Recessive Lethal Nonsense Suppressor in *Escherichia coli* which inserts Glutamine

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A new amber suppressor which can exist only in *su*$^+$/*su*$^-$ heterozygotes inserts glutamine in response to the UAG triplet.

CERTAIN suppressor mutations in bacteria give rise to altered transfer RNA molecules which can translate the normally chain terminating amber and ochre codons as amino-acids. Recently we described a new class of suppressor mutations in *E. coli*[1]. These suppressors are unique because they cannot be isolated in haploid cells; however, they are readily recovered in F'14 merodiploids which also contain the corresponding *su*$^-$ allele. One of our recessive lethal amber suppressors, *su7*, is much more efficient (76 per cent) than any other known amber suppressor. We have now identified the amino-acid inserted by *su7* as glutamine, by analysis of the α-subunit of tryptophan synthetase (α-Ts'ase) isolated from a strain containing both an amber mutation at the forty-eighth codon of the Ts-ase A gene and the suppressor mutation, *su7*.

The mutant *trpA88* has a UAG codon in place of a glutamic acid codon (presumably GAG) at position 48 from the amino terminus[2]. We constructed the suppressed strain LS409 (W3110 R$^-$*trp* *trpA88 his29 ilva 1*/*su7 ilva*$^+$) from LS268 (W3110 R$^-$*trp* *trpA9605 his29 ilva 1*) using P1 phage transduction to exchange the *trp* alleles and to introduce *su7* (ref. 1). LS409 is phenotypically Trp$^-$ because the amino-acid inserted by *su7* does not restore enzymatic activity to the α-subunit; *su7* cannot therefore insert glutamic acid.

The suppressed protein can be assayed because the α-CRM complements the β-subunit of Ts'ase in the conversion of indole to tryptophan. To obtain the α-CRM produced by suppression, cultures were grown to a limit on indole (5 μg/ml.) in minimal medium containing 0·5 per cent glucose[3] and the protein purified[4]. The final product had a specific activity of 4,000 units/mg as measured by the rate of conversion of indole to tryptophan in the presence of an excess of the β-protein; the corresponding activity of purified wild-type α-Ts'ase is 4,700 (ref. 4).

Fig. 1 shows the mobility on polyacrylamide gel electrophoresis of the protein isolated from *trpA88 su7* (gel *a*) and from *trpA*$^+$ (gel *b*), and a mixture of the two (gel *c*). Clearly the wild-type subunit is more anionic than the suppressed protein; because glutamic acid is the wild-type amino-acid at position 48, the amino-acid inserted by suppression must be either uncharged or positively charged. The mutant *trpA11* is a missense mutant of α-Ts'ase in which glutamine replaces glutamic acid at position 48 (ref. 2). The gel electrophoresis pattern of a partially purified preparation of the *trpA11* (provided by Dr C. Yanofsky) (gel *d*), shows a major band which is less anionic than the *trpA*$^+$ protein (see also the mixture in gel *f*), but the *trpA11* protein has the same mobility as the *su7* suppressed protein (see gel *e* for the mixture of the *trpA11* and *trpA88 su7* proteins). We conclude that *su7* promotes insertion of a neutral amino-acid for the amber codon.

Previously[1], we compared the suppression pattern of *su7* with the known amber suppressors *su1* (serine), *su2*

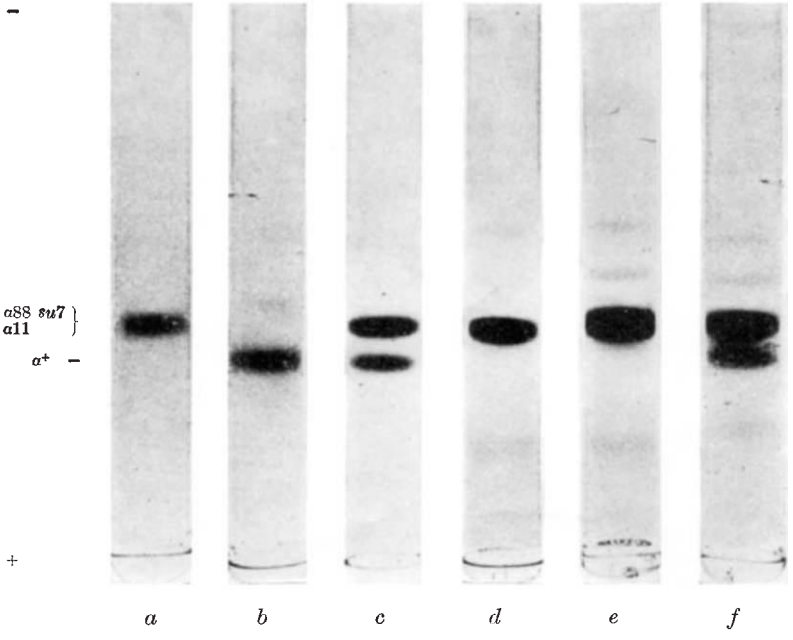

Fig. 1. *a*, *a88 su7*; *b*, *a*$^+$; *c*, *a*$^+$ and *a88 su7*; *d*, *a11*; *e*, *a11* and *a88 su7*; *f*, *a11* and *a*$^+$. Electrophoresis was performed according to Ornstein[17] in a *tris*-glycinate buffer system (resolving gel *p*H 9·6) using approximately 25 μg of each protein per gel.

(glutamine), *su3* (tyrosine), *su6* (leucine)[5] and the two ochre suppressors *su4* (tyrosine) and *su5* (lysine) on a collection of phage nonsense mutants. The pattern obtained with *su7* was readily distinguishable from all but *su2* which inserts glutamine[6]. The only difference between the suppression patterns of *su2* and *su7* was observed with a phage having the nonsense mutation in a structural protein (λ sus J27) and can be ascribed to the higher efficiency of suppression of *su7*. This result suggests that the neutral amino-acid inserted by *su7* is probably not tyrosine, serine or leucine, but it could well be glutamine.

To obtain more direct evidence on the amino-acid inserted by *su7*, the α-protein, isolated from *trpA88 su7* was digested with trypsin to yield the tryptic peptide containing the forty-eighth amino-acid (TP15). Twenty mg of purified *α88 su7* protein (the preparation shown in gel *a* of Fig. 1) was denatured (100° C, 5 min), the insoluble protein was incubated in 2 ml. of 6 M urea containing 0·3 N $NaHCO_3$, and after the solution was diluted with 4 ml. of water, 2·5 mg of trypsin was added. After 2·5 h at 29° C, the mixture was filtered over a column of 'Sephadex *G*-25' (2·5 cm × 100 cm) in 0·1 N NH_4OH. TP15 is the largest tryptic cleavage product and appears in the first ultraviolet absorbing peak[7]. These fractions were pooled and chromatographed on a 'Dowex-1-*X*2' column using the stream splitting accessory of the Beckman automatic amino-acid analyser to locate the eluted peptides[8]. TP15, which was identified by its amino-acid composition, was further purified by filtration over a 'Sephadex *G*-25' column as described.

Table 1. AMINO-ACID COMPOSITION OF TP15 FROM WILD-TYPE AND MUTANT α-TS'ASE ACID HYDROLYSIS

Amino-acids	TP15 from *α88 su7*	*α*+[7]	*α11*[2]
Lysine	0·50	—	0·60
Arginine	0·88	1·00	0·91
Aspartic acid	4·32	5·00	4·80
Threonine	2·46	2·70	2·60
Serine	1·00	1·06	1·37
Glutamic acid	*3·36*	*3·06*	*3·21*
Proline	3·19	3·01	3·38
Glycine	2·86	3·07	3·00
Alanine	4·72	5·10	5·10
Valine	0·74		0·75
Isoleucine	3·47	4·15	3·60
Leucine	4·75	4·75	4·75
Tyrosine	0·34	—	—
Phenylalanine	1·43	0·97	1·07

Table 1 shows the amino-acid analysis (determined with a Beckman automatic amino-acid analyser after hydrolysis in 6 N HCl for 18 h *in vacuo*) of TP15 isolated from the *α88 su7* protein, along with published values for the α+ and *α11* subunits. Allowing for possible differences in the purity of the TP15 peptide preparations, the amino-acid analysis of the three peptides is indistinguishable. In particular, they contain the same number of glutamic acid residues: because we have already eliminated glutamic acid as the amino-acid inserted by *su7*, and because a substitution of glutamic acid by glutamine would not alter the amino-acid analysis (compare the analysis of TP15 from α+ which contains glutamic acid at position 48 and that of TP15 from *α11* which contains glutamine at the same position), this result indicates that *su7* inserts glutamine at position 48.

To confirm this assignment, TP15 from *α88 su7* and from α+ were enzymatically digested to yield free amino-acids and then analysed. Approximately 100 μg of each peptide was incubated with 100 μg of amino-peptidase M (Henley and Co., New York) and 2 μg of chymotrypsin in a final volume of 0·30 ml. of 0·05 M *tris* pH 7·4 at room temperature for 18 h. Digestion was at least 90 per cent complete. The free amino-acids were resolved in the lithium buffer system[9] which permits determination of glutamine and asparagine in addition to the other amino-acids. The values for the neutral and acidic amino-acids (Table 2) show that the tryptic peptide from *α88 su7* has 0·70 glutamic acid residues less and 1·12 glutamine residues more than α+; the amounts of the other amino-acids are only insignificantly different. This analysis confirms that glutamine is the amino-acid inserted at position 48 of the *α88 su7* subunit and therefore that *su7* causes the incorporation of glutamine for the amber codon.

Table 2. AMINO-ACID ANALYSIS OF AN ENZYMATIC DIGEST OF TP15 FROM WILD-TYPE AND *α88 su7* PROTEINS

Amino-acid	TP from *α88 su7*	TP from α+
Aspartic acid	3·45	3·68
Threonine	2·83	2·75
Serine	1·05	1·06
Asparagine	0·93	0·67
Glutamic acid	*1·48*	*2·18*
Glutamine	*2·04*	*0·92*
Proline	2·40	2·34
Glycine	2·96	2·92
Alanine	5·00	5·02
Isoleucine	4·60	5·19
Leucine	4·44	4·96
Tyrosine	0·35	0
Phenylalanine	1·45	1·29

The most widely accepted explanation of efficient suppression of the amber codon is that it results from creation of a new coding specificity of *t*RNAs by mutation. Transfer RNAs isolated from strains carrying *su1* (refs. 10 and 11), *su2* (ref. 12), *su3* (ref. 13) and *su6* (ref. 14) suppress the UAG codon *in vitro*. More to the point, Goodman *et al.* established that the *su3* mutation changes the anticodon of a $tRNA^{Tyr}$ thereby permitting it to translate UAG instead of its former codons UA^{U}_{C} (ref. 15). Clearly, this model of amber suppressor mutations demands that there be more than one *t*RNA species (or at least duplicate *t*RNA structural genes) for translating the original set of codons; and, if an organism has only a single *t*RNA species to translate a set of codons, any mutational modification which permits that *t*RNA to decode only UAG would be lethal.

Can that argument explain the recessive lethality of *su7*? We know that both *su7* and *su2* allow translation of UAG as glutamine; moreover, Primakoff and Cordes (unpublished results) have found that suppression by *su7* *in vitro* is mediated by *t*RNA. If we accept that the *su2* mutation changes the anticodon of a $tRNA^{Gln}$ it follows that there must still be a $tRNA^{Gln}$ able to translate the glutamine codon CAG; very likely the product of the wild-type *su7* gene could perform that function. But strains carrying *su7* should also be able to translate CAG with the *t*RNA coded for by the *su2*⁻ gene. If a deficiency of *t*RNA for decoding CAG is not the explanation, perhaps the lethality is the result of a quantitative difference in the amount of *t*RNA coded by *su2* and *su7*. Because *su7* is much more efficient than *su2*, the *su7* suppressor may be a major $tRNA^{Gln}$ species while the *su2* suppressor corresponds to a minor species which cannot translate CAG fast enough to support growth. A similar argument could be made if there were differences in the charging efficiency of these two $tRNA^{Gln}$ species. It is also possible that the *t*RNA coded for by the normal *su7* gene (*su7*⁻) has another biosynthetic or regulatory function in addition to its role in protein synthesis. In this case, it is the loss of this function *per se* and not the inability to translate the CAG codon which would explain the lethality.

Su7 is the second suppressor mutation shown to have lethal or deleterious effects on the host cell when present in the haploid state. A missense suppressor mutation, *su159*, which caused modification of a $tRNA^{Gly}$ (for decoding GG^{A}_{G}), and thereby permits insertion of glycine for the arginine codon, AGA, is lethal in enriched media if the organism does not also contain the *su159*⁻ gene[16]. And yet, in this case also, there is evidence for the existence of another gene, which can supply the wild-type form of the $tRNA^{Gly}$ for translating the GG^{A}_{G} codon (unpublished results of J. Carbon and C. W. Hill).

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Iron–Sulphur Proteins

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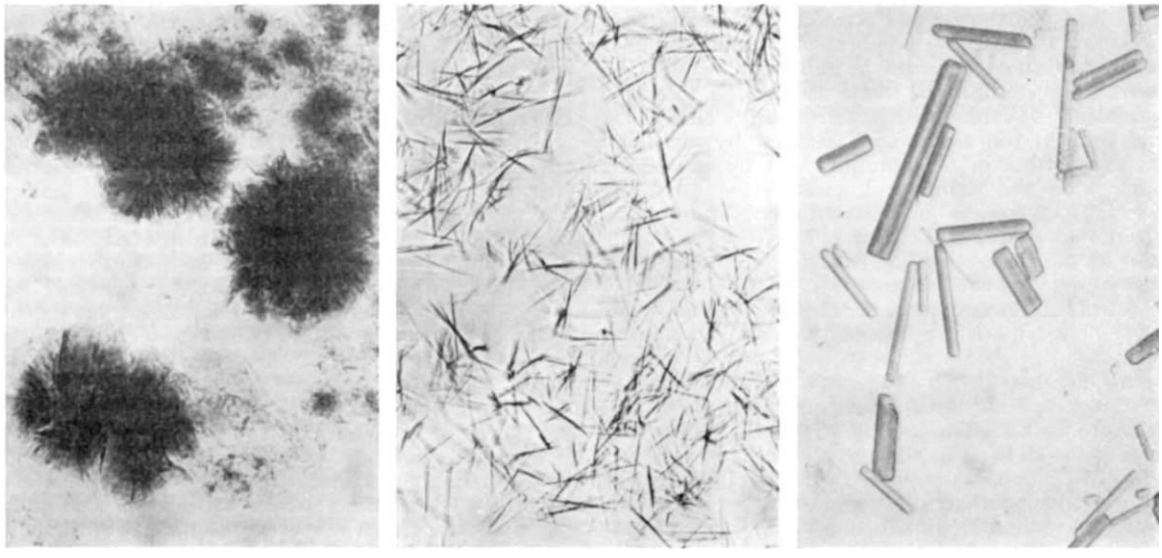
These electron transfer agents often have unusually low redox potentials. Eighteen are known from higher plants and bacteria, and are involved in nitrogen fixation as well as photosynthesis.

In the past year or so it has become apparent that iron-sulphur (Fe-S), or non-haem iron, proteins occur widely, in animals, plants and bacteria[1,2]. Their role in respiration, photosynthesis, nitrogen fixation, hormone synthesis and sulphur and carbon metabolism has been recognized as central. There are eighteen recognized Fe-S proteins which either occur uniquely in specific organisms or are widely distributed in various organisms. The number will undoubtedly increase as further searches are made with the correct analytical approaches.

Both Fe-S proteins and cytochromes are electron carriers and contain iron, but their active centres are entirely different. The Fe in cytochromes occurs in a haem environment while the Fe-S proteins have a non-haem Fe active centre with structure unknown, except that inorganic sulphur and possibly cysteine-sulphurs are involved in the iron binding. Unfortunately, in the case of Fe-S proteins we cannot use the classical destructive techniques of organic chemistry to investigate the structure of the active site and must rely largely on non-destructive physical techniques, which are not only more complex but also require relatively large amounts of material for analysis.

Properties of Fe-S Proteins

These proteins are electron carriers which have so far been recognized to have the following basic characteristics. (*a*) They contain non-haem iron and inorganic (acid-labile)



(*a*) (*b*) (*c*)

Fig. 1. Crystalline ferredoxins from (*a*) spinach, (*b*) *Nostoc* and (*c*) *Chromatium*[6,37].