PHENOTYPIC INSTABILITY IN A *tif*-1 MUTANT
OF *ESCHERICHIA COLI*

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TABLE OF CONTENTS

	Page
SUMMARY	1
1. INTRODUCTION	3
2. MATERIALS AND METHODS	4
3. THE EFFECT OF GROWTH CONDITIONS UPON THE	5
A OF STREPTOMYCIN (Sm) BY T44(A)	
This study was carried out by me under the	
direction of Professor Erela Ephrati-Elizur.	
OF GROWTH ON Sm ON THE T11	9
PHENOTYPE	13
POLYPEPTIDE SYNTHESIS IN AN IN VITRO SYSTEM	13
THE EFFECT OF Sm ON IN VITRO POLYPEPTIDE SYNTHESIS	16
THE EFFECT OF ETHANOL	19
ON T11	21
THE EFFECT OF RIBOSOMAL ALTERATION ON THE PHENOTYPE	23
OF T44(A)	
VARIANTS TO TEMPERATURE RESISTANCE	26
THE EFFECT OF YEAST EXTRACT (YE), GUANOSINE AND	30
CYTIDINE (GC) AND ETHANOL UPON T44(A) AND ITS	
Spc-R AND Ts ⁺ DERIVATIVES	32
MODIFICATION OF THE Ts ⁺ PHENOTYPE BY INTRODUCTION	36
OF A spo ^r MUTATION	
DERIVATIVES OF T44(A) WITH UNUSUAL PHENOTYPES ('S'	39
STRAINS)	
TRANSDUCTION EXPERIMENTS	43
PROTEIN SYNTHESIS IN THE 'S' STRAINS	43
DISCUSSION	49
REFERENCES	54
ACKNOWLEDGEMENTS	59

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TABLE OF CONTENTS

	<u>Page</u>
SUMMARY	1
1. INTRODUCTION	3
2. MATERIALS AND METHODS	4
3. THE EFFECT OF GROWTH CONDITIONS UPON THE TOLERANCE OF STREPTOMYCIN (Sm) by T44(λ) CULTURES	9
4. THE EFFECT OF GROWTH ON Sm ON THE Tif PHENOTYPE	9
5. POLYPEPTIDE SYNTHESIS IN AN <i>In Vitro</i> SYSTEM	13
6. THE EFFECT OF Sm on <i>In Vitro</i> POLYPEPTIDE SYNTHESIS	16
7. THE EFFECT OF ETHANOL ON T44(λ) <i>In Vivo</i> AND <i>In Vitro</i>	19
8. THE EFFECT OF RIBOSOMAL ALTERATION ON THE PHENOTYPE OF T44(λ)	23
9. 'REVERTANTS' TO TEMPERATURE RESISTANCE	28
10. THE EFFECT OF YEAST EXTRACT (YE) GUANOSINE AND CYTIDINE (GC) AND ETHANOL UPON T44(λ) AND ITS Spc-R AND Ts ⁺ DERIVATIVES	30
11. MODIFICATION OF THE Ts ⁺ PHENOTYPE BY INTRODUCTION OF A <i>spc^r</i> MUTATION	36
12. DERIVATIVES OF T44(λ) WITH UNUSUAL PHENOTYPES ('S' STRAINS)	38
13. TRANSDUCTION EXPERIMENTS	39
14. PROTEIN SYNTHESIS IN THE 'S' STRAINS	43
15. DISCUSSION	49
REFERENCES	54
ACKNOWLEDGEMENTS	59

SUMMARY

This study deals with the pleiotropic nature of the *tif-1* (temperature induced filaments) mutation in *Escherichia coli* K-12 T44(λ). Results of *in vivo* and *in vitro* experiments show that the expression of this mutation involves ribosomal function. *In vivo*, growth at 37° in the presence of adenine, which leads to partial expression of *tif-1*, increased the tolerance of streptomycin (Sm) by the culture. Growth on Sm enabled the cells to grow subsequently at the non-permissive temperature of 42°. Thermoinduction of λ could be prevented by addition of ethanol to the defined growth medium. Sm and ethanol are known to act at the ribosomal level, and impairment of ribosomal function after growth at high temperature was demonstrated by *in vitro* experiments. The level of incorporation of amino acids in an *in vitro* polypeptide-synthesising system was reduced when the culture had been grown at 37°, particularly in the presence of adenine, with both natural and synthetic messenger RNA. In addition there was a marked decrease in the misincorporation of isoleucine with Polyuridilic acid as messenger, which could be corrected by the addition of Sm to the *in vitro* system.

Introduction of a ribosomal mutation giving rise to spectinomycin resistance (Spc-R) rendered the strain less sensitive to high temperature, but each mutation to Spc-R affected the phenotype of the cell differently. *tif-1* is expressed only in defined medium. The addition of yeast extract to

the medium in small amounts (0.05%) prevented λ induction and in large amounts (0.5%) enabled the cells to grow normally at 42° in the presence of adenine. The presence of guanosine and cytidine (GC) protected the culture from lysis, but did not suppress the division defect of T44(λ) at high temperature.

Temperature-resistant (Ts^+) strains isolated were shown not to be true revertants. Their Ts^+ phenotype was suppressed by factors (ethanol, YE and GC) which enable T44(λ) to grow at high temperature, and by the introduction of a mutation to Spc-R.

Several derivatives of T44(λ) which showed an unusual capacity for simultaneous suppression of auxotrophic mutations were isolated. Some of them also displayed multiple resistance to antibiotics. Protein synthesis in the latter group was drastically impaired, indicating that this suppressor mutation(s) may also be involved with ribosomal function. Preliminary genetic analysis has tentatively located the mutation at or near the *recA* region.

The results presented may throw some light on the mode of action of *recA*, since *tif-1* is a mutation within this gene, and point to the possibility that the translation process has a role in regulatory functions controlled by the *recA* gene.

1. INTRODUCTION

The *tif* mutation (temperature induced filaments) was first described by Goldthwait & Jacob [1] in a temperature-sensitive mutant of *Escherichia coli* K12 (C600(λ)). It was found that induction of inducible prophages occurred when the growth temperature was raised to 40°, and that non-lysogens could not divide, but formed filaments [2]. The expression of these phenotypes was potentiated by the addition of adenine to the medium, and inhibited by the addition of guanosine, cytidine or pantooyl lactone [1, 2].

The effects of the *tif* mutation in the cell at high temperature are similar to those of UV-irradiation, causing filamentation, or induction of prophage in lysogens, induction of protein X [3], a protein thought to be associated with cell division [4, 5] and shown recently to be coded for by the *recA* gene [6], the induction or activation of DNA repair enzymes [7], and an increase in mutator activity [8, 9]. The only way in which *tif*-mediated phenomena differed from those observed after UV-irradiation was that no damage to the DNA or changes in the nucleotide pool were observed in cells carrying the *tif*-1 mutation [2].

The influence of growth medium on the expression of Tif phenotypes was found in the early studies on Tif cultures [2], and Castellazzi *et al.* found that *tif*-1 is poorly expressed in rich medium [7]. Early in the present study, a great heterogeneity was observed among colonies of T44(λ), particularly when the strain was plated on Luria agar. This observation suggested that the strain had either a high

mutation rate, even at 30°, or a degree of phenotypic instability that had not previously been described. The investigation that was undertaken showed that phenotypic instability is a feature of the behaviour of T44(λ) and evidence will be presented which suggests that impairment of ribosomal function is responsible for this and other Tif phenomena.

2. MATERIALS AND METHODS

(a) Bacterial Strains

The strains used in this study were C600 (*thi*, *thr*, *leu*) and its *tif*-1 derivative T44(λ), KL163 (Hfr, *recA*), CAJ64 *Thy*⁻ (*lac*, *thy*, *sup*), and JM1 (*thr*, *leu*, *arg*, *pro*, *his*, *rpsL*). KL163 was used to construct a non-lysogenic *tif*-1 strain, S44, by P₁kc transduction of the *recA* region from T44(λ).

(b) Media and Growth Conditions

Minimal medium M9 [10] supplemented with 1 μ g/ml thiamine and containing 0.4% glucose (Min B₁) was supplemented with 20 μ g/ml each L-threonine and L-leucine (MinTL) when minimal medium was required. 0.2% vitamin-free casamino acids (Difco) was usually added to the minimal medium, and the glucose content reduced to 0.2% (MinCA). Supplementation with adenine (MinCA-adenine) or guanosine (MinCA-guanosine) was 100 μ g/ml unless otherwise stated. Luria medium [10] was the rich medium used. Yeast extract (Difco) was used at between 0.05% and 0.5% to supplement MinCA-

adenine in various experiments. Solid media were the same as above with 1.5% Difco agar.

For growth of P_1kc for transductions, Luria agar plates supplemented with 0.002 M $CaCl_2$ were used as bottom agar, and R-top agar [10] as the top layer agar.

All strains were maintained on MinCA-guanosine agar.

(c) Experimental Method for Quantitative Analysis of Phenotype

A cell suspension was made in phosphate buffer pH 7.0 [10] to about 10^7 cells/ml. Ten- or one hundred-fold dilutions were made in phosphate buffer, and 10 μ l drops placed on plates. The number of colonies able to grow under different conditions could then be determined as a fraction of the total viable count.

(d) *In vitro* Polypeptide Synthesising System

Cell extracts (S-30) were prepared from mid-log cultures harvested at 4° and washed twice in a buffer containing 10 mM Tris-acetate pH 8.2, 14 mM Mg-acetate, 60 mM KCl and 1 mM dithiothreitol (DTT) (buffer A). The washed cells were stored in liquid nitrogen, and thawed at 4° for S-30 preparation. They were mixed 1:1 weight:volume with buffer B (as buffer A, but with 60 mM K-acetate instead of KCl) and disrupted by passage twice through a French pressure cell at 18,000 psi. Additional DTT (10 μ M to 8 g cells) was added after the second passage through the cell. The extract was spun twice at 30,000 g for 30' at 4° to remove cell debris, and the extract preincubated at 37° for 80'. The preincubation mixture contained (for 8 g cells): 800 μ M

disodium adenosine triphosphate (ATP), 70 mM phospho-enol-pyruvate (PEP) pH 7.0, 900 mM of each amino acid, 10 μ M DTT and 24 μ g pyruvate kinase (PK). After preincubation, the S-30 was dialysed against 4 x 1 ℓ . of buffer B overnight at 4°, spun for 15' at 4° at 30,000 g (with another 10 minute spin if the extract was cloudy) and stored in 100 μ l aliquots in liquid nitrogen. S-100 fractions were prepared by spinning the S-30 fraction, before preincubation, at 160,000 g for 2 hrs. The supernatant fraction, excluding the ribosomal pellet its overlying layer, was dialysed and stored as for S-30 extracts.

For use, the S-30 extract was thawed on ice and used immediately. 50 μ l incubation mixtures contained 2 mM Tris-acetate pH 8.2, 50 mM Tris-HCl pH 7.8, 75 mM NH_4Cl , 12 mM K-acetate, 5 mM PEP, 0.15 μ g PK, 25 μ M unlabelled amino acids (AA) (all 20 AA were included when only one amino acid was labelled; when a mixture of labelled AA was used, these were omitted from the cold mixture), 0.025 μ Ci ^{14}C -AA mixture (14AA mixture, Amersham) or 0.0125-0.125 μ Ci of a single ^{14}C -labelled amino acid (Amersham), 10 μ g of Polyuridilic acid (PolyU Boehringer) or 50 μ g of MS2 m-RNA, 10 μ l of S-30 preparation and between 6 and 24 mM Mg-acetate. Other additions are noted in the legends to Figures. The MS2 m-RNA was prepared from purified MS2 bacteriophage by phenol extraction as the K^+ salt and stored in 10 mM Tris-HCl pH 7.6 at 5 mg/ml in liquid nitrogen. The incubations were carried out at 34° for 30' and 20 μ l samples processed as follows: they were allowed

to air dry on to filter paper, washed with 10% trichloroacetic acid (TCA) at 0°C for 30', twice with 5% TCA at 0°, for 10', once with 5% TCA at 90° for 15', twice with 5% TCA at room temperature for 5', once with a 1:1 mixture of ether:ethanol at 37° for 10', once with ether at 37° for 10', and air-dried. The precipitated radioactivity was counted in 2.5 ml of a toluene-PPO scintillation fluid (toluene + 2 g/l 2,5-diphenyloxazole) in a Beckman LS250 scintillation counter for 10'. (This cell-free polypeptide synthesising system is modified after Modolell, 1971 [11]).

(e) Isolation of Thermoresistant and Spectinomycin-Resistant Strains

Spontaneously arising thermoresistant (Ts^+) strains were isolated from MinTL plates spread with approximately 10^8 cells from independent cultures and incubated for 3 days at 42°. Spectinomycin-resistant (Spc-R) strains were isolated from MinCA plates containing 100 or 200 µg/ml spectinomycin sulphate (Spc) spread with approximately 10^8 cells and incubated for 2 days at 30°.

(f) Transduction Procedure

Recipient cells were grown overnight at 30° in MinCA, spun down and resuspended in MC buffer (0.1 M $MgSO_4$ + 0.005 M $CaCl_2$ [9]). 1 ml aliquots of cell suspension were incubated with 0.01 or 0.1 ml P_1kc or P_1cml (10^9 - 10^{10} plaque forming units/ml) which had been grown on the donor strain. After 20 minutes' incubation at 30° or 37° (depending upon the thermo-sensitivity of the recipient) 0.2 ml 1M Na-citrate was added. Samples were plated on selective medium immediately or after dilution with Luria broth (1:6) and

growth for 90'-3 hrs. Colonies were picked after 48 hours' incubation and retested on the selective medium before use.

(g) Assay of Thermal Stability of β -galactosidase

Cells were grown to mid-log phase in MinCA at 30° and incubated at 30° for 30 minutes in the presence of 10^{-3} M IPTG (isopropyl thiogalactoside). The cells were washed and resuspended in Minimal Medium A + B₁, and 0.2 ml chloroform and 2 mls Z buffer [10] added to 20 mls of cell suspension. Open flasks were shaken gently at 30° for 20 minutes to allow the chloroform to evaporate. Cell debris was spun down and the supernatant divided into 1 ml aliquots. Duplicate 1 ml samples were placed for various times in a waterbath at 57.5°, cooled by immersion in a waterbath at 20° for 1 minute, and 0.2 ml of a 4 mg/ml solution of ONPG (o-nitrophenyl- β -D-galactopyranoside) in Minimal Medium A + B₁ was added. Tubes were allowed to stand overnight at room temperature, 0.5 ml 1M Na₂CO₃ was added, and the optical density at 420 nm measured.

Abbreviations

Streptomycin sulphate - Sm

Spectinomycin sulphate - Spc

Chloramphenicol - Cml

Ultraviolet light - UV

Resistant/Sensitive to ultraviolet light - UV-R/UV-S

Thiamine (vitamin B₁) - B₁

Thermosensitive - Ts

Thermoresistant - Ts⁺

Streptomycin- or spectinomycin-resistant - Sm-R or Spc-R

Streptomycin-sensitive/dependent - Sm-S/Sm-D

MS2 viral RNA - MS2 m-RNA

Polyuridilic acid - PolyU

Yeast extract - YE

Guanosine + cytidine, 100 μ g/ml each (unless otherwise stated) - GC

3. THE EFFECT OF GROWTH CONDITIONS UPON THE TOLERANCE
OF STREPTOMYCIN (Sm) BY T44(λ)

T44(λ) is genetically a streptomycin-sensitive strain, but a small proportion of cells in a normal culture (about 0.01%) can form colonies on plates containing 5 μ g/ml Sm, about ten times that found in C600. The fraction of cells which could tolerate Sm increased by a factor of between 100 and 1000 when the cells were cultured overnight in MinCA-adenine at 37°. This fraction was further increased when the culture was allowed to stand at room temperature for several days. When *E. coli* C600 was similarly treated, there was no change in the proportion of cells able to form colonies on plates containing 5 μ g/ml Sm. Table I shows the increase in tolerance of Sm with time in a culture of T44(λ) standing in MinCA-adenine and the contrasting decrease when the culture was left standing in MinCA-guanosine. (See also plate 1).

4. THE EFFECT OF GROWTH ON Sm ON THE Tif PHENOTYPE

Single colonies growing on MinTL Sm5 were tested for expression of the Ts phenotype. It was found that they were able to grow to titre on MinCA-adenine at 42°, but at the same time lost their tolerance of Sm. The cells returned to their usual Ts phenotype on further transfer. The physiological changes occurring in the cells are transient, but of sufficient duration for single cells to form colonies under restrictive conditions (at 42°).

A more unusual occurrence was the ability of cells to

TABLE I

The Effect of Adenine and Guanosine During Growth
and in the Stationary Phase on the Tolerance of Sm
by T44(λ)

Culture medium	Plate	Day 0		Day 1		Day 2		Day 3		Day 6	
		a	b	a	b	a	b	a	b	a	b
MinCA-adenine	Min TL	$5 \cdot 10^7$		10^9		10^9		10^9		10^9	
	Min TL Sm5		$4 \cdot 10^{-3}$		10^{-2}		10^{-1}		10^{-1}		10^{-1}
		$2 \cdot 10^5$		10^7		¹ 10^8		¹ 10^8		10^8	
MinCA-guanosine	Min TL	$2 \cdot 10^8$		10^9		10^9		10^9		10^9	
	Min TL Sm5		$4.5 \cdot 10^{-3}$		10^{-4}		10^{-6}		10^{-6}		0
		$9 \cdot 10^5$		10^{5^1}		10^{3^1}		10^{3^1}		0	

Columns a give the viable count (on MinTL) and number of colonies able to grow on MinTL + 5 μ g/ml Sm (MinTL Sm5). Columns b give the proportion of viable cells able to form colonies on MinTL Sm5.

¹ Small colonies, mucoid.

Plate 1. Cultures were grown in MinCA-adenine (T44 + Ad, C600 + Ad) or MinCA-guanosine for 18 hours at 37° and allowed to stand at room temperature for two days. 0.01 ml of one hundred-fold dilutions were plated on MinTL Sm5 and the plate incubated at 30° for 48 hours. Viable counts of all cultures were comparable ($2 \cdot 10^9$ /ml).

form colonies on media containing 100 μ g/ml Sm or on minimal medium with B₁ lacking the required amino acids threonine and leucine. These colonies, like those which frequently appeared on MinTL Sm5, were phenotypic in most cases, and here, too, growth on Sm enabled the cells to grow subsequently at 42°. In one experiment colonies were isolated after 6 hrs' growth in MinCA-adenine on MinB₁ or MinTL Sm100. (In experiments where these cells arose, there were about 4 or 5 colonies formed for every 10⁷ cells plated on selective media. Both Sm-tolerant and threonine-, leucine-independent cells arose in the same culture and in similar numbers. Further testing showed that all cells were phenotypically both Sm-R and prototrophic.) The cells grown on MinCA Sm100 in some cases temporarily became Sm-dependent. Growth on minimal medium did not have a suppressive effect on thermosensitivity, showing that this effect was due to growth in the presence of Sm.

The antibiotic Sm is known to interact with the S12 protein of the 30S ribosomal subunit [12, 13]. The fact that growth on Sm enabled cells of T44(λ) to form colonies at 42° suggested that this suppression might be connected with changes in ribosomal function. By analogy, it also suggested that high temperature (37°) and adenine might act upon the ribosomes to increase tolerance of Sm. Sm is known to increase translational ambiguity during protein synthesis [14] and this can be detected by *in vitro* studies. Therefore, the pattern of translation in T44(λ) under various

growth conditions was investigated.

5. POLYPEPTIDE SYNTHESIS IN AN *IN VITRO* SYSTEM

Crude cell extracts (S-30) were prepared (see Materials and Methods) from cultures of T44(λ) and their ability to incorporate amino acids into polypeptides when directed by natural or synthetic messenger RNA was examined. Figure 1 shows the levels of incorporation of a mixture of amino acids directed by MS2 m-RNA, using S-30 extracts from T44(λ) cultures grown at 30° or 37°. The incorporation of amino acids by the 37° extract was markedly less efficient than by the 30° extract, and this level was further reduced when adenine was present either during growth, before preparation of the S-30 extract, or in the *in vitro* system during incubation. The fact that addition of adenine *in vitro* gave the same result as its presence *in vivo* indicates that adenine need not undergo any metabolic change to exert its effect, and suggests that it directly affects the translation process. There was no adverse effect upon amino acid incorporation when adenine was added to S-30 extracts from cells grown at 30° (not shown). At this temperature adenine has little effect *in vivo* on the expression of Tif phenotypes.

In the *in vitro* system using the synthetic messenger PolyU, incorporation of phenylalanine and misincorporation of isoleucine were measured. As can be seen in Figure 2, the 30° extract was significantly more efficient in incorporation of phenylalanine than the extracts from cultures

¹ Incorporation directed by MS2 m-RNA using S-30 from C600 grown at 30° and 37° with adenine are also shown.

Figure 1. Incorporation of amino acids directed by MS2 m-RNA as a function of Mg^{++} concentration

Incubations were carried out as described in Materials and Methods.

Symbols: S-30 from T44(λ) grown in MinCA at 30° (○—○), in MinCA at 37° (●—●), in MinCA-adenine at 37° (▲—▲), in MinCA at 37° with 100 μ g/ml of adenine present during the *in vitro* incubation (□—□); S-30 from C600 grown in MinCA at 30° (■—■), in MinCA-adenine at 37° (▼—▼). S-30 extracts contained about 5-6 mg/ml of ribosomes.

Figure 2. Incorporation of phenylalanine directed by PolyU as a function of Mg^{++} concentration.

Incubations were carried out as described in Materials and Methods using $0.0125 \mu Ci$ ^{14}C -phenylalanine (225 mCi/mmol).

Symbols: S-30 from T44(λ) grown in MinCA at 30° (o—o), in MinCA at 37° (●—●), in MinCA-adenine at 37° (▲—▲), in MinCA at 37° with $100 \mu g/ml$ of adenine present during the *in vitro* incorporation (□----□). S-30 extracts were the

same as those used in Figure 1. Incorporation of phenylalanine by extracts of C600 grown at 30° in MinCA or MinCA-adenine at 37° was identical.

grown at 37°. (The level of activity of the 30° extract was similar to that of extracts of C600 cells grown at either 30° or 37°, not shown). In addition, in most experiments the 37° extract required a higher Mg^{++} concentration for maximal incorporation than the 30° extract. The presence of adenine during growth at 37° reduced the incorporation of phenylalanine. In normal bacterial cells, there is a very small degree of misincorporation of wrong amino acids (translational ambiguity) during protein synthesis [15, 16]. Measurement of the level of misincorporation of isoleucine with PolyU showed that there was considerably less misincorporation using an extract from T44(λ) (or a non-lysogenic Tif strain, S44, Figure 4) cells grown at 37° than that observed in the 30° extracts.¹ This level was further reduced when adenine was present during growth at 37° or *in vitro*, and abolished when adenine was present both during growth at 37° and in the *in vitro* incubation (Figure 3).

These results indicate that the *tif*-1 mutation at elevated temperature may interfere directly with the translation process, and that this effect is amplified by the presence of adenine.

6. THE EFFECT OF Sm ON *IN VITRO* POLYPEPTIDE SYNTHESIS

Since Sm increases translational ambiguity *in vivo* and *in vitro* [14, 15] it was of interest to test its effect in the *in vitro* system with Tif extracts where misincorporation of isoleucine was shown to be very low.

¹This is not the case in the wild-type strain C600 (Figure 3).

Figure 3. Misincorporation of isoleucine directed by PolyU as a function of Mg⁺⁺ concentration

Incubations were carried out as described in Materials and Methods using 0.0125 μ Ci of 14 C isoleucine (225 mCi/mmol).

Symbols: S-30 from T44(λ) grown in MinCA at 30° (○—○), in MinCA at 37° (●—●), in MinCA-adenine at 37° (▲—▲), in MinCA at 37° 100 μ g/ml of adenine present during the *in vitro* incubation (□---□), in MinCA-adenine at 37° with 100 μ g/ml of adenine present during the *in vitro* incubation (▲---▲); S-30 from C600 grown in MinCA at 30° (■—■), in MinCA-adenine at 37° (▼—▼). S-30 extracts were the same as those used in Figure 1.

Figure 4. Incorporation of phenylalanine and misincorporation of isoleucine directed by PolyU as a function of Mg^{++} concentration using S-30 from S44.

Incubations were carried out as described using 0.0125 μ Ci of either ^{14}C -phenylalanine or ^{14}C -isoleucine.

Symbols: S-30 from S44 grown in MinCA at 30°, phenylalanine (o—o), isoleucine (Δ — Δ), grown in MinCA at 37°, phenylalanine ($\bullet$ — $\bullet$), isoleucine ($\blacktriangle$ — $\blacktriangle$).

Table II shows that the addition of Sm to the extract of T44(λ) cells which had been grown in MinCA-adenine at 37° raised the level of misincorporation to that of the 30° extract. This result indicated that the increased restriction imposed by growth at 37° with adenine, which can be relieved by Sm, is probably due to alterations in ribosomal function. Addition of Sm to the 30° extract increased misincorporation of isoleucine with PolyU, but the increase was not as dramatic as with the 37° extract, being similar to that reported for other strains [14, 15, 17].

The experiments described indicate that growth at high temperature particularly in the presence of adenine may modify ribosomal function in Tif strains. It was therefore of interest to examine whether factors which, like Sm, are known to affect ribosomal function, could alter *tif* expression.

7. THE EFFECT OF ETHANOL ON T44(λ) *IN VIVO* AND *IN VITRO*

Ethanol, like Sm, is known to increase translational ambiguity *in vivo* and *in vitro* [18, 19]. Figure 5a shows that the presence of 2% ethanol prevented induction of λ in MinCA-adenine at 40°. It can be seen that ethanol had to be present at 40° to be effective; pre-growth in ethanol delayed, but did not prevent lysis. Figure 5b shows that while T44(λ) rapidly lost viability at 40° in MinCA-adenine, in the presence of ethanol the culture doubled twice. The division defect of *tif* is not suppressed by ethanol, and on

TABLE II

The Effect of Sm on Incorporation of Phenylalanine and Misincorporation of Isoleucine Directed by PolyU

Concentration of Sm ($\mu\text{g/ml}$)	S-30 from T44(λ) grown in MinCA at 30°			S-30 from T44(λ) grown in MinCA-adenine at 37°		
	phe (cpm $\times 10^2$)	ile (cpm $\times 10^2$)	phe/ile	phe (cpm $\times 10^2$)	ile (cpm $\times 10^2$)	phe/ile
0	320	22	14.6	240	0.5	480
0.5	320	60	5.4	240	68	3.2
0.1	300	80	3.8	210	79	2.7
0.2	260	134	1.9	190	72	2.6

The incubation mixtures contained 15 mM Mg^{++} , and 0.125 μCi of either ^{14}C -phenylalanine or ^{14}C -isoleucine. Both S-30 extracts contained about 6 mg/ml of ribosomes.

The effect of ethanol on growth and viability of T44(λ)
in MinCA-adenine at 40°

Cells were grown to log phase in MinCA-guanosine at 30° with or without 2% ethanol before transfer to 40° in MinCA-adenine with or without 2% ethanol.

- a. Growth curves at 40°: no ethanol added (●——●),
ethanol present during growth at 30° (○——○),
ethanol present during growth at 40° (▲——▲),
ethanol present throughout growth (△——△)
- b. Viable count at 40°: no ethanol added (●——●),
ethanol present during growth at 40° (○——○).

The growth of C600 (λ) in the presence of ethanol at 40° is normal, reaching an OD of 120, and viable count of $1.5 \cdot 10^9$ cells/ml in 240 minutes.

Figure 5a

Figure 5b

solid media there was no growth of single colonies at 42° when 2, 4, or 6% ethanol was added to MinCA-adenine or MinTL plates. In the *in vitro* polypeptide synthesising system, ethanol approximately doubled the misincorporation of isoleucine with PolyU as messenger irrespective of the growth conditions used to prepare the S-30 extract (not shown). Both ethanol and Sm increase translational ambiguity, but although ethanol prevented thermoinduction, it did not alter the cells' Ts phenotype in the same way as did growth on Sm. (Pre-growth on ethanol did not allow subsequent growth at 42°, not shown). Thus it seems likely that the suppressive effects of Sm and ethanol are not due simply to their ability to increase translational ambiguity. Induction of λ due to treatment with mitomycin C could not be reversed by ethanol, indicating that ethanol does not interfere with the normal induction process, nor with phage maturation.

8. THE EFFECT OF RIBOSOMAL ALTERATION ON THE PHENOTYPE OF T44(λ)

In view of the evidence that factors which act at the ribosomal level (namely Sm and ethanol) affected the phenotype of T44(λ) it was relevant to examine the effect of mutations in ribosomal proteins on *tif* expression. Alterations in ribosomal proteins can affect the normal translation process by altering the degree of translational ambiguity [20, 21, 22]. The *spcA* (*rps E*) gene codes for the 30S

ribosomal protein S5, which is the site of resistance to spectinomycin (Spc) [13]. Mutations in the *rpsE* gene can relieve the translational restriction imposed by mutations in the *strA* (*rpsL*) gene which codes for the S12 protein, the site of resistance to Sm [23, 13]. Since translational restriction appears to be one aspect of the Tif phenotype as it is of the Sm-R phenotype [15] the effect of the introduction of a change in protein S5 on *tif* expression was examined. The phenotypes of ten mutants resistant to 100-150 µg/ml Spc and one mutant resistant to 200 µg/ml Spc were studied.

Figure 6a shows the growth curves of Spc-R mutants of T44(λ) in MinCA-adenine at 40°. It can be seen that all the mutants isolated on 100 µg/ml Spc lyse later or to a lesser degree than T44(λ). Strain Spc^r(211) which is resistant to 200 µg/ml Spc was more sensitive to thermo-induction than the parent strain. This indicated that different mutations in the *rpsE* gene affect *tif* expression differently. Figure 6b shows that the viable count corresponds closely to the growth curves. The effect of ethanol upon the Spc-R mutants *in vivo* is similar to its effect on T44(λ), preventing lysis completely.

In vitro studies with two Spc-R mutants (one shown in Figure 7) showed that using extracts from cells grown at 37°, the level of misincorporation of isoleucine with PolyU as messenger was similar to that found when using extracts of T44(λ) grown at 30°. With MS2 m-RNA, there was about 10% less amino acid incorporation using the Spc-R S-30 than

Growth and Viability of Spc-R Mutants of T44(λ) in
MinCA-adenine at 40°

Cells were grown to log phase in MinCA-guanosine before transfer to 40° in MinCA-adenine. The effect of addition of 2% ethanol during growth at 40° is shown (Δ ----- Δ), and its presence similarly prevented lysis in the other strains. All Spc-R strains are resistant to 100-150 μ g/ml Spc except Spc^r(211) which is resistant to 200 μ g/ml Spc.

- a. Growth curves at 40°.
- b. Viable count at 40°.

Figure 6a

Figure 6b

Figure 7. Misincorporation of isoleucine directed by PolyU as a function of Mg^{++} using extracts of Spc-R and Ts^+ derivatives of T44(λ).

Incubations were carried out as described using 0.0125 μ Ci ^{14}C -isoleucine (225 mCi/mmol).

Symbols: S-30 from T44(λ) grown in MinCA at 30° (○—○), from T44 grown in MinCA at 37° (●—●), from a Spc-R strain grown in MinCA at 37° (■—■), from a Ts^+ strain grown in MinCA at 37° (□—□).

using the T44(λ) 30° extract (not shown).

All the Spc-R mutants resistant to 100 μ g/ml Spc suppress filamentation to some extent. The quantitative determination of this suppression in different Spc-R mutants was done as described in Materials and Methods. The absolute fraction of cells able to form colonies at 42° varied slightly in different experiments, but the pattern was reproducible. In Table III the first three columns show the effect of 42° alone and of a combination of 42° and adenine on the colony-forming ability of the Spc-R strains and T44(λ). All strains except Spc^r(211) are more resistant to the effects of temperature and adenine than T44(λ). It can be seen that different *spc*^r mutations (resistance to 100 μ g/ml Spc) suppress *tif* expression to different degrees.

The *in vitro* and *in vivo* results show that by altering the structure of a ribosomal protein involved in the translational machinery, some Tif phenotypes, namely λ induction, filamentation and translational restriction, can be suppressed. The degree of suppression depends upon the mutation involved, all of which presumably cause different alterations in the S5 protein.

9. 'REVERTANTS' TO TEMPERATURE RESISTANCE

Thermoresistant (Ts⁺) mutants of T44(λ) were selected on MinTL at 42° (MinCA-adenine tends to give a high background of phenotypic colonies). Colonies thus isolated which grew to titre when re-tested on MinTL and MinCA-adenine at 42° were considered true Ts⁺ strains. None of

TABLE III

The Colony-forming Ability of Spc-R Mutants of T44(λ) at 42°; the Suppressive Effect of YE and GC on the Ts phenotype

Strain	MinTL 30°	MinCA 42°	MinCAA ¹ 42°	MinCAA + 0.05% YE 42°	MinCAA + GC 42°	λ induction in MinCAA at 40°
T44(λ)	1.0	$8 \cdot 10^{-3}$	$1 \cdot 10^{-4^2}$	$3 \cdot 10^{-4}$	$2 \cdot 10^{-3}$	++ ³
Spc ^r (3)	1.0	$2 \cdot 10^{-3}$	$3 \cdot 10^{-5}$	$2 \cdot 10^{-2}$	$5 \cdot 10^{-4}$	-
Spc ^r (12)	1.0	$1 \cdot 10^{-3}$	$1 \cdot 10^{-5}$	$1 \cdot 10^{-4}$	$5 \cdot 10^{-5}$	+
Spc ^r (21)	1.0	0.1	$7 \cdot 10^{-4}$	$7 \cdot 10^{-2}$	$3 \cdot 10^{-3}$	$\pm$
Spc ^r (22)	1.0	0.1	$1 \cdot 10^{-4}$	$2 \cdot 10^{-3}$	0.7	-
Spc ^r (24)	1.0	1.0	$1 \cdot 10^{-2}$	$1 \cdot 10^{-2}$	0.3	-
Spc ^r (25)	1.0	1.0	$1 \cdot 10^{-2}$	0.5	0.1	$\pm$
Spc ^r (26)	1.0	$1 \cdot 10^{-3}$	$1 \cdot 10^{-4}$	0.7	0.1	-
Spc ^r (27)	1.0	$2 \cdot 10^{-3}$	$2 \cdot 10^{-4}$	1.0	1.0	-
Spc ^r (28)	1.0	0.2	$4 \cdot 10^{-4}$	$3 \cdot 10^{-2}$	$3 \cdot 10^{-2}$	++
Spc ^r (211)	1.0	0	0	$4 \cdot 10^{-5}$	$3 \cdot 10^{-5}$	++

¹ - MinCA-adenine

² - On MinCA-adenine at 42°, T44(λ) is able to form minute colonies only (see plate 2), while the other strains form colonies of normal size.

³ - ++, complete lysis; +, partial lysis; $\pm$, trace of lysis; -, no lysis.

the Ts^+ strains showed signs of lysis at 40° in MinCA-adenine, although all strains produced some λ . Ts^+ mutants isolated by other workers [7, 24] fall into at least two categories: UV-sensitive ($Rec A^-$, $Lex A^-$, $Lex B^-$ and Zab^-) and UV-resistant (considered by them to be true revertants). The former category was found to comprise about 30% of 'revertants'. A similar proportion was found among Ts^+ mutants in this study. Of the eight mutants studied extensively, three are UV-sensitive but not wholly recombination deficient. This means that none are $Rec A^-$ and are probably $Lex A^-$, $Lex B^-$ or Zab^- [7, 24].

10. THE EFFECT OF YEAST EXTRACT (YE), GUANOSINE AND CYTIDINE (GC) AND ETHANOL UPON T44(λ) AND ITS Spc-R and Ts^+ DERIVATIVES

It has been noted that growth in rich medium suppresses *tif* expression [7]. During this study the component of rich medium responsible for this suppression was identified as yeast extract (YE). Figure 8 shows the effect of the addition of various amounts of YE to cultures of T44(λ) in MinCA-adenine at 40° . The cultures were grown to log phase in MinCA at 30° before transfer to 40° in the stated media. The viable counts at the end of the experiment are shown in brackets. The curves show that small amounts of YE (0.05%) can protect against lysis but that cell division is still inhibited. Addition of 0.5% YE allowed normal growth at 42° . The nature of the active factor in YE has not been elucidated, but some components have been ruled out. The vitamins contained

Figure 3. The effect of yeast extract (YE) and guanosine + cytidine (GC) on the growth and viability of T44(λ) in MinCA-adenine at 40°

Cells were grown to log phase in MinCA at 30° before transfer to 40° in MinCA. YE and GC were present at 40° in the amounts stated. The viable counts at the end of the experiment are shown in brackets. The effect of addition of the vitamins found in YE (according to Difco analysis) to a concentration equal to that found in 0.5% YE is also shown.

in YE (according to Difco analysis) singly or in combination had no effect on the growth of T44(λ). Nor is it the GC content of YE since adding 100 $\mu\text{g/ml}$ GC to the MinCA-adenine had only a little suppressive effect and this could not be enhanced by increasing the concentration to 150 $\mu\text{g/ml}$. However, there seems to be some interaction between GC and another component of YE, as a slight improvement could be made by adding GC to 0.1% YE. The molecule(s) involved is dialysable, and addition of dialysate had the same effect as addition of whole YE (not shown).

Table III shows the effect of YE and GC on T44(λ) and Spc-R mutants on solid medium at 42°. The addition of one of these factors suppressed filamentation to some degree in T44(λ) and strain Spc^r(211) (a mutant resistant to 200 $\mu\text{g/ml}$ Spc) and much more markedly in strains resistant to 100-150 $\mu\text{g/ml}$ Spc. Some Spc-R mutants were affected more by YE than by GC and vice versa, suggesting that the mode of action of these two factors may be different. Table IV shows that the presence of YE or GC or a combination of both (see plates 2 and 3) had an inhibitory effect on the Ts⁺ strains at 42°, the degree of which varied with the strain. Ethanol also inhibited the growth of these strains at 42°. These factors have no inhibitory effect on the colony-forming ability of the Ts⁺ strains at 30°, nor on the growth of C600(λ) at either temperature. The suppression of the Ts⁺ character by ethanol, YE and GC indicates that the Ts⁺ 'revertants' are still harbouring the *tif*-1 mutation and are not true revertants. The fact that the Ts⁺ character is suppressed

TABLE IV

The Colony-forming Ability of Ts⁺ 'Revertants' of T44(λ) at 42°; the Effect of YE, GC and Ethanol on the Expression of the Ts⁺ Phenotype

Strain	MinTL 30°	MinCAA ¹ or MinTL 42°	MinCAA + 0.05% YE 42°	MinCAA + GC 42°	MinCAA + GC + 0.05% YE 42°	MinCAA + 2% ethanol 42°	λ induction in MinCAA at 40°
ts ⁺ 7	1.0	1.0	$6 \cdot 10^{-4}$	$1 \cdot 10^{-2}$	$8 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	+ ³
ts ⁺ 9	1.0	0.5	$3 \cdot 10^{-4}$	0.1	$1 \cdot 10^{-3}$	$2 \cdot 10^{-4}$	+
ts ⁺ 10	1.0	1.0	0.3	$3 \cdot 10^{-2}$	$1 \cdot 10^{-3}$	$5 \cdot 10^{-3}$	-
ts ⁺ 11	1.0	1.0	$1 \cdot 10^{-2}$	$1 \cdot 10^{-2}$	$5 \cdot 10^{-3}$	$4 \cdot 10^{-3}$	-
ts ⁺ 12 ²	1.0	1.0	0.5	$2 \cdot 10^{-2}$	$2 \cdot 10^{-4}$	$2 \cdot 10^{-2}$	-
ts ⁺ 13 ²	1.0	1.0	1.0	0.1	$2 \cdot 10^{-4}$	0.5	+
ts ⁺ 14	1.0	1.0	1.0	$3 \cdot 10^{-3}$	$4 \cdot 10^{-4}$	$4 \cdot 10^{-3}$	-
ts ⁺ 15 ²	1.0	1.0	$3 \cdot 10^{-4}$	$3 \cdot 10^{-2}$	$7 \cdot 10^{-3}$	$5 \cdot 10^{-2}$	+
C600(λ)	1.0	1.0	1.0	1.0	1.0	1.0	-

¹ - MinCA-adenine

² - UV-sensitive

³ - See Table III

Plate 2.

The appearance of colonies of T44(λ) and Ts⁺ strains grown for 3 days at 42° on a MinCA-adenine plate.

Plate 3.

The same cell suspensions grown for 3 days at 42° on a MinCA-adenine plate containing 0.05% YE and 100 μ g/ml each of guanosine and cytidine. (For plating procedure see Materials and Methods).

Plate 2

Plate 3

by factors which enable the Ts strains to grow at 42° shows that their effect is not a nutritional one.

11. MODIFICATION OF THE Ts⁺ PHENOTYPE BY INTRODUCTION OF A *spc^r* MUTATION

It has been demonstrated that a mutation in one protein of the 30S ribosomal subunit can suppress the effect of a mutation in another 30S protein [22, 23, 25, 26, 27]. Furthermore, introduction of a third ribosomal mutation can reverse this suppression, again allowing expression of the original phenotype [28]. The suppression of *tif* by mutation to Spc-R may be an analogous phenomenon; so too may be the suppression in the Ts⁺ strains. If this were the case, then introduction of a third factor involving ribosomal function (such as Spc resistance) into the Ts⁺ strains might alter their phenotype.

To test this, all the Ts⁺ strains listed (except ts⁺13 which could not be transduced to Spc-R) were transduced to Spc-R using Spc^r(211) (resistant to 200 µg/ml Spc) as the donor. Other Spc-R strains resistant to only 100-150 µg/ml Spc could not be used as donors because selection for transductants was complicated by background growth. Table V shows the effect of the newly-introduced Spc-R mutation on the Ts⁺ phenotype. It can be seen that suppression of filamentation was lost and the cells could not form colonies at 42°, although thermoinduction was still suppressed. The two UV-sensitive strains were less affected by the presence of the *spc^r* mutation than the UV-

TABLE V

The Colony-forming Ability of Spc-resistant Ts⁺ 'Revertants' of T44(λ); the Effect of the *spc*^r Mutation from Spc^r (211) on the Expression of the Ts⁺ Phenotype

Strain	MinTL 30°	MinCAA ¹ 42°	MinTL 42°	λ-induction in MinCAA at 40°
ts ⁺ 7 (Spc ^r 211)	1.0	0	0	- ³
ts ⁺ 9 (Spc ^r 211)	1.0	0	0	-
ts ⁺ 10 (Spc ^r 211)	1.0	5.10 ⁻⁴	0	-
ts ⁺ 11 (Spc ^r 211)	1.0	3.10 ⁻⁴	0	-
ts ⁺ 12 (Spc ^r 211) ²	1.0	1.10 ⁻³	0.7 ⁴	-
ts ⁺ 14 (Spc ^r 211)	1.0	2.10 ⁻⁴	0	-
ts ⁺ 15 (Spc ^r 211) ²	1.0	1.10 ⁻³	0.8 ⁴	-
Spc ^r (211)	1.0	0	0	++

1 - MinCA-adenine

2 - UV-S

3 - See Table III

4 - These strains were able to form colonies almost to titre at 42° on MinTL after 3-4 days' incubation.

resistant strains. The presence of adenine seems necessary for the reversal of suppression in the UV-sensitive strains since they were capable of forming single colonies almost to titre in MinTL at 42°. The mode of suppression of the Ts phenotype by the UV-sensitive strains may thus be different from that of the UV-resistant strains. The interference of a ribosomal mutation with the suppression of *tif* by another mutation (expressed as a Ts⁺ phenotype), particularly in the presence of adenine, implicates the ribosome as the site of action of both adenine and the Ts⁺ suppressor mutations. *In vitro* studies showed that the degree of misincorporation by extracts from cultures grown at 37° was similar to that observed using S-30 from T44(λ) grown at 30° or from a Spc-R mutant grown at 37° (Figure 7).

12. DERIVATIVES OF T44(λ) WITH UNUSUAL PHENOTYPES ('S' STRAINS)

During the work described on T44(λ), several unusual derivatives were isolated. In an experiment similar to that described in section 4, colonies were isolated which were able to grow without threonine, leucine and thiamine, on 100 µg/ml Sm or Spc, and were resistant to infection by lysogenic or virulent bacteriophage. The level of Sm tolerance varied between 20 µg/ml and 100 µg/ml depending upon the physiological state of the culture, but the Spc resistance was stable. As with resistance to Sm, the temperature resistance was variable. Although lysis was observed at 40° in MinCA-adenine, or after UV-irradiation, no plaque-forming units of λ were produced. A number of

derivatives showing all or some of these characteristics were independently isolated, as can be seen in Table VI. (In all, 11 derivatives were isolated). All the characteristics of each strain appeared simultaneously when the strain was isolated, regardless of the medium upon which the isolation was made; this makes it likely that at least some of the characteristics are due to a single mutational event. Some of the strains show resistance to other drugs associated with the ribosome in addition to Sm and Spc, for instance neomycin and chloramphenicol (data not shown).

An attempt was made to ascertain whether the ability to grow without nutritional requirements or to resist antibiotic drugs was due to a mutator effect or to a form of suppression. This was done by transduction. If the loss of nutritional requirements, or the resistance to antibiotics were due not to mutations in the respective genes, but to a single mutation, then some or all of these characters should be transferrable in a single transduction. Since none of the strains showing all of the unusual phenotypes was able to plate P_1 cml or P_1 kc, similar derivatives of T44(λ) (P_1 cml) were isolated, and one, C8(P_1 cml), whose phenotype is shown in Table VI, was used as the donor for the transduction experiments.

13. TRANSDUCTION EXPERIMENTS

In Table VII it can be seen that the transfer of the simultaneous suppression of several nutritional markers

TABLE VI

The Correlation between Aspects of the 'S' Phenotype and Stability of β -galactosidase at 57.5°

Parent Strain	Derivative Strains	β -galactosidase ¹	Ability to plate P_1 kc or P_1 cml ²	Resistance to Sm^3	Resistance to Spc^4
T44(λ)	C1, C3, C4, C5	Thermosensitive	-	+	+
T44(λ) (P_1 cml)	C6 (P_1 cml)	As T44(λ)	+	+	-
	C8 (P_1 cml)	As T44(λ)	+	-	+
T44(λ)	TR1	None detectable	-	+	+
JM1	SC18	As T44(λ)	+	-	-
KL163	SC9	None detectable	-	+	+

1 - See Figure 9.

2 - +, ability, -, inability to form plaque-forming units of P_1 kc or P_1 cml.

3 - +, resistance to up to 100 μ g/ml Sm (fluctuating with culture conditions); -, resistance to <20 μ g/ml Sm .

4 - +, resistance to 100 μ g/ml Spc ; -, resistance to <50 μ g/ml Spc .

All the strains listed are protrophic.

TABLE VII

Transduction Experiments with Derivative Strains of T44(λ)

Donor	Donor phenotype	Transducing phage	Recipient	Recipient genotype	Selection medium	Transductant phenotype	Name of Transductant
C8(λ) (P ₁ cml)	Thr ⁺ , Leu ⁺	P ₁ cml	C600	<i>thr</i> , <i>leu</i> , <i>thi</i>	Min B ₁	Thr ⁺ , Leu ⁺	SC8
C8(λ) (P ₁ cml)	Thr ⁺ , Leu ⁺	"	JM1	<i>thr</i> , <i>leu</i> , <i>pro</i> , <i>arg</i> , <i>his</i> , <i>str</i> ^r	Min B ₁	Thr ⁺ , Leu ⁺ , Pro ⁺ , Arg ⁺ , His ⁺ , Sm-S	SC18
SC8	Thr ⁺ , Leu ⁺	P ₁ kc	KL163	<i>recA</i>	Luria + 0.5 μ g/ml mitomycin C	Sm-R, Spc-R, RecA ⁺ , Ts	SC9

could be carried out by transduction. Using C8(P₁cml) as the DNA donor, and C600 as the recipient, Thr⁺, Leu⁺ colonies were obtained by selection on MinB₁. With Jm1 as recipient, it was possible to obtain colonies independent of five nutritional requirements whose genetic sites are distributed around the chromosome. At the same time, resistance to Sm was lost. The transductant strains, SC8 and SC18 respectively, grew more slowly on MinB₁ than on amino acid-supplemented medium. (Single colonies took 48 hrs to appear.)

An attempt was made to find out whether the mutation responsible for the unusual suppression was located at or near the *recA* gene, within which the *tif-1* mutation has been mapped [7, 24]. SC8 was used as the donor to transduce KL163 (*recA*⁻) to RecA⁺. The transductant, SC9, became partially Ts, forming some filaments at 42° (like *tif-1* strains) and acquired resistance to 100 µg/ml of Sm and Spc, a characteristic of some of the 'S' strains. By introduction of the *recA* region from SC8 it was thus possible to obtain a strain with an 'S' phenotype, although it is not clear why the drug resistance was not expressed in SC8. It was not possible to use SC9 as the donor for further transduction experiments as it would not plate P₁cml or P₁kc.

In order to show that the prototrophy transferred into C600 and Jm1 in these experiments was due to suppression and not to a mutator effect, the following experiment was carried out: Resistance to sodium azide is closely linked

to the *leu* locus [12]. An azide-resistant strain of SC8 was isolated and this resistance was transferred into a *leu*⁺ strain (CAJ64 Thy⁻) by transduction. Of 34 transductants tested, 28 required leucine, thus demonstrating that the Leu⁻ character had been cotransduced with the azide resistance from SC8. This shows that SC8 is still genotypically *leu*⁻ and that the suppressor mutation is not cotransducible with azide resistance.

In one of several spontaneous Spc-R mutants of SC18, resistance to Sm was regained, suggesting that the loss of resistance to Sm in SC18 was probably not due to a change in the *rpsL* gene. This Spc-R strain also grew as fast on minimal agar as on supplemented medium, indicating that alteration of the S5 ribosomal protein had had an effect on the suppression of nutritional requirements as well as on the expression of Sm resistance.

14. PROTEIN SYNTHESIS IN THE 'S' STRAINS

In view of the unusual suppressive capacity observed in the 'S' strains, it was of interest to study their translation pattern. The ability to suppress mutations could involve the incorporation of wrong amino acids during protein synthesis, thus producing faulty proteins. This can be tested by examining the thermolability of an enzyme since one containing incorrect amino acids may more rapidly lose activity at high temperature than the wild-type protein [29, 30]. Figure 9 shows the thermolability of the β -galactosidase produced by some of the 'S' strains at

Figure 9. The effect of incubation at 57.5° upon the β-galactosidase produced by T44(λ) and its 'S' derivatives

Duplicate 1 ml cell extracts of log-phase cells which had been induced to produce β-galactosidase as described in Materials and Methods were heated for various times at 57.5°, the activity of the enzyme measured and plotted as a % of the activity of the unheated extract.

57.5° measured as described in Materials and Methods. It can be seen that there is a very rapid inactivation of the enzyme from some of the 'S' strains, and this correlates with their concomitant resistance to streptomycin, spectinomycin, and resistance to infection with P_1 phage (see Table VI). The amount of β -galactosidase produced by some derivative strains is larger than that produced by T44(λ), although the protein is evidently faulty, as shown by its high rate of thermal inactivation, but in some 'S' strains, no β -galactosidase activity could be detected after induction with IPTG (strains TR1 and SC9). In spite of their production of faulty proteins, some of the 'S' strains grow faster than T44(λ), as shown in Figure 10, and reach a higher optical density and viable count (5.0^9 cells/ml for C1, $1-2.10^9$ cells/ml for T44(λ)).

In vitro polypeptide synthesis was studied using S-30 extracts from C1 cultures grown at 30°. It can be seen in Figures 11 and 12 that the level of amino acid incorporation, using either MS2 or PolyU as messenger, was much lower than found using extracts of T44(λ) grown at 30°, and there was no misincorporation of isoleucine with PolyU (not shown).

It can be seen that the incorporation of amino acids directed by MS2 m-RNA was improved by addition of S-100 from either source, while only the S-100 from T44(λ) significantly improved the incorporation of phenylalanine with PolyU as messenger. Addition of S-100 did not

Figure 10. The growth rate of T44(λ) and two 'S' derivatives in MinCA at 30°

A fresh overnight culture grown on a MinCA-guanosine plate was used to inoculate 10 mls of MinCA in 250 ml erlenmeyer flasks. Curves represent optical density measurements.

Figure 11. The effect of addition of S-100 on the incorporation of ^{14}C -phenylalanine directed by PolyU *in vitro* using an S-30 extract of Cl.

Incubations were carried out as described in Materials and Methods using $0.0125 \mu\text{Ci } ^{14}\text{C}$ -phenylalanine. Symbols S-30 from Cl (o—o) and T44(λ) ($\blacksquare$ — $\blacksquare$) grown in MinCA at 30° ; the same Cl S-30 with the addition of $10 \mu\text{l}$ of S-100 from Cl ($\bullet$ — $\bullet$) or T44(λ) ($\square$ — $\square$) grown in MinCA at 30° .

Figure 12. The effect of addition of S-100 on the incorporation of amino acids directed by MS2 m-RNA using an S-30 extract of Cl

Incubations were carried out as described in Materials and Methods using $0.025 \mu\text{Ci } ^{14}\text{C}$ -amino acid mixture.

Symbols: S-30 from Cl (o—o) and T44(λ) (■—■) grown in MinCA at 30° ; the same Cl S-30 with the addition of $10 \mu\text{l}$ of S-100 from Cl (●—●) or T44(λ) (□—□) grown in MinCA at 30° .

increase the misincorporation of isoleucine with PolyU as messenger (not shown). These observations indicate that in the 'S' strains there is a component(s) of the S-100 fraction which is either faulty or reduced in quantity. However, the lack of translational ambiguity even in the presence of normal S-100 may mean that the structure of the ribosome itself has been altered.

15. DISCUSSION

Evidence has been presented that expression of the *tif*-1 mutation can interfere with ribosomal function. It has been shown that the presence of Sm or ethanol, both of which are known to increase translational ambiguity [15, 18, 19] can alter the phenotype of a T44(λ) culture. Growth on Sm enabled the culture to grow subsequently at the non-permissive temperature of 42°. This indicated that growth in the presence of Sm can alter the cells' physiology for many generations after the drug has been removed. The prospective effect of Sm may be due to its irreversible binding to the ribosomes [17] as it will remain in the cell after removal of the drug from the medium. The expression of the *tif* mutation is temporarily suppressed, enabling the cell to grow at high temperature in the presence of adenine. In contrast, ethanol has to be present to suppress thermoinduction, and it cannot suppress filamentation. Thus these factors affect *tif* expression differently although both are presumably acting at the translational level. *In vitro* studies supported the idea that growth at

high temperature affects ribosomal function, showing that the pattern of protein synthesis in T44(λ) was altered by growth at 37°: translation of MS2 and PolyU was impaired and there was a reduction in translational ambiguity as measured by the misincorporation of isoleucine with PolyU as messenger RNA.

Adenine, a most potent promoter of *tif* expression, has been shown to act at the ribosomal level by reducing the degree of translational ambiguity. This could partially account for the suppressive effects of ethanol at high temperature in the presence of adenine since it counteracts translation restriction [18]. Translational restriction imposed by growth in MinCA-adenine at 37° might similarly account for the increased tolerance of Sm observed. However, the data indicate that the low level of translational ambiguity seen at high temperature in T44(λ) is only one aspect of the impairment in ribosomal function; although both Sm and ethanol increase translational ambiguity, they do not suppress Tif phenotypes in the same manner, as is the case for the highly-restricted Sm-D strains in which Sm and ethanol enable the cells to grow equally well [18].

The expression of a ribosomal mutation can be suppressed by a mutation in a different ribosomal protein. This has been demonstrated by several workers [22, 26, 27], for proteins in the 30S subunit. Mutations in the S5 ribosomal protein can mask streptomycin-resistance or -dependence which are due to mutations in the S12 protein [25, 26, 27]. The introduction of a mutation in S5 (Spc-R) into T44(λ)

partially suppressed *tif*, substantiating the other observations that *tif* expression is involved with ribosomal function. The growth of the Spc-R strains at high temperature in the presence of adenine was greatly improved by the addition of YE or GC to the medium.

The suppression of thermoinduction and filamentation in T44(λ) requires the presence of a very high concentration of YE (0.5%), and since YE and GC have an adverse effect upon the Ts⁺ strains at 42°, as does ethanol, it can be assumed that the suppression is not nutritional. There may be some interaction between a minor component of YE and a ribosomal factor which counteracts the effects of *tif*, although no effect of YE or GC was observed *in vitro* (not shown).

The Ts⁺ strains described here are not true revertants but rather carry suppressor mutations. This is known to be so in UV-S 'revertants' [7, 24] but has also been demonstrated here for the UV-R strains. The Ts⁺ character is abolished when the culture is grown in the presence of ethanol or YE and GC, or when a Spc-R mutation is introduced. It has been shown that in a Sm-D strain suppressed by a mutation in S5 the introduction of a third ribosomal mutation, in the S4 protein, can restore the original Sm-D phenotype [28]. The suppression of *tif* in the Ts⁺ strains may involve similar interactions among ribosomal subunits, an idea which is supported by the reversal of the Ts⁺ phenotype by the Spc-R mutation.

Several of the known suppressors of *tif* reside in the *recA* gene [7, 24]. The mode of action of the *recA* gene

(within which *tif*-1 maps [24]) is not yet understood, but it was shown recently [31] that *recA* mutants show very little translational ambiguity *in vitro*, indicating the involvement of the ribosome in *recA* function.

A current model for the role of the *recA* gene product has been reviewed by Witkin [32]. It is proposed that a *recA-tif* complex is activated as a result of DNA damage or *tif* expression and that this activated complex removes the *lexA*⁺ product from 'SOS' repressors, thus allowing proteases to cleave the repressors and induce the 'SOS' functions. The cleavage of λ repressor has been shown to occur during *tif* expression [33] and protease inhibitors can block *tif* expression [34]. The data presented, showing that the ribosome is involved with *tif* expression, suggest that a change in the pattern of translation could be affecting the synthesis of regulatory molecules involved with the expression of 'SOS' functions, probably at a stage prior to the proteolytic cleavage of repressors.

The changes brought about by the suppressor mutation(s) in the 'S' strains described in sections 12, 13 and 14 also appear to involve ribosomal function. The suppression of auxotrophic markers is probably translational; the thermal sensitivity of the β -galactosidase activity, the simultaneous appearance of resistance to several drugs, and the low level of amino acid incorporation *in vitro* all point to an alteration in ribosomal function. However, the fact that addition of S-100 improved *in vitro* translation may mean that the major fault does not reside in the ribosome itself.

Transduction experiments suggest that the mutation lies at or near the *recA* region and not in the ribosomal region, so the effect of this mutation must be via soluble factors. The behaviour of the 'S' strains presents some paradoxes: in spite of the fact that the proteins synthesised by some of these strains are faulty (the β -galactosidase activity is thermolabile), they can grow faster than T44(λ); the ability to suppress auxotrophic mutations does not correlate with the lack of translational ambiguity displayed. It must be assumed that this is a novel mutation whose activity cannot be understood in terms of the present knowledge of ribosomal function.

* * *

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Ephrati-Elizur, E., Luther-Davies, S., Hayes, W.: Phenotypic instability in a *tif-1* mutant of *Escherichia coli*. I. Impairment in ribosomal function. Molec. gen. Genet. 147, 59-65 (1975).

Ephrati-Elizur, E., Luther-Davies, S.: Phenotypic instability in a *tif-1* mutant of *Escherichia coli*. III. Suppression of thermoinduction and filamentation. Molec. gen. Genet. 155, 163-169 (1977).

A third manuscript is in preparation for submission to the same journal:

Ephrati-Elizur, E., Luther-Davies, S.: Phenotypic instability in a *tif-1* mutant of *Escherichia coli*. IV. An unusual suppressive capacity in derivatives of T44(λ).

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