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REVIEW ARTICLE

Endless possibilities: translation termination and stop codon recognition

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Overview

The process of protein synthesis can be divided into three main phases: initiation, during which the ribosomal subunits join the mRNA and locate the AUG initiator codon; elongation, during which sense codons are decoded and the bulk of the polypeptide is made; and termination, during which a stop codon directs the release of the completed polypeptide from the ribosome. Whereas the general principles of sense codon decoding by transfer RNAs are well established, a clear picture of translation termination and stop codon recognition has hitherto been lacking. Recently however, the use of optimized, complex, reconstituted *in vitro* termination reactions to identify the roles of key termination factors, and the solution of tertiary structures of termination factors, has allowed a reappraisal of termination factor structure and function. This review will describe these recent advances, many of which have resulted from studies of termination in micro-organisms, and in the light of the new information, discuss models for the mechanism of termination and recognition of the stop codon. While the mechanism of peptide chain termination is normally effective, stop codons are not always recognized efficiently, and during the translation of certain viral RNAs can be suppressed or read through, resulting in the expression of additional coding information. How stop codons are reassigned to encode 'sense' at a given frequency in some RNAs will be reviewed in the context of current understanding of termination and stop codon recognition mechanisms.

The translation termination apparatus in eukaryotes and prokaryotes

During translation termination, a stop codon located in the ribosomal A-site is recognized by a release factor or release factor complex, which binds the ribosome and triggers release of the nascent peptide. In eukaryotes, translation is terminated by a heterodimer consisting of two proteins, release factors eRF1 and eRF3, which interact *in vivo* (Frolova *et al*., 1994; Zhouravleva *et al*., 1995; Stansfield *et al*., 1995). eRF1 recognizes all three stop codons and triggers peptidyl-tRNA hydrolysis by the ribosome, releasing the nascent peptide (Frolova *et al*., 1994; Drugeon *et al*., 1997). Eukaryote termination efficiency is enhanced by the GTPase release factor eRF3, the second component of the heterodimer eRF complex. In response to a stop codon in the ribosomal A-site, formation of a quaternary complex comprising the ribosome, eRF1, GTP and eRF3 triggers GTP hydrolysis and enhances the rate of peptidyl release (Zhouravleva *et al*., 1995; Frolova *et al*., 1994; Fig. 1). In yeast, eRF1 and eRF3 are encoded by essential genes (*SUP35* and *SUP45*, respectively), mutations in which produce nonsense suppression phenotypes (Stansfield & Tuite, 1994).

In contrast to eukaryotes, the role of stop codon recognition during translation termination in eubacteria is divided between two so-called class 1 release factors, RF1 and RF2, which in *Escherichia coli* are encoded by the essential *prfA* and *prfB* genes, respectively (Scolnick *et al*., 1968; Caskey *et al*., 1984; Weiss *et al*., 1984). RF1 catalyses translation termination at UAA and UAG codons, and RF2 at UAA and UGA codons (Craigen *et al*., 1990; Fig. 1). Since RF2 bound to the ribosomal A-site can be chemically cross-linked to an artificial mRNA containing a UAA stop codon, this recognition must be achieved through direct physical contact (Lang *et al*., 1989; Brown & Tate, 1994). The mRNA bases following the stop codon can also be cross-linked to RF2, implying the release factor has a footprint broader than a single codon span (Poole *et al*., 1998). Consistent with this, 3′ nucleotide stop codon context has a marked influence on the efficiency of stop codon recognition in bacteria, yeast and human cells (Poole *et al*., 1995; Bonetti *et al*.,

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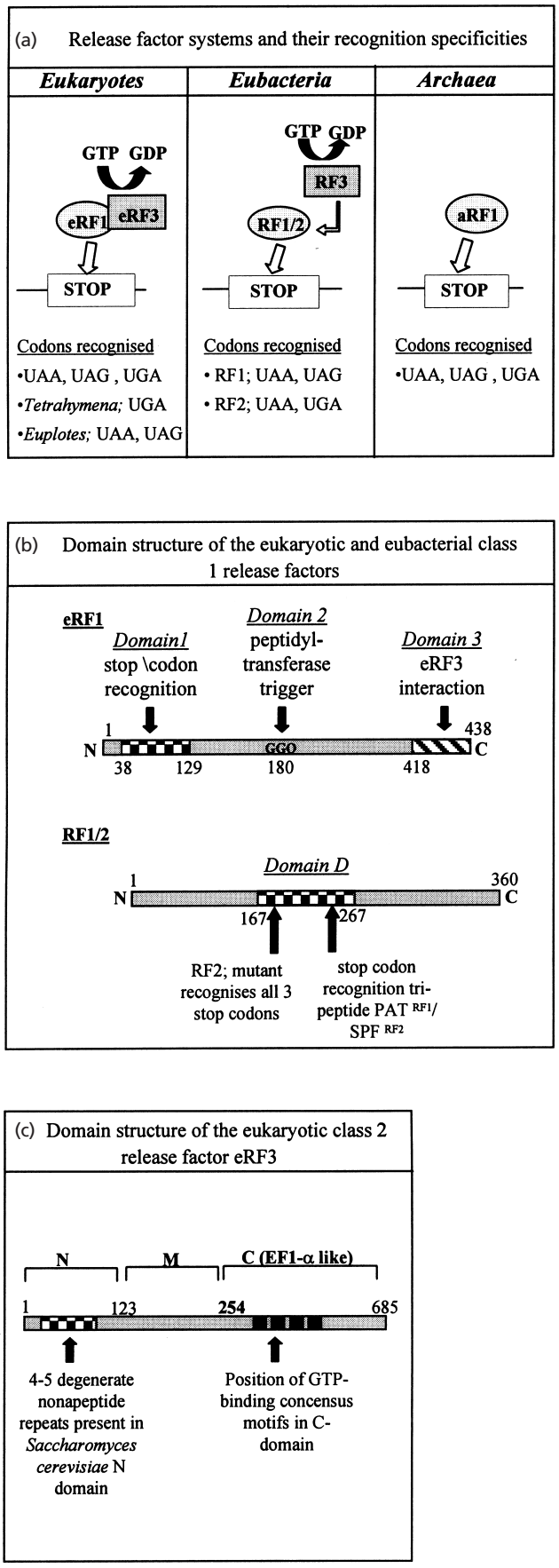

1995; McCaughan *et al.*, 1995). In prokaryotes, dissociation of RF1 and RF2 from the ribosome is stimulated by the addition of a GTPase release factor, RF3, analogous to eukaryote eRF3 (Freistroffer *et al.*, 1997). However, unlike eRF3, RF3 is non-essential and does not form stable complexes with RF1 and RF2, implying these latter RFs can function in the absence of RF3 (Grentzmann *et al.*, 1994; Mikuni *et al.*, 1994).

The role of GTPase release factors in termination

eRF3 and RF3 show limited sequence similarity to one another at the amino acid level, and while the eRF3 C-terminal domain shows most sequence similarity to eukaryotic elongation factor EF-1α (which brings aminoacyl tRNAs to the A-site), RF3 is most similar to prokaryotic elongation factor EF-G (the ribosome translocase), implying their precise functions may differ. A study of *in vitro* termination reactions has shown that in single turnover reactions, peptidyl release is independent of RF3, and that the rate of RF1/2 association with the ribosome is independent of RF3. This evidence supports a model in which RF3 may stimulate termination efficiency by ejecting the class 1 release factors RF1/2 from the A-site following peptidyl-tRNA hydrolysis (Freistroffer *et al.*, 1997). RF3 also enhances the efficiency of RF2-mediated termination at less preferred (UGA) stop codons, or at stop codons in weak contexts (Crawford *et al.*, 1999; Grentzmann *et al.*, 1995). However, there is as yet no evidence that eRF3 functions in a similar way to eject eRF1 from the A-site following termination. Instead, some observations suggest that eRF1 and eRF3 might act as a complex to mediate peptidyl release; in yeast and mammals, eRF1 and eRF3 form a complex *in vivo* and *in vitro* (Zhouravleva *et al.*, 1995), and in yeast, overexpression of both eRF3 and eRF1 together is required to cause large increases in translation termination efficiency and thereby outcompete suppressor tRNAs for stop codon binding (Stansfield *et al.*, 1995). However, the demonstration that eRF1 alone has peptidyl-release activity *in vitro* argues that, at least in a reconstituted system, an (EF1-α-like) eRF3 system to deliver eRF1 to the A-site is not a

Fig. 1. Release factors and their domain structures. (a) Release factor complexes in eukaryotes, eubacteria and archaea. Some eukaryote genera, such as *Tetrahymena* and *Paramecium* (UGA signals stop) and *Euplotes* (UAR signals stop) use a restricted set of stop codons. This may result from an altered stop codon recognition ability of the eRF1s in these organisms. (b) Domain structure of class 1 release factors from eukaryotes and eubacteria, showing the functionally important domains (see text for detailed discussion). (c) Domain structure of the eukaryote class 2 release factors. The three assigned N, M and C domains are shown, as are the approximate position of the nonapeptide repeat motifs present in the *S. cerevisiae* eRF3 sequence which are required for inheritance of the [*PSI*$^+$] prion state (see text for details). Figures next to rectangles representing the release factor polypeptides denote amino acid numberings.

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requirement (Frolova *et al*., 1994). Secondly, in mammalian cells and *in vitro* systems, overexpression of eRF1 alone is sufficient to outcompete suppressor tRNAs for stop codon binding (Legoff *et al*., 1997). Recent results have also cast doubt on the early models suggesting that eRF1 and eRF3 interaction is a requirement for function; the *in vivo* interaction of yeast eRF1 and eRF3 is mediated by the C-terminal-most 6–11 amino acids of eRF1 (Eurwilaichitr *et al*., 1999; Ito *et al*., 1998a). However, it is possible to trim off the yeast eRF1 C-terminal residues to a point where eRF3 interaction is no longer detectable, and yet still maintain yeast viability, although stop codon recognition is somewhat impaired, producing a nonsense suppression phenotype (Eurwilaichitr *et al*., 1999; Ito *et al*., 1998a). It may therefore be unnecessary to form an eRF1–eRF3 complex in order to terminate translation, although it remains possible that eRF3 may still have affinity for a ribosome–eRF1–stop codon complex. Subsequently, eRF3 could perform a central (essential) role in termination, either enhancing peptidyl release, or perhaps recycling eRF1 from post-peptidyl-release ribosomes in an RF3-like manner. Such models are speculative however, and much more detailed biochemical evidence is required before the mechanism of eukaryote termination can be elucidated. Interestingly, archaea also have eukaryote-like eRF1s (aRF1), although intriguingly, without exception they lack the eRF1 C-terminal amino acids which in yeast interact with eRF3 (Inagaki & Doolittle, 2000). However, it is not clear that archaea even have an eRF3 counterpart, although for instance *Archeoglobus fulgidus* possesses two EF1α/EF-Tu-like proteins, one of which could conceivably have class 2 release factor function. These observations support the hypothesis that eRF1–eRF3 interaction may not be required for the stop codon recognition and termination reactions (Fig. 1).

In mouse, two eRF3 variants have been identified which differ in their N-terminal domain sequences and expression patterns in this organism (Hoshino *et al*., 1998). However, whether or not they have distinct activities in termination remains to be addressed. Intriguingly, one mouse eRF3 is known to interact with the C terminus of mammalian poly(A)-binding protein PABP (Hoshino *et al*., 1999), raising a number of questions about the breadth of potential functions this protein may carry out, including involvement in the regulation of mRNA stability and, potentially, recycling post-termination ribosomes back to the 5′ end of the mRNA to participate in new rounds of translation initiation (Hoshino *et al*., 1999).

RF recognition of the stop codon

One important, as yet unanswered question concerns the mechanism of stop codon recognition by the release factor. The ability to cross-link RF2 to a stop codon indicates that the class 1 release factors may exhibit a tRNA-like activity (Poole *et al*., 1998). One hypothesis is that release factors may mimic a tRNA both structurally and functionally. This model was prompted by the discovery by Nissen and colleagues that the structure of elongation factor EF-G (GDP) from *Thermus thermophilus* resembles that of a tRNA–EF-Tu–GTP complex, with EF-G domain IV mimicking major portions of a tRNA, including the T-stem and anticodon loop (Fig. 2; Nissen *et al*., 1995; Ito *et al*., 1996). This hypothesis was developed by Ito and co-workers, who suggested that prokaryotic class 1 release factors, which share weak sequence homology across their central D and E domains to EF-G domain IV, may also 'mimic' a tRNA molecule. *prfB* (RF2) mutants were subsequently isolated which could complement conditional-lethal RF1 and RF2 mutations, i.e. an RF2 molecule capable of recognizing all three stop codons; the mutation (E167K) mapped within the central D domain of RF2 (Ito *et al*., 1998b). More recently, elegant experiments swapping domains between RF1 and RF2 have exchanged stop codon recognition specificity between the two prokaryote RFs. In this way, an RF domain responsible for stop codon specificity was subsequently narrowed down to a single tripeptide (RF1, Pro Ala Thr; RF2, Ser Pro Phe), again in the central domain D, where the weak similarity to EF-G domain IV was located (Ito *et al*., 2000). However, final confirmation that this tripeptide represents the actual site of stop codon interaction will require chemical cross-linking data (stop codon–RF), or co-crystallization of the stop codon and RF.

The RF-tRNA mimicry proposal is also supported by the recent determination of the crystal structure of eukaryote eRF1 (human) revealing a Y-shaped molecule with similar dimensions to a tRNA (Song *et al*., 2000; Fig. 2). The eRF1 GGQ motif, which triggers peptidyl release once the release factor binds to the stop codon (Frolova *et al*., 1999), is located in one of the arms of the Y, in domain 2. Domain 2 lies 80 Å from eRF1 domain 1 (comprising the N-terminal third of the protein) in the stem of the Y (Fig. 2). This distance is very similar to the 75 Å which separates the CCA acceptor stem of a tRNA molecule (which triggers peptidyl transferase during elongation) and the tRNA anticodon. eRF1 domain 1 is thus a likely candidate to represent the anticodon-like structural element in the release factor (Song *et al*., 2000). Consistent with this, a series of mutations have recently been identified in yeast eRF1 domain 1 which alter the stop codon recognition specificity of the release factor, and which define pockets potentially capable of binding stop codon trinucleotides (Bertram *et al*., 2000). These studies, and the tRNA-like shape of eRF1, provide evidence that this domain could play some role in recognizing the stop codon. The N-terminal location of this putative anticodon-like region is dissimilar to the central location of the discriminator tripeptide in RF1/2 (Ito *et al*., 2000), inferring eukaryote and prokaryote class 1 release factors may have very dissimilar protein folds, although both may have an overall tRNA-like shape. Again, confirmation of models for eukaryote eRF1–stop codon interaction requires stop codon/eRF1 co-crystallization or cross-linking data, and the functional relevance of the putative eRF1-tRNA structural similarity remains to be established.

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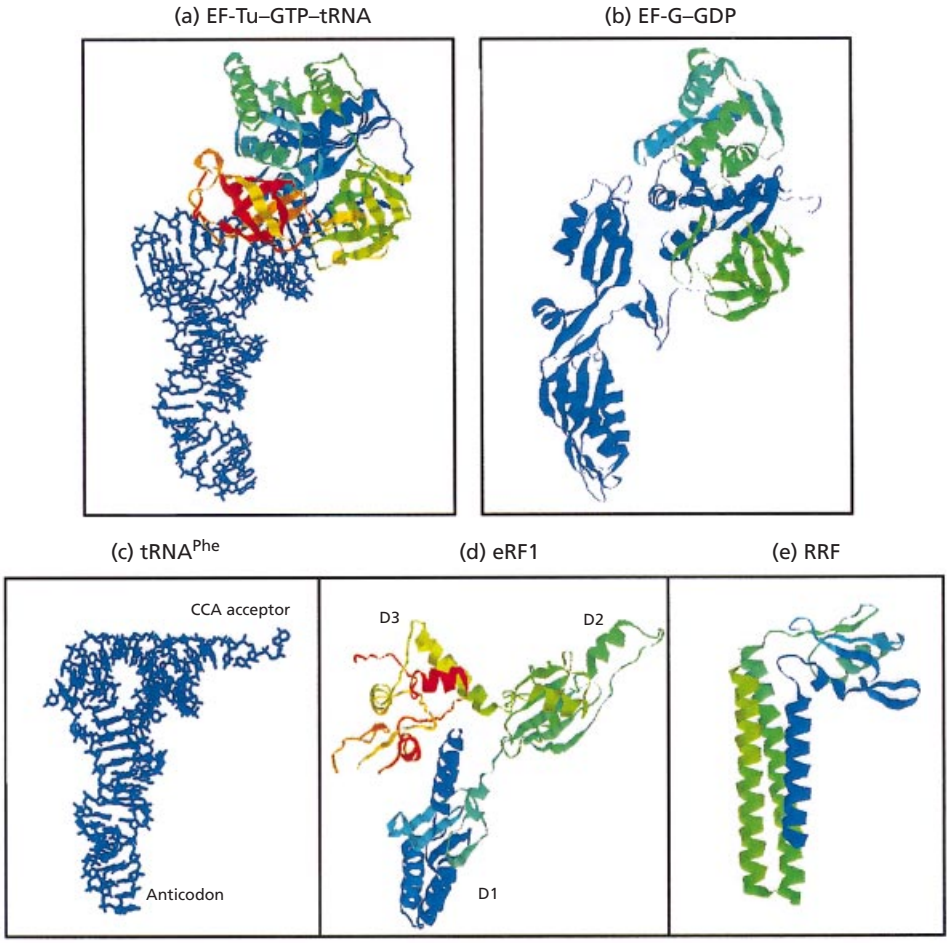

Fig. 2. Molecular mimicry amongst translation elongation and termination factors from prokaryotes and eukaryotes. (a) EF-Tu–tRNA–GTP complex (Nissen *et al*., 1995), with tRNA in stick format coloured blue and protein in ribbon format. (b) EF-G in the GDP-bound state (Al Karadaghi *et al*., 1996), with domains III, IV and V acting as a tRNA mimicry structure and coloured blue. (c) Crystal structure of yeast $tRNA^{Phe}$, coloured blue (Robertus *et al*., 1974). The anticodon and CCA acceptor are labelled. (d) Crystal structure of human eRF1 in ribbon format, aligned to show tRNA-like overall shape and dimensions (Song *et al*., 2000). The three domains referred to in the text and in Fig. 1 are labelled D1–D3. (e) Secondary structure ribbon diagram of the crystal structure of *Thermus thermatoga* ribosome recycling factor (RRF; Selmer *et al*., 1999) aligned to show the tRNA-like shape and dimensions. Except where stated, molecule chains are coloured using a continuous spectrum starting at blue (N terminus), passing through green, yellow and orange, to red at the C terminus.

Release factors and the reassignment of stop codons to sense

Deviations from the universal code have been identified in both the mitochondrial and the nuclear genomes of various organisms. In the prokaryotes *Mycoplasma genitalium*, *Mycoplasma capricolum*, *Mycoplasma pneumoniae and Mycoplasma gallisepticum*, the UGA codon is not used for termination but instead encodes tryptophan (Inamine *et al*., 1990; Yamao *et al*., 1985) due to the presence of a $tRNA_{ACU}$, which translates both UGA and UGG codons. While the genome of *M. genitalium* contains a *prfA* gene encoding RF1, there is no homologue of the gene encoding UGA-decoding RF2 (*prfB*; Fraser *et al*., 1995).

Amongst eukaryote micro-organisms, the ciliate species of the genera *Paramecium* and *Tetrahymena* use UAA and UAG to encode glutamine (Caron & Meyer, 1985), while *Euplotes* species signal stop using UAA and UAG only, with UGA encoding cysteine (Meyer *et al*., 1991). Recently, an unclassified diplomonad species from the *Hexamitidae* has been discovered with an apparent stop codon reassignment to glutamine (Keeling & Doolittle, 1997). These discoveries have led to the search for the corresponding tRNAs which decode the stop codons as sense. In *Tetrahymena*, a $tRNA^{Gln}_{CUA}$ and a $tRNA^{Gln}_{UmUA}$ (where Um represents 2′-*O*-methyluridine) have anticodons cognate for UAG and UAA, respectively (Hanyu *et al*., 1986; Kuchino *et al*., 1985; Table 1). A third $tRNA^{Gln}_{UmUG}$ decodes both the glutamine codons CAA and CAG. The diplomonads with reassigned stop codons use similar $tRNA^{Gln}_{UUA}$ and $tRNA^{Gln}_{CUA}$ (Keeling & Doolittle, 1997).

In contrast to these stop codon cognate tRNAs, a *Euplotes* cysteinyl-tRNA decoding UGA has the non-

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Table 1. Properties of example stop-codon-decoding and natural suppressor tRNAs from different species

	tRNA	Species	Anticodon (5′–3′)	Codon(s) naturally decoded	Stop codon decoded	Conditions required for stop codon decoding*
Stop codon cognate tRNAs from non-universal code species						
	$tRNA^{Gln}$	*Tetrahymena thermophila*	UmUA	UAA	UAA	—
	$tRNA^{Gln}$	*Tetrahymena thermophila*	CUA	UAG	UAG	—
	$tRNA^{Cys}$	*Euplotes octocarinatus*	GCA	UGG, UGA	UGA	—
Natural suppressor tRNAs from universal genetic code species						
UAA, UAG	$tRNA^{Gln}$	*Saccharomyces cerevisiae*	UUG	CAA	UAA	Overexpression
	$tRNA^{Gln}$	*Saccharomyces cerevisiae*	CUG	CAG	UAG	Overexpression
	$tRNA^{Tyr}$	*Nicotiana rustica*	GΨA	UGG	UAA, UAG	TMV leaky stop context
UGA	$tRNA^{Cys}$	*Nicotiana rustica*	GCA	UGC	UGA	TMV leaky stop context
	$tRNA^{Arg}$	Wheat	U*CG	CGA	UGA	TRV/PEMV leaky stop context
	$tRNA^{Trp}$	*Nicotiana rustica*	CmCA	UGG	UGA	TRV/TMV/PEMV leaky stop context

* TMV, tobacco mosaic virus; TRV, tobacco rattle virus; PEMV, pea enation mosaic virus. References are given in the text.

UGA cognate anticodon GCA, requiring a G:A mispairing event at the third codon position. It appears to be the only $tRNA^{Cys}$ in the *Euplotes* genome, also decoding the canonical cysteine codon UGC (Grimm *et al*., 1998). While this is not an obvious solution to the UGA decoding problem, a plant $tRNA^{Cys}_{GCA}$ is known with UGA suppressor activity, although this is only efficient where the UGA codon is placed in a poor nucleotide context (Urban & Beier, 1995). It is not clear whether to achieve efficient UGA decoding, *Euplotes* employs an altered release factor disabled for UGA recognition, or a ciliate $tRNA^{Cys}$ with unique decoding properties, or both. However, the *Euplotes* $tRNA^{Cys}$ does not appear to be unusual structurally when compared to other known $tRNA^{Cys}$, and UGA sense codons in *Euplotes* genes are not found in nucleotide contexts unfavourable for eRF1 binding (see below), suggesting eRF1 altered stop specificity may be an important component of codon reassignment (Grimm *et al*., 1998). It is also feasible that a UGA-cognate *Euplotes* tRNA still remains to be discovered. Recently, the eRF1 gene sequence from *Tetrahymena thermophila* has been isolated; it encodes an eRF1 protein highly homologous at the amino acid level to other eukaryotic and archaeal release factors (e.g. 57% identity with human eRF1), but it is unclear which, if any, amino acid substitutions are responsible for altered stop codon specificity (Karamyshev *et al*., 1999). However, it is known that the *Tetrahymena* eRF1 is unable to complement a yeast temperature-sensitive mutation in the *SUP45* gene (encoding eRF1), consistent, among other possibilities, with a restricted stop codon recognition specificity (Karamyshev *et al*., 1999).

The termination signal: stop codons and the role of flanking nucleotides

It is well established that not only is the relative usage of the three types of stop codon biased, but also the nucleotide contexts in which they are found is non-random, particularly for those genes that are highly expressed. The identity of the base directly following the termination codon (the +1 base) is subject to particular bias, but nucleotides as far downstream as nucleotide position +3 are also non-random; experiments measuring competition between termination and other non-standard translational events, such as frame-shifting, nonsense suppression or selenocysteine incorporation, reveal that the base in this position affects termination efficiency *in vivo* in bacteria, yeast and humans, respectively (Major *et al*., 1996; Bonetti *et al*., 1995; McCaughan *et al*., 1995). The stop codon then has characteristics of a tetranucleotide, where the identity of the fourth base determines the efficiency with which termination occurs. Contexts favouring termination are predominantly used by highly expressed genes in all micro-organisms so far examined (Bonetti *et al*., 1995; Poole *et al*., 1995). Conversely, viral genomes containing stop codons whose suppression is required to direct translation of extended proteins have stop codons in poor contexts, favouring suppression. The tetranucleotide stop codon hypothesis has been strengthened considerably by studies showing that bacterial release factor RF-2 can be directly cross-linked to the nucleotide in the +4 position, as well as nucleotides further downstream, supporting the idea that as many as three succeeding nucleotides may contact the release factor

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and have roles as termination efficiency determinants (Poole *et al*., 1998).

The 5′ nucleotide context of a stop codon also influences how efficiently a stop codon directs termination. In *E. coli*, this operates partly through the identity of the penultimate amino acid residue in the nascent peptide, which influences termination efficiency by up to 30-fold (Mottagui-Tabar *et al*., 1994). Efficient termination events are associated with penultimate amino acid residues that are basic in nature. The nature of the C-terminal (ultimate) amino acid residue, whose codon is located immediately 5′ to the termination signal, also modulates termination efficiency in *E. coli* (Bjornsson *et al*., 1996). Termination efficiency is stimulated synergistically by pairings of the last two (ultimate and penultimate) amino acids of a polypeptide chain, which increase the propensity of α-helix or β-sheet formation, pairings which are represented most frequently in highly expressed genes in both *E. coli* and *Bacillus subtilis* (Bjornsson *et al*., 1996; Mottagui-Tabar & Isaksson, 1998). The 5′ codon context effect in *E. coli* is also partly mediated by the identity of the tRNA isoacceptor in the ribosomal P-site (Bjornsson *et al*., 1996). In *Saccharomyces cerevisiae*, termination by eRF1 is less influenced by the penultimate amino acid in the peptide chain; the greater effect is exerted by the P-site tRNAs. While a P-site CAG-decoding glutamine tRNA is not particularly antagonistic to release factor binding, CAA-decoding $tRNA^{Gln}_{UUG}$, when in the P-site, increases suppression of an A-site stop codon far more (Mottagui-Tabar *et al*., 1998). These effects almost certainly occur through stearic interactions between the P-site tRNA and the release factor (Bjornsson *et al*., 1996). Context effects thus have profound influences on termination efficiency in both prokaryotic and eukaryotic systems, and stop codon contexts which are poor substrates for eRF1 recognition are employed by viruses to direct stop codon suppression, as will be discussed below.

Programmed stop codon readthrough

While in organisms using the standard genetic code efficient recognition of the three stop codons is required for viability, in some viral genomes and cellular genes readthrough of stop codons is an integral part of the control of gene expression. Thus at a certain frequency, alternative translation elongation events take place instead of the default termination process. Regulation of this type controls the ratio of correctly terminated translation product to that of the polypeptide generated by stop codon readthrough. The readthrough of in-frame stop codons is driven by various *cis*-acting signals, almost always including a stop codon context which is suboptimal for release factor recognition, but which in some cases can include readthrough-enhancing RNA secondary structure elements downstream of the stop codon. How such secondary structures downstream of the stop codon can negatively influence successful eRF decoding is at present unknown, but clearly elements comparatively remote from the stop codon can have important effects on eRF-codon recognition.

Viral and retrotransposon stop codon suppression

Translation of tobacco mosaic virus (TMV) main genomic RNA results in the synthesis of two polypeptides of 126 kDa and 183 kDa with a molar ratio of 20:1. Synthesis of the larger polypeptide results from the translational readthrough of a UAG stop codon, possibly by a $tRNA^{Tyr}_{AUG}$ which has known *in vitro* suppression activity (Pelham, 1978; Beier *et al*., 1984). This readthrough process is essential for viability of the virus and controls the level of RNA replicase (Ishikawa *et al*., 1986). The five nucleotides following the stop codon with the consensus sequence UAG CAR YYA, including the cytosine nucleotide immediately 3′ to the UAG stop codon, play an important role in readthrough of the TMV stop coding signal, stimulating 2–5% suppression (Skuzeski & Atkins, 1990; Goelet *et al*., 1982; Skuzeski *et al*., 1991; Table 2). Since no RNA secondary structural elements have been identified in the leaky stop codon environment (Goelet *et al*., 1982), it seems likely that the 3′ stop codon context alone directs readthrough (Skuzeski *et al*., 1991). Programmed readthrough of the Qβ bacteriophage coat protein gene UGA codon by a $tRNA^{Trp}$ is also driven by nucleotide context 3′ of the stop codon (Weiner & Weber, 1973; Engelberg-Kulka, 1981; Table 2).

Table 2. Regulatory recoding signals directing the sense translation of viral and cellular gene stop codons

See text for details. ND, Not determined.

System	Secondary structure	5′ codon	Stop codon	3′ codon	Spacer region size	Other sequence requirements
Moloney murine leukaemia virus (Mo-MuLV)	Pseudoknot	GAC	UAG	GGA	8 nt (purine-rich)	Possible involvement of stem–loop structure which potentially competes with pseudoknot for formation
Tobacco mosaic virus (TMV)	None found	CAA	UAG	CAA	ND	The nucleotide 3′ of the UGA is very important to readthrough
Barley yellow dwarf virus (BYDV)	ND	AAA	UAG	GUA	ND	CN AAA UAG GUA GAC Stop codon environment found in all luteoviruses; additional CCN repeat region required, plus downstream sequences. For additional sequences see text
pCal (*Candida albicans* retrotransposon)	Potential pseudoknot	GAA	UGA	AAA	8 nt (purine-rich)	Potential pseudoknot?
Skipper (*Dictyostelium* retrotransposon)	Potential pseudoknot	CAA	UGA	CUC	Proposed to have no spacer as UGA forms part of stem I	Potential pseudoknot?

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Barley yellow dwarf virus (BYDV) belongs to the luteovirus group, which use a variety of translational control mechanisms for gene expression. The BYDV coat protein (CP) ORF is separated from a second reading frame, RT, by a stop codon, whose suppression is required to form a fusion protein (Dinesh Kumar *et al*., 1992). The CP stop codon environment is conserved between different luteoviruses, although unique among stop codon readthrough signals (see Table 2). A 6–15 base spacer separates the stop codon and a downstream block of 7–16 tandem proline-encoding repeats. This, together with a sequence 700 bases downstream from the stop codon, drives CP-stop codon readthrough (Brown *et al*., 1996; Guilley *et al*., 1994). The stop codon distal nature of this *cis* sequence is unusual, although it is well conserved between the luteoviruses and functions at different distances from the stop codon.

Not all stop codon readthrough signals are solely dependent on primary sequence; some also have an RNA structural component (see Fig. 3). Moloney murine leukaemia virus (Mo-MuLV) is a well-studied example of a mammalian type C retrovirus that uses UAG stop codon readthrough, with glutamine insertion at the stop codon position to express the *gag* and *gag–pol* fusion proteins in the 5 % ratio required for virion production (Yoshinaka *et al*., 1985). *Cis*-acting factors directing nonsense suppression include the sequence downstream of the stop codon, which has the ability to form an RNA pseudoknot structure with a minimal sequence requirement of 60 nt (Wills *et al*., 1991; Feng *et al*., 1992). While RNA pseudoknots are common *cis* signals which promote programmed ribosomal frameshifting in viral genomes (Brierley *et al*., 1989), they cannot be regarded as generic RNA secondary structures which can trigger any recoding event; the mouse mammary tumour virus (MMTV) pseudoknot, found downstream of the MMTV frameshift site, while similar to the Mo-MuLV pseudoknot, is unable to functionally substitute for the Mo-MuLV structure (Gesteland & Atkins, 1996).

Retrotransposons have two long ORFs homologous to the *gag* and *pol* ORFs of retroviruses, and the yeast retrotransposon Ty1 employs ribosomal frameshifting at the *gag–pol* junction to regulate *gag*:*gag–pol* translation products (Clare *et al*., 1988). However, in the case of both the *Candida albicans* retrotransposon pCal and the *Dictyostelium discoideum* retrotransposon *skipper*, *gag* and *pol* ORFs are in the same reading frame, separated by a UGA termination codon (Matthews *et al*., 1997; Leng *et al*., 1998), a novel retrotransposon coding arrangement. In both cases, the UGA stop codon separating *gag* and *pol* is followed by a potential pseudoknot-forming sequence, similar to the Mo-MuLV readthrough signal, an indication that readthrough of the UGA stop codon may be programmed and represent a common solution to the problem of regulating retrotransposon gene expression (Matthews *et al*., 1997; Leng *et al*., 1998).

The mechanism by which an RNA pseudoknot can trigger stop codon readthrough or ribosomal frameshifting is unknown. Evidence from initiation-synchronized *in vitro* translation reactions suggests that pseudoknots pause ribosomes at the infectious bronchitis virus (IBV) frameshift site, although not all RNA secondary structures which cause pausing are sufficient to trigger frameshifting (Somogyi *et al*., 1993). During programmed stop codon readthrough, however, pseudoknots must perform a different overall function to that in stop codon readthrough, although there may still be a role for pseudoknots in pausing the ribosome. Clearly, by definition, readthrough of a stop codon implies a tRNA-mediated translation elongation event at the expense of release factor recognition of the stop codon. Interaction of the pseudoknot with the ribosome may favour tRNA interaction over eRF1 binding at the A-site, perhaps by distorting the mRNA structure, or constraining its flexibility. This might in turn restrict the necessary 'presentation' of a stop codon to the release factor for recognition. Such presentation effects could operate synergistically with the unfavourable nucleotide context of the viral stop codon being read through to reduce the efficiency of eRF1-stop codon recognition.

Programmed stop codon readthrough in cellular genes

CFA/II strains of enterotoxigenic *E. coli* (ETEC) express three types of surface-associated hair-like fimbriae (coli surface antigens) known as CS1, CS2 and CS3. Expression of the genes required for biosynthesis and assembly of CS3 pili requires the suppression of an amber stop codon to produce a 104 kDa protein (Jalajakumari *et al*., 1989). While CFA/II strains have a nonsense suppressor mutant $tRNA^{Gln}$ (*supE*), it is believed that in a wild-type tRNA background, the extended protein is still produced although its reduced abundance does not allow pilus synthesis (Jalajakumari *et al*., 1989). The amino acid (glutamine) inserted by suppression at the stop codon is specifically required for protein activity, since when tyrosine is inserted by the *supF* $tRNA^{Tyr}$, protein function is lost.

During translation in a range of species studied, UGA codons in cellular mRNAs can direct the insertion of a twenty-first amino acid, selenocysteine. This recoding event occurs in response to a specific set of *trans* factors and *cis* signals, which differ between eukaryotes and prokaryotes. Prokaryotes direct selenocysteine insertion at specific UGA codons using a 40 nt structured RNA sequence or SECIS element (Bock *et al*., 1991). The *E. coli* SelB protein, a homologue of the translation elongation factor EF-Tu, binds to the SECIS element using its C-terminal domain, bringing a UGA-decoding selenocysteine $tRNA^{Sec}$ to the UGA codon at the ribosomal A-site (Bock *et al*., 1991; Kromayer *et al*., 1996). In eukaryote systems, the SECIS element is located in the 3′ untranslated region (UTR) of the mRNA encoding the selenoprotein, distal to the UGA codon directing selenocysteine incorporation, and the *trans* factors are less well characterized (Berry *et al*., 1993, 1991). Obviously, the key to selenocysteine UGA

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recoding is the elimination of release factor termination at the UGA selenocysteine signal. In eukaryote systems, this is in part achieved by the poor nucleotide context in which the selenocysteine UGA codons are found. Improving this context reduces selenocysteine incorporation at the expense of termination (McCaughan *et al*., 1995). However, readthough efficiencies of 4–5 % at the *E. coli fdhF* UGA selenocysteine codon and 75 % at the mammalian deiodinase internal UGA codon indicate that recoding has variable efficiency (Suppmann *et al*., 1999; McCaughan *et al*., 1995). Apart from nucleotide context, the structured SECIS elements in both eukaryote and prokaryote systems are essential for UGA recoding. In prokaryotes, SECIS elements do not act simply to increase the local concentration of $tRNA^{Sec}$–SelB complex at the UGA codon, thus outcompeting RF2; rather the SECIS element is required for delivery of the $tRNA^{Sec}$–SelB–GTP ternary complex to the ribosome, and overexpressing the SelB–tRNA combination does not increase the efficiency of incorporation (Suppmann *et al*., 1999). This delivery mechanism, together with context effects, seems to be sufficient to much reduce alternative termination reactions. It seems likely the eukaryote SECIS elements function in the same way, although the mechanism used to achieve $tRNA^{Sec}$ UGA decoding when the SECIS element is located distally in the 3′ UTR has not been established.

Modulating termination efficiency using release factor concentration: eRF3 and [*PSI*]

Programmed stop codon readthrough is principally employed by viruses and retrotransposons to control gene expression, and although some cellular gene examples are known, all seem to utilize *cis* mRNA elements such as stop codon context and downstream secondary structures. It is also possible to direct stop codon readthrough by lowering the cellular concentration of release factor (Stansfield *et al*., 1996). Intriguingly, in *S. cerevisiae*, lowered available concentrations of yeast eRF3 (the product of the *SUP35* gene, Sup35p) can exist naturally, since the yeast GTPase release factor eRF3 has prion properties. It can thus exist in alternative states, soluble or aggregated, known as [psi^-] or [PSI^+], respectively (Wickner, 1994). A [PSI^+] state causes an enhanced nonsense suppression phenotype and thus readthough of natural stop codons at a certain frequency. Such readthrough events will C-terminally lengthen a small proportion of proteins, perhaps altering their turnover, localization or function. However, it is at present unknown whether a [PSI^+] state enhances the readthrough of the stop codon of any cellular gene sufficient to produce a detectable phenotype. However, [PSI^+]-specific phenotypes other than nonsense suppression are known, and could possibly arise through such a C-terminal extension mechanism, discussed below (Eaglestone *et al*., 1999).

eRF3 (Sup35p) in the [PSI^+], aggregated state exists predominantly as cytoplasmic, high-molecular-mass oligomers, causing a weak nonsense suppressor phenotype. Release factor eRF1 (Paushkin *et al*., 1996) and Upf1p, a component of the mRNA surveillance complex which regulates nonsense-mediated decay, are both proteins which bind eRF3, and both are found incorporated into [*PSI*] eRF3 aggregates (Czaplinski *et al*., 1998).

Inheritance of [PSI^+] eRF3 occurs by cytoplasmic mixing, primarily through yeast mating, and results in the conversion of soluble eRF3 (Sup35p) into the aggregated [PSI^+] form. The propagation of [*PSI*] is dependent upon protein–protein interaction, and shows a spore (meiotic product) inheritance pattern of 4:0 [PSI^+]:[psi^-], characteristic of cytoplasmic, rather than nuclear, inheritance (Cox, 1965). The eRF3 [PSI^+] prion exhibits many similarities to mammalian PrP prions, best illustrated by experiments to recreate *de novo* eRF3 (Sup35p) prion generation *in vitro*. Highly ordered amyloid-like fibres can be induced to form *in vitro* with purified eRF3, and fragments comprising the eRF3 N-terminal and middle domains (NM) or the N-terminal domain alone (Fig. 1; Glover *et al*., 1997). [PSI^+]-form eRF3 fibres bind Congo red, are rich in β-sheet and can adopt different conformations, which are maintained throughout the fibre once self-perpetuation is initiated (Glover *et al*., 1997).

The eRF3 (Sup35p) protein is 685 amino acids in length and consists of three domains, each with different functions and properties (Fig. 1). The essential C-terminal domain of eRF3 is required for interaction with eRF1 in *S. cerevisiae* and *Schizosaccharomyces pombe* (Paushkin *et al*., 1997a). A second eRF1-binding site may be present on eRF3 in *S. cerevisiae*, spanning the N-terminal domain and middle (M) domain of the release factor (Paushkin *et al*., 1997a). As of yet, no designated function has been ascribed to the middle domain of eRF3.

The N-terminal domain, comprising the first 114 amino acid residues, contains four glutamine-rich degenerate nonapeptide repeats, and is not required for release factor activity (Teravanesyan *et al*., 1993). In spite of this, the domain has been conserved in all eukaryotic eRF3 proteins, although not all N-domains contain defined nonapeptide repeats. A number of lines of evidence indicate that the N-domain, with its peptide repeats, is centrally involved in [*PSI*] inheritance: (i) overexpression of eRF3 (Sup35p) fragments comprising either the N- and M-domains or N-terminal domain of eRF3 alone can induce a [PSI^+] state *de novo* (Paushkin *et al*., 1997a); (ii) single amino acid substitutions in one of the N-domain nonapeptide repeats results in mutant eRF3 (Sup35p) unable to propagate or support the [PSI^+] state (Doel *et al*., 1994; DePace *et al*., 1998); (iii) increasing or decreasing the number of nonapeptide repeats in the eRF3 N-domain markedly increases or decreases, respectively, the spontaneous generation of the [PSI^+] state in a [psi^-] yeast (Liu & Lindquist, 1999); and finally (iv) the fusion of *SUP35* NM domains to GFP in a [PSI^+] genetic background results in GFP aggregation *in vivo* (Patino *et al*., 1996).

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Environmental and genetic factors influence the loss and reacquisition of [PSI]

The ease with which aggregated [*PSI*$^+$] eRF3 is inherited is mirrored by the efficiency with which a [*PSI*$^+$] yeast strain can be converted to [*psi*$^-$]. Known as curing, growth of a [*PSI*$^+$] yeast in the presence of specific chemicals, such as guanidine hydrochloride (5 mM) or glycerol (2 M) can liberate soluble eRF3 (Sup35p) from pre-established [*PSI*$^+$] aggregates, and hence produce an isogenic yeast which is [*psi*$^-$] (Tuite *et al*., 1981). A [*PSI*$^+$] state can be re-established by eRF3 overexpression (Chernoff *et al*., 1993), although this phenomenon is strain dependent. Overexpression of the eRF3 NM domain is much more efficient at re-establishing a [*PSI*$^+$] state (Paushkin *et al*., 1997b).

Proteins that adopt aberrant conformations can be disaggregated *in vivo* by the activity of molecular chaperones (Parsell *et al*., 1994), and the activity of the heat-shock 'disaggregase' protein Hsp104p can also play a role in the process of [*PSI*] curing, evidence that [*PSI*$^+$] defines an altered aggregation state of eRF3 (Chernoff *et al*., 1995; Schirmer & Lindquist, 1997; Newnam *et al*., 1999). Both overexpression and disruption of the *HSP104* gene result in the loss of [*PSI*] (Chernoff *et al*., 1995). Following Hsp104p overexpression, the [*PSI*$^+$] state can be re-established by eRF3 (Sup35p) overexpression.

In addition to nonsense suppression itself, other discernible [*PSI*$^+$] phenotypes, that of resistance to extreme temperatures and raised ethanol concentrations, have been detected (Eaglestone *et al*., 1999). Interestingly, the level of [*PSI*$^+$]-mediated nonsense suppression was reduced when the [*PSI*$^+$] strains were exposed to raised ethanol concentrations. Stress imposition thus produced transient reductions in the degree of eRF3 (Sup35p) aggregation in a [*PSI*$^+$] strain, and thereby reduced nonsense suppression, without curing the [*PSI*$^+$] state (Eaglestone *et al*., 1999). The reduced suppression phenotype observed was reversible, and a [*PSI*$^+$] state restored upon ethanol removal. Such reversible control of nonsense suppression frequencies may indicate that under certain stress conditions *S. cerevisiae* may use the [*PSI*$^+$] state to control readthrough of a natural stop codon(s) with the consequent C-terminal extension of the polypeptide. However, no ORFs subject to [*PSI*]-programmed readthrough have yet been identified.

tRNA suppressors of nonsense codons

The failure of a release factor to recognize a stop codon normally triggers a default, alternative elongation event mediated by a suppressor tRNA. Stop codon recognition by the RF and termination competes directly with suppressor tRNAs, which can be cognate or mis-cognate for the termination codon (Fig. 3). Their affinity for the stop codon and A-site termination environment, relative to that of the RF, determines the stop codon readthrough frequency. Suppressors can be wild-type tRNAs which are miscognate for stop codons, and wobble base pair with the stop codon at the third codon position, or cognate for a stop codon, either through mutation, or because in a given non-universal genetic code organism a stop codon has been reassigned to sense (discussed previously). A final category contains tRNAs which can be defined as P-site effectors of nonsense suppression. These tRNAs modulate A-site decoding events either through antagonism of release factor binding, or promotion of suppressor tRNA binding; in doing so, they contribute to the 5′ context effects on termination efficiency. Natural suppressor tRNAs will be considered in more detail below.

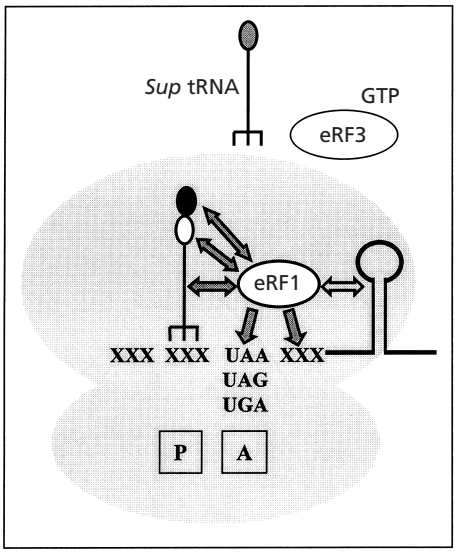

Fig. 3. Schematic representation of factors determining the efficiency of stop codon recognition by the class 1 release factors eRF1, RF1 and RF2. *Cis*-acting factors which influence termination efficiency include the nucleotides immediately downstream of the stop codon, particularly at the +1 and +3 positions, the stop codon itself, the P-site tRNA, and the ultimate and penultimate amino acids in the nascent polypeptide chain (dark grey arrows). The relative importance of these factors varies between eukaryotes and prokaryotes (see text for details). Other important *cis* factors, particularly in viral programmed stop codon readthrough signals, are downstream secondary structures, such as stem–loops and pseudoknots (light grey arrow). These may have indirect, as opposed to direct stearic or stereochemical effects on RF–stop codon interaction. *Trans* factors modulating competitive advantage of the RF over suppressor tRNAs include firstly the tRNAs themselves, which may be cognate or miscognate for stop codons, and secondly the abundance of the class 2 GTPase release factor eRF3.

Sense codon-cognate suppressor tRNAs

Ochre (UAA) mutations can be suppressed in yeast *in vivo* by increasing the copy number of a wild-type CAA-decoding $tRNA^{Gln}_{U^*UG}$ (where U* represents modified uridine) (Pure *et al*., 1985). A second yeast CAG-decoding $tRNA^{Gln}_{CUG}$, can suppress amber UAG codons when expressed on a multi-copy plasmid (Weiss & Friedberg, 1986). In both these cases, UAA and UAG suppression requires a first anticodon position G::U wobble base pairing (Table 2). In another study, peptide

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sequencing identified amino acids inserted at the position of a premature UAG stop codon of a nonsense yeast *STE6* allele. This stop codon, found in a nucleotide context unfavourable for eRF1 recognition, was suppressed by tyrosine, lysine and tryptophan tRNAs, although not by a $tRNA^{Gln}$ as expected (Fearon *et al.*, 1994).

Many plant viruses, together with some animal viruses, rely for propagation on programmed stop codon readthrough events, generally of stop codons in nucleotide contexts unfavourable for eRF recognition. This ensures correct relative proportions of structural and enzymic virus translation products. This has stimulated the search for the naturally suppressing tRNAs responsible for the stop codon readthrough. In plants, a wheat cytoplasmic $tRNA^{Arg}_{U*CG}$ has been purified with *in vitro* UGA suppressor activity, a decoding event requiring a first codon position G:U wobble pairing (Baum & Beier, 1998). A UGA stop codon in various virus programmed readthrough contexts was also efficiently suppressed *in vitro* by a plant $tRNA^{Trp}_{CmCA}$, involving a C:A mispairing at the third codon position (Urban *et al.*, 1996). A third plant tRNA, a $tRNA^{Cys}_{GCA}$ from tobacco, was also able to decode UGA in either the TMV or tobacco rattle virus (TRV) leaky contexts (Urban & Beier, 1995). Finally, a plant $tRNA^{Tyr}_{G\psi A}$ (where ψ represents pseudouridine) has been isolated that has both UAA and UAG suppression activity *in vitro* (Beier *et al.*, 1984). Decoding of UAA and UAG requires non-canonical G:A or G:G pairings at the third codon position, respectively.

In mammalian cells, the identity of natural suppressor tRNAs involved in programmed readthrough of the Mo-MuLV) has been inferred by identifying the amino acid inserted at the position corresponding to the suppressed stop codon. This revealed that both UAG and UAA can direct glutamine incorporation as well as termination events, while UGA directs arginine, cysteine and tryptophan insertion (Feng *et al.*, 1990).

Clearly, similar isoacceptor tRNA species act as natural tRNA suppressors in different systems studied via first or third codon position wobble base pairing. In all cases, however, nonsense suppression frequency directed by these miscognate tRNAs is only detectable when the stop codon is placed in a nucleotide context unfavourable for eRF binding. Clearly the structural properties of the suppressor tRNAs themselves will contribute to their mis-decoding of stop codons, as well as their nucleoside modifications, which are known to affect nonsense suppression efficiency in yeast and other systems (Dihanich *et al.*, 1987).

Post-termination ribosomal fates

Following termination in both prokaryotes and eukaryotes, ribosomes must be released from the mRNA to take part in new rounds of translation initiation, a recycling activity which resides in specific protein factors. However, at least in eukaryote cells, the activity of such factors may be regulated; during the translation of certain small, upstream ORFs (uORFs), ribosomes may not be immediately released following termination, but may instead resume scanning. Eukaryote ribosomes which have resumed scanning can subsequently re-initiate at downstream AUG codons, or alternatively can play a role in complex pathways controlling mRNA stability, which are only now being slowly unravelled. These 'recycling alternative' events constitute novel forms of regulation of gene expression at a post-transcriptional level.

Ribosomes are released from the mRNA and recycled post-termination

Following termination, cells must recycle ribosomes, release factors and tRNAs to allow new rounds of protein synthesis. In prokaryotes, post-termination ribosome release requires the activity of the essential ribosome recycling factor (RRF; Janosi *et al.*, 1998). A post-termination ribosomal complex becomes a pre-initiation complex via a two-step mechanism. Firstly, RRF and GTP-complexed EF-G together split the post-peptidyl-release ribosome into its component subunits. In the second step, the initiation factor IF3 acts to remove the deacylated tRNA from the ribosomal P-site (Freistroffer *et al.*, 1997; Pavlov *et al.*, 1997; Karimi *et al.*, 1999). *In vivo*, following termination and in the absence of RRF, prokaryote 70S ribosomes can remain on the mRNA and continue to 'slide' downstream, and eventually reinitiate protein synthesis at any codon between 17 and 45 nt downstream in a frame-independent manner (Janosi *et al.*, 1998). In addition, RRF acting with EF-G can also reduce frameshift errors *in vitro*, and reduce missense translation error during peptide elongation (Janosi *et al.*, 1996), although the mechanism of this activity is not well understood. The recently solved crystal structure of RRF from *Thermotoga maritima* has revealed that this protein, like eRF1, is a tRNA mimic, although a much more exact mimic than is eRF1 (Fig. 2; Selmer *et al.*, 1999). RRF-tRNA mimicry is almost certainly a central feature of its activity in dissociating ribosomes. It has been proposed that following termination and peptidyl release, RRF binds to the A-site and is translocated by EF-G in the same way as peptidyl-tRNA is translocated. P-site occupancy by RRF is then the trigger for ribosomal dissociation (Selmer *et al.*, 1999). This model has certain features which distinguish it from the proposal that IF3 acts to remove deacylated tRNA from the P-site following RRF-catalysed ribosome dissociation from the mRNA (Karimi *et al.*, 1999). However, it might be that in bacteria, post-termination mechanisms differ dependent upon the ribosome proximity to a ribosome-binding site (Selmer *et al.*, 1999).

The analogous ribosome recycling step in eukaryotes is in contrast completely uncharacterized; a homologous nucleus-encoded gene encoding an RRF-like protein is found in eukaryotes, but appears to be an organelle protein; in yeast, the *RRF1* gene has a mitochondrial targeting sequence, and disruption of the gene is non-lethal, generating a *petite* phenotype typical of disrupted

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mitochondrial function (I. Stansfield & M. F. Tuite, unpublished). While other eukaryote RRF-encoding genes have been identified, they are all targeted to organelles such as chloroplasts (Rolland *et al*., 1999). No equivalent cytoplasmic form has yet been identified. One recent suggestion is that eRF3 may represent a combined RF3 and RRF activity, thus representing a eukaryote recycling function, although hard evidence to support this is lacking at present (Buckingham *et al*., 1997).

Alternative post-termination events: resumed scanning

In eukaryotes, post-termination ribosomes can experience different fates, regulated by the nucleotide context of the stop codon. This is best exemplified by the post-transcriptional regulation mechanisms of the *S. cerevisiae GCN4* gene, which encodes a transcription factor involved in responses to amino acid starvation. The *GCN4* mRNA 5′ UTR contains four uORFs, of which only uORF1 and uORF4 are required for wild-type translational control of *GCN4* expression (Mueller & Hinnebusch, 1986). The precise details of this mechanism, and its relation to amino acid starvation responses, are reviewed elsewhere (Hinnebusch, 1997). Of importance to this discussion of regulation of (post-) termination events are the findings that ribosomes can resume scanning after terminating translation at uORF1, and that they can subsequently acquire competence to reinitiate at downstream AUG codons (Hinnebusch, 1997). In contrast, ribosomes terminating translation at the uORF4 termination codon are released from the mRNA (Abastado *et al*., 1991; Dever *et al*., 1992). This key difference between the behaviour of post-termination ribosomes at uORFs 1 and 4 results from the character of the nucleotide context immediately preceding (3 nt) and following (10 nt) the respective uORF stop codons (Grant & Hinnebusch, 1994). An AU-rich nucleotide bias around the uORF1 stop codon triggers resumed scanning, whereas the GC-rich character of the corresponding uORF4 nucleotides promotes ribosome release and recycling. While the mechanism of action of these *cis* sequences is not fully understood, it is clear that no single sequence confers these properties, and that a wide variety of contexts can achieve the same effect (Grant & Hinnebusch, 1994). uORFs in the 5′ UTRs of the *YAP1* and *YAP2* genes, while having different sequences surrounding their stop codons, nevertheless have very similar properties to *GCN4* uORFs 1 and 4, respectively, again an indication that primary sequence itself is not the prime determinant of the ability to promote resumed scanning (Vilela *et al*., 1998). It has been suggested that the GC-rich environment around the uORF4 stop codon may pause a terminating ribosome (perhaps via rRNA interaction) long enough to allow a putative recycling factor to bind, an event prevented by rapid termination at uORF1 (Grant & Hinnebusch, 1994). The region 5′ of uORF1 is also important for the reinitiation stimulating ability of uORF1, although this mRNA region lies outside that which would be occluded by a ribosome terminating at uORF1 (Grant *et al*., 1995).

In prokaryotic systems, small ORFs have peculiar properties, first highlighted by studies of the λ bar mini-genes, which encode dipeptides. During translation of such mini-genes, translation termination does not always take place, and in some instances, peptidyl-tRNA is released, stimulated by RRF, RF3 and the elongation factor EF-G; this reaction is detrimental to peptidyl hydrolase mutants expressing a mini-gene (peptidyl hydrolase breaks the tRNA nascent peptide bond in peptidyl tRNAs inappropriately released from the ribosome; Heurgue-Hamard *et al*., 1998). Translation initiation factors IF1 and IF2 also play a role in stimulating release of nascent peptidyl-tRNA without a prior termination reaction during short ORF (mini-gene) translation *in vitro*, and *in vivo*, overexpression of IF1 and IF2 cause growth defects in a peptidyl hydrolase mutant (Karimi *et al*., 1998). It is unclear at present whether these prokaryote termination-alternative reactions stimulated by mini-genes or uORFs have any significance for the regulatory properties conferred by uORF1 in the yeast *GCN4* 5′ UTR. Certainly there is no direct evidence that translation termination actually takes place at either uORF 1 or uORF4, and the possibility that termination-alternative events take place has not been excluded.

Conclusion and perspectives

A skeleton outline of the mechanism of translation termination is now emerging from recent research. Arguably, two of the most important advances have been the solution of the eRF1 and RRF crystal structures, revealing their tRNA-like shapes and dimensions (Selmer *et al*., 1999; Song *et al*., 2000). The eRF1 structure solution now allows stop codon recognition, and eRF3–eRF1 and eRF1–ribosome interactions to be addressed in the context of structure (e.g. Bertram *et al*., 2000). However, despite similarities between eRFs and tRNAs, the detail of RF–stop codon interaction may be fundamentally different from that of tRNA anticodon–codon interaction; certainly the RF–stop codon recognition process is up to 60 times slower than sense codon decoding (Freistroffer *et al*., 2000). A recent model for eRF1 stop codon recognition also proposes that the stop codon bases may not be stacked as is probably the case in a sense codon interacting with cognate tRNA (Bertram *et al*., 2000). The involvement of nucleotides 3′ to the stop codon in class 1 RFs stop codon recognition also argues that stop codon recognition has unique features in comparison to sense codon decoding, which can now be addressed using the eRF1 structural information.

RF-tRNA mimcry also raises some intriguing questions, at least in prokaryote systems, where apparently an EF-Tu like molecule is not required to bring the RF to the A-site (Freistroffer *et al*., 1997). What then provides the codon–'anticodon' fidelity checking mechanism which is fulfilled by EF-Tu when an aminoacyl tRNA is

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presented to the A-site? Termination at non-stop codons occurs at very low frequencies, so fidelity is obviously preserved by some mechanism (Freistroffer *et al*., 2000). It is also unclear how the release factors (which unlike tRNAs and EF-Tu/EF1-α, are present in the cell at relatively low abundance) are selected quickly by the ribosome when a stop codon is located at the A-site.

The use of reconstituted translation systems using highly purified protein *trans*-acting factors has allowed an increasingly detailed picture to emerge, not just of termination itself, but also of the events which follow it. Eukaryote termination is less well defined; although *in vitro* termination reactions have been reconstituted, the precise role of the GTPase eRF3 is unclear, and our knowledge of how eukaryote release factors are removed from the ribosome, and the ribosomes subsequently removed from the mRNA, are hazy. The need to recycle ribosomes or subunits back to the 5′ end of the mRNA makes it likely that a specialized set of eukaryote factors exist for this purpose; either none have been identified to date, or factors already known have as yet undiscovered functions. The prospect of a fuller understanding emerging of the mechanism of both eukaryote and prokaryote termination in the near future is encouraging, providing a clear picture of how the last three codons in the genetic code are accurately decoded. It will also open the door to understanding how control of gene expression is exerted at the termination stage, and post-termination.

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