CMLS, Cell. Mol. Life Sci. 60 (2003) 2115–2130
1420-682X/03/102115-16
DOI 10.1007/s00018-003-2334-2

CMLS Cellular and Molecular Life Sciences

Review

Yeast as a sensor of factors affecting the accuracy of protein synthesis

L. Valente [a] and T. G. Kinzy [a,b,*]

[a] Department of Molecular Genetics, Microbiology and Immunology, UMDNJ Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, New Jersey 08854 (USA), Fax: +1 732 235 5223, e-mail: kinzytg@umdnj.edu
[b] The Cancer Institute of New Jersey, UMDNJ Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, New Jersey 08854 (USA)

Received 27 November 2002; received after revision 16 April 2003; accepted 25 April 2003

Abstract. The cell monitors and maintains the fidelity of translation during the three stages of protein synthesis: initiation, elongation and termination. Errors can arise by multiple mechanisms, such as altered start site selection, reading frame shifts, misincorporation or nonsense codon suppression. All of these events produce incorrect protein products. Translational accuracy is affected by both cis- and trans-acting elements that insure the proper peptide is synthesized by the protein synthetic machinery. Many cellular components are involved in the accuracy of translation, including RNAs (transfer RNAs, messenger RNAs and ribosomal RNAs) and proteins (ribosomal proteins and translation factors). The yeast *Saccharomyces cerevisiae* has proven an ideal system to study translational fidelity by integrating genetic approaches with biochemical analysis. This review focuses on the ways studies in yeast have contributed to our understanding of the roles translation factors and the ribosome play in assuring the accuracy of protein synthesis.

Key words. Fidelity; translation factor; protein synthesis; frameshift; missense; nonsense; ribosome.

The protein synthesis process in eukaryotic cells

The transformation of the genetic information from messenger RNA (mRNA) to protein occurs in three steps: initiation, elongation and termination. In general, the overall process is conserved between eukaryotes and prokaryotes, although the eukaryotic process described in this review is more complex. During initiation, mRNA is recruited to the ribosome by the action of a series of soluble protein factors termed eukaryotic initiation factors or eIFs (fig. 1A and reviewed in [1]). The end result is an 80S ribosome positioned with the Met-transfer RNA $(tRNA)_i^{Met}$ in the P-site. The eukaryotic elongation factor 1 (eEF1) complex then directs delivery of each aminoacyl-tRNA (aa-tRNA) to the A-site of the ribosome in the elongation phase of protein synthesis (fig. 1B and reviewed in [2]). Following peptide bond formation by the ribosome, the translocase eEF2 repositions the new peptidyl-tRNA in the P-site and helps assure the three-base shift in reading frame. A protein unique to fungi, eEF3, is essential in vivo and required for elongation to occur in vitro [3–5]. eEF3 interacts with eEF1A and promotes release of the deacylated tRNA from the E-site [6–8]. The termination event occurs when the A-site becomes occupied by one of the three stop or nonsense codons (UAA, UAG or UGA). This process is facilitated by the action of eukaryotic release factors 1 and 3 (eRF1 and eRF3, fig. 1C and reviewed in [9]). The resulting synthesis of a protein is the end product of the careful regulation of gene expression in the cell. Those components linked to fidelity and discussed in the review are shown in figure 1.

* Corresponding author.

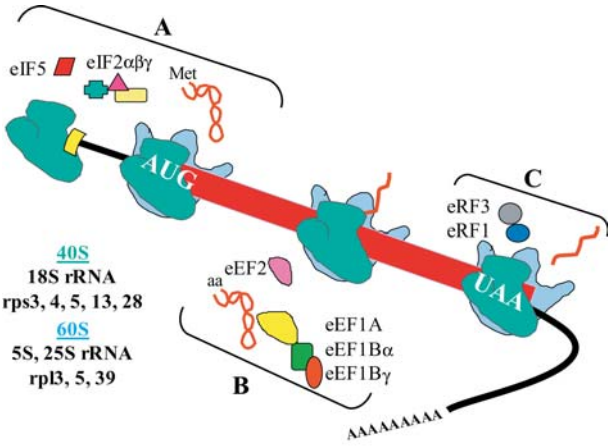

Figure 1. The process of protein synthesis monitors fidelity during all three stages of translation. (*A*) During initiation soluble translation initiation factors (eIFs) bind the mRNA, deliver the initiator Met-tRNA$_i^{Met}$ to the first AUG codon and assemble to form a complete 80S ribosome from the 40S and 60S subunits. (*B*) Elongation is the repeated delivery of each subsequent aa-tRNA to the ribosome, peptide bond formation, movement of the mRNA and the growing peptidyl-tRNA in a process using both soluble elongation factors (eEFs) and the activity of the ribosome. (*C*) When a stop or nonsense codon is encountered, soluble release factors (eRFs) facilitate hydrolysis of the peptide from the tRNA and release. Shown are those translational components linked to fidelity in yeast.

Importance of translational fidelity

Translational fidelity is critical to the production of full-length, functional proteins. Thus, the translational apparatus carefully monitors and helps maintain accuracy at every point during protein synthesis. During initiation, fidelity can refer to the selection of the appropriate AUG or the use of an AUG as opposed to another codon. During elongation, an even wider range of events can occur. After start site selection, the correct three-base reading frame must be maintained. A shift in reading frame of one or two bases in either the 5′ or 3′ direction will lead to an encounter with a premature stop codon. This results in termination to produce an incomplete polypeptide and may trigger the nonsense-mediated mRNA decay (NMD) pathway [10]. Estimates of the frequency of frameshift errors are 3×10^{-5} to $^{-4}$ [11]. Many viruses utilize specific programmed ribosomal frameshift (PRF) sites within the mRNAs to produce significantly higher frequencies of this event. This mechanism is important for the virus to express the proper proportions of viral proteins. This is exemplified in the yeast *Saccharomyces cerevisiae* by the –1 signal in the L-A virus [12] and the +1 signal in the Ty retrotransposons [13]. Work in yeast has identified the cis-acting sequences of the viral mRNA, cellular proteins and drugs such as anisomycin, preussin and sparsomycin that affect the PRF process [14–18]. A change in frameshifting efficiencies affects viral maintenance and propagation and thus represents a possible therapeutic target (reviewed in [19]). Insertion of each aa-tRNA is also monitored to assure that the correct amino acid is incorporated into the protein. Misincorporation of the wrong amino acid can happen by mischarging a tRNA. Errors can also occur if the ribosome fails to reject an improper codon-anticodon interaction, allowing the deposit of an incorrect aa-tRNA in the A-site. Missense error frequencies in yeast are also low, estimated from experimental data to be 10^{-5} [20]. An innaccurate termination process can result in nonsense suppression, or readthrough of a stop codon. This allows incorporation of an amino acid and extension of the polypeptide chain. Analysis of the steps of protein synthesis has led to the development of a series of elegant approaches to characterize translational fidelity in eukaryotes using *S. cerevisiae*.

Mechanisms and assays of altered translational fidelity

There are multiple methods to analyze the effects of site-directed or null mutations of specific genes, to screen for new mutations in novel genes or to define cis-acting elements in the mRNA that alter fidelity. Some take advantage of the ability to select for reduced fidelity by the ability to ‘mistranslate’ a mutant allele of a metabolic gene in yeast. Others introduce mutations in the *lacZ* gene, such that any factor that reduces fidelity will result in production of a blue color in cells grown on 5-bromo-4-chloro-3-indoyl-β-D-galactose (*X*-gal) for screening. This second approach can also be utilized to quantitate effects on fidelity [21]. The yeast viral systems L-A and Ty*1* provide a functional window on the consequences of altered fidelity on a biological process in the cell. One important point in all fidelity assays in vivo is that mutations that introduce nonsense codons or shift the reading frame result in a premature stop codon. Screens for factors that affect fidelity can also lead to mutations that increase or alter the mRNA. Mutations that alter transcription of the reporter mRNA or inactivate the nonsense-mediated mRNA decay pathway have been identified in this manner [22, 23]. The following sections describe the approaches to analyze fidelity utilized in the studies highlighted in this review.

Analysis of start site selection

The application of yeast to the analysis of genes whose products affect start codon identification has proven an effective method to identify the genes encoding initiation factors. The reader is referred to an excellent and detailed review by Donahue [24]. A classic analysis was performed by the Donahue laboratory utilizing a dual reporter system demonstrating the use of both selection and

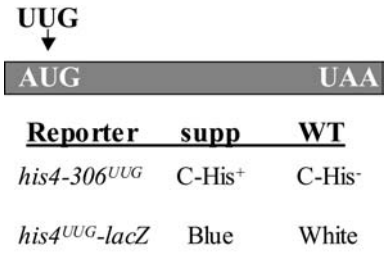

Figure 2. Assays for alterations in start site selection. Replacement of the authentic AUG codon with a near-cognate codon allows for selection of mutations that result in reduced fidelity of start site selection. Mutations are monitored for growth on medium lacking histidine for a *his4-306*^{UUG} mutation [149] or expression of the reporter gene *lacZ* (*his4*^{UUG}*-lacZ*, [49]) by blue color on X-gal plates.

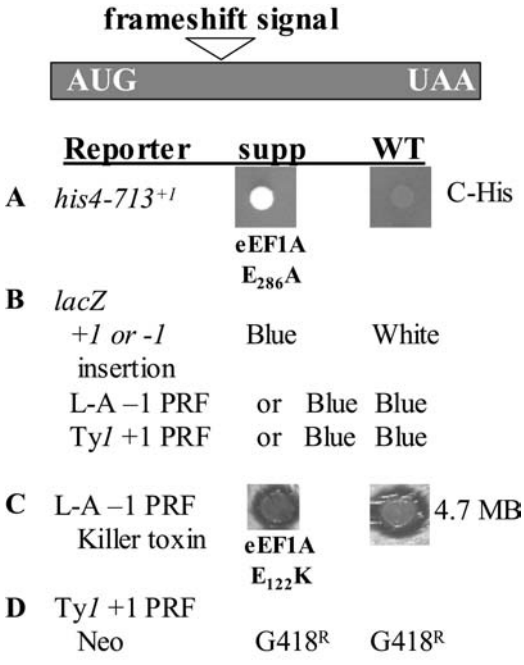

Figure 3. Assays for alterations in nonprogrammed (insertion) and PRF (viral-like +1 and –1) reading frame shifts. (*A*) A +1 insertion in a gene required for synthesis of an amino acid, for example histidine with the *his4-713* allele, results in death on cells lacking histidine. The reduced fidelity caused by the eEF1A $E_{286}A$ mutation allows suppression of the frameshift and growth on C-His. (*B*) A simple insertion or deletion in the *lacZ* gene results in a blue color on X-gal when frameshifting is increased. Insertion of the entire PRF signal from L-A or Ty*1* results in an increase in blue color with increased frameshifting or a lighter/white color with reduced frameshifting. (*C*) Altered frameshifting at the L-A PRF signal results in reduced ability to maintain the yeast M_1 satellite virus and produce the killer toxin. Wild-type L-A M_1 cells create a zone of killing on a lawn of sensitive yeast plated on 4.7-MB medium. Altered PRF by eEF1A E122K results in less maintenance of M_1 and a reduced halo size. (*D*) Altered frameshifting on the yeast Ty*1* signal reduces the frequency of retrotransposition. Induction of the expression of a Ty*1* element marked with a reporter gene (such as neo^R) allows quantification of the extent of retrotransposition. A wild-type cell produces geneticin-resistant colonies, and mutations that alter the frequency of frameshifting reduce the frequency of transposition (% geneticin-resistant colonies).

screening in vivo [25]. A strain was used where both the *HIS4* gene and a *HIS4-lacZ* fusion were engineered to remove or alter the authentic AUG codon. These cells die on complete media lacking histidine (C-His, fig. 2). Following mutagenesis of the strain, selection on C-His identified mutations that allow an alternative start site selection mechanism. Screening for those colonies that were also blue on X-gal confirmed the effect was due to altered initiation. When cloned and analyzed, these *sui* (*su*ppressors of *i*nitiator codon) mutations were identified in genes encoding several translation initiation factors (see below).

Identification of alterations in maintenance of frame and the effectiveness of cis-acting viral mRNA elements in yeast

Maintenance of the proper reading frame is essential for production of the correct protein. In yeast, both nondirected (simple insertion or deletions in a reporter system) and programmed (viral-like) signals for frameshifting are used for in vivo analysis. Screening for factors involved in maintenance of reading frame can be accomplished by suppression of a +1 insertion in an auxotrophic marker. A specific example is the *his4-713* +1 insertion allele, which when present in a cell results in death on C-His medium (fig. 3A). Mutations that suppress the +1 mutation allow growth on this medium. The effect on frameshifting in either the +1 or –1 direction can be quantified by the use of a *lacZ* reporter-based system (fig. 3B). Suppression of the frameshift results in blue colonies on media containing X-gal or increased β-galactosidase activity.

Yeast is very amendable to the study of programmed ribosomal frameshifting (PRF) events that many viruses exploit, such as the –1 PRF in the L-A killer virus [12] and the +1 PRF utilized by the Ty retrotransposons [13]. Work in yeast has identified the cis-acting sequences of the viral mRNA required for the high levels of frameshifting seen at these sites, typically 2% for the L-A signal [12] and 40% for the Ty*1* signal [13]. Briefly, the L-A signal is characterized by the presence of a slippery site (fig. 4A), and an RNA pseudoknot. The Ty*1* element has a rare codon for the Arg-tRNA^{Arg} (fig. 4B) as well as sequences flanking this site that promote frameshifting [13]. Mutations that affect these processes can be characterized by the insertion of either signal into a *lacZ* reporter (fig. 3B). The maintenance of frame (*mof*) yeast mutants, encoding translation and mRNA decay pathway components, were identified by a screen for mutations with altered expression of *lacZ* with the L-A signal [26].

The yeast viruses themselves can be assayed in vivo. The L-A virus has a satellite virus M_1 which produces a toxin. In order for M_1 to propagate, the efficiency of frameshifting on the L-A –1 signal must remain ~2% [12]. When

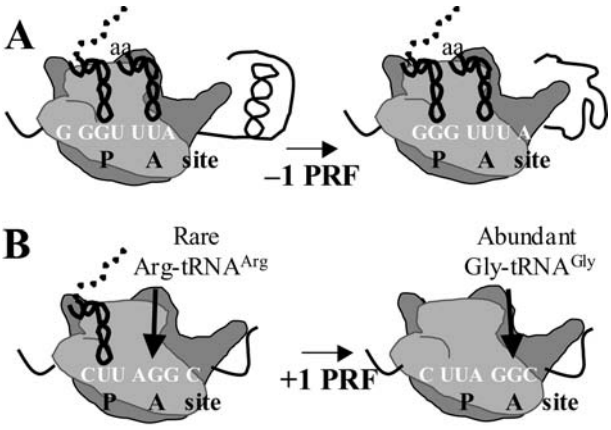

Figure 4. Different cis-acting signals in the yeast L-A virus and Ty*1* retrotransposon direct PRF. (*A*) In the L-A virus, a pseudoknot 3′ of the 'slippery sequence' causes the ribosome to pause with both the A and P sites occupied by tRNAs. A slip in the –1 direction retains 2 of 3 for each tRNA. (*B*) In the Ty*1* retrotransposon, an AGG codon recognized by the rare Arg-tRNAArg(CCU) causes a pause such that the peptidyl-tRNA (Leu) can allow the mRNA to slip one base in the +1 direction. This places a GGC codon, decoded by an abundant Gly-tRNA^{Gly}, in the A-site and allows translation to continue.

cells infected with the virus are plated on a lawn of sensitive cells [27], maintenance of the killer virus is seen by the zone of killing around the colony (fig. 3C). For Ty*1*, the retrotransposition frequency is affected by alterations in the frequency of the +1 frameshift. A set of reporter plasmids have been produced that can monitor the frequency of retrotransposition [28, 29]. For example, a *URA3*-based plasmid contains a reporter gene (neo^R) within the Ty*1* element and under a galactose-inducible promoter. The neo^R gene is transcribed and translated to produce active protein only after a round of transposition. Following transformation of the plasmid and induction of Ty*1* expression for several days, and loss of the plasmid monitored by growth on 5-fluoroorotic acid [30]. The frequency of colonies that now grow on media with geneticin/G418 quantifies the retrotransposition frequency (fig. 3D). The various assays developed to study and dissect ribosomal frameshifting in yeast has allowed a mechanistic analysis of the translation process and in particular the role of elongation factors as described below [31].

Misincorporation

Misincorporation of the wrong amino acid into the polypeptide chain can occur if the tRNA is aminoacylated with the wrong amino acid or if an improper codon-anticodon match allows an incorrect aa-tRNA to enter the A-site. There is no in vivo selection for mutations that cause misincorporation, so the study of fidelity in the area of missense mutations does have its limitations. Many mutants that affect nonsense suppression or frameshifting efficiencies are subsequently shown to affect missense rates in vitro [32, 33]. An assay was developed where affinity-tagged type III chloramphenicol acetyl transferase (CAT) was altered at His$_{196}$(CAC) to Tyr(UAC) [20]. The protein is catalytically inactive unless His is misincorporated. This construct can be expressed in wild type and cells bearing mutations likely to affect fidelity. Purification and analysis of CAT activity provides an estimate of altered misincorporation. Alternatively, one can analyze this process in vitro by utilizing a cell-free system and measuring the amount of the misincorporation of the near-cognate Leu-tRNA^{Leu} relative to the cognate Phe-tRNA^{Phe} using a poly (U)-dependent translation assay [34]. Both of these assays can take a substantial amount of time as compared with the in vivo assays; thus missense errors have not been studied as extensively as nonsense or frameshifting.

Nonsense suppression

Nonsense suppression results when a near-cognate aa-tRNA or a mutant suppressor aa-tRNA delivered by eEF1A competes with the release factors at a stop codon (reviewed in [10, 35]). For the analysis of nonsense suppression, a plasmid containing a *lacZ* reporter gene with an inserted nonsense codon (fig. 5A) or an auxotrophic allele caused by a premature stop codon (fig. 5B, C) is used. There are many useful nonsense alleles for all three stop codons, and common alleles are shown in table 1. One commonly used allele is *lys2-801* (UAG), which dis-

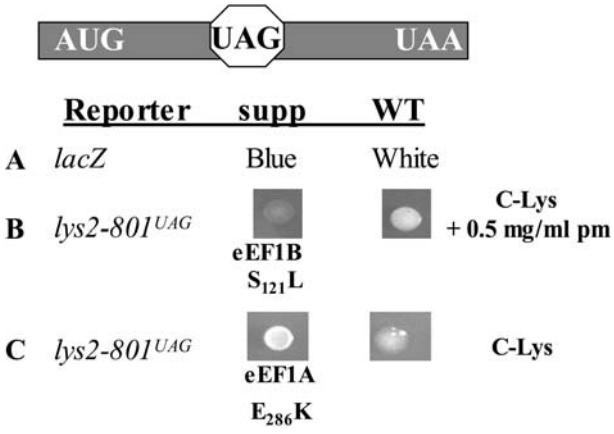

Figure 5. Assays for nonsense suppression. (*A*) Stop codon readthrough is quantitated with a reporter *lacZ* construct containing an in-frame stop codon or by increased blue color on X-gal plates. Insertion of a stop codon in the reading frame of the *LYS2* gene (*lys2-801^{UAG}*) allows for selection of mutations that alter fidelity. (*B*) The presence of low concentrations of paromomycin (pm) increases misreading, such that mutations with more efficient reading of a stop codon (eEF1Bα S_{121}L) show less growth. (*C*) Without paromomycin wild-type cells die on media lacking lysine, however, mutations that increase stop codon readthrough (eEF1A E_{286}K) show increased growth.

Table 1. Common auxotrophic alleles used for analysis of fidelity.

UAA	UAG	UGA	AUG (start)	+1 insertion
ade2-101, ade2-1	*can1-132, lys2-801*	*his4-260, his4-166*	*his4-305^{GUG}*	*met2-1*
lys1-1, lys2-1	*tyr7-1, trp1-1*	*leu2-2, lys2-101*	*his4-306^{UUG}*	*his4-713*
leu2-1, can1-100	*trp1-7, met8-1*	*ade1-14*		
his7-1	*lys2-864*			

rupts the lysine biosynthesis pathway. It is of note that the drug paromomycin increases the frequency of translation errors, and can be used to increase the level of suppression [36, 37]. Thus, with paromomycin present wild-type cells read through the stop codon more efficiently, and a mutation that increases fidelity shows reduced growth (fig. 5B). Without the drug, wild-type cells cannot grow on C-Lys unless suppression of the stop codon occurs (fig. 5C). Thus, mutants that increase nonsense suppression can be selected on this medium. The *can1-100* (UAA) mutation produces resistance to the compound canavanine on C-Arg. This allows an opposite approach, such that suppression results in sensitivity to this drug. The level of nonsense suppression in a wild-type strain can be very low, usually $<0.1\%$. The baseline level can be increased in a strain bearing a suppressor tRNA for a specific stop codon (i.e. the UAA suppressor *SUQ5*).

Taken together, these assays have provided the basis for an approach to the processes involved in assuring fidelity. These approaches have led to the identification and analysis of components of the translational apparatus. There likely remain other cellular components, such as the actin cytoskeleton, whose roles may be identified by these approaches. The following sections discuss the RNAs and proteins linked to translational accuracy in yeast.

Role of tRNA in fidelity

The critical role of aa-tRNA in correctly interpreting the genetic code means there are many ways tRNA can affect the accuracy of this process. Elegant mechanisms have been identified in other systems to account for proofreading by the synthetases [38]. The effects of altered aminoacylation are outside the scope of this review. The tRNAs themselves are also important components of the coding process. There is a long history of the isolation of mutant tRNAs as suppressors of all types of cis-elements in the RNA (for review see [39]). Additionally, the use of a start site AUG to AGG mutation in the *HIS4* gene, coupled with a system to express sufficient amounts of an aminoacylated UCC-tRNA$_i^{Met}$ mutant in yeast, allowed the dissection of the role of the codon-anticodon interaction in start site selection [40]. Mutant tRNAs have also been used to dissect the contributions of base pairing in the maintenance of reading frame [41, 42].

Initiation and start site selection: fidelity of AUG codon recognition

The ternary complex of eIF2•GTP•Met-tRNA$_i^{Met}$ recognizes the first AUG codon of the mRNA coding sequence, which marks the start of translation. Elements of the Met-tRNA$_i^{Met}$ recognized by eIF2 that assure discrimination from the 20 elongator tRNAs have been identified in yeast [43, 44] and other systems. The ribosome scans down from the m^{7}G cap in the 5′ to 3′ direction on the mRNA (reviewed in [45]). Predominantly, translation initiation occurs by proper placement of the Met-tRNA$_i^{Met}$ by eIF2 on the first AUG start codon. The selection of the five *sui* mutations by the method described earlier identified the critical role of the three eIF2 subunits α (*SUI2*) [46], β (*SUI3*) [25] and γ (*SUI4/GCD11*) [47] in start site selection. In addition, the two other *sui* mutations show interesting links to this process. The *SUI5* mutation encodes a form of eIF5 [48]. This has led to biochemical analysis of the interplay between eIF2 and eIF5 in the initiation process. The eIF5 protein stimulates the GTPase activity of the eIF2γ subunit, and links GTP-dependent events with fidelity, as will be seen below for another G protein eEF1A. The *sui1* mutation is a form of eIF1 that affects start site selection [49]. Another allele of this gene, however, also affects the efficiency of suppression of an L-A –1 PRF and mRNA decay [50, 51]. eIF1 is known to stimulate formation of the initiation complex on the 40S subunit [52, 53] and modulate GTPase activity by eIF5 [24], although its exact function remains to be determined. It is of interest that these factors are part of a large multifactor complex including eIF1, eIF2, eIF3, eIF5 and Met-tRNA$_i^{Met}$ [54]. Other communications within the complex that affect fidelity, especially those involving the many subunits of eIF3, may yet become apparent.

Role of elongation factors in translational fidelity

After initiation, three nucleotide-binding proteins that hydrolyze GTP (eEF1 and eEF2) or ATP (eEF3) play critical roles in efficient and accurate protein synthesis. The hydrolysis of nucleotides is stimulated on all three eEFs by their interaction with the ribosome. The elongation factors affect maintenance of proper reading frame, cor-

rect amino acid placement and accurate stop codon recognition.

eEF1A influences multiple types of translational fidelity

All aa-tRNAs, except the initiator Met-tRNA$_i^{Met}$, are delivered to the ribosome by the G protein eEF1A. The kinetics of aa-tRNA delivery to the A-site have been extensively studied in prokaryotes for the elongation factor Tu (EF-Tu, reviewed in [55]). It is hypothesized that there is a 'kinetic proofreading' step for the proper placement of the cognate aa-tRNA into the A-site by EF-Tu/eEF1A [56, 57]. This involves eEF1A delivering the aa-tRNA to the A-site in a GTP-dependent manner. Sensing a proper codon-anticodon interaction, GTP hydrolysis occurs, aa-tRNA is released from eEF1A and aa-tRNA is accommodated in the A-site. Yeast has proven an excellent system to address fidelity in a eukaryotic organism. The X-ray crystal structure of the yeast eEF1A protein in complex with the guanine nucleotide exchange factor eEF1Bα [58] has allowed testing of the models for the roles of nucleotide and aa-tRNA in providing signals that assure accurate protein synthesis (fig. 6).

The central role of eEF1A in the delivery of aa-tRNA makes it an important component of the accuracy maintenance system of the cell. Yeast eEF1A is encoded by two genes, *TEF1* and *TEF2*, which have identical coding regions. The use of the *met2-1* +1 insertion allele led to the isolation of nine mutant alleles in the *TEF2* gene that function as dominant frameshift suppressors of growth on C-Met [59]. Not all the mutant forms of eEF1A are functional as the only form of the protein in vivo, however. Further analysis of the effects of those mutant forms that are functional as the only form of eEF1A has shown that not all mutants that read through an insertion signal affect a PRF signal (fig. 6B). The *TEF2-4* allele, which alters E_{122} to K results in a significant reduction in –1 PRF, with lesser effects seen for *TEF2-9* (E_{295}K), *TEF2-7* (T_{142}I) and *TEF2-10* (E_{122}Q) [60]. Altered +1 PRF efficiencies are seen for strains bearing the *TEF2-2* (E_{317}K) and *TEF2-3* (E_{40}K) alleles. The dominant +1 PRF phenotypes of these mutants on the yeast Ty*1* element are different between alleles and in comparison to the recessive effects [61]. Thus, unique alleles that affect +1 versus –1 PRF indicate specific functions of eEF1A required for the two very different cis-acting signals used in these two viral systems.

The key role of GTP hydrolysis in signaling a correct codon-anti-codon interaction predicts mutations that affect nucleotide binding and hydrolysis will also affect fidelity. Substitution of N_{153}T in the NKXD GTP-binding consensus element increases Leu misincorporation measured in vitro in the poly (U) assay by 2.3-fold, while N_{153}T/D_{156}E and D_{156}N show 1.5–1.9-fold effects [32]. In vitro, the N_{153}T mutant shows a 4.6-fold increase in intrinsic GTPase activity. Since decoding and termination occur at the A-site, mutations that affect one process could also affect the other [62]. In vivo, all three mutants show increased omnipotent nonsense suppression (readthrough at all three stop codons) by as much as 4-fold [33]. It remains to be determined whether nonsense suppression is completely due to misreading versus a direct effect on the ter-

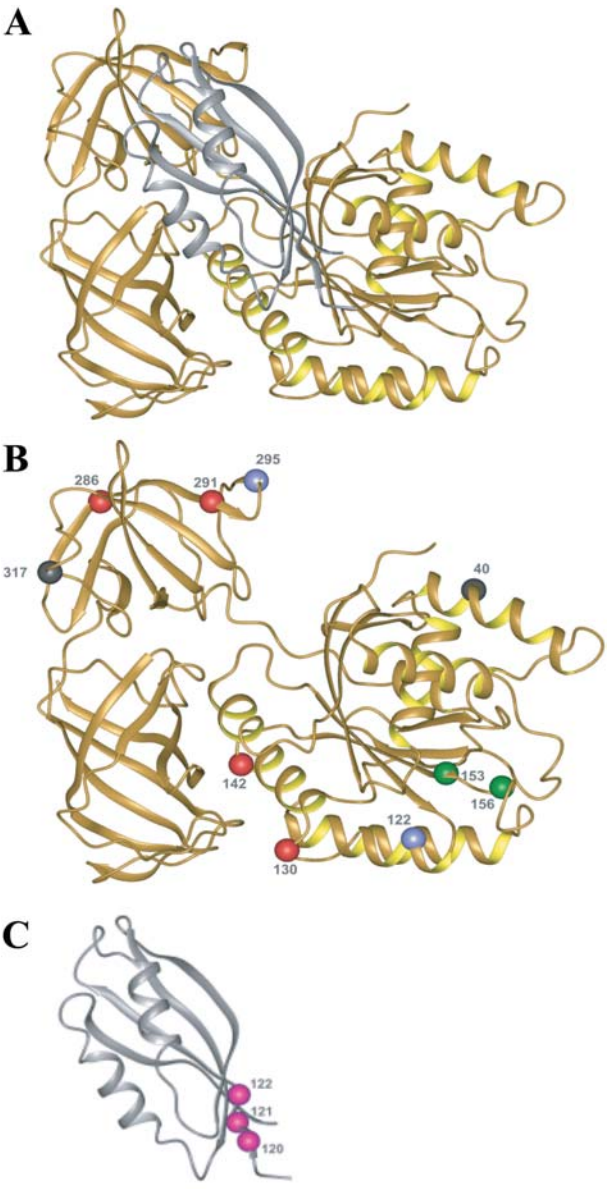

Figure 6. (*A*) The X-ray crystal structure of *S. cerevisiae* eEF1A (gold) complexed with the catalytic fragment of the exchange factor eEF1Bα (silver) [58]. Mutations in *S. cerevisiae* eEF1A (*B*) and the catalytic fragment of eEF1Bα (*C*) that affect the fidelity of protein synthesis. Red spheres indicate mutations that result in dominant suppression of a +1 insertion [59], blue dominant suppression of a +1 insertion and a recessive –1 PRF defect [59, 60], black dominant suppression of a +1 insertion and a recessive +1 PRF defect [59, 60], green increased nonsense suppression and misincorporation in vitro [33], purple reduced nonsense suppression [64].

mination process. This is of particular importance since both misreading and nonsense suppression are affected in the NKXD mutations. Studies have also indicated that a reduction in the gene dosage of eEF1A from the normal complement of two genes to one results in enhanced fidelity at a nonsense mutation [62].

The co-crystal structure of yeast eEF1A and the catalytic fragment of eEF1Bα led to a model for the role of domain II of eEF1A in aa-tRNA binding [58]. The $E_{291}K$ (*TEF2-16*) and $E_{286}K$ (*TEF2-1*) mutations confer dominant +1 frameshift suppression, and map to a site predicted within the aa-tRNA binding pocket. An $E_{291}A$, but not an $E_{286}A$ mutant can suppress a +1 insertion, although less effectively than the $E_{286}K$ mutation [63]. The charge reversal substitution is likely to be important for the dramatic fidelity phenotype, and may reflect an electrostatic effect with RNA. Identification of mutations in rRNA or ribosomal proteins that suppress these effects may help elucidate the connection between ribosomal components and the ability of eEF1A to aid in maintaining translational accuracy.

The guanine nucleotide exchange factor eEF1Bα affects translation fidelity

The role of eEF1Bα in the cell is to regulate the activity of eEF1A by catalyzing the release of GDP in exchange for GTP. Based on the co-crystal structure of yeast eEF1A and the catalytic fragment of eEF1Bα [58], it is clear that mutations in eEF1Bα behave differently depending upon whether they interact with domain I (GTP binding) or domain II (aa-tRNA binding, fig. 6A). The K_{120} end of eEF1Bα is positioned in domain I of eEF1A and helps define a region important for exchange. An analysis of 21 different conditional alleles in residues $K_{120}S_{121}I_{122}$ of eEF1Bα [64] demonstrated antisuppression of all three stop codons (fig. 6C). Analysis of the F_{163} residue of eEF1Bα, proposed to overlie the position of the aminoacyl moiety of the aa-tRNA in domain II and on the opposite face, gives quite different results. While a strain expressing the $F_{163}A$ mutant shows reduced growth, reduced total translation rates and sensitivity to translation elongation inhibitors, no effect is seen on nonsense suppression [58]. The lack of correlation between reduced total translation and altered fidelity in eEF1Bα mutants indicates nonsense suppression is not a result of slowed elongation. Further analysis is needed to determine whether eEF1Bα affects fidelity through eEF1A or via another mechanism.

Altered eEF1Bγ levels and fidelity, through or around eEF1Bα

In all eukaryotic organisms studied to date, eEF1Bα is associated with the eEF1Bγ subunit. The *Artemia salina* eEF1Bγ protein stimulates eEF1Bα's activity in vitro [65]. In yeast, the genes encoding eEF1Bγ, *TEF3* and *TEF4,* are not essential [66]. Similar to eEF1A, eEF1Bγ levels can affect the accuracy of the translation process. Overexpression of the *TEF4* gene from a *GAL* promoter results in reduced readthrough of a stop codon [67]. This phenotype is similar to the effect of eEF1Bα mutations on the catalytic face of the protein. Deletion of both eEF1Bγ genes results in an approximately twofold increase in nonsense suppression [O. Olarewaju et al., unpublished]. Thus, while eEF1Bγ is not essential in vivo, it may help modulate the activity of eEF1Bα and as a result affect nonsense suppression.

eEF2 and the maintenance of reading frame

The translocation of the peptidyl-tRNA from the P/A hybrid site to the P-site by eEF2 can produce fidelity errors. The kinetics and molecular mechanics of translocation of the peptidyl-tRNA by the homologous prokaryotic factor EF-G have been studied by the Rodnina and Wintermeyer labs (reviewed in [68]). eEF2 catalyzes the GTP-dependent translocation of the peptidyl-tRNA, and the mRNA proceeds by the next three bases to expose the next codon in the A-site (reviewed in [2, 69, 70]). Unlike EF-G, eEF2 undergoes a posttranslational diphthamide modification at residue His699 by a multistep conversion. This modification is the site of diphtheria-toxin-mediated ADP ribosylation which inactivates eEF2, but no biological function has been assigned to the diphthamide modification [71]. The recent X-ray structure of eEF2 and cryo electron microscopy (EM) data for the yeast ribosome indicate this residue is located near the mRNA [72, 73]. Also, eEF2 undergoes a reversible phosphorylation at residue Thr57 located prior to one of the GTP binding motifs [74–77]. The key location of these residues in eEF2 suggests they may play a role in maintaining fidelity.

A connection between eEF2 and translational fidelity comes from recent work connecting peptidyltransferase inhibitors to altered frameshifting levels [14,19]. Some of the compounds which affect the process of PRF are anisomycin, preussin and sparsomycin for a –1 signal and sordarin for a +1 signal [14–18]. Sordarin inhibits the translocation step by binding to eEF2 directly, although high-affinity binding requires 80S ribosomes [78]. Cryo-EM studies with a sordarin derivative indicate that it prevents release of eEF2 from the 80S posttranslocation ribosome [73]. Biochemical analysis demonstrated that sordarin blocks the translocation step preceding GTP hydrolysis [79]. A study of sordarin-resistant eEF2 mutants indicates three classes with altered Ty*1* retrotransposition frequencies [18, 78, 80]. First, eEF2 mutants with the smallest effects on Ty*1* retrotransposition were generally most sensitive to sordarin ($R_{180}G$, $V_{187}F$, $Y_{521}N$ and $I_{529}T$).

The second class encompassed eEF2 mutants that were highly resistant to sordarin, but completely defective in Ty*1* retrotransposition ($P_{559}R$, $V_{774}F$ and $G_{790}\Delta$). The third class showed strong Ty*1* retrotransposition and sordarin resistance, including $Q_{490}E$, $S_{523}F$, $S_{523}P$, $P_{559}L$ and $P_{727}S$. One eEF2 mutant, $A_{562}P$, did not follow this trend, as it was highly resistant to sordarin but had no significant effect on Ty*1* retrotransposition. The recently derived crystal structure of eEF2 bound to sordarin shows the drug binds in a pocket well defined by these mutations and will help further dissect the mechanism of how these residues are involved in reading frame maintenance and translocation [72].

Analysis of the effect of the L-A –1 PRF inhibitor preussin indicated clusters of eEF2 mutants resistant to this drug [15]. A correlation was observed between the resistance of eEF2 mutants to preussin and sordarin, consistent with increased intrinsic GTP-hydrolysis rates. This suggests that preussin, like sordarin, may allow eEF2 to bind the ribosome but impair its GTP-hydrolyzing function. In the 'integrated model' of PRF, it is observed that –1 PRF is not affected by any of the mutants or drugs assayed that are involved in the translocation step [31]. After peptidyltransfer has occurred, the ribosome cannot undergo a –1 ribosomal frameshift. This analysis demonstrates the strength of integrated biochemical studies with in vivo analyses of multiple types of fidelity defects.

eEF3, a fungal specific factor

The unique and essential eEF3 protein utilizes ATP, as opposed to the other elongation factors eEF1 and eEF2, which possess ribosome-dependent GTPase activities. Evidence indicates there may be a putative eEF3-like protein in prokaryotes [81]. There is no homolog in higher eukaryotes, although metazoan ribosomes have an intrinsic ATPase activity [82–84] and eEF3 is required with yeast but not metazoan ribosomes [85]. This has led to models that eEF3, or its proposed function in E-site release of deacylated-tRNA and allosteric interactions to A-site function, were later incorporated into the ribosome [86]. In vitro, eEF3 stimulates the delivery of cognate aa-tRNA by eEF1A in preference to non-cognate aa-tRNA [87], thus suggesting a link to translational fidelity in vivo. A temperature-sensitive (Ts–) ATPase defective mutant eEF3 ($F_{650}S$) displays increased sensitivity to aminoglycosides [6]. However, no effect was observed for –1 or +1 ribosomal frameshifting or nonsense suppression in vivo. Typically, aminoglycosidic drug sensitivities correlate with altered translational fidelity; however, it is possible that the effect of this one allele may be specific to misincorporation. In vivo selection of more eEF3 mutants that show fidelity defects may help answer this question.

eRF1, eRF3 and the [PSI+] prion, an alternate form of eRF3, affect the fidelity of termination

The readthrough of stop codons is affected by the ribosome, suppressor tRNAs and soluble elongation factors. However, the two most prominent factors affecting this process are eRF1 and eRF3, which recognize the stop codons and are required for efficient translation termination (for review see [88]). These two essential yeast factors are highly conserved among eukaryotes [89] and are encoded by the *SUP45* and *SUP35* genes, respectively [90–93]. These gene products interact to form a functional translation termination complex and mediate the release of the nascent polypeptide chain [94–96]. Perturbing the functions of eRF1 or eRF3 has serious consequences on stop codon recognition. Indeed, mutations in the genes encoding eRF1 and eRF3 were identified as strong omnipotent nonsense suppressors [97].

eRF1 recognizes all three stop codons in the A-site of the ribosome and its peptidyl-tRNA hydrolysis activity releases the newly formed protein [98,99]. This suggests a direct interaction of eRF1 with the mRNA and the peptidyl transferase center of the ribosome to transduce the termination signal [100, 101]. As suggested by the crystal structure of eRF1 [102], the domain 2 tripeptide GGQ is critical for peptidyl-tRNA hydrolysis [101,103], and the NIKS tetrapeptide of domain 1 is essential for function and appears to mimic the anticodon loop of the aa-tRNA [104,105]. Studies have shown that the efficiency of termination can be altered by the levels of eRF1 [106,107]. In a strain bearing a nonsense suppressor tRNA, the simultaneous overexpression of both the *SUP45* and *SUP35* genes produced an antisuppressor effect [94]. Deletion of the C-terminal eRF1 residues required for the eRF3 interaction reduced the efficiency of complex formation and nonsense suppression significantly, demonstrating the importance of this association [108,109]. A novel yeast screen was used to identify specific eRF1 mutants designated as 'unipotent' for the defective reading of one stop codon, and not the other two, to define a potential stop codon recognition site/domain of eRF1 [110]. This screen utilized a strain of yeast that employed the use of three auxotrophic markers (*ade1-14*^{UGA}, *lys2-864*^{UAG} and *his7-1*^{UAA}), each containing one of the three nonsense mutations, and was verified by secondary screening and a *lacZ* reporter system. Nine of the ten nonsense suppressor mutations map to conserved residues within domain 1 of eRF1 [110]. The mutants identified had a codon bias but not complete unipotence. The $I_{32}F$, $M_{48}I$, $V_{68}I$, $V_{68}A$, $L_{123}V$ and $H_{129}R$ suppressor mutations are located at three pockets and define the molecular surface for eRF1 domain 1 into which a stop codon can be modeled. The *sup45-R2*^{ts} $P_{86}A$ mutation, also in domain I [111], has been used for the analysis of interactions between ribosonal RNA (rRNA) mutations and eRF mutations in vivo (see below, table 2). The power of this genetic analy-

Table 2. rRNA mutants linked to fidelity.

Allele	Yeast	Helix	Non	Sup35	*PSI+*	Sup45	FS	Mis	Ref.
rdn2	G517A 18S	18	anti	supp	supp		↓Ty*1*		[122, 124, 125]
rdn12A	C526A 18S	18	anti		supp	supp			[124]
rdn8	G886A 18S	27	anti			supp Ts$^-$		↓	[124, 126]
rdn6	G888A 18S	27	anti			supp		↓	[111, 121, 124, 126]
rdn4	U912C 18S	27	anti	supp		supp	↓Ty*1*	↓	[122,124–126]
rdn1A	C1054A 18S	34	supp				↓Ty*3*		[111,125]
rdn1G	C1054G 18S	34	supp						[111]
rdn1T	C1054T 18S	34	anti	supp	supp	supp	↓Ty*1*, ↓Ty*3*		[111, 121, 124–126]
rdn15	A1491G 18S	44	anti	supp Ts$^-$		supp Ts$^-$			[124]
hyg1	U1495C 18S	44			supp	supp Ts$^-$			[124]
rdn5	C3022U 25S	S/R	supp				↓Ty*1*, supp +1		[125, 127]
mof9	5S	III IV, loops, BCDE	supp				↓L-A		[130, 131]

sis in combination with the known eRF1 structure [102] has further defined the necessary elements in reading the individual stop codons and the high fidelity required for discrimination in the termination process.

eRF3 is an eEF1A-like protein whose GTPase activity is stimulated by eRF1 in a stop codon-dependent manner [100,112–114]. The C-terminal eEF1A-like region of eRF3 is necessary for viability, while the nonessential N-terminal domain has been implicated in the formation of the [*PSI*$^+$] prion (discussed below), indicating that there are two nonoverlapping regions of this protein [93]. In yeast it is clear that that eRF3 has an essential function in the termination process and is important for the fidelity of stop codon recognition mediated through eRF1. The *sup35-R8*^{ts} ($R_{419}G$) mutation is located in the GTP binding domain and results in nonsense suppression, perhaps linking hydrolysis with fidelity. Mutations in the genes encoding both release factors have proven useful tools for the genetic analysis in combination with ribosomal component mutations.

The [*PSI*$^+$] genetic element is the prion form of eRF3 that self-propagates in a non-Mendelian manner (reviewed in [115]). The eRF3 protein forms aggregates that reduce the efficiency of translation termination by lowering the pool of soluble, active eRF3, resulting in omnipotent nonsense suppression in [*PSI*$^+$] strains [93,116]. It also appears that the [*PSI*$^+$] element sequesters eRF1 into these aggregates, which may be another mechanism for reduced translation termination [117]. [*PSI*$^+$] can be induced by the overexpression of eRF3 [118,119]. This effect can be neutralized by eRF1 overexpression, indicating that the relative levels of these two proteins are important [106]. Overexpression of the C-terminal portion of eRF3 generates an antisuppressor phenotype to counteract the effects of its prion form in [*PSI*$^+$] strains [93]. The N-terminal domain of eRF3 is sufficient to induce [*PSI*$^+$] but not required in vivo for cell viability [93,120]. Thus, prion formation is separate from the essential role in termination.

Yeast ribosomal protein and rRNA components are involved in fidelity

The ribosome is at the center of translational accuracy. Both cis-acting factors such as rRNA and ribosomal proteins and trans-acting factors that interact with the ribosome as described above can affect translational fidelity. Unraveling the function of eukaryotic ribosomes by a genetic approach has contributed to understanding the regions of this machine that are involved in maintaining fidelity. Since rRNA has been highly conserved throughout evolution, analyses performed in prokaryotes shed clues on how to approach the study of the yeast ribosome (reviewed in [121]). Yeast is an excellent system due to the ease with which one can delete or mutate genes encoding ribosomal proteins. The capability to delete the wild-type copy of the rRNA locus from the chromosome allows for the analysis of mutant ribosomes in a homogeneous population [122,123].

rRNA plays a key role in fidelity

Based on the role of the 40S subunit in decoding, many small subunit 18S rRNA mutations have been isolated or studied to address fidelity. In helix 18 of the 18S rRNA, the *rdn2* (G517A) mutation causes resistance to paromomycin, suggesting a link to fidelity. It functions as an antisuppressor mutation and further reduces both *sup35-R8*^{ts} and [*PSI*$^+$]-mediated suppression [122,124]. The *rdn2* mutation decreases Ty*1* +1 PRF about 20-fold without affecting Ty*3* frameshifting [125]. The *rdn12A* (C526A) mutation is also an antisuppressor that strongly inhibits stop-codon readthrough mediated by [*PSI*$^+$] or *sup45-R2*^{ts} [124]. It is important to note that suppression of stop-codon readthrough in [*PSI*$^+$] strains is not due to curing of the prion.

A series of mutations in helix 27 of the 18S rRNA also function as antisuppressors. *rdn4* (U912C) causes resistance to paromomycin and inhibits nonsense readthrough

induced by *sup45-R2*^{ts} and *sup35-R8*^{ts} release factor mutations or paromomycin exposure [122, 124]. *rdn4* decreases Ty*1* PRF sevenfold without affecting Ty*3* PRF [125]. The *rdn6* (G888A) mutation strongly inhibits *sup45-R2*^{ts}-mediated nonsense suppression but fails to restore growth for the Ts$^-$ phenotype [111, 121, 124]. Thus, restoration of stop-codon recognition does not completely allow restoration of the growth defect of an eRF1 mutant. The *rdn8* (G886A) mutant rescues the *sup45-R2*^{ts} lethal mutation and decreases paromomycin-mediated stop-codon readthrough [124, 126]. Corresponding to enhanced fidelity at the A-site, *rdn4, rdn6* and *rdn8* all increase the accuracy of tRNA decoding, as measured in vitro by Leu misincorporation in the poly (U) assay [126].

Nucleotide 1054 in helix 34 of the18S rRNA has yielded a series of interesting and surprisingly diverse fidelity mutants. The *rdn1A* (C1054A) mutation causes dominant nonsense suppression and is extremely sensitive to paromomycin [111]. This mutant decreases PRF from the yeast Ty*3* retrotransposon signal three-fold without affecting Ty*1* [125]. The *rdn1G* (C1054G) mutant also causes dominant nonsense suppression [111]. Surprisingly, the *rdn1T* (C1054T) mutation is an antisuppressor. Unlike *rdn1A, rdn1T* decreases both Ty*1* and Ty*3* PRF [125]. This mutant inhibits stop-codon readthrough in [*PSI*$^+$], *sup45-R2*^{ts} and *sup35-R8*^{ts} strains as well as UAA-specific suppression caused by a mutant tRNA^{Gln} that decodes UAA (*SLT3*) [111, 121, 124, 126]. However, it fails to restore the Ts$^-$ phenotype of *sup45-R2*^{ts}. Thus, while this mutant reverses multiple types of nonsense suppressor mutations, this is not sufficient to restore growth defects by an eRF mutant, again supporting that these phenotypes may not be linked.

Last, *rdn15* (A1491G) in helix 44 of the 18S rRNA was found as a suppressor of the Ts$^-$ growth defect of the *sup45-R2*^{ts} mutation. This mutant only slightly reduces *sup45-R2*^{ts}-mediated nonsense suppression; thus, as described above, these two phenotypes are not always linked. The *rdn15* mutation partially compensates for inactivation of eRF3 resulting from [*PSI*$^+$], and unlike the case for *sup45-R2*^{ts}, it suppresses both the Ts$^-$ and nonsense suppression phenotypes of the *sup35-R8*^{ts} mutation. The *rdn15* mutation creates a C1409-G1491 base pair analogous to that seen in the prokaryotic rRNA that is important for high-affinity paromomycin binding. While yeast cells are normally resistant to the killing effect of aminoglycosides yet still susceptible to the misreading effect of these drugs [36, 37], *rdn15* makes yeast sensitive to paromomycin. This indicates that the naturally high resistance to paromomycin is due to the missing C1409-G1491 base pair in yeast. These results have led to the hypothesis that *rdn15* decreases the translational pause at the stop codon by allowing eRF1 to recognize stop codons more effectively [124]. The *hyg1* (U1495C) mutant is resistant to another aminoglycoside, hygromycin [127]. *hyg1* rescues a *sup45-R2*^{ts} mutation as well as partially compensates for inactivation of eRF3 resulting from [*PSI*$^+$]. Consistent with this effect in [*PSI*$^+$] strains, this mutant enables [*PSI*$^+$] strains to accumulate larger Sup35p aggregates upon Sup35p overproduction, suggesting lower toxicity in the presence of *hyg1* [124]. *hyg1* does not rescue all alterations of eRF3, such as the *sup35-R8*^{ts} mutant, indicating allele specificity to the suppression effects.

While many 18S rRNA mutations affect fidelity, the 25S rRNA is also important for the accuracy of translation. The *rdn5* (C3022U) mutant is in the sarcin/ricin domain, a universally conserved stem loop containing a GAGA tetraloop. The *rdn5* mutation changes the wild-type CG closing pair of the tetraloop to a weaker UG pair. The sarcin/ricin loop has also been shown to play an important role in binding elongation factors [128, 129], providing a logical place to analyze the link between the eEFs and the ribosome. The *rdn5* mutant displays multiple fidelity defects: suppression of several stop codons and a nonprogrammed +1 insertion, resistance to the aminoglycosides paromomycin, G418 and hygromycin, and a five-fold decrease in Ty*1* PRF [125,127]. Based on the link to elongation factors, this mutation may cause reduced affinity for the release factors, while at the same time enhancing the ability of noncognate tRNA to decode the A-site codon.

Another large ribosomal subunit rRNA that has been shown to affect the process of translational fidelity is the 5S rRNA, which sits at the crown of the 60S large subunit. The 5S rRNA was initially linked to fidelity when identified as the *mof9* mutation [130]. More recently a highly detailed mutagenesis of the 5S rRNA was undertaken [131]. This analysis demonstrated 44 out of 239 5S rRNA alleles tested suppressed the *ade2-1*$_{UAA}$ mutation and 47 out of 223 suppressed the *can1-100*$_{UAA}$ mutation. Suppression occurred with a threefold greater frequency for mutations in conserved nucleotides. Of 229 alleles examined for –1 PRF by the killer virus assay, over one-third had discernible effects. Mapping the mutant 5S RNA alleles with regard to nonsense suppression and –1 PRF revealed fidelity defects clustered in helicies III and IV and loops B, C, D and E. It is proposed that one of the major functions of 5S rRNA may be to enhance translational fidelity by acting as a physical transducer of information between the different functional centers of the ribosome.

Ribosomal proteins are involved in the fidelity of translation

In yeast, as in *Escherichia coli*, most ribosomal proteins tested have been shown to be essential for the function of the ribosome. Data show that even though rRNA mainly

occupies the accuracy center of the ribosome, proteins also play a role and have been conserved through out evolution ([132], reviewed in [121]). Since decoding takes place in the small subunit, many small ribosomal subunit proteins (rps) are also involved in the process of quality control during translation. The rpS3 mutant allele *suf14-1* ($K_{108}E$) is resistant to paromomycin and suppresses a series of +1 insertion and stop-codon mutations [133]. The rpS2 (formerly rpS4) *sup44-1* allele is a paromomycin-sensitive omnipotent suppressor [134–136]. Isolated mutant 40S ribosomal subunits misread at an increased frequency in the poly (U) assay, indicating that it optimizes translational accuracy [137]. Mutations in rpS5 and rpS9 (formerly rpS13) are also known to affect the efficiency of stop-codon recognition and are important for maintaining the fidelity of termination (reviewed in [35]). The rpS9A *sup46-2* allele is a paromomycin-sensitive omnipotent suppressor that also causes misreading at an increased frequency in a poly (U) assay [134, 135, 138]. Interestingly, rpS28 can alter translational accuracy in both directions. For example, *rps28-5* (G_8C) is a suppressor mutation while *rps28-12* ($A_{113}V$) is an antisuppressor mutation [139], although neither affect Ty*1* PRF [125]. Various rpS28 mutations ($K_{62}N$, $K_{62}T$, $K_{62}Q$) were shown to optimize translational accuracy in the in vitro poly (U) misincorporation assay [137]. These rpS28 point mutations reduce the sensitivity to paromomycin and show an antisuppressor effect against omnipotent *SUP44* (rpS2) and *SUP46* (rpS9) suppressor alleles [132]. Interestingly, rpS28 $K_{62}R$ behaves oppositely by decreasing translational accuracy and is a strong omnipotent suppressor. The *E. coli* equivalents to rpS28, rpS2 and rpS9 (S12, S5 and S4) are essential for the control of translational accuracy, showing the conservation of these functions.

The large ribosomal subunit protein L3 (rpL3) is located near the peptidyltransferase center of the ribosome. rpL3 was isolated as *mak8-1* (*ma*intenance of *k*iller, $W_{255}C$ $P_{257}T$) by the inability of mutant strains to maintain the M_1 double-stranded RNA (dsRNA) virus. This mutant promotes approximately three- to fourfold increases in –1 PRF efficiencies. Strains harboring the *mak8-1* allele are resistant to the effects of peptidyltransferase inhibitors on –1 PRF [17]. Another rpL3 mutant tested, *L3Δ*, lacking the N-terminal 100 amino acids exerts a dominant effect on –1 PRF and killer virus maintenance [17].

Ribosomes prepared from strain deleted for *RPL39* or carrying the *spb2-1* mutant form of this gene display a fourfold increase in Leu misincorporation in the poly (U) assay [140]. These rpL39 mutants have a substantial increase in A-site binding, as is typical for error-prone mutants. This fidelity effect may arise from a higher affinity for noncognate tRNAs, which leads to translational errors. These error-prone mutants, particularly the *RPL39* null allele, are hypersensitive to paromomycin, which again correlates with a fidelity defect.

A link between rRNA and ribosomal protein effects on fidelity are seen with rpL5 (formally rpL1 or YL3), a protein that binds 5S rRNA. HA epitope-tagged rpL5 alleles *rpl5-HA1* ($K_{27}E$), *rpl5-HA2* ($T_{28}A$), *rpl5-HA3* ($V_{53}G$), *rpl5-HA4* ($G_{91}R$), *rpl5-HA5* ($K_{289}E$) and *rpl5-HA9* (lacking the last 2 aa) promote increased frameshifting efficiencies in both the –1 and +1 directions [141]. No correlation was observed between the alteration in rpL5 and 5S rRNA association and PRF. For example, *rpl5-HA2* has the greatest effect on –1 and +1 frameshifting but binds 5S RNA better than some of the other mutants. Biochemically, these mutants have decreased affinities for the peptidyl-tRNA in the ribosome. Analysis of sparsomycin resistance, a P-site-specific peptidyltransferase inhibitor that specifically increases the binding of peptidyl-tRNA with ribosomes, correlated with the severity of the frameshift defect in all of the mutants tested. In the long term more studies of ribosomal proteins and the rRNA with which they associate may link functions of the two components of the ribosome in fidelity.

Other links to fidelity: the NMD pathway and the actin cytoskeleton

While this review has focused on the translational components that affect the fidelity of protein synthesis, other cellular factors are linked to this process. The NMD pathway, with its emphasis on identifying mRNAs with premature stop codons, has a logical link to the termination process. An increasing body of work shows physical links between components of the two processes as well as alleles of mutations in the NMD pathway that can separate nonsense suppression effects from mRNA stability effects (reviewed in [10]). Additionally, translational components, including the ribosome [142] and some of the soluble protein synthesis factors including eEF2 [143, 144], eEF1Bγ [145] and eEF1A [146, 147], interact with cytoskeletal components. A set of 25 isogenic yeast actin mutants were examined for links to translational fidelity [148]. The *act1-2* ($A_{58}T$) and *act1-122* ($D_{80}A$ $D_{81}A$) mutant strains showed a significant increase in nonsense suppression. These mutants do not affect –1 PRF, indicating specificity for effects on the readthrough of stop codons. In the presence of excess eEF1A, the *act1-2* and *act1-122* showed increased and reduced sensitivity to paromomycin, respectively. Overall, these results support the biological significance of the interaction between eEF1A and the actin cytoskeleton. Since nonsense suppression can occur as a result of reduced fidelity by elongation or termination factor mutants, the role of actin in this process remains to be determined.

Summary

Yeast has proven an excellent model system for studies of both the mechanism and regulation of protein synthesis. The genetic tools employed have highlighted the roles of key players in translational fidelity such as eEF1A. With the emerging molecular view of the ribosome, systems to manipulate not only ribosomal protein genes but also the rRNA itself are likely to shed light on the important effects of both ribosomal components on fidelity. This is leading to further functional links between the soluble protein synthesis factors and ribosome-associated functions on fidelity.

Acknowledgements. We would like to acknowledge members of the Kinzy laboratory, Paul Copeland and Anne Carr-Schmid for helpful comments and Gregers Andersen for the preparation of Figure 6. T.G.K. is supported by NIH GM57483 and NSF MCB 9983565 and L.V. by NIH F31 GM20445.

1 Hershey J. W. B. and Merrick W. C. (2000) The pathway and mechanism of initiation of protein synthesis. In: Translational Control of Gene Expression, pp. 33–88, Sonenberg N., Hershey J. W. B. and Mathews M. B. (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harborn, NY
2 Merrick W. C. and Nyborg J. (2000) The protein biosynthesis elongation cycle. In: Translational Control of Gene Expression, pp. 89–125, Sonenberg N., Hershey J. W. B. and Mathews M. B. (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
3 Dasmahapatra B. and Chakraburtty K. (1981) Purification and properties of elongation factor 3 from *Saccharomyces cerevisiae*. J. Biol. Chem. **256:** 9999–10004
4 Hutchinson J. S., Feinberg B., Rotwell T. C. and Moldave K. (1984) Monoclonal antibody specific for yeast elongation factor 3. Biochemistry **23:** 3055–3063
5 Skogerson L. and Wakatama E. (1976) A ribosome-dependent GTPase from yeast distinct from elongation factor 2. Proc. Natl. Acad. Sci. USA **73:** 73–76
6 Anand M., Chakraburtty K., Marton M. J., Hinnebusch A. G. and Kinzy T. G. (2003) Functional interactions between yeast translation eukaryotic elongation factors (eEF) 1A and eEF3. J. Biol. Chem. **278:** 6985–6991
7 Triana-Alonso F. J., Chakraburtty K. and Nierhaus K. H. (1995) The elongation factor 3 unique in higher fungi and essential for protein biosynthesis is an E site factor. J. Biol. Chem. **270:** 20473–20478
8 Kovalchuke O., Kambampati R., Pladies E. and Chakraburtty K. (1998) Competition and cooperation amongst yeast elongation factors. Eur. J. Biochem. **258:** 986–993
9 Welch E. M., Wang W. and Peltz S. W. (2000) Translation termination: it's not the end of the story. In: Translational Control of Gene Expression, vol. 2, pp. 467–485, Sonenberg N., Hershey J. W. B. and Mathews M. B. (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
10 Czaplinski K., Ruiz-Echevarria M. J., Gonzalez C. I. and Peltz S. W. (1999) Should we kill the messenger? The role of the surveillance complex in translation termination and mRNA turnover. Bioessays **21:** 685–696
11 Atkins J. F., Weiss R. B., Thompson S. and Gesteland R. F. (1991) Towards a genetic dissection of the basis of triplet decoding, and its natural subversion: programmed reading frame shifts and hops. Annu. Rev. Genet. **25:** 201–228
12 Dinman J. D. (1995) Ribosomal frameshifting in yeast viruses. Yeast **11:** 1115–1127
13 Farabaugh P. J. (1995) Post-transcriptional regulation of transposition by Ty retrotransposons of *Saccharomyces cerevisiae*. J. Biol. Chem. **270:** 10361–10364
14 Dinman J. D., Ruiz-Echevarria M. J., Czaplinski K. and Peltz S. W. (1997) Peptidyl-transferase inhibitors have antiviral properties by altering programmed –1 ribosomal frameshifting efficiencies: development of model systems. Proc. Natl. Acad. Sci. USA **94:** 6606–6611
15 Kinzy T. G., Harger J. W., Carr-Schmid A., Kwon J., Shastry M., Justice M. et al. (2002) New targets for antivirals: the ribosomal A-site and the factors that interact with it. Virology **300:** 60–70
16 Pestka S. (1977) Inhibitors of protein synthesis. In: Molecular Mechanisms of Protein Biosynthesis, vol. 10, pp. 467–553, Weissbach H. and Pestka S. (eds.), Academic Press, New York
17 Peltz S. W., Hammell A. B., Cui Y., Yasenchak J., Puljanowski L. and Dinman J. D. (1999) Ribosomal protein L3 mutants alter translational fidelity and promote rapid loss of the yeast killer virus. Mol. Cell. Biol. **19:** 384–391
18 Harger J. W., Meskauskas A., Nielsen J., Justice M. C. and Dinman J. D. (2001) Ty1 retrotransposition and programmed +1 ribosomal frameshifting require the integrity of the protein synthetic translocation step. Virology **286:** 216–224
19 Dinman J. D., Ruiz-Echevarria M. J. and Peltz S. W. (1998) Translating old drugs into new treatments: ribosomal frameshifting as a target for antiviral agents. Trends Biotechnol. **16:** 190–196
20 Stansfield I., Jones K. M., Herbert P., Lewendon A., Shaw W. V. and Tuite M. F. (1998) Missense translation errors in *Saccharomyces cerevisiae*. J. Mol. Biol. **282:** 13–24
21 Casadaban M. J., Martinez-Arias A., Shapira S. K. and Chou J. (1983) Beta-galactosidase gene fusions for analyzing gene expression in *Escherichia coli* and yeast. Methods Enzymol. **100:** 293–308
22 Hampsey M., Na J. G., Pinto I., Ware D. E. and Berroteran R. W. (1991) Extragenic suppressors of a translation initiation defect in the cyc1 gene of *Saccharomyces cerevisiae*. Biochimie **73:** 1445–1455
23 Culbertson M. R., Underbrink K. M. and Fink G. R. (1980) Frameshift suppression *Saccharomyces cerevisiae*. II. Genetic properties of group II suppressors. Genetics **95:** 833–853
24 Donahue T. F. (2000) Genetic approaches to translation initiation in *Saccharomyces cerevisiae*. In: Translational Control, pp. 487–502, Sonenberg N., Hershey J. W. B., Mathews M. B. (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
25 Donahue T. F., Cigan A. M., Pabich E. K. and Valavicius B. C. (1988) Mutations at a Zn(II) finger motif in the yeast eIF-2β gene alter ribosomal start-site selection during the scanning process. Cell **54:** 621–632
26 Dinman J. D. and Wickner R. B. (1994) Translational maintenance of frame: mutants in *Saccharomyces cerevisiae* with altered –1 ribosomal frameshifting efficiencies. Genetics **136:** 75–86
27 Wickner R. B. and Leibowitz M. J. (1976) Two chromosomal genes required for killing expression in killer strains of *Saccharomyces cerevisiae*. Genetics **82:** 429–442
28 Boeke J. D., Xu H. and Fink G. R. (1988) A general method for the chromosomal amplification of genes into yeast. Science **239:** 280–282
29 Curcio M. J. and Garfinkel D. J. (1991) Single step selection for Ty*1* element retrotransposition. Proc. Natl. Acad. Sci. USA **88:** 936–940
30 Boeke J. D., Trueheart J., Natsoulis G. and Fink G. R. (1987) 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. Methods Enzymol. **154:** 164–175
31 Harger J., Meskauskas A. and Dinman J. (2002) An 'integrated model' of programmed ribosomal frameshifting. Trends Biochem. Sci. **27:** 448–454

32 Cavallius J. and Merrick W. C. (1998) Site-directed mutagenesis of yeast eEF1A: viable mutants with altered nucleotide specificity. J. Biol. Chem. **273:** 28752–28758
33 Carr-Schmid A., Durko N., Cavallius J., Merrick W. C. and Kinzy T. G. (1999) Mutations in a GTP-binding motif of eEF1A reduce both translational fidelity and the requirement for nucleotide exchange. J. Biol. Chem. **274:** 30297–30302
34 Merrick W. C. (1979) Assays for eukaryotic protein synthesis. Methods Enzymol. **60:** 108–123
35 Stansfield I. and Tuite M. F. (1994) Polypeptide chain termination in *Saccharomyces cerevisiae*. Curr. Genet. **25:** 385–395
36 Palmer E., Wilhelm J. M. and Sherman F. (1979) Phenotypic suppression of nonsense mutants in yeast by aminoglycoside antibiotics. Nature **277:** 148–150
37 Singh A., Ursic D. and Davies J. (1979) Phenotypic suppression and misreading in *Saccharomyces cerevisiae*. Nature **277:** 146–148
38 Ibba M. and Soll D. (2000) Aminoacyl-tRNA synthesis. Annu. Rev. Biochem. **69:** 617–650
39 Murgola E. J. (1985) tRNA, suppression and the code. Annu. Rev. Genet. **19:** 57–80
40 Cigan A. M., Feng L. and Donahue T. F. (1988) tRNAi(met) functions in directing the scanning ribosome to the start site of translation. Science **242:** 93–97
41 Farabaugh P. J., Qian Q. and Stahl G. (2000) Programmed translational frameshifting, hopping and readthrough of termination codons. In: Translational Control of Gene Expression, vol. 2, pp. 33–88, Mathews M. B. (ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
42 Qian Q., Li J. N., Zhao H., Hagervall T. G., Farabaugh P. J. and Bjork G. R. (1998) A new model for phenotypic suppression of frameshift mutations by mutant tRNAs. Mol. Cell. **1:** 471–482
43 von Pawel-Rammingen U., Astrom S. and Bystrom A. S. (1992) Mutational analysis of conserved positions potentially important for initiator tRNA function in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:** 1432–1442
44 Forster C., Chakraburtty K. and Sprinzl M. (1993) Discrimination between initiation and elongation of protein biosynthesis in yeast: identity assured by a nucleotide modification in the initiator tRNA. Nucleic Acids Res. **21:** 5679–5683
45 Kozak M. (1999) Initiation of translation in prokaryotes and eukaryotes. Gene **234:** 187–208
46 Cigan A. M., Pabich E. K., Feng L. and Donahue T. F. (1989) Yeast translation initiation suppressor *sui2* encodes the α subunit of eukaryotic initiation factor 2 and shares sequence identity with the human α subunit. Proc. Natl. Acad. Sci. USA **86:** 2784–2788
47 Hannig E. M., Cigan A. M., Freeman B. A. and Kinzy T. G. (1993) *GCD11*, a negative regulator of *GCN4* expression, encodes the γ subunit of eIF-2 in yeast. Mol. Cell. Biol. **13:** 506–520
48 Huang H. K., Yoon H., Hannig E. M. and Donahue T. F. (1997) GTP hydrolysis controls stringent selection of the AUG start codon during translation initiation in *Saccharomyces cerevisiae*. Genes Dev. **11:** 2396–2413
49 Yoon H. and Donahue, T.F. (1992) The *sui1* suppressor locus in *Saccharomyces cerevisiae* encodes a translation factor that functions during tRNAiMet recognition of the start codon. Mol. Cell. Biol. **12:** 248–260
50 Cui Y., Dinman J. D., Kinzy T. G. and Peltz S. W. (1998) The Mof2/Sui1 protein is general monitor of translational accuracy. Mol. Cell. Biol. **18:** 1506–1516
51 Cui Y., Gonzales C. I., Kinzy T. G., Dinman J. D. and Peltz S. W. (1999) Mutations in the *MOF2/SUI1* gene affect both translation and nonsense-mediated mRNA decay. RNA **5:** 794–804
52 Trachsel H., Erni B., Schreier M. H. and Staehelin T. (1977) Initiation of mammalian protein synthesis. II. The assembly of the initiation complex with purified initiation factors. J. Mol. Biol. **116:** 755–767
53 Benne R. and Hershey J. W. (1978) The mechanism of action of protein synthesis initiation factors from rabbit reticulocytes. J. Biol. Chem. **253:** 3078–3087
54 Asano K., Clayton J., Shalev A. and Hinnebusch A. G. (2000) A multifactor complex of eukaryotic initiation factors, eIF1, eIF2, eIF3, eIF5 and initiator tRNA(Met) is an important translation initiation intermediate in vivo. Genes Dev. **14:** 2534–2546
55 Rodnina M. V. and Wintermeyer W. (2001) Fidelity of aminoacyl-tRNA selection on the ribosome: kinetic and structural mechanisms. Annu. Rev. Biochem. **70:** 415–435
56 Hopfield J. J. (1974) Kinetic proofreading: a new mechanism for reducing errors in biosynthetic processes requiring high specificity. Proc. Natl. Acad. Sci. USA **71:** 4135–4139
57 Burgess S. M. and Guthrie C. (1993) Beat the clock: paradigms for NTPases in the maintenance of biological fidelity. Trends Biochem. Sci. **18:** 381–384
58 Andersen G. R., Pedersen L., Valente L., Chatterjee I., Kinzy T. G., Kjeldgaard M. et al. (2000) Structural basis for nucleotide exchange and competition with tRNA in the yeast elongation factor complex eEF1A:eEF1Bα. Mol. Cell. **6:** 1261–1266
59 Sandbaken M. G. and Culbertson M. R. (1988) Mutations in elongation factor EF-1α affect the frequency of frameshifting and amino acid misincorporation in *Saccharomyces cerevisiae*. Genetics **120:** 923–934
60 Dinman J. D. and Kinzy T. G. (1997) Translational misreading: mutations in translation elongation factor 1α differentially affect programmed ribosomal frameshifting and drug sensitivity. RNA **3:** 870–881
61 Farabaugh P. J. and Vimaladithan A. (1998) Effect of frameshift-inducing mutants of elongation factor 1α on programmed +1 frameshifting in yeast. RNA **4:** 38–16
62 Song J. M., Picologlou S., Grant C. M., Firoozan M., Tuite M. F. and Liebman S. (1989) Elongation factor EF-1α gene dosage alters translational fidelity in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:** 4571–4575
63 Anand M., Valente L., Carr-Schmid A., Munshi R., Olarewaju O., Ortiz P. A. et al. (2001) Translation elongation factor 1 functions in the yeast *Saccharomyces cerevisiae*. Cold Spring Harbor Symposia on Quantitative Biology **66:** 439–448
64 Carr-Schmid A., Valente L., Loik V. I., Williams T., Starita L. M. and Kinzy T. G. (1999) Mutations in elongation factor 1β, a guanine nucleotide exchange factor, enhance translational fidelity. Mol. Cell. Biol. **19:** 5257–5266
65 Janssen G. M. C. and Moller W. (1988) Kinetic studies on the role of elongation factors 1β and 1γ in protein synthesis. J. Biol. Chem. **263:** 1773–1778
66 Kinzy T. G., Ripmaster T. R. and Woolford J. L. Jr (1994) Multiple genes encode the translation elongation factor EF-1γ in *Saccharomyces cerevisiae*. Nucleic Acids Res. **22:** 2703–2707
67 Benko A. L., Vaduva G., Martin N. C. and Hopper A. K. (2000) Competition between a sterol biosynthetic enzyme and tRNA modification in addition to changes in the protein synthesis machinery causes altered nonsense suppression. Proc. Natl. Acad. Sci. USA **97:** 61–66
68 Rodnina M. V., Savelsbergh A. and Wintermeyer W. (1999) Dynamics of translation on the ribosome: molecular mechanics of translocation. FEMS Microbiol. Rev. **23:** 317–333
69 Noller H. F., Yusupov M. M., Yusupova G. Z., Baucom A. and Cate J. H. (2002) Translocation of tRNA during protein synthesis. FEBS Lett. **514:** 11–16
70 Ramakrishnan V. (2002) Ribosome structure and the mechanism of translation. Cell **108:** 557–572

71 Fendrick J. L., Iglewski W. J., Moehring J. M. and Moehring T. J. (1992) Characterization of the endogenous ADP-ribosylation of wild-type and mutant elongation factor 2 in eukaryotic cells. Eur. J. Biochem. **205:** 25–31

72 Jorgensen R., Ortiz P. A., Carr-Schmid A., Nissen P., Kinzy T. G. and Andersen G. R. (2003) Two crystal structures demonstrate very large conformational changes of the eukaryotic ribosomal translocase. Nature Struct. Biol. **10:** 379–385

73 Gomez-Lorenzo M. G., Spahn C. M. T., Agrawal R. K., Grassucci R. A., Penczek P., Chakraburtty K. et al. (2000) Three-dimensional cryo-electron microscopy localization of EF2 in the *Saccharomyces cerevisiae* 80S ribosome at 17.5 Å resolution. EMBO J. **19:** 2710–2718

74 Nairn A. and Palfrey H. (1987) Identification of the major Mr 100,000 substrate for calmodulin- dependent protein kinase III in mammalian cells as elongation factor-2. J. Biol. Chem. **262:** 17299–17303

75 Palfrey H. C., Nairn A. C., Muldoon L. L. and Villereal M. L. (1987) Rapid activation of calmodulin-dependent protein kinase III in mitogen-stimulated human fibroblasts. Correlation with intracellular Ca2+ transients. J. Biol. Chem. **262:** 9785–9792

76 Price N. T., Redpath N. T., Severinov K. V., Campbell D. G., Russell J. M. and Proud C. G. (1991) Identification of the phosphorylation sites in elongation factor-2 from rabbit reticulocytes. FEBS Lett. **282:** 253–258

77 Donovan M. G. and Bodley J. W. (1991) *Saccharomyces cerevisiae* elongation factor 2 is phosphorylated by an endogenous kinase. FEBS Lett. **291:** 303–306

78 Justice M. C., Hsu M. J., Tse B., Ku T., Balkovec J., Schmatz D. et al. (1998) Elongation factor 2 as a novel target for selective inhibition of fungal protein synthesis. J. Biol. Chem. **273:** 3148–3151

79 Dominguez J. M., Gomez-Lorenzo M. G. and Martin J. J. (1999) Sordarin inhibits fungal protein synthesis by blocking translocation differently to fusidic acid. J. Biol. Chem. **274:** 22423–22427

80 Justice M. C., Ku T., Hsu M.-J., Carniol K., Schmatz D. and Nielsen J. (1999) Mutations in ribosomal protein L10e confer resistance to the fungal-specific eukaryotic elongation factor 2 inhibitor sordarin. J. Biol. Chem. **274:** 4869–4875

81 Kiel M. C., Aoki H. and Ganoza M. C. (1999) Identification of a ribosomal ATPase in *Escherichia coli* cells. Biochimie **81:** 1097–1108

82 Rodnina M. V., Serebryanik A. I., Ovcharenko G. V. and El'Skaya A. V. (1994) ATPase strongly bound to higher eukaryotic ribosomes. Eur. J. Biochem. **225:** 305–310

83 Kovalchuke O. and Chakraburtty K. (1994) Comparative analysis of ribosome-associated adenosinetriphosphatase (ATPase) from pig liver and the ATPase of elongation factor 3 from *Saccharomyces cerevisiae*. Eur. J. Biochem. **226:** 133–140

84 El'skaya A. V., Ovcharenko G. V., Palchevskii S. S., Petrushenko Z. M., Triana-Alonso F. J. and Nierhaus K. H. (1997) Three tRNA binding sites in rabbit liver ribosomes and role of the intrinsic ATPase in 80S ribosomes from higher eukaryotes. Biochemistry **36:** 10492–10497

85 Skogerson L. and Engelhardt D. (1977) Dissimilarity in protein chain elongation factor requirements between yeast and rat liver ribosomes. J. Biol. Chem. **252:** 1471–1475

86 Belfield G. P., Ross-Smith N. J. and Tuite M. F. (1995) Translation elongation factor-3 (EF-3): an evolving eukaryotic ribosomal protein? J. Mol. Evol. **41:** 376–387

87 Uritani M. and Miyazaki M. (1988) Role of the yeast peptide elongation factor 3 (EF-3) at the AA-tRNA binding step. J. Biochem. **104:** 118–126

88 Bertram G., Innes S., Minella O., Richardson J. P. and Stansfield I. (2001) Endless possibilities: translation termination and stop codon recognition. Microbiology **147:** 255–269

89 Urbero B., Eurwilaichitr L., Stansfield I., Tassan J. P., Le Goff X., Kress M. et al. (1997) Expression of the release factor eRF1 (Sup45p) gene of higher eukaryotes in yeast and mammalian tissues. Biochimie **79:** 27–36

90 Himmelfarb H. J., Maicas E. and Friesen J. D. (1985) Isolation of the *sup45* omnipotent suppressor gene of *Saccharomyces cerevisiae* and characterization of its gene product. Mol. Cell. Biol. **5:** 816–822

91 Breining P. and Piepersberg W. (1986) Yeast omnipotent suppressor SUP1 (SUP45): nucleotide sequence of the wildtype and a mutant gene. Nucleic Acids Res. **14:** 5187–5197

92 Kushnirov V. V., Ter-Ananesyan M. D., Telckov M. V., Surguchov A. P., Smirnov V. N. and Inge-Vechtomov S. G. (1988) Nucleotide sequence of the *sup2* (*sup35*) gene of *Saccharomyces cerevisiae*. Gene **66:** 45–54

93 Ter-Avanesyan M. D., Kushnirov V. V., Dagkesamanskaya A. R., Didichenko S. A., Chernoff Y. O., Inge-Vechtomov S. G. et al. (1993) Deletion analysis of the SUP35 gene of the yeast *Saccharomyces cerevisiae* reveals two non-overlapping functional regions in the encoded protein. Mol. Microbiol. **7:** 683–692

94 Stansfield I., Jones K. M., Kushnirov V. V., Dagkesamanskaya A. R., Paushkin S. V., Nierras C. R. et al. (1995) The products of the *SUP45* (eRF1) and *SUP35* genes interact to mediate translation termination in *Saccharomyces cerevisiae*. EMBO J. **14:** 4365–4373

95 Frolova L. Y., Simonsen J. L., Merkulova T. I., Litvinov D. Y., Martensen P. M., Rechinsky V. O. et al. (1998) Functional expression of eukaryotic polypeptide chain release factors 1 and 3 by means of baculovirus/insect cells and complex formation between the factors. Eur. J. Biochem. **256:** 36–44

96 Merkulova T. I., Frolova L. Y., Lazar M., Camonis J. and Kisselev L. L. (1999) C-terminal domains of human translation termination factors eRF1 and eRF3 mediate their in vivo interaction. FEBS Lett. **443:** 41–47

97 Hawthorne D. C. and Leupold U. (1974) Suppressors in yeast. Curr. Top. Microbiol. Immunol. **64:** 1–47

98 Ito K., Uno M. and Nakamura Y. (2000) A tripeptide 'anticodon' deciphers stop codons in messenger RNA. Nature **403:** 680–684

99 Kervestin S., Frolova L., Kisselev L. and Jean-Jean O. (2001) Stop codon recognition in ciliates: Euplotes release factor does not respond to reassigned UGA codon. EMBO Rep. **2:** 680–684

100 Frolova L., Le Goff X., Zhouravleva G., Davydova E., Philippe M. and Kisselev L. (1996) Eukaryotic polypeptide chain release factor eRF3 is an eRF1- and ribosome-dependent guanosine triphosphatase. RNA **2:** 334–341

101 Frolova L. Y., Tsivkovskii R. Y., Sivolobova G. F., Oparina N. Y., Serpinsky O. I., Blinov V. M. et al. (1999) Mutations in the highly conserved GGQ motif of class 1 polypeptide release factors abolish ability of human eRF1 to trigger peptidyl-tRNA hydrolysis. RNA **5:** 1014–1020

102 Song H., Mugnier P., Das A. K., Wedd H. M., Evans D. R., Tuite M. F. et al. (2000) The crystal structure of human eukaryotic release factor eEF1- mechanism of stop codon recognition and peptidyl-tRNA hydrolysis. Cell **100:** 311–321

103 Seit-Nebi A., Frolova L., Justesen J. and Kisselev L. (2001) Class-1 translation termination factors: invariant GGQ minidomain is essential for release activity and ribosome binding but not for stop codon recognition. Nucleic Acids Res. **29:** 3982–3987

104 Frolova L., Seit-Nebi A. and Kisselev L. (2002) Highly conserved NIKS tetrapeptide is functionally essential in eukaryotic translation termination factor eRF1. RNA **8:** 129–136

105 Chavatte L., Seit-Nebi A., Dubovaya V. and Favre A. (2002) The invariant uridine of stop codons contacts the conserved NIKSR loop of human eRF1 in the ribosome. EMBO J. **21:** 5302–5311

106 Derkatch I. L., Bradley M. E. and Liebman S. W. (1998) Overexpression of the SUP45 gene encoding a Sup35p-binding protein inhibits the induction of the de novo appearance of the [PSI+] prion. Proc. Natl. Acad. Sci. USA **95:** 2400–2405

107 Stansfield I., Eurwilaichitr L., Akhmaloka and Tuite M. F. (1996) Depletion in the levels of the release factor eRF1 causes a reduction in the efficiency of translation termination in yeast. Mol. Microbiol. **20:** 1135–1143

108 Eurwilaichitr L., Graves F. M., Stansfield I. and Tuite M. F. (1999) The C-terminus of eRF1 defines a functionally important domain for translation termination in *Saccharomyces cerevisiae*. Mol. Microbiol. **32:** 485–496

109 Ito K., Ebihara K. and Nakamura Y. (1998) The stretch of C-terminal acidic amino acids of translational release factor eRF1 is a primary binding site for eRF3 of fission yeast. RNA **4:** 958–972

110 Bertram G., Bell H. A., Ritchie D. W., Fullerton G. and Stansfield I. (2000) Terminating eukaryote translation: domain 1 of release factor eRF1 functions in stop codon recognition. RNA **6:** 1236–1247

111 Chernoff Y. O., Newnam G. P. and Liebman S. W. (1996) The translational function of nucleotide C1054 in the small subunit rRNA is conserved throughout evolution: genetic evidence in yeast. Proc. Natl. Acad. Sci. USA **93:** 2517–2522

112 Wilson P. G. and Culbertson M. R. (1988) *SUF12* suppressor protein of yeast: a fusion protein related to the EF-1 family of elongation factors. J. Mol. Biol. **199:** 559–573

113 Moreira D., Kervestin S., Jean-Jean O. and Philippe H. (2002) Evolution of eukaryotic translation elongation and termination factors: variations of evolutionary rate and genetic code deviations. Mol. Biol. Evol. **19:** 189–200

114 Zhouravleva G., Frolova L., Le Goff X., Le Guellec R., Inge-Vechtomov S., Kisselev L. et al. (1995) Termination of translation in eukaryotes is governed by two interacting polypeptide chain release factors, eRF1 and eRF3. EMBO J. **14:** 4065–4072

115 Wickner R. B., Edskes H. K., Roberts B. T., Pierce M. and Baxa U. (2002) Prions of yeast as epigenetic phenomena: high protein 'copy number' inducing protein 'silencing'. Adv. Genet. **46:** 485–525

116 Paushkin S. V., Kushnirov V. V., Smirnov V. N. and Ter-Avanesyan M. D. (1996) Propagation of the yeast prion-like [psi+] determinant is mediated by oligomerization of the SUP35-encoded polypeptide chain release factor. EMBO J. **15:** 3127–3134

117 Paushkin S., Kushnirov V., Smirnov V. and Ter-Avanesyan M. (1997) Interaction between yeast Sup45p (eRF1) and Sup35p (eRF3) polypeptide chain release factors: implications for prion-dependent regulation. Mol. Cell. Biol. **17:** 2798–2805

118 Chernoff Y. O., Derkach I. L. and Inge-Vechtomov S. G. (1993) Multicopy SUP35 gene induces de-novo appearance of psi-like factors in the yeast *Saccharomyces cerevisiae*. Curr. Genet. **24:** 268–270

119 Derkatch I. L., Chernoff Y. O., Kushnirov V. V., Inge-Vechtomov S. G. and Liebman S. W. (1996) Genesis and variability of [PSI] prion factors in *Saccharomyces cerevisiae*. Genetics **144:** 1375–1386

120 Kochneva-Pervukhova N. V., Poznyakovski A. I., Smirnov V. N. and Ter-Avanesyan M. D. (1998) C-terminal truncation of the Sup35 protein increases the frequency of de novo generation of a prion-based [PSI+] determinant in *Saccharomyces cerevisiae*. Curr. Genet. **34:** 146–151

121 Liebman S. W., Chernoff Y. O. and Liu R. (1995) The accuracy center of a eukaryotic ribosome. Biochem. Cell Biol. **73:** 1141–1149

122 Chernoff Y. O., Vincent A. and Liebman S. W. (1994) Mutations in eukaryotic 18S ribosomal RNA affect translational fidelity and resistance to aminoglycoside antibiotics. EMBO J. **13:** 906–913

123 Wai H. H., Vu L., Oakes M. and Nomura M. (2000) Complete deletion of yeast chromosomal rDNA repeats and integration of a new rDNA repeat: use of rDNA deletion strains for functional analysis of rDNA promoter elements in vivo. Nucleic Acids Res. **28:** 3524–3534.

124 Velichutina I. V., Hong J. Y., Mesecar A. D., Chernoff Y. O. and Liebman S. W. (2001) Genetic interaction between yeast *Saccharomyces cerevisiae* release factors and the decoding region of 18 S rRNA. J. Mol. Biol. **305:** 715–727

125 Burck C. L., Chernoff Y. O., Liu R., Farabaugh P. J. and Liebman S. W. (1999) Translational suppressors and antisuppressors alter the efficiency of the Ty1 programmed translational frameshift. RNA **5:** 1451–1457

126 Velichutina I. V., Dresios J., Hong J. Y., Li C., Mankin A., Synetos D. et al. (2000) Mutations in helix 27 of the yeast *Saccharomyces cerevisiae* 18S rRNA affect the function of the decoding center of the ribosome. RNA **6:** 1174–1184

127 Liu R. and Liebman S. W. (1996) A translational fidelity mutation in the universally conserved sarcin/ricin domain of 25S yeast ribosomal RNA. RNA **2:** 254–263

128 Moazed D., Robertson J. M. and Noller H. F. (1988) Interaction of elongation factors EF-G and EF-Tu with a conserved loop in 23S RNA. Nature **334:** 362–364

129 Brigotti M., Rambelli F., Zamboni M., Montanaro L. and Sperti S. (1989) Effect of alpha-sarcin and ribosome-inactivating proteins on the interaction of elongation factors with ribosomes. Biochem. J. **257:** 723–727

130 Dinman J. D. and Wickner R. B. (1995) 5S rRNA is involved in fidelity of translational reading frame. Genetics **141:** 95–105

131 Smith M. W., Meskauskas A., Wang P., Sergiev P. V. and Dinman J. D. (2001) Saturation mutagenesis of 5S rRNA in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **21:** 8264–8275

132 Alksne L. E., Anthony R. A., Liebman S. W. and Warner J. R. (1993) An accuracy center in the ribosome conserved over 2 billion years. Proc. Natl. Acad. Sci. USA **90:** 9538–9541

133 Hendrick J. L., Wilson P. G., Edelman I. I., Sandbaken M. G., Ursic D. and Culbertson M. R. (2001) Yeast frameshift suppressor mutations in the genes coding for transcription factor Mbf1p and ribosomal protein S3: evidence for autoregulation of S3 synthesis. Genetics **157:** 1141–1158

134 Eustice D. C., Wakem L. P., Wilhelm J. M. and Sherman F. (1986) Altered 40 S ribosomal subunits in omnipotent suppressors of yeast. J. Mol. Biol. **188:** 207–214

135 Masurekar M., Palmer E., Ono B.-I., Wilhelm J. M. and Sherman F. (1981) Misreading of the ribosomal suppressor *SUP46* due to an altered 40S subunit in yeast. J. Mol. Biol. **147:** 381–390

136 All-Robyn J. A., Brown N., Otaka E. and Liebman S. W. (1990) Sequence and functional similarity between a yeast ribosomal protein and the *E.coli* S5 *ram* protein. Mol. Cell. Biol. **10:** 6544–6553

137 Synetos D., Frantziou C. P. and Alksne L. E. (1996) Mutations in yeast ribosomal proteins S28 and S4 affect the accuracy of translation and alter the sensitivity of the ribosomes to paromomycin. Biochim. Biophys. Acta **1309:** 156–166

138 Vincent A. and Liebman S. W. (1992) The yeast omnipotent suppressor *SUP46* encodes a ribosomal protein which is a functional and structural homolog of the *Escherichia coli* S4 *ram* protein. Genetics **132:** 375–386

139 Anthony R. A. and Liebman S. W. (1995) Alterations in ribosomal protein RPS28 can diversely affect translational accuracy in *Saccharomyces cerevisiae*. Genetics **140:** 1247–1258

140 Dresios J., Derkatch I. L., Liebman S. W. and Synetos D. (2000) Yeast ribosomal protein L24 affects the kinetics of protein synthesis and ribosomal protein L39 improves translational accuracy, while mutants lacking both remain viable. Biochemistry **39:** 7236–7244

141 Meskauskas A., Harger J. W. and Dinman J. D. (2001) Ribosomal protein L5 helps anchor peptidyl-tRNA to the P-site in *Saccharomyces cerevisiae*. RNA **7:** 1084–1096
142 Hesketh J. E. and Pryme I. F. (1991) Interaction between mRNA, ribosomes and the cytoskeleton. Biochem. J. **277:** 1–10
143 Shestakova E. A., Motuz L. P., Minin A. A., Gelfand V. I. and Gavrilova L. P. (1991) Some of eukaryotic elongation factor 2 is colocalized with actin microfilament bundles in mouse embryo fibroblasts. Cell. Biol. Int . Rep. **15:** 75–84
144 Bektas M., Nurten R., Gurel Z., Sayers Z. and Bermek E. (1994) Interactions of eukaryotic elongation factor 2 with actin: a possible link between protein synthetic machinery and cytoskeleton. FEBS Lett. **356:** 89–93
145 Janssen G. M. C. and Moller W. (1988) Elongation factor 1$\beta\gamma$ from *Artemia*: purification and properties of its subunits. Eur. J. Biochem. **171:** 119–129
146 Yang F., Demma M., Warren V., Dharmawardhane S. and Condeelis J. (1990) Identification of an actin-binding protein from *Dictyostelium* as elongation factor 1α. Nature **347:** 494–496
147 Moore R. C., Durso N. A. and Cyr R. J. (1998) Elongation factor-1alpha stabilizes microtubules in a calcium/calmodulin-dependent manner. Cell. Motil. Cytoskeleton **41:** 168–180
148 Kandl K. A., Munshi R., Ortiz P. A., Andersen G. R., Kinzy T. G. and Adams A. E. (2002) Identification of a role for actin in translational fidelity in yeast. Mol. Genet. Genomics **268:** 10–18.
149 Castilho-Valavicius B., Yoon H. and Donohue T. F. (1990) Genetic characterization of the *Saccharomyces cerevisiae* translational initiation suppressors *sui1, sui2* and *SUI3* and their effects on *HIS4* expression. Genetics **124:** 483-495