCC instead of CCC found in wildtype. N4316 might also have an altered tRNA with four bases on the anti-codon which can read four bases and therefore suppress the frameshift mutation. As shown in Table 8, there could be a sequence of four or five adjacent adenines at the 43rd and the 44th triplet of the lysozyme mRNA. Normally, an AAA codon is translated by lysyl-tRNA with anti-codon UUU. In *E. coli* lysyl-tRNA, the adjacent base of the anticodon is a modified U (Gauss, Gruter and Sprinzl, 1979). If the suppressor mutation in N4316 prevents the modification of this U, then the lysyl-tRNA may be able to read the sequence of AAAA in the lysozyme message thereby restoring the reading frame to normal and the frameshift mutation will be suppressed. This model is diagrammatically presented in Figure 2.

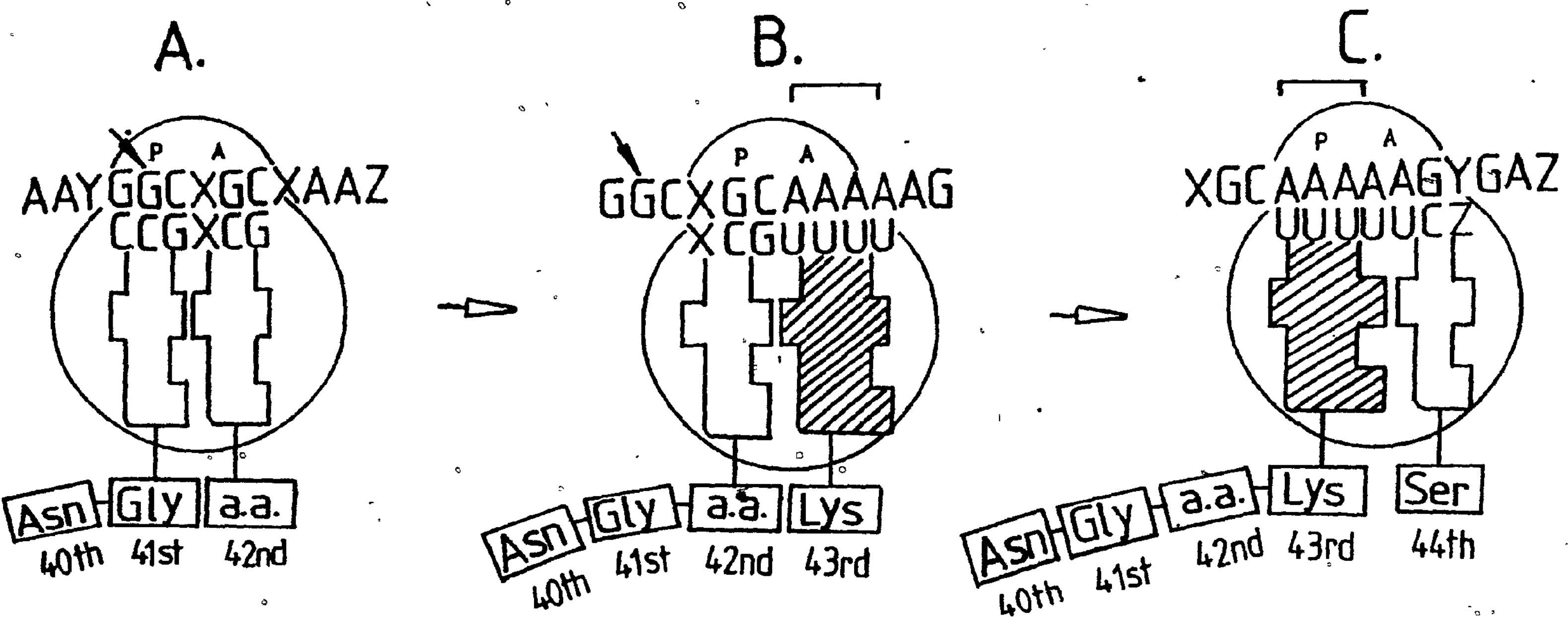


FIGURE 2. Possible mechanism of frameshift suppression by an under-modified t-RNA recognizing AAAA

A. J44 mutation results in an addition of a G residue as indicated by the dark arrow. The reading frame is shifted. The amino acids are numbered according to their positions in the lysozyme protein.

B. As translation propagates, assuming that XAAZ is AAAA (now at the A site), it is recognized by the under-modified lysyl-tRNA. The translation of the protein could be continued.

C. The undermodified lysyl-tRNA is now at the P site. By reading four nucleotides AAAA, the original reading frame is restored. Lysine and serine are inserted at the 43rd and 44th positions of the polypeptide chain.

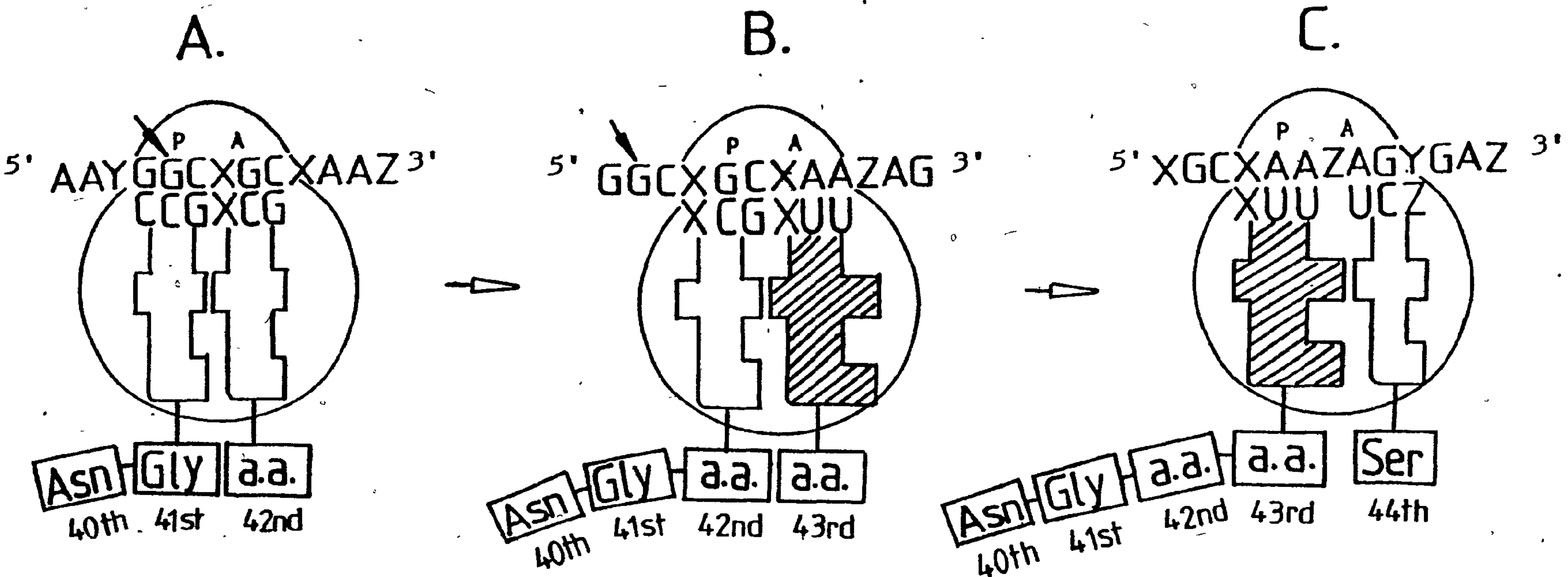


FIGURE 3. Possible mechanism of frameshift suppression by an under-modified tRNA recognizing nonsense codon

A. J44 mutation results in an addition of a G residue as indicated by the dark arrow. The reading frame is shifted. The amino acids are numbered according to their positions in the lysozyme protein.

B. As translation propagates, assuming that XAA(at the A site) is the nonsense codon UAA, it is recognized by the undermodified t-RNA. The translation of the protein could be continued.

C. The undermodified t-RNA is now at the P site. Its conformation has caused the translation to skip the Z residue. The reading frame is therefore restored. Serine is inserted at the 44th position of the polypeptide chain.

Another possible mechanism of frameshift suppression by N4316 involving tRNA modification might require nonsense codon recognition with simultaneous shifting of the reading frame. As shown in Table 8, the shifted reading frame had resulted in many possible nonsense codons. The altered tRNA might recognize one of these possible nonsense codons and the reading frame could be restored if the spatial conformation between the altered tRNA and the ribosome interact in such a way that one base is skipped during translation. This situation is represented diagramatically in Figure 3. These two models predict some change of amino acid sequence near the J44 mutation. This should not interfere with the activity of the lysozyme since double mutants carrying an addition of a base pair and a deletion of a base pair in this region of the lysozyme gene produce active lysozyme. This indicates that alteration of amino acid sequence near the J44 mutation is allowable (Streisinger et al., 1966). More frameshift mutants have to be tested before one could conclude whether N4316 can suppress only this particular mutant J44, or if N4316 has a particular pattern of frameshift suppression.

To conclude, the most plausible suppression mechanism of N4316 might involve tRNA modification. A gene coded for a tRNA modifying enzyme might be mutated in N4316 such that several altered tRNA species are produced and so one species might be responsible for the recognition and suppression of UAA mutations, another species might be responsible for the

suppression of UAG mutations, another for UGA and another for frameshift mutation.

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APPENDIX 1

F-test* for difference between two regression coefficients:

$$F_s = \frac{(b_1 - b_2)^2}{\frac{\sum x_1^2 + \sum x_2^2}{(\sum x_1^2)(\sum x_2^2)} \left\{ \frac{\sum y_1^2 - \frac{(\sum xy)_1^2}{\sum x_1^2} + \sum y_2^2 - \frac{(\sum xy)_2^2}{\sum x_2^2}}{(a_1 + a_2 - 4)} \right\}}$$

* Adapted from Sokal and Rohlf (1969)

where a = number of points for line
b = slope of line

$$\sum x^2 = \sum X^2 - \frac{(\sum X^2)}{n}$$

$$\sum y^2 = \sum Y^2 - \frac{(\sum Y^2)}{n}$$

$$\sum xy = \sum XY - \frac{(\sum X)(\sum Y)}{n}$$

in this assay: X = minutes of incubation
Y = log of the survival fraction of phage
n = 13