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REVIEW ARTICLE

Informational suppression as a tool for the investigation of gene structure and function

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Introduction

Changes in the genetic material that generate nonsense, missense or frameshift mutations may be suppressed by second site lesions in unlinked genes. When partial or total restoration of the wild phenotype is achieved by alteration of the translational apparatus, the process is termed informational suppression. [Phenotypic suppression, on the other hand, refers to suppression by extracellular changes, for example, the presence of streptomycin in the growth medium; reviewed by Gorini (1974).]

Informational suppression functions primarily through changes in tRNA species (but see below). Thus, nonsense suppressor tRNAs carry, in general, mutant anticodons capable of base pairing with translation-terminating triplets (*sup9* is an exception, see Table 1). They, therefore, prevent premature termination of translation at internal chain-terminating triplets (Fig. 1). In missense suppression, the altered anticodon allows pairing of a tRNA with non-cognate triplets [e.g. $tRNA^{Gly}_{supT}$ inserts glycine residues at GAG (Glu) codons; reviewed by Hill (1975)]. Of those frameshift suppressor tRNAs sequenced, there is an additional ribonucleotide in the anticodon loop which restores, presumably, the correct reading-frame [reviewed by Roth (1974)].

This ability to perturb the course of gene expression allows both the analysis of the translation decoding mechanism and the creation of protein variants carrying known amino acid substitutions at defined sites. This review is concerned with such applications of nonsense suppression. Suppression has been previously reviewed by Garen (1968), Brammar (1969), Gorini (1970), Hartman & Roth (1973), Smith (1979) and Steege & Söll (1979) (prokaryotes), and by Hawthorne & Leupold (1974), Piper (1980), Olson *et al.* (1980, Kohli *et al.* (1980) and Sherman (1982) (eukaryotes).

Prokaryotic nonsense suppressors

Dissection of the translational machinery

tRNA biosynthesis and function. tRNA molecules are small RNA species consisting, on average, of about 76 ribonucleotides [see Gauss & Sprinzl (1981) for a compilation of tRNA sequences]. These stable RNAs are synthesized as precursors which are processed by specific RNases and base modifying enzymes [reviewed by Apirion & Gegenheimer (1981) and McCloskey & Nishimura (1977)] to yield the mature tRNA. Study of mutant tRNAs, in particular *supF* (an allele of a $tRNA^{Tyr}$ gene, *tyrT*), has greatly contributed to our current knowledge of tRNA gene organization and expression, since lesions that interfere with the production of nonsense suppressor tRNA lead to a suppressor-negative (Su^-) phenotype.

Secondary mutations which reduce suppressor activity were found to accumulate suppressor tRNA in its precursor form containing extra bases on the 5′ and 3′ side (Altman & Smith, 1971). Using such precursors as substrate, novel RNases involved in tRNA processing were identified. Moreover, the position of the lesions responsible for defects in processing allow an understanding of the process itself. Mutations which severely depress RNase P activity in the generation of the mature 5′ terminus are located in the amino acid acceptor stem, e.g. *supF* A2 [reviewed by Altman (1979)]. A base change which increases processing of *supF* A2 maps four bases upstream from the RNase P cleavage site (Smith *et al.*, 1971). Interestingly, this processed suppressor tRNA is temperature-sensitive for function and is mischarged with glutamine (see below). Both ribonucleotide sequence and conformation of the precursor molecule appear to govern the efficiency of processing.

The problem of aminoacyl-tRNA synthetase–tRNA recognition is of interest from two viewpoints: the mechanisms involved in maintaining accuracy in charging (Fersht, 1979; Hopfield & Yamane, 1980) and the protein–nucleic acid interactions involved. To date, there is no unifying model which explains synthetase–tRNA discrimination. Analysis of secondary mutations in *sup*$_c$ alleles which yield tRNA species with altered charging properties has been useful in determining the tRNA regions involved.

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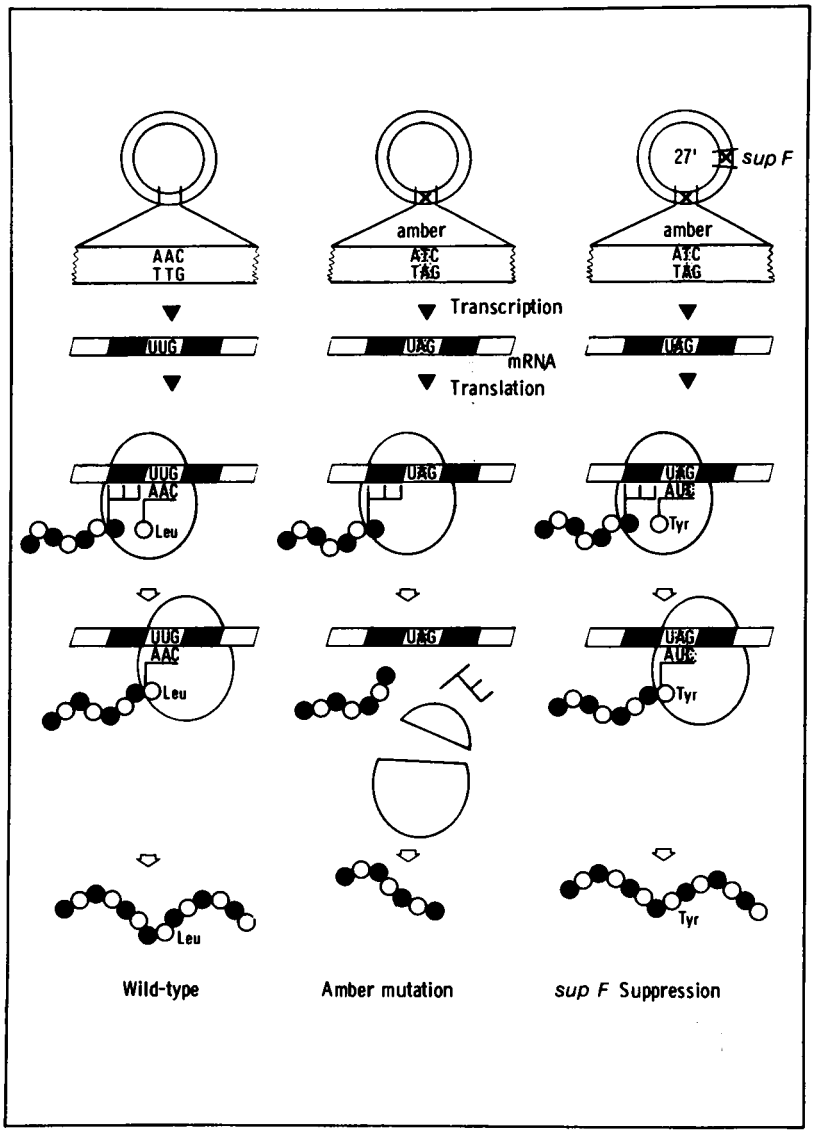

Fig. 1. *Schematic representation of the process of nonsense suppression*
Suppression of an amber nonsense codon (UAG) by *supF* is shown in the right-hand panel (the wild-type situation and the amber case alone are given in the left and centre panels); other nonsense suppressors are listed in Table 1. From Glass (1982) with permission of the publishers.

For example, mutations in the amino acid acceptor stem of *supF* (Celis *et al.*, 1973) and the anticodon stem of *supE* (Ozeki *et al.*, 1980) yield mischarged suppressor derivatives. Although these tRNAs have altered anticodons, the data imply that two distinct regions on a tRNA molecule are essential for correct recognition by glutamyl- and tyrosyl-tRNA synthetases. Some mischarged *supF* derivatives are also temperature sensitive for function, apparently due to disruption of the base pairing capacity of the amino acid acceptor stem [reviewed by Smith (1979)]. That the anticodon arm plays a role in the synthetase reaction is suppported by the nature of *supU* $tRNA^{Trp}$. Mischarging of $tRNA^{Trp}$, unlike the cases cited above, is achieved by a single base change in the anticodon; this mutant $tRNA^{Trp}$ species inserts glutamine (and tryptophan) residues at amber codons (Yaniv *et al.*, 1974). There is no

clear similarity between $tRNA^{Trp}$ and $tRNA^{Gln}$ that could explain the switch in tRNA recognition [but see Ivanov (1975)].

The aminoacyl group of a charged tRNA has been thought to be redundant during decoding of the mRNA. Recent evidence from studies on mutant $tRNA^{Trp}$ species suggests otherwise. Although the amber suppressor derivative is charged by glutamine and tryptophan at approximately equivalent levels, and the two charged species are equally stable *in vivo* (Knowlton *et al.*, 1980), glutamine is preferentially inserted at amber sites (Celis *et al.*, 1976). There, therefore, appears to be a discrimination mechanism against tryptophanyl-$tRNA^{Trp}_{UAG}$ which acts subsequent to tRNA charging, apparently after formation of the EF-Tu·GTP·aminoacyl-tRNA ternary complex (Knowlton & Yarus, 1980).

Decoding the message. That anticodon–codon interaction is not the only event in the maintenance of translational specificity is highlighted in the case of *sup9*, an allele of *trpT*. The mutant tRNA is able to read UGA codons but retains the wild-type anticodon of $tRNA^{Trp}$ (Hirsh, 1971). Instead, the

Table 1. *E. coli nonsense suppressors*
Abbreviation: n.k., not known

Suppressor*	Map location* (min)	tRNA	Codon(s) suppressed	Anticodon† Suppressor	Anticodon† Wild-type	Amino acid inserted‡	References
Amber							
supD	43	Ser	UAG	AUC	AGC	Ser	Stretton & Brenner, 1965; Weigert & Garen, 1965; D. A. Steege, personal communication
supE	15	Gln2 (*glnU*)	UAG	AUC	GUC	Gln	Kaplan *et al.*, 1965; Weigert *et al.*, 1965; Inokuchi *et al.*, 1979
supF	27	Tyr1 (*tyrT*)	UAG	AUC	AUQ	Tyr	Kaplan *et al.*, 1965; Weigert *et al.*, 1965; Goodman *et al.*, 1968
supP	96	n.k.	UAG	n.k.	n.k.	Leu	Chan & Garen, 1969; Arnardøttir *et al.*, 1980
supU	84	Trp (*trpT*)	UAG	AUC	ACC	Gln/Trp§	Yaniv *et al.*, 1974; Celis *et al.* 1976
Ochre							
supB	15	Gln1 (*glnV*)	UAA/G	AUN	GUN	Gln	Ozeki *et al.*, 1980
supC	27	Tyr1 (*tyrT*)	UAA/G	n.k.	AUQ	Tyr	Weigert *et al.*, 1967
supG	15	n.k.	UAA/G	n.k.	n.k.	Lys	Gallucci & Garen, 1966; Garen, 1968
supL	16	Lys (*lysT*)	UAA/G	AUN	UUN	Lys	Kaplan, 1971; Ozeki *et al.*, 1980
UGA							
sup9	84	Trp (*trpT*)	UGA	G-24→A‖		Trp	Chan & Garen, 1970; Hirsh, 1971

* A varied nomenclature has been applied to suppressor genes. In general, *sup* refers to the mutant suppressor allele and Su^+ indicates the resultant suppressor phenotype. A wild-type strain is phenotypicaly Su^-. Sul, 2, 3, 6, 7, B, C or 4, G or 5, *β* and 9 are the phenotypic symbols for the suppressors listed. Map locations and information on other less well characterized suppressors are listed by Bachmann & Low (1980).

† The anticodon base that is changed to allow pairing with a nonsense triplet is underlined. Although the anticodons of some suppressors have not been determined, they are expected to be complementary to the codons with which they interact. Note that anticodons are written 3′→5′ and codons 5′→3′. Q is a guanosine derivative and N is a modified uridine residue.

‡ Suppressor efficiency refers to the frequency of translational readthrough frequency past a nonsense codon. The mean absolute efficiency of *supD*, *supE*, *supF*, *supP* and *supU*, determined at two unique sites in *rpoB*, is 48%, 19%, 68%, 33% and 43%, respectively [Nene & Glass (1981); V. Nene & R. E. Glass, unpublished work]. Suppressor efficiencies as determined by other methods for the UGA and ochre suppressors indicate that the former is efficient while ochre suppressors are very inefficient [references cited in Garen (1968), Brammar (1969) and Gorini (1970)]. Note that efficiency is very much dependent upon reading context.

§ *supU* tRNA exhibits an ambivalent charging property. Both glutamine and tryptophan are inserted, albeit in the ratio 9:1 (Celis *et al.*, 1976).

‖ Although *sup9* harbours a mutation in the D stem of the tRNA, it is able to suppress UGA codons (Hirsch, 1971).

suppressor harbours a mutation in the dihydrouracil stem (Table 1). Presumably, alterations in the tRNA tertiary structure [the D arm interdigitates with the TΨC arm in tRNA folding; reviewed by Rich (1977)] create this ambivalent specificity. Wild-type $tRNA^{Trp}$ is also able to read UGA, albeit less efficiently (Hirsh & Gold, 1971). Genetic context (see later) greatly influences the efficiency of $tRNA^{Trp}$ misreading; the presence of an adenine to the 3′ side of UGA is correlated with an increase in translational readthrough frequency (Engelberg-Kulka, 1981).

Misreading of the genetic code is not limited to wobble (Crick, 1966) of the 5′ base of the anticodon. Analysis of the fidelity of nonsense suppressors provides evidence for misreading at the middle base: UGA is suppressed at low efficiency by ochre suppressors (Stringini & Brickman, 1973). [Misreading at the 3′ base is exemplified by the capacity of initiator $tRNA^{fMet}$ to function at AUG, GUG and UUG codons; see Steitz (1979) and Young *et al.* (1981)].

Mistranslation may also be effected by alterations in other components of the translational apparatus. For example, the UGA codon that terminates the ribosomal protein S7 message (Post & Nomura, 1980) is less efficient in the presence of certain mutations in *rpsD*, the structural gene for the S4 r-protein (Olson & Isaksson, 1980). Other *rpsD* mutations increase translational readthrough past both UGA and UAG triplets.

Base modification of tRNAs is essential for function. *supF* tRNA lacking a highly modified adenosine residue (2-methylthio-N^6-isopentyladenosine) 3′ adjacent to the anticodon is inactive in a translation system *in vitro* despite being correctly charged with tyrosine (Gefter & Russell, 1969): it is unable to bind to ribosomes. $tRNA^{Trp}$ carries the same adenosine derivative and exhibits a partial loss of function in mutant strains lacking a component of the modification process. Undermodified $tRNA^{Trp}_{UGA}$, however, remains functional (Eisenberg *et al.*, 1979; Buck & Griffiths, 1981). Base modification is essential in maintaining accuracy in decoding [reviewed by McCloskey & Nishimura (1977)]. For example, lack of a tRNA methylase has been reported to give rise to UGA suppression [cited in Bachmann & Low (1980)].

Protein structure–function relationships

Six different but known amino acids can be inserted at an amber site through the use of nonsense suppression (Fig. 2). Thus, six of the seven amino acids encoded at potential amber sites can be

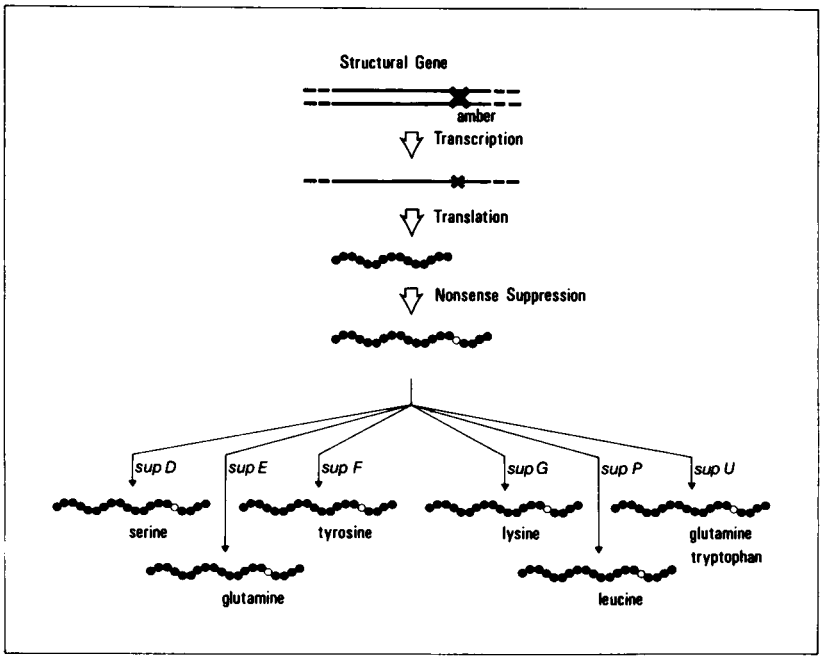

Fig. 2. *Insertion of six different but known amino acids at each amber site*
Nonsense mutants are isolated as strains exhibiting a conditional mutant phenotype. The lesions may be mapped by classical means or from the size of *N*-terminal fragments produced. Note that *supU* inserts both glutamine and tryptophan (see Table 1).

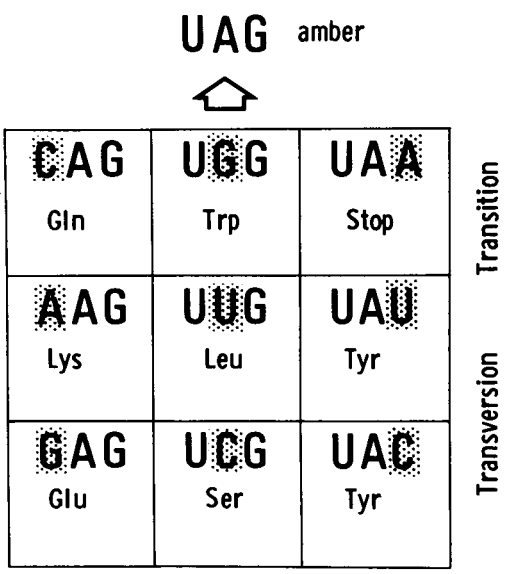

Fig. 3. *Sense codons that can mutate to amber triplets in a single base change*

Six of the seven amino acids encoded by amber mutable codons may be inserted by specific nonsense suppressors (Table 1). Note that not all mutational events necessarily occur at equal frequency (see the text). From Glass (1982) with permission of the publishers.

inserted (Fig. 3). (A glutamate-inserting suppressor has not as yet been obtained and, hence, some nonsense mutants are not suppressed by the available suppressors.) In this manner, it is possible both to re-create the wild-type polypeptide and to create novel protein variants, identical except for a particular set of substitutions at one site (Fig. 2). Tyrosine insertions are particularly useful since they allow spectral analysis of protein structure [see, for example, Sommer *et al.* (1976) and Jarema *et al.* (1981)]. In summary, the ability to isolate nonsense mutants, to locate accurately the site of these lesions and to use a battery of nonsense suppressors is a powerful tool in the investigation of protein structure–function relationships.

Certain cytoplasmic and exported proteins have been studied in some detail. Examples considered below include RNA polymerase (that is, the β subunit encoded by *rpoB*), *lac* repressor (*lacI* gene product), *lamB* receptor, *btuB* receptor and phage M13 coat protein (gene VIII product). Although not discussed, mention should be made of similar investigations on phage T4 DNA polymerase (Reha-Krantz & Bessman, 1977, 1981), *lac* permease (Hobson *et al.*, 1977) and aspartate carbamoyltransferase (Kantrowitz *et al.*, 1980, 1981) among others. In this context, it is first necessary to consider two aspects of nonsense suppression: the possible occurrence of hotspot formation in the generation of nonsense mutations and the efficiency (the translational readthrough frequency or transmission) of the suppressors employed.

Distribution of nonsense mutations. Of the nine codons that can give rise, for instance, to an amber mutation in a single base change, eight are sense triplets encoding seven different amino acids (Fig. 3). In the absence of specific mutagenic processes, therefore, the distribution of nonsense mutations in a gene is dictated by codon usage. [A fairly accurate prediction can be made from the amino acid composition of the protein encoded if *Escherichia coli* codon usage is taken into consideration (Nene & Glass, 1980).]

Even spontaneous amber mutations may occur at hotspots as exemplified by the large proportion of UAG codons generated at two sites in *lacI* (Coulondre *et al.*, 1978). Each site comprises the sequence CCAGG, which is the recognition signal for methylation of the second cytosine (Boyer *et al.*, 1973). This sequence does not appear to be the sole determinant of hotspot sites, since a third CCAGG in *lacI* is relatively silent (Coulondre *et al.*, 1978). Indeed, *rpoB* has four such sequences, yet does not appear to contain hotspots for amber mutations (see below). Spontaneous deamination of methylcytosine to thymine seems to be the mechanism for mutational hotspots at CAG (Gln) codons (Coulondre *et al.*, 1978; Duncan & Miller, 1980). In addition to hotspots generated at CCAGG, G·C→A·T transitions (and G·C→T·A transversions) occur most frequently. Hence a structural gene that lacks the pentanucleotide sequence is more likely to carry lesions at CAG (Gln) and UGG (Trp) codons (although it should be noted that tryptophan is a rare amino acid, that is, the codon is present infrequently).

Suppressor efficiency. Translational readthrough past a nonsense codon does not restore biological activity *per se*. Not only must the inserted residue be compatible with protein structure for that particular site, but also the efficiency of suppression (the translational readthrough frequency) must be high enough to maintain an adequate supply of the suppressed polypeptide. In short, both the amino acid inserted and the efficiency of insertion determines protein function. Moreover, both are dependent on genetic context, i.e. the neighbouring nucleotide sequence.

In general, UGA and amber suppressors (other than *supE*) are classified as efficient suppressors (>30%), *supE* as being of intermediate strength (10–30%) and the ochre suppressors as being the least efficient (≤5%) (Garen, 1968; Brammar, 1969; Gorini, 1970). It is important to stress that these are

consensus groupings. For instance, the efficiency of *supE* in reading the same UAG codon in *Salmonella typhimurium* varies over a 10-fold range depending on the nature of the base adjacent to the 3′ side of the nonsense triplet (Bossi & Roth, 1980). A two-fold variation in translational readthrough frequency has been noted for *supE* in *E. coli* (Nene & Glass, 1981). Although the strongest suppressor, *supF*, appears to promote readthrough independently of context (Nene & Glass, 1981), there may be strict limitation in the sites that accept the bulky aromatic residue inserted [see, for example, Kantrowitz *et al.* (1980, 1981) and Table 2 below].

The integrity of the components of the translation machinery also plays an important role in suppressor function. Secondary mutations in suppressor alleles, themselves, may lead to aberrant processing or charging [reviewed by Smith (1979)]. Certain *rpsL* (Str-r) (the structural gene for the S12 r-protein) alleles are known to restrict suppressor efficiency; such restrictive alleles may be antagonized by streptomycin [reviewed by Gorini (1974)]. Finally, it should be noted that competition between translational termination (release) factors [reviewed by Caskey (1980)] and suppressor tRNAs dictates efficiency (Capecchi & Klein, 1970).

Cytoplasmic proteins. (i) RNA polymerase. *E. coli* RNA polymerase, an essential heterooligomer consisting of at least four non-identical subunits, $\alpha_2\beta\beta'\sigma$ (Burgess, 1976), is responsible for the bulk of cellular RNA synthesis (Pato & von Meyenberg, 1970). The role of the individual subunits in enzyme biosynthesis, assembly and activity may be investigated by making use of informational suppression.

A detailed study, for example, has been initiated on the β polypeptide (Nene & Glass, 1980). The *rpoB* gene product consists of 1342 residues (150,618 daltons) and analysis of the DNA sequence (Ovchinnikov *et al.*, 1980) indicates that there are a total of 163 potential amber sites. About one hundred independent, spontaneously occurring mutants carrying amber lesions that affect *rpoB* expression have been screened with six different nonsense suppressors, *supD*, *supE*, *supF*, *supG*, *supP* and *supU* (responsible for the insertion of serine, glutamine, tyrosine, lysine, leucine and glutamine/tryptophan, Table 1). With RNA polymerase (unlike the *lac* repressor, see below), most amino acid substitutions are non-neutral. This is exemplified by the fact that only 331 of the possible 570 variants impart viability (Table 2) and of these, approximately two-thirds exhibit altered properties, thermolability, aberrant transcription termination and a changed stringent response.

It is not possible as yet to determine the initial sense codon that give rise to each *rpoB* amber mutation (but see *lacI*, below) and, hence, suitability of amino acid substitutions cannot be considered in terms of the wild-type residue replaced. However, the high incidence of *supD* and *supP* suppressible *rpoB*(Am) strains, when less than 6% of the amber-mutable codons encode serine or leucine, suggests that these amino acid substitutions are

Table 2. *Suppression pattern of amber mutations affecting rpoB expression*

Each of the 95 amber mutants were tested with the six nonsense suppressors for viability (+). Lack of suppression is symbolized by '–'. Of a potential 570 suppressed derivatives, only 331 are viable. All 95 mutants are suppressed by *supU*, 82 by *supD*, 49 by *supE*, 10 by *supF*, 32 by *supG* and 63 by *supP*.

	Nonsense suppressor						
Class	*supU* (Gln/Trp)	*supD* (Ser)	*supE* (Gln)	*supF* (Tyr)	*supG* (Lys)	*supP* (Leu)	Number
A	+	+	+	+	+	+	4
B	+	+	+	+	–	+	3
C	+	+	+	+	–	–	2
D	+	+	+	–	+	+	13
E	+	+	+	–	+	–	8
F	+	+	+	–	–	+	12
G	+	+	+	–	–	–	5
H	+	+	–	+	–	–	1
I	+	+	–	–	+	+	3
J	+	+	–	–	+	–	2
K	+	+	–	–	–	+	23
L	+	+	–	–	–	–	6
M	+	–	+	–	–	+	2
N	+	–	–	–	+	–	2
O	+	–	–	–	–	+	3
P	+	–	–	–	–	–	6

generally acceptable. This contension is supported by the wild phenotype of the majority of serine-substituted strains; half the leucine insertions yield polypeptides of unaltered function. The observation that all *rpoB* mutants are suppressed by *supU* (Table 2) appears to reflect the high incidence and even distribution of CAG (Gln) codons. There do not appear to be mutational hotspots in *rpoB* although there are four potential CCAGG hotspot sites (see above).

It is worthwhile considering the relative contribution of suppressor efficiency in the maintenance of cell viability. The translational readthrough frequency has been determined at two unique sites in *rpoB* in the presence of the five amber suppressors employed in this study (Nene & Glass, 1981; Table 1). As might be expected, since *E. coli* possesses a mechanism for increasing β subunit synthesis under conditions of poor translation of the β mRNA (Glass *et al.*, 1976), there is little correlation between efficiency and the ability of a nonsense suppressor to impart viability. *supF*, for instance, consistently the most powerful of the amber suppressors employed, is particularly limited in its use (Table 2). This restriction presumbly reflects the incompatibility of a bulky aromatic group at the majority of sites in β. However, the differences between *supE* and *supU* may be ascribed to *supE* inefficiency, since *rpoB* encodes only four tryptophan residues and some temperature-sensitive *supE* derivatives are amenable to phenotypic suppression.

These studies on the β subunit of *E. coli* RNA polymerase have helped to define functional domains. Thus, amino acid substitutions that perturb transcription termination and the stringent response appear to map in the proximal two-thirds of β: single-site changes in the *C*-terminal one-third do not seem to affect these functions.

(ii) *lac* Repressor. Transcriptional repression of the *lac* operon is mediated through binding of the *lac* repressor at the *lac* operator site. Repressor binding is, itself, controlled allosterically by allolactose and other galactosides [see Barkley & Bourgeois (1980)]. The elegant studies of Miller and co-workers on *lacI* nonsense mutations and their suppression have considerably advanced our understanding of repressor function [reviewed by Miller (1980)].

The *lacI* gene encodes a 38600-dalton polypeptide consisting of 360 amino acid residues (Farabaugh, 1978). More than 80 of the potential 91 nonsense mutable codons have been identified (Coulondre & Miller, 1977; Miller *et al.*, 1978). Suppression of these mutants, therefore, provides a unique opportunity for the study of structure–function relationships, since the precise map position, wild-type residue and inserted amino acid residue(s) are known [reviewed by Miller *et al.* (1979*a*)].

The majority of substitutions are neutral; approximately 40% of the suppressed repressors exhibit altered properties [reviewed by Miller (1980)]. Table 3 summarizes the effects of substitution of the wild-type amino acid residue by glutamine, lysine, leucine, serine and tyrosine [*supE* (or *supB*), *supG*, *supP*, *supD* and *supF* (or *supC*) were employed]. As a generalization, replacement of glutamine, glutamate or lysine is less sensitive than substitution of leucine, serine or tyrosine. Replacement of tryptophan is not tolerated. Insertion of leucine, serine or tyrosine is generally more suitable than insertion of glutamine, whereas replacement by lysine is extremely sensitive. That the latter observation does not reflect inefficient suppression is indicated by the lack of *lac* operon induction, i.e. the mutant repressor permanently 'switches off' operon expression. Where insertion of tryptophan by *sup9* was possible, half the repressors exhibited altered properties. These effects correlate well with the initial observations of genetic variants of haemoglobin. Namely, polar residues can normally be replaced by both polar and non-polar amino acid residues, while non-polar residues are sensitive to substitution by polar amino acids (Perutz & Lehmann, 1968).

The properties of missense mutations and various

Table 3. *Suitability of amino acid substitutions in lacI*

The effect of amino acid substitutions on *lac* repressor function is tabulated. S and U (suitable and unsuitable) indicate that the majority of insertions yield wild-type and altered phenotypes, respectively. S/U refers to insertions which give an equal chance of either event. Adapted from Miller (1980).

Wild-type residue	Amino acid inserted				
	Glutamine	Leucine	Lysine	Serine	Tyrosine
Glutamine	S	S	S/U	S	S
Glutamate	S	S	U	S	S
Leucine	S/U	S	U	S/U	S/U
Lysine	S	S	S	S	S
Serine	S/U	S/U	U	S	S/U
Tyrosine	S/U	S/U	U	S/U	S
Tryptophan	U	U	U	U	U

studies *in vitro* on isolated repressor and its cleaved products indicate that there are two major domains for repressor function [reviewed by Weber & Geisler (1980)]. The first 60 residues constitute the DNA binding region, while the rest of the polypeptide chain carries sites for inducer binding and monomer aggregation to the active tetramer. Studies with nonsense suppressors substantiate these results. Moreover, they point to certain residues important in repressor function (Miller *et al.*, 1979*b*; Miller, 1979). Thus, the tyrosine residues at positions 17 and 47, and to a smaller extent at position 7, are essential for DNA binding. Substitution of these residues generates *lacI* mutants while tyrosine at position 12 is insensitive to amino acid replacement. Substitution of residues beyond position 77 give rise to super-repressors of varying strength which exhibit either reduced affinity for inducer, increased affinity for *lac* operator DNA or lack of conformational change on inducer binding. Substitution of Ser-262 for glutamine yields a thermolabile repressor while Tyr-282 appears to be essential for tetramer formation.

Function of the *lacI* gene product may be successfully dissected as described above. Such studies highlight the importance of particular residues as well as the role of defined regions in higher-order structure. More importantly, mutations giving a particular phenotype tend to be clustered, suggesting the presence of additional domains. There appear to be a few regions on *lacI* which are 'silent' with respect to function. Whether these areas play a subtle role in repressor function or are purely structural 'bridges' remains to be seen.

Exported proteins. Informational suppression has been used to investigate structure–function relationships of two *E. coli* cell-surface receptor proteins, those encoded by *lamB* and *btuB*, as well as an exported bacteriophage protein. The *lamB* gene product is involved in the binding and uptake of phage λ (Randall-Hazelbauer & Schwartz, 1973; Thirion & Hofnung, 1972) as well as being implicated in maltose transport (Szmelcman & Hofnung, 1975). The *btuB* protein is responsible for vitamin B_{12}, bacteriophage BF23 and E colicin uptake (Kadner & Liggins, 1973; Bassford & Kadner, 1977). The coat protein of coliphage M13 is made in a precursor form (Pieczenik *et al.*, 1974; Konings *et al.*, 1975; Sugimoto *et al.*, 1977) which is processed proteolytically during phage infection (Chang *et al.*, 1978). The mature coat protein (50 rather than 75 amino acids long) is inserted into the bacterial inner membrane prior to DNA encapsidation (Chang *et al.*, 1979).

In the case of *lamB*, Hofnung and co-workers examined the growth of phage λ and some of its host range mutants on strains harbouring lesions at this locus (Hofnung *et al.*, 1976). Over 60 mutants were isolated and the strains were grouped according to their ability to plate λ host range derivatives. Most of those mutants which showed a totally non-functional receptor were of the nonsense type, as indicated by suppression with *supC*, *supF* and *supU*. Mutants producing a partially functional receptor were not suppressed by any of these suppressors and, thus, appeared to be of the missense type. The results suggest that missense mutants are less likely to be detrimental to receptor function than nonsense mutations (or multisite lesions) (and see below).

The entrance of the three disparate ligands which bind the *btuB* receptor has been investigated by making use of informational suppression of a collection of *btuB* amber and ochre mutations (Hunter & Glass, 1981). The effect of six different but known amino acid substitutions at each nonsense site on receptor function was studied. Such work has shown that the majority of amino acid substitutions in the *btuB* product are neutral, indicating that most missense mutants of this receptor are unlikely to interfere with receptor activity (in agreement with the data of Hofnung and co-workers). A small number of amino acid replacements restore B_{12} uptake without imparting sensitivity to phage BF23 or colicin E3. This is evidence for the overlapping of binding sites for the two proteinaceous, lethal agents.

A conditional M13 coat protein mutant carrying both an amber mutation in Glu-2 and a Pro-6→Ser-6 change (Pratt *et al.*, 1969) has been characterized. (Note that residue positions refer to the processed polypeptide species.) This mutant grows on *supD* (Ser) but not on *supE* (Gln), *supF* (Tyr) or *supP* (Leu) strains (Boeke & Model, 1979) (but see below). Studies on pseudorevertants, in conjunction with nonsense suppression (Boeke *et al.*, 1980), suggest that failure of *supE* and *supF* to permit viable phage formation may be due to inefficient suppression, perhaps leading to a low ratio of coat protein to phage DNA. Insertion of leucine at position 2 seems to affect processing. [Growth of the amber phage on *supP* (Leu) strains could be achieved when a second-site compensatory mutation generated an Asn-12→Asp-12 change.] Such work is useful for determining the contribution of sequences downstream from the signal peptidase cleavage site in processing.

Eukaryotic nonsense suppressors

Suppressor mutants that act on nonsense triplets have been isolated from *Saccharomyces cerevisiae* [reviewed in Hawthorne & Leupold (1974), Olson *et al.* (1980), Piper (1980) and Sherman (1982)], *Schizosaccharomyces pombe* [reviewed in Kohli *et al.* (1980)], *Neurospora crassa* (Seale *et al.*, 1977), *Aspergillus nidulans* (Bal *et al.*, 1979), *Drosophila melanogaster* [discussed in Steege & Söll (1979)]

and *Caernorhabditis elegans* (Waterston & Brenner, 1978) among others. For example, there are several *C. elegans* suppressor genes that suppress specific alleles of *unc*-54, encoding a major myosin heavy chain present only in the body wall musculature of this soil nematode; their mode of action suggests that they act as informational suppressors (Waterston & Brenner, 1978). The *N. crassa* suppressor mutation *ssu*-1 is responsible for the insertion of tyrosine at the site of a glutamate residue in the NADP-specific glutamate dehydrogenase, also suggesting it acts at some stage in information transfer (Seale *et al.*, 1977). Preliminary evidence that $tRNA^{Tyr}$ from the recessive suppressor of *sable* in *D. melanogaster*, *su(s)*[2], is different from wild-type (Twardzik *et al.*, 1971; Warner & Jacobson, 1976) may reflect non-specific changes in the levels of the modified nucleoside Q (found at the first position of the anticodon) rather than the involvement of a specific tRNA molecule in suppression (White *et al.*, 1973; Wosnick & White, 1977).

Our knowledge of eukaryotic informational suppression is most advanced in yeast, in particular *S. cerevisiae* and *S. pombe* (see Table 4). Characterization of yeast suppressors has proceeded in two main ways: priming of translation systems *in vitro* with tRNA fractions from mutant and wild-type strains and the sequencing of suppressor tRNAs and their genes. These approaches have established that suppressor genes can arise, as in prokaryotes, through alterations in sequences encoding the anticodon region of tRNAs. Both *S. cerevisiae* amber and ochre suppressor derivatives have been isolated; UGA suppressor tRNAs have been obtained in *S. pombe* (Table 4). There are eight unlinked loci in *S. cerevisiae* which give rise to dominant, tyrosine-inserting suppressors [reviewed by Olson *et al.* (1980), Piper (1980) and Sherman (1982)]. To date, genetic analysis has revealed four different loci capable of mutating to give serine-inserting nonsense suppressors and at least seven loci responsible for the insertion of leucine [see Ono *et al.* (1979, 1981), Olson *et al.* (1980) and Piper (1980)]. Interestingly, ochre suppressors in yeast act only on UAA codons, unlike their bacterial counterparts which recognise both UAA and UAG (Table 1). Moreover, these UAA-specific suppressors function at high efficiency.

The efficiencies of these yeast suppressors have been estimated largely by suppression of nonsense mutations in the structural gene for iso-1-cytochrome *c*, *cyc1* [see, for example, Gilmore *et al.* (1971), Brandriss *et al.* (1976), Liebman *et al.* (1977) and Ono *et al.* (1981)]. Most efficient are the tyrosine-inserting species (Gilmore *et al.*, 1971; Liebman *et al.*, 1975; Ono *et al.*, 1979, 1981); a similar situation is found in *E. coli* (Table 1). *SUP-RL1* and *SUP4* have been shown to be about 50% and 63% efficient, respectively, in a heterologous cell-free protein-synthesizing system pro-

Table 4. *Yeast nonsense suppressors*

Only sequenced suppressors are shown; anticodons and codons are written 3′→5′ and 5′→3′, respectively. Abbreviation; n.k., not known.

Suppressor*†	Chromosome	tRNA	Codon(s) suppressed	Anticodon: Suppressor	Anticodon: Wild-type	Amino acid inserted	References
SUP 4	X R	$tRNA^{Tyr}$	UAA	AΨ$\underline{\overset{*}{U}}$	AΨ$\underline{G}$	Tyrosine	Gilmore *et al.*, 1971; Olson *et al.*, 1979; Goodman *et al.*, 1977
SUP 5	F8	$tRNA^{Tyr}$	UAG	AΨ$\underline{C}$	AΨ$\underline{G}$	Tyrosine	Piper *et al.*, 1976
SUP 16 (*SUQ5*)	XVI R	$tRNA^{Ser}_{UCA}$	UAA	A$\underline{U}\overset{*}{U}$‡	A$\underline{G}\overset{*}{U}$‡	Serine	Cox, 1965; Ono *et al.*, 1979
SUP-RL1 (*SUP61*)	III R	$tRNA^{Ser}_{UCG}$	UAG	A$\underline{U}$C	A$\underline{G}$C	Serine	Brandriss *et al.*, 1975, 1976; Piper, 1978, Olson *et al.*, 1981
sup-3	I	$tRNA^{Ser}_{UCA?}$§	UGA	AC$\underline{\overset{*}{U}}$‡	n.k.	Serine	Kohli *et al.*, 1977, 1979
sup-8	II	$tRNA^{Leu}_{UAA?}$§	UGA	AC$\underline{\overset{*}{U}}$‡	n.k.	Leucine	Kohli *et al.*, 1977; Wetzel *et al.*, 1979; Rafalski *et al.*, 1979

* *S. cerevisiae* (*SUP*) and *S. pombe* (*sup*) suppressor loci; *SUP-RL1* is recessive-lethal

† Amber and ochre (and opal, in the case of *SUP5*; Hawthorne, 1976) derivatives of each suppressor are available. Note that yeast ochre suppressors only suppress ochre mutations.

‡ The modified bases (denoted $\overset{*}{U}$) are probably derivatives of 5-carboxymethyluridine (Wetzel *et al.*, 1979; Rafalski *et al.*, 1979; Piper, 1980; Waldron *et al.*, 1981).

§ Identity inferred: the wild-type sequences have not as yet been obtained.

grammed with coliphage RNA (Gestland *et al.*, 1976). As in prokaryotes, context effects have been observed. The absolute efficiency of *SUP52*, for example, a leucine-inserting suppressor, varies over a ten-fold range for different amber sites in *cyc1* (Liebman *et al.*, 1977).

There is a factor, sometimes present in *S. cerevisiae*, which potentiates ochre (and frameshift) suppressors several-fold (Cox, 1965; Liebman *et al.*, 1975; Ono *et al.*, 1979) as well as suppressing certain UAA markers *per se* (Liebman & Sherman, 1979). This psi$^+$ extrachromosomal determinant is inherited in a non-Mendelian fashion [though it is not associated with mitochondrial DNA nor the 2 μm circular DNA (McCready & McLaughlin, 1977)]. psi$^+$ factor increases the efficiency of several different ochre suppressors, to an extent that they generally become lethal to the organism (Liebman *et al.*, 1975). Despite its lack of specificity towards a particular ochre suppressor, psi$^+$ action is apparently not mediated by suppressor tRNA modification (Piper, 1980). [That modification is an important factor in eukaryotic tRNA suppressor function has been shown by anti-suppressor mutants, of both *S. cerevisiae* and *S. pombe*, that contain approx. 1% of the normal tRNA complement of isopentenyladenosine (reviewed in Laten *et al.* (1980).]

Yeast nonsense suppressors have been used extensively in the study of viral gene expression. Thus, translation *in vitro* of mRNA isolated from infected cells has helped identify nonsense lesions in two DNA viruses, an Adeno(2)–SV40 hybrid virus (Gestland *et al.*, 1977) and Herpes simplex virus (in the thymidine kinase gene in the latter case; Cremer *et al.*, 1979). Comparable studies suggest that a UAG codon terminates synthesis of the *gag* protein of several different retroviruses (Philipson *et al.*, 1978; Weiss *et al.*, 1978; Murphy *et al.*, 1980). Finally, investigation of the readthrough of leaky termination triplets has suggested similarities in the strategies of expression of the non-coat protein genes of tobacco rattle virus and tobacco mosaic virus (Pelham, 1979).

What about structure–function studies in eukaryotes? The following yeast genes are under study, those for: iso-1-cytochrome *c* and iso-2-cytochrome *c* (Stewart & Sherman, 1973; Zitomer & Hall, 1976; Liebman *et al.*, 1977; Downie *et al.*, 1977; Boss *et al.*, 1980); the positive regulator of galactose-catabolizing enzymes (Hopper & Rowe, 1978; Hopper *et al.*, 1978; Matsumoto *et al.*, 1980); the regulator for methionine biosynthesis (Masselot & De Robichon-Szulmajster, 1972); enzymes involved in arginine (Minet *et al.*, 1979) and pyrimidine (Losson & Lacroute, 1979; Exinger & Lacroute, 1979) biogenesis and developmental functions (Rothstein *et al.*, 1977; Nurse *et al.*, 1979; Rai *et al.*, 1981). Nonsense mutations themselves have been used in fine-structure analysis, for example, of loci involved in isoleucine and valine production (Thuriaux *et al.*, 1971) and aromatic amino acid biosynthesis (Strauss, 1979). Finally, nonsense lesions have been partially characterized in mouse immunoglobulin mutants (Adetugo *et al.*, 1977) and mouse hypoxanthine–guanine phosphoribosyltransferase (Capecchi *et al.*, 1977).

Overview

Informational suppression, in particular nonsense suppression, has played a key role in the elucidation of the mechanism of protein biosynthesis in prokaryotes: the nature of the genetic code, structure–function relationships in tRNA and aminoacyl-tRNA synthetase reactions. Moreover, it is now possible to study protein structure and function at a highly sophisticated level through the use of a battery of different nonsense suppressors. In eukaryotes, only yeast nonsense suppressors are well characterized (although nonsense mutations have been identified in other species, including mouse). There is no doubt that these mutant tRNAs will prove as important in the investigation of eukaryotic translation machineries. Despite the fact that nonsense suppressors have not been unambiguously identified in animals cells, the availability of tyrosine-, serine- and leucine-inserting UAG, UAA and UGA yeast suppressors allows detailed analysis of both fungi and higher organisms by making use of translation systems *in vitro*.

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