proteases, and, in eukaryotes, the ubiquitin conjugating system, to distinguish nonnative from native proteins allows a kinetic partitioning of misfolded proteins between these systems, leading to preferential degradation of those proteins that cannot readily fold into native conformations. Degradation of properly folded proteins is avoided because the motifs recognized by the regulatory components of the degradative machinery have characteristics of regions normally buried within folded proteins and because the proteolytic sites themselves are sequestered within internal chambers that are not directly accessible to proteins in the surrounding medium.

References and Notes

1. S. Gottesman, M. R. Maurizi, S. Wickner, *Cell* **91**, 435 (1997).
2. S. Gottesman, S. Wickner, M. R. Maurizi, *Genes Dev.* **11**, 815 (1997).
3. S. A. Teter *et al.*, *Cell* **97**, 755 (1999).
4. K. L. Ewalt, J. P. Hendrick, W. A. Houry, F. U. Hartl, *Cell* **90**, 491 (1997).
5. C. Yen, L. Green, C. G. Miller, *J. Mol. Biol.* **143**, 21 (1980).
6. A. L. Goldberg, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 422 (1972).
7. S. Gottesman and M. R. Maurizi, *Microbiol. Rev.* **56**, 592 (1992).
8. R. R. Kopito, *Physiol. Rev.* **79**, S167 (1999).
9. P. B. Sigler *et al.*, *Annu. Rev. Biochem.* **67**, 581 (1998).
10. B. Bukau and A. L. Horwich, *Cell* **92**, 351 (1998).
11. M. Kessel *et al.*, *J. Mol. Biol.* **250**, 587 (1995).
12. E. C. Schirmer, J. R. Glover, M. A. Singer, S. Lindquist, *Trends Biochem. Sci.* **21**, 289 (1996).
13. E. U. Weber-Ban, B. G. Reid, A. D. Miranker, A. L. Horwich, *Nature* **401**, 90 (1999).
14. E. Deuerling, A. Schulze-Specking, T. Tomoyasu, A. Mogk, B. Bukau, *Nature* **400**, 693 (1999).
15. J. Wang, J. A. Hartling, J. M. Flanagan, *Cell* **91**, 447 (1997).
16. R. Grimaud, M. Kessel, F. Beuron, A. C. Stevens, M. R. Maurizi, *J. Biol. Chem.* **273**, 12476 (1998).
17. S. Wickner *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12218 (1994).
18. J. R. Hoskins, M. Pak, M. R. Maurizi, S. Wickner, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 12135 (1998).
19. M. Pak, J. R. Hoskins, S. K. Singh, M. R. Maurizi, S. Wickner, *J. Biol. Chem.* **274**, 19316 (1999).
20. M. W. Thompson, S. K. Singh, M. R. Maurizi, *J. Biol. Chem.* **269**, 18209 (1994).
21. S. Gottesman, *Annu. Rev. Genet.* **30**, 465 (1996).
22. J. M. van Dijl *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 10584 (1998).
23. W. Schumann, *FEMS Microbiol. Rev.* **23**, 1 (1998).
24. M. R. Maurizi, *Adv. Mol. Cell Biol.* **27**, 1 (1998).
25. M. Bochtler, L. Ditzel, M. Groll, C. Hartmann, R. Huber, *Annu. Rev. Biophys. Biomol. Struct.* **28**, 295 (1999).
26. G. N. DeMartino and C. A. Slaughter, *J. Biol. Chem.* **274**, 22123 (1999).
27. A. Lupas, J. M. Flanagan, T. Tamura, W. Baumeister, *Trends Biochem. Sci.* **22**, 399 (1997).
28. A. F. Neuwald, L. Aravind, J. L. Spouge, E. V. Koonin, *Genome Res.* **9**, 27 (1999).
29. M. H. Glickman *et al.*, *Cell* **94**, 615 (1998).
30. B. C. Braun *et al.*, *Nature Cell Biol.* **1**, 221 (1999).
31. S. Rudiger, L. Germeroth, J. Schneider-Mergener, B. Bukau, *EMBO J.* **16**, 1501 (1997).
32. M. W. Thompson and M. R. Maurizi, *J. Biol. Chem.* **269**, 18201 (1994).
33. C. K. Smith, T. A. Baker, R. T. Sauer, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 6678 (1999).
34. M. Gonzalez, E. G. Frank, A. S. Levine, R. Woodgate, *Genes Dev.* **12**, 3889 (1998).
35. M. Gonciarz-Swiatek *et al.*, *J. Biol. Chem.* **274**, 13999 (1999).
36. A. Varshavsky, *Genes Cells* **2**, 13 (1997).
37. S. Gottesman, E. Roche, Y.-N. Zhou, R. T. Sauer, *Genes Dev.* **12**, 1338 (1998).
38. C. Herman, D. Thevenet, P. Bouloc, G. C. Walker, R. D'Ari, *Genes Dev.* **12**, 1348 (1998).
39. K. C. Keiler, P. R. H. Waller, R. T. Sauer, *Science* **271**, 990 (1996).
40. M. Ibba and D. Soll, *Science* **286**, 1893 (1999).
41. M. Hochstrasser, *Annu. Rev. Genet.* **30**, 405 (1996).
42. A. Hershko and A. Ciechanover, *Annu. Rev. Biochem.* **67**, 425 (1998).
43. S. Sadis, C. J. Atienza, D. Finley, *Mol. Cell Biol.* **15**, 4086 (1995).
44. J. D. Laney and M. Hochstrasser, *Cell* **97**, 427 (1999).
45. T. Yura and K. Nakahigashi, *Curr. Opin. Microbiol.* **2**, 153 (1999).
46. R. I. Morimoto, *Genes Dev.* **12**, 3788 (1998).
47. D. A. Harris, *Clin. Microbiol. Rev.* **12**, 429 (1999).
48. J. B. Martin, *N. Engl. J. Med.* **340**, 1970 (1999).
49. R. L. Levine, B. S. Berlett, J. Moskovitz, L. Mosoni, E. R. Stadtman, *Mech. Ageing Dev.* **107**, 323 (1999).
50. J. R. Glover and S. Lindquist, *Cell* **94**, 73 (1998).
51. K. Motohashi, Y. Watanabe, M. Yohda, M. Yoshida, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 7184 (1999).
52. M. Zolkiewski, *J. Biol. Chem.* **274**, 28083 (1999).
53. J. A. Johnston, C. L.Ward, R. R. Kopito, *J. Cell Biol.* **143**, 1883 (1998).
54. S. B. Prusiner, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 13363 (1998).
55. Y. O. Chernoff, S. L. Lindquist, B. Ono, S. G. Inge-Vechtomov, S. W. Liebman, *Science* **268**, 881 (1995).
56. G. P. Newnam, R. D. Wegrzyn, S. L. Lindquist, Y. O. Chernoff, *Mol. Cell. Biol.* **19**, 1325 (1999).
57. S. K. DebBurman, G. J. Raymond, B. Caughey, S. Lindquist, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 13938 (1997).
58. C. J. Cummings *et al.*, *Nature Genet.* **19**, 148 (1998).
59. D. L. Stenoien *et al.*, *Hum. Mol. Genet.* **8**, 731 (1999).
60. F. Beuron *et al.*, *J. Struct. Biol.* **123**, 248 (1998).
61. J. Walzet *al.*, *J. Struct. Biol.* **121**, 19 (1998).

62. Y. A. Lam, W. Xu, G. N. DeMartino, R. E. Cohen, *Nature* **385**, 737 (1997).
63. We thank C.-C. Li for comments on the manuscript.

REVIEW

Quality Control Mechanisms During Translation

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Translation uses the genetic information in messenger RNA (mRNA) to synthesize proteins. Transfer RNAs (tRNAs) are charged with an amino acid and brought to the ribosome, where they are paired with the corresponding trinucleotide codon in mRNA. The amino acid is attached to the nascent polypeptide and the ribosome moves on to the next codon. The cycle is then repeated to produce a full-length protein. Proofreading and editing processes are used throughout protein synthesis to ensure the faithful translation of genetic information. The maturation of tRNAs and mRNAs is monitored, as is the identity of amino acids attached to tRNAs. Accuracy is further enhanced during the selection of aminoacyl-tRNAs on the ribosome and their base pairing with mRNA. Recent studies have begun to reveal the molecular mechanisms underpinning quality control and go some way to explaining the phenomenal accuracy of translation first observed over three decades ago.

Translation is the process by which the genetic information contained in mRNA is used to determine the sequential order of amino acids in a protein (Fig. 1). Translation is a key facet of the Central Dogma of molecular biology (*1*) and must be relatively error free in order to allow the accurate flow of genetic information. Experimental measurements have suggested that, overall, an amino acid is misincorporated at about 1 in every 10,000 codons under normal growth conditions (*2*). This high level of accuracy is seemingly at odds with the limited ability of enzymes to distinguish structurally similar molecules such as, for example, the amino acids valine and isoleucine, both of which are substrates for translation (*3*). This particular problem is solved by the enzyme isoleucyl-tRNA synthetase, which is able to almost completely prevent the misincorporation of valine at isoleucine codons during translation (*4*). While this represents the first identified, and perhaps best understood, example of quality control during translation, numerous other

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mechanisms have since been identified at almost every step of the process. These mechanisms can now be seen to function on several levels: The integrity of the nucleic acid substrates (tRNA and mRNA) is rigorously checked; the exact matching of tRNAs with the appropriate amino acid is carefully controlled; and finally, the precise pairing of aminoacylated tRNAs with the corresponding mRNA codon is mediated by the ribosome.

Preparing the Substrates for Translation

The two key substrates of translation are mRNA and aminoacyl-tRNA; it is their eventual pairing on the ribosome that determines which amino acid is inserted at a particular point in the nascent polypeptide chain. After the transcription of tRNA genes, the resulting RNAs undergo numerous changes before a mature translation-competent species is produced. These have been found to include terminal processing, intron splicing, editing, deamination, and addition on the nucleotide level (*5*). Furthermore, tRNAs also contain extensive nucleotide modifications that can be essential for their function (*6*). Given the need for many steps in the production of mature tRNAs, it is to be expected that some form of quality control must operate to ensure that all of the necessary changes have been completed before tRNAs are used in translation. This function is principally performed by the aminoacyl-tRNA synthetases, although the strict requirement by the processing enzymes for the correct three-dimensional structure of tRNAs suggests that these steps are also used for quality control (*5*, *6*). The aminoacyl-tRNA synthetases are a family of enzymes (one for each amino acid) that catalyze the attachment of a particular amino acid to the 3′ end of tRNAs containing the anticodon corresponding to that amino acid.

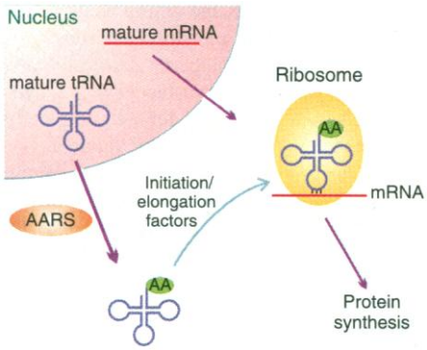

Fig. 1. An overview of translation in eukaryotic cells. Messenger RNA and tRNA are synthesized and processed in the nucleus and then exported to the cytoplasm. In prokaryotic cells, mRNA and tRNA are made in the cytoplasm. AA, the aminoacyl moiety attached to the 3′ end of mature tRNAs; AARS, aminoacyl-tRNA synthetase. Before translation initiation in bacteria and organelles, the initiator methionyl-tRNA must first be formylated.

The aminoacyl-tRNA synthetases make extensive contacts with tRNAs over a large area (2470 to 5650 Å^2) (*7*), allowing numerous sequence-specific interactions during RNA recognition (*8*). These extensive interactions ensure that only mature tRNAs are selected as bona fide substrates for translation (the specificity of this process will be discussed later). The point at which aminoacyl-tRNA synthetases execute their tRNA quality control function is not the same in prokaryotes and eukaryotes. In prokaryotes the maturation of tRNAs occurs in the cytoplasm, and this step is directly followed by their aminoacylation, with the product of this reaction then being used for ribosomal translation. In eukaryotes, tRNA transcription and maturation occur in the nucleus and may also be followed by aminoacylation—not to provide substrates directly for translation, but to facilitate interaction with specific factors necessary for export to the cytoplasm (Fig. 2) (*9*). In this way, the aminoacyl-tRNA synthetases ensure that mature, fully functional tRNAs (but not unprocessed or misfolded tRNAs) are exported to the cytoplasm where they can participate in translation. It has also been found that some tRNAs are exported from the nucleus without prior aminoacylation. In this case, their structural integrity is monitored by the Ran-GTPase (guanosine triphosphatase) exportin-t before their transport to the cytosol (*10*) and again by the aminoacyl-tRNA synthetases directly before their use in translation.

The accuracy of mRNA synthesis is carefully monitored during transcription elongation and termination in prokaryotes and eukaryotes by the same general mechanisms (*11*). However, the subsequent fate of these primary transcripts is markedly different. In prokaryotes, the transcripts of protein-coding genes can usually be directly used as substrates for translation. In eukaryotes, the primary transcript must normally be processed to generate a mature mRNA that is a template for translation. As with tRNA, maturation occurs in the nucleus and is subjected to quality control before export of the mature mRNA, primarily through the association of various factors with the 3′ polyadenylate tail (*12*). A second process of mRNA quality control has recently come to light: mRNA surveillance (also known as nonsense-mediated decay). This process is fundamentally different from that seen for the quality control of tRNAs, because a faulty mRNA must first be at least partially exported and used for translation before it can be destroyed. The role of mRNA surveillance is to detect and destroy mRNAs containing premature translation termination signals that would otherwise result in the synthesis of truncated proteins (*13*). Although the exact mechanism of nonsense-mediated decay has yet to be fully resolved, it represents a critical quality control step during eukaryotic translation and explains why nonsense mutations rarely lead to the synthesis of truncated proteins in eukaryotes.

Aminoacylation of tRNAs: Matching Nucleic and Amino Acids

The sequential pairing of codons in mRNA with tRNA anticodons determines the order of amino acids in a protein. Thus, it is imperative for accurate translation that tRNAs are only coupled to amino acids corresponding to the RNA anticodon. This is chiefly, but not exclusively, achieved by the direct attachment of the appropriate amino acid to the 3′ end of the corresponding tRNA by the aminoacyl-tRNA synthetases (Fig. 3A) (*14*). Extensive structural, biochemical, and genetic studies have shown that an intricate network of sequence-specific protein-RNA interactions ensures the accurate selection of the correct (cognate) tRNA and discrimination against other (noncognate) tRNAs (*8*, *15*). The structural diversity presented by the different combinations of bases, both modified and unmodified, in tRNAs ensures that the cognate molecules can be specifically selected by the appropriate aminoacyl-tRNA synthetase without recourse to proofreading (*16*). The accuracy of tRNA selection is further enhanced in vivo by competition be-

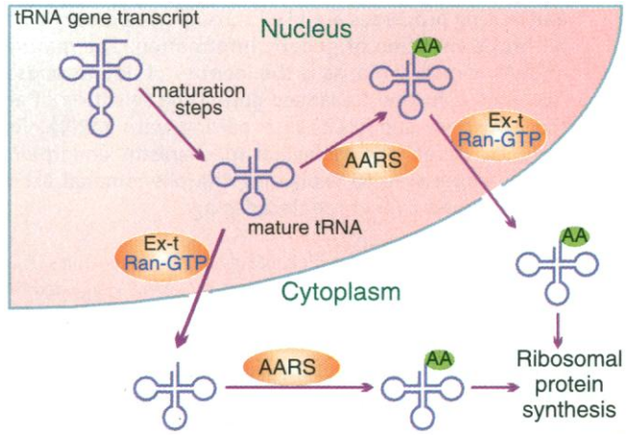

Fig. 2. Quality control in eukaryotic tRNA maturation. Transcripts of tRNA genes are first processed to produce a mature tRNA. Some mature tRNAs can then directly associate with exportin-t (Ex-t) and Ran-GTP, which mediate their export to the cytoplasm, whereas others must first be aminoacylated before they can be exported. AA, the aminoacyl moiety attached to the 3′ end of mature tRNAs.

tween synthetases for their cognate tRNAs (*17*) and in some cases by the recruitment of additional proteins that enhance binding (*18*).

Amino acids, being considerably less complex in structure, present a more challenging problem. Numerous examples have been reported of in vitro activation of noncognate amino acids by aminoacyl-tRNA synthetases (*19*), sometimes with a frequency as high as 1 in 150 compared with the cognate amino acid [the recognition of valine versus isoleucine by isoleucyl-tRNA synthease (*3*)]. The principal reason that this inability to discriminate similar amino acids does not compromise the fidelity of translation is that the respective aminoacyl-tRNA synthetases have proofreading activities (*4*). These activities have been found to operate at two levels: Most commonly, the activated noncognate aminoacyl-adenylate is hydrolyzed before transfer to tRNA can occur; less frequently, a noncognate aminoacyl-tRNA may be synthesized that is then deacylated (Fig. 3B). The molecular mechanisms underlying these proofreading activities have recently been elucidated for isoleucyl-tRNA synthetase. This enzyme contains two distinct catalytic sites that present a double sieve during substrate selection (*16*, *20*). The first sieve serves to exclude amino acids larger than isoleucine from the active site but is unable to exclude valine, and consequently valyl-AMP (adenosine monophosphate) is synthesized. The second sieve then acts by hydrolyzing valyl-AMP at a structurally distinct "editing" site. The proofreading activity of isoleucyl-tRNA synthetase is dependent on specific sequences in cognate isoleucine tRNA species, which trigger the translocation of misactivated valine from the catalytic to the editing site, further enhancing the accuracy of isoleucyl-tRNA synthesis by the enzyme (*4*, *21*). Isoleucyl-tRNA synthetase provides a highly effective point of quality control, as seen from the observation that only about 1 in 3000 isoleucine codons are misread as valine during protein synthesis (*22*).

Despite the existence of highly refined quality control mechanisms in many of the aminoacyl-tRNA synthetases, these enzymes are not the sole providers of aminoacyl-tRNA for translation. It has become increasingly apparent in recent years that, paradoxically, several aminoacyl-tRNA synthetases must first attach their cognate amino acids to apparently noncognate tRNAs as an essential step in translation (*14*). These misacylated tRNAs do not compromise the fidelity of translation as they are not substrates for elongation factors and hence are not delivered to the ribosome (*23*). Instead, the noncognate amino acid moieties are enzymatically modified by nonsynthetase proteins to yield correctly charged aminoacyl-tRNAs that can then be used in protein synthesis (Fig. 3C). These tRNA-dependent amino acid transformation pathways provide the only known means of synthesizing selenocysteinyl-tRNA [from Ser-tRNA^{Sec} (*24*)] and formylmethionyl-tRNA [from Met-tRNA$_i^{Met}$ (*25*)] and are solely responsible for the synthesis of asparaginyl-tRNA (from Asp-tRNA^{Asn}) or glutaminyl-tRNA (from Glu-tRNA^{Gln}) in many bacteria, archaea, and organelles (*26*). A final problem that must be addressed during aminoacyl-tRNA synthesis is the stereospecificity of amino acid recognition. Although most aminoacyl-tRNA synthetases can adequately discriminate D- from L-amino acids, some, such as tyrosyl-tRNA synthetase, readily recognize both enantiomers of their cognate substrates. However, D-tyrosine is prevented from being incorporated into proteins by a proofreading enzyme, D-Tyr-tRNA^{Tyr} deacylase, that specifically deacylates D-Tyr-tRNA^{Tyr} but not L-Tyr-tRNA^{Tyr}, and to a lesser extent by the preference of elongation factors for L-Tyr-tRNA^{Tyr} (*27*).

Synthesizing Proteins from mRNA and Aminoacyl-tRNA

Once an aminoacyl-tRNA has been released by its respective aminoacyl-tRNA synthetase, it must associate with elongation factor Tu (EF-Tu, EF-1α in eukaryotes) before it can participate in ribosomal protein synthesis (*28*). The primary function of EF-Tu, to deliver a broad range of aminoacyl-tRNAs to the ribosome, dictates that it has a wide substrate specificity. At the same time, EF-Tu is more than simply a carrier and is essential for quality control during translation. EF-Tu helps to maintain translational fidelity by rejecting a number of tRNA species including uncharged tRNAs, naturally occurring mischarged tRNAs (*23*), and aminoacyl-tRNAs that are substrates for other translation factors (*29*). Aminoacyl-tRNA associates with EF-Tu in a ternary complex with guanosine triphosphate (GTP), which can then bind to the ribosome where anticodon:codon pairing occurs. The primary determinant of anticodon:codon pairing is classical Watson-Crick base pairing. Modified nucleotides in tRNA are often also essential—for example, in modulating the degree of wobble at the third position in the codon (*30*) and in restricting shifts of the reading frame to those essential for recoding events (*31*). The correct pairing of bases between the

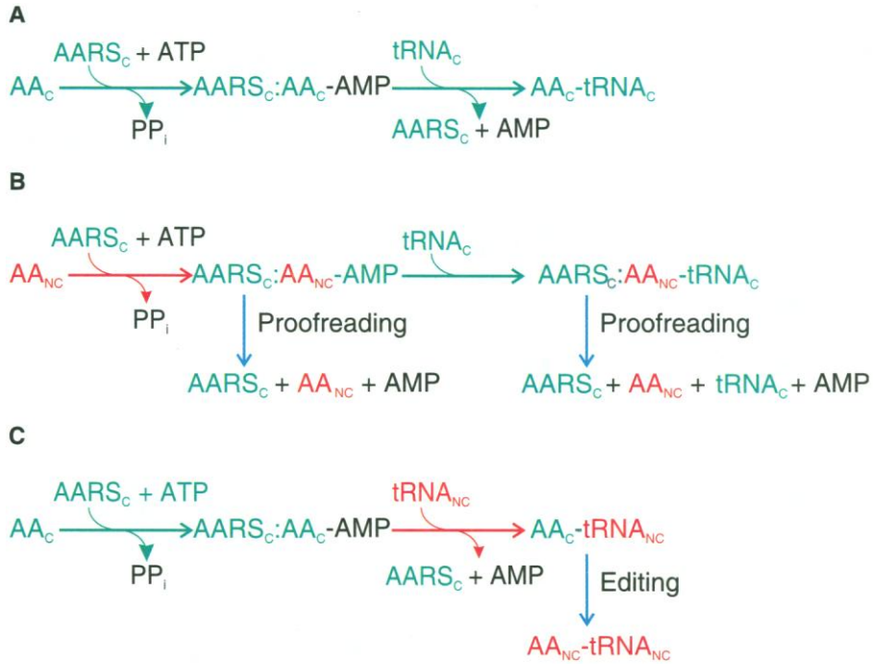

Fig. 3. Proofreading and editing pathways in aminoacyl-tRNA synthesis. (**A**) Pathway for direct synthesis of a cognate aminoacyl-tRNA by the corresponding aminoacyl-tRNA synthetase. The cognate amino acid (AA$_C$) is first activated in the presence of ATP, leading to the synthesis of an enzyme-bound aminoacyl-adenylate (AA$_C$-AMP). The aminoacyl moiety is then transferred to the 3′ end of cognate tRNA (tRNA$_C$), leading to the release of aminoacyl-tRNA (AA$_C$-tRNA$_C$). AARS$_C$, cognate aminoacyl-tRNA synthetase. (**B**) Proofreading of noncognate amino acids by aminoacyl tRNA synthetases. The noncognate amino acid (AA$_{NC}$) is first activated, leading to the formation of a complex between a cognate aminoacyl-tRNA synthetase and a noncognate aminoacyl-adenylate. This complex may then either be proofread, resulting in the release of the noncognate amino acid, or it may transfer the noncognate aminoacyl moiety to the 3′ end of cognate tRNA. When a noncognate aminoacyl-tRNA (AA$_{NC}$-tRNA$_C$) is synthesized, it is not released but is instead proofread by the cognate aminoacyl-tRNA synthetase. (**C**) Editing of noncognate aminoacyl-tRNAs. After activation, the cognate aminoacyl moiety is transferred to the 3′ end of an apparently noncognate tRNA (tRNA$_{NC}$). The resulting aminoacyl-tRNA is then released and used as a substrate in a separate editing reaction that generates an aminoacyl moiety corresponding to the identity of the tRNA. Cognate pathways and components are shown in green, noncognates in red.

aminoacyl-tRNA and mRNA results in the hydrolysis of GTP and the release of EF-Tu:GDP, the aminoacyl-tRNA enters the ribosomal A site, and finally, the aminoacyl moiety is transferred to the nascent polypeptide chain. It has long been known that this stage of translation is subjected to quality control by passive kinetic proofreading of noncognate anticodon:codon interactions (*32*), although there has been some disagreement as to the exact mechanism (*33*). Recent studies suggest that cognate anticodon:codon pairings more efficiently induce a conformational change in the ribosome than noncognate or near-cognate pairings, possibly by contacts between 16*S* ribosomal RNA and the codon-anticodon complex (*34*). This is predicted to have two consequences: (i) Selection of a cognate EF-Tu:GTP:aminoacyl-tRNA ternary complex will be favored and (ii) GTP hydrolysis and A site entry will occur more efficiently. The overall effect is that cognate anticodon:codon pairings will be kinetically more favorable than noncognate pairings for protein synthesis, providing an important quality control step in translation.

The accurate synthesis of a protein is not only dependent on consecutively translating each mRNA codon as the correct amino acid. The protein must also start and finish in the right place. The site of translation initiation is determined by specialized initiator tRNAs that, when aminoacylated, form part of a higher order complex that exclusively recognizes start codons (*35*). The completion of protein synthesis is determined by the recognition of stop codons by release factors, leading to termination of translation, polypeptide release, and ribosome recycling (*36*). Initiation and termination are dependent on the sequence context of start and stop codons, respectively (*37*), thereby enhancing the accuracy of both processes. Despite these various levels of control, nascent peptidyl-tRNAs may dissociate from the elongating ribosome before termination. The accumulation of these potentially toxic peptidyl-tRNAs, which can interfere with translation by disrupting initiation and sequestering tRNAs, is prevented by the recycling activity of the enzyme peptidyl-tRNA hydrolase (*38*). As described above, eukaryotes use an additional mechanism (RNA surveillance) to detect misplaced stop codons. Although a similar pathway has not been found to date in prokaryotes, bacteria contain a means of dealing with a different kind of termination problem, the absence of stop codons resulting from damage to the 3′ ends of mRNAs.

tmRNA, the Twist in the Tale of Bacterial Translation

mRNAs that lack stop codons present two potential problems for translation: They interfere with the ribosomal termination and reinitiation cycle and they give rise to truncated polypeptides. Bacteria overcome both of these obstacles by using a pathway mediated by a unique tRNA-mRNA hybrid, tmRNA (*39*) (Fig. 4). tmRNA contains two distinct functional domains, one that mimics part of $tRNA^{Ala}$ and one that encodes a short polypeptide. tmRNA is first charged with alanine by alanyl-tRNA synthetase, after which it associates with EF-Tu and binds at the A site of stalled ribosomes. The alanyl moiety is then transferred to the nascent polypeptide chain, while at the same time the mRNA-like domain of tmRNA replaces the truncated mRNA on the ribosome. tmRNA is then used as the template to add a further 10 amino acids to the nascent polypeptide before translation terminates and a tagged protein is released. Proteins synthesized in this way are subsequently degraded, as the 11–amino acid tag is a recognition sequence for a number of proteases (*40*). This remarkable quality control mechanism ensures that ribosomes do not get stuck on terminator-less mRNAs and that prematurely truncated proteins do not accumulate.

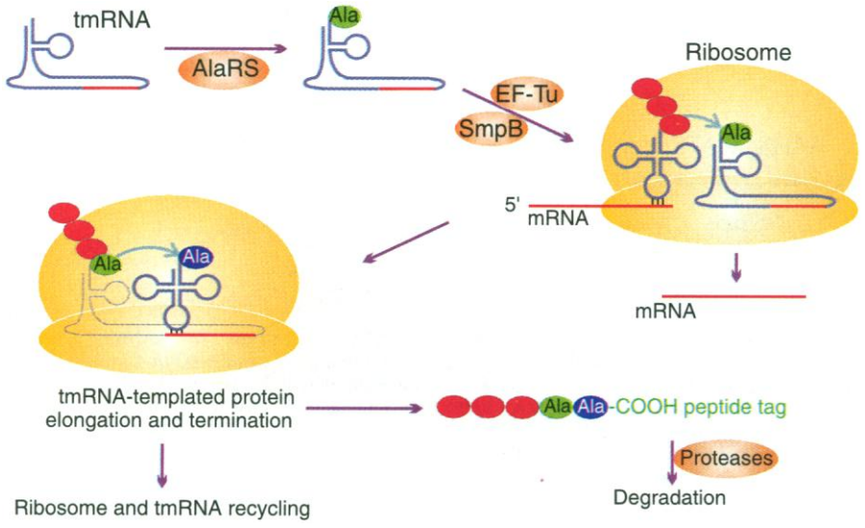

Fig. 4. The tmRNA pathway. tmRNA is first aminoacylated with alanine by alanyl-tRNA synthetase (AlaRS). Ala-tmRNA is then taken to stalled ribosomes in a pathway dependent on the proteins SmpB and EF-Tu. The "stalled" polypeptide chain is then transferred to the Ala of Ala-tmRNA and protein synthesis resumes, but now using tmRNA as its template. tmRNA-templated elongation and termination result in the release of a polypeptide with an 11–amino acid COOH-terminal tag. This tagged polypeptide is subsequently recognized and degraded by COOH-terminal–specific proteases.

Conclusions and Perspectives

The cell places a high priority on ensuring that translation produces proteins that accurately reflect the corresponding genetic information. To this end, quality control can be seen at every step in translation where errors might accumulate. These mechanisms share a common feature: Their activities are adapted to prevent naturally occurring mistakes. While this might seem obvious, it means that the translational machinery can be subverted with unnatural substrates. For example, while EF-Tu can discriminate against a number of naturally occurring mischarged tRNAs, it recognizes a vast range of synthetic aminoacyl moieties that are subsequently incorporated into proteins (*41*). This has already led to the rational design of in vitro translation systems able to use a significantly expanded range of amino acids and raises the tantalizing possibility that the same goal may one day be achieved in vivo (*42*). Advances in our understanding of quality control during translation will facilitate this goal. Our knowledge of the mechanisms underlying quality control has increased enormously in recent years. In some cases, such as the rejection of valine by isoleucyl-tRNA synthetase, the process of quality control is understood at near-atomic resolution and it seems likely that other steps, in particular those involving the ribosome, will also be clarified at the same resolution (*43*). For other aspects of quality control, such as nuclear aminoacylation and RNA surveillance, recent studies have advanced our knowledge from the anecdotal to the mechanistic. It now seems likely that the combined knowledge obtained from these very different experimental approaches may allow us to finally answer one of modern molecular biology's very first questions: How does the cell manage to make so few errors during protein synthesis?

References and Notes

1. F. Crick, *Nature* **227**, 561 (1970).
2. M. Yarus, *Prog. Nucleic Acid Res. Mol. Biol.* **23**, 195 (1979); C. G. Kurland, *Annu. Rev. Genet.* **26**, 29 (1992).
3. L. Pauling, in *Festschrift Arthur Stoll* (Birkhäuser Verlag, Basel, Switzerland, 1958), p. 597; R. B. Loftfield, *Biochem. J.* **89**, 82 (1963).
4. A. N. Baldwin and P. Berg, *J. Biol. Chem.* **241**, 839 (1966).
5. S. Altman and L. Kirsebom, in *The RNA World*, R. F.

Gesteland, T. R. Cech, J. F. Atkins, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, ed. 2, 1999), pp. 351–380; C. R. Trotta and J. Abelson, in *The RNA World*, R. F. Gesteland, T. R. Cech, J. F. Atkins, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, ed. 2, 1999), pp. 561–584; Z. Li and M. P. Deutscher, *Cell* **86**, 503 (1996); G. V. Börner, S. Yokobori, M. Mörl, M. Dörner, S. Pääbo, *FEBS Lett.* **409**, 320 (1997); A. P. Gerber and W. Keller, *Science* **286**, 1146 (1999), wherein an evolutionary link between eukaryotic pre-mRNA editing and tRNA modification is revealed; P. Y. Shi, N. Maizels, A. M. Weiner, *EMBO J.* **17**, 3197 (1998).
6. G. R. Björk, *Prog. Nucleic Acid Res. Mol. Biol.* **50**, 263 (1995).
7. K. Nadassy, S. J. Wodak, J. Janin, *Biochemistry* **38**, 1999 (1999).
8. S. Cusack, *Curr. Opin. Struct. Biol.* **7**, 881 (1997); J. G. Arnez and D. Moras, in *RNA Structure and Function*, R. W. Simons and M. Grunberg-Manago, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1998), pp. 465–494. A more general review of this and other examples of RNA recognition by proteins is given by T. A. Steitz, in *The RNA World*, R. F. Gesteland, T. R. Cech, J. F. Atkins, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, ed. 2, 1999), pp. 427–450.
9. E. Lund and J. E. Dahlberg, *Science* **282**, 2082 (1998); G. Simos and E. Hurt, *Curr. Biol.* **9**, R238 (1999).
10. G.-J. Arts, S. Kuersten, P. Romby, B. Ehresmann, I. W. Mattaj, *EMBO J.* **17**, 7430 (1998).
11. Reviewed in P. H. von Hippel, *Science* **281**, 660 (1998).
12. J. Zhao, L. Hyman, C. Moore, *Microbiol. Mol. Biol. Rev.* **63**, 405 (1999).
13. M. R. Culbertson, *Trends Genet.* **15**, 74 (1999); M. W. Hentze and A. E. Kulozik, *Cell* **96**, 307 (1999); P. Hillern and R. Parker, *RNA* **5**, 711 (1999).
14. M. Wilcox and M. Nirenberg, *Proc. Natl. Acad. Sci. U.S.A.* **61**, 229 (1968); M. Ibba, A. W. Curnow, D. Söll, *Trends Biochem. Sci.* **22**, 39 (1997).
15. R. Giegé, M. Sissler, C. Florentz, *Nucleic Acids Res.* **26**, 5017 (1998).
16. A. R. Fersht, in *Transfer RNA: Structure, Properties and Recognition*, P. R. Schimmel, D. Söll, J. N. Abelson, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1979), pp. 247–254.
17. R. Swanson *et al.*, *Science* **242**, 1548 (1988); J. M. Sherman, M. J. Rogers, D. Söll, *Nucleic Acids Res.* **20**, 2847 (1992).
18. G. Simos, A. Sauer, F. Fasiolo, E. C. Hurt, *Mol. Cell* **1**, 235 (1998).
19. A. Fersht, *Enzyme Structure and Mechanism* (Freeman, New York, 1985); H. Jakubowski and E. Goldman, *Microbiol. Rev.* **56**, 412 (1992); H. Jakubowski, *Biochemistry* **38**, 8088 (1999).
20. E. Schmidt and P. Schimmel, *Science* **264**, 265 (1994); O. Nureki *et al.*, *Science* **280**, 578 (1998).
21. S. P. Hale, D. S. Auld, E. Schmidt, P. Schimmel, *Science* **276**, 1250 (1997); L. F. Silvian, J. Wang, T. A. Steitz, *Science* **285**, 1074 (1999); T. K. Nomanbhoy, T. L. Hendrickson, P. Schimmel, *Mol. Cell* **4**, 519 (1999).
22. R. B. Loftfield and M. A. Vanderjagt, *Biochem. J.* **128**, 1353 (1972).
23. M. Stanzel, A. Schön, M. Sprinzl, *Eur. J. Biochem.* **219**, 435 (1994); J. Rudinger, R. Hillenbrandt, M. Sprinzl, R. Giegé, *EMBO J.* **15**, 650 (1996); H. D. Becker and D. Kern, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 12832 (1998).
24. S. Commans and A. Böck, *FEMS Microbiol. Rev.* **23**, 335 (1999). Ser-tRNA^{Sec} denotes tRNA specific for selenocysteine aminoacylated with serine.
25. U. L. RajBhandary, *J. Bacteriol.* **176**, 547 (1994); E. Schmitt, M. Panvert, S. Blanquet, Y. Mechulam, *EMBO J.* **17**, 6819 (1998). Met-tRNA$_i^{Met}$ denotes initiator methionine tRNA aminoacylated with methionine.
26. A. Schön, C. G. Kannangara, S. Gough, D. Söll, *Nature* **331**, 187 (1988); A. W. Curnow, M. Ibba, D. Söll, *Nature* **382**, 589 (1996); A. W. Curnow *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 11819 (1997). Asp-tRNA^{Asn} denotes tRNA specific for asparagine aminoacylated with aspartate, Glu-tRNA^{Gln} denotes tRNA specific for glutamine aminoacylated with glutamate.
27. R. Calendar and P. Berg, *J. Mol. Biol.* **26**, 39 (1967); J. Soutourina, P. Plateau, F. Delort, A. Peirotes, S. Blanquet, *J. Biol. Chem.* **274**, 19109 (1999); T. Yamane, D. L. Miller, J. J. Hopfield, *Biochemistry* **20**, 7059 (1981). D-Tyr-tRNA^{Tyr} and L-Tyr-tRNA^{Tyr} denote tRNA specific for tyrosine aminoacylated with D-tyrosine or L-tyrosine, respectively.
28. P. Nissen *et al.*, *Science* **270**, 1464 (1995); J. Czworkowski and P. B. Moore, *Prog. Nucleic Acid Res. Mol. Biol.* **54**, 293 (1996); B. F. C. Clark, S. Thirup, M. Kjeldgaard, J. Nyborg, *FEBS Lett.* **452**, 41 (1999).
29. Selenocysteinyl-tRNA and (formyl)methionyl-initiator tRNA do not interact with EF-Tu, but with the translation factor SELB [K. Forchhammer, W. Leinfelder, A. Böck, *Nature* **342**, 453 (1989)] and initiation factors [S. Brock, K. Szkaradkiewicz, M. Sprinzl, *Mol. Microbiol.* **29**, 409 (1998)], respectively.
30. G. R. Björk, in *tRNA: Structure, Biosynthesis, and Function*, D. Söll and U. L. RajBhandary, Eds. (American Society for Microbiology, Washington, DC, 1995), pp. 165–205; S. Yokoyama and S. Nishimura, in *tRNA: Structure, Biosynthesis, and Function*, D. Söll and U. L. RajBhandary, Eds. (American Society for Microbiology, Washington, DC, 1995), pp. 207–223.
31. P. J. Farabaugh and G. R. Björk, *EMBO J.* **18**, 1427 (1999); J. F. Atkins, A. Böck, S. Matsufuji, R. F. Gesteland, in *The RNA World*, R. F. Gesteland, T. R. Cech, J. F. Atkins, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, ed. 2, 1999), pp. 637–673.
32. M. Ehrenberg *et al.*, in *Structure, Function and Genetics of Ribosomes*, H. Boyd and G. Kramer, Eds. (Springer-Veralg, New York, 1986), pp. 573–585; R. C. Thompson, *Trends Biochem. Sci.* **13**, 91 (1988); M. Yarus and D. Smith, in *tRNA: Structure, Biosynthesis, and Function*, D. Söll and U. L. RajBhandary, Eds. (American Society for Microbiology, Washington, DC, 1995), pp. 443–469.
33. K. H. Nierhaus, *Biochemistry* **29**, 4997 (1990).
34. T. Pape, W. Wintermeyer, M. Rodnina, *EMBO J.* **18**, 3800 (1999); S. Yoshizawa, D. Fourmy, J. D. Puglisi, *Science* **285**, 1722 (1999).
35. U. L. RajBhandary and C. M. Chow, in *tRNA: Structure, Biosynthesis, and Function*, D. Söll and U. L. RajBhandary, Eds. (American Society for Microbiology, Washington, DC, 1995), pp. 511–528; D. E. Draper, in Escherichia coli *and* Salmonella typhimurium*: Cellular and Molecular Biology*, F. C. Neidhardt *et al.*, Eds. (American Society for Microbiology, Washington, DC, 1996), pp. 902–908.
36. L. Janosi, R. Ricker, A. Y. Kaji, *Biochimie* **78**, 959 (1996); Nakamura, K. Ito, *Genes Cells* **3**, 265 (1998); R. Karimi, M.Y. Pavlov, R. H. Buckingham, M. Ehrenberg, *Mol. Cell* **3**, 601 (1999).
37. M. Kozak, *Gene* **234**, 187 (1999); M. Y. Pavlov *et al.*, *J. Mol. Biol.* **284**, 579 (1998).
38. E. Schmitt, Y. Mechulam, M. Fromant, P. Plateau, S. Blanquet, *EMBO J.* **16**, 4760 (1997); V. Heurgue-Hamard *et al.*, *EMBO J.* **17**, 808 (1998); M. Fromant, P. Plateau, E. Schmitt, Y. Mechulam, S. Blanquet, *Biochemistry* **38**, 4982 (1999).
39. K. C. Keiler, P. R. H. Walter, R. T. Sauer, *Science* **271**, 990 (1996); A. W. Karzai, M. M. Susskind, R. T. Sauer, *EMBO J.* **18**, 3793 (1999); E. D. Roche and R. T. Sauer, *EMBO J.* **18**, 4579 (1999); K. P. Williams, K. A. Martindale, D. P. Bartel, *EMBO J.* **18**, 5423 (1999); J. Rudinger-Thirion, R. Giegé, B. Felden, *RNA* **5**, 989 (1999).
40. S. Gottesman, E. Roche, Y. Zhou, R. T. Sauer, *Genes Dev.* **12**, 1338 (1998); S. Wickner, M. R. Maurizi, S. Gottesman, *Science* **286**, 1888 (1999).
41. T. Hohsaka, K. Sato, M. Sisido, K. Takai, S. Yokoyama, *FEBS Lett.* **335**, 47 (1993); D. Mendel, V. W. Cornish, P. G. Schultz, *Annu. Rev. Biophys. Biomol. Struct.* **24**, 435 (1995).
42. D. R. Liu and P. G. Schultz, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 4780 (1999).
43. W. M. Clemons *et al.*, *Nature* **400**, 833 (1999); N. Ban *et al.*, *Nature* **400**, 841 (1999); J. H. Cate, M. M. Yusupov, G. Z. Yusupova, T. N. Earnest, H. F. Noller, *Science* **285**, 2095 (1999).
44. We thank W. Keller and P. Schimmel for providing preprints of unpublished manuscripts, and P. Lengyel, P. B. Moore, H. Nielsen, M. Prætorius-Ibba, and U. L. RajBhandary for their many insightful suggestions and helpful comments on the manuscript. Supported by an Investigator Fellowship from the Alfred Benzon Foundation (M.I) and by grants from the National Institute of General and Medical Sciences and the Department of Energy (D.S.).

REVIEW

Quality Control by DNA Repair

Tomas Lindahl and Richard D. Wood

Faithful maintenance of the genome is crucial to the individual and to species. DNA damage arises from both endogenous sources such as water and oxygen and exogenous sources such as sunlight and tobacco smoke. In human cells, base alterations are generally removed by excision repair pathways that counteract the mutagenic effects of DNA lesions. This serves to maintain the integrity of the genetic information, although not all of the pathways are absolutely error-free. In some cases, DNA damage is not repaired but is instead bypassed by specialized DNA polymerases.

The large genomes of mammalian cells are vulnerable to an array of DNA-damaging agents, of both endogenous and environmental origin. This situation requires constant excision and replacement of damaged nucleotide residues by DNA repair pathways to counteract potentially mutagenic and cytotoxic accidents. Consequently, DNA exhibits very slow but substantial turnover in vivo, despite its role as carrier of stable genetic information. No correction procedure is going to be absolutely exact and error-free, but repair of common DNA lesions clearly demands highly accurate performance. In practice, an altered nucleotide residue is usually replaced after the removal of a short segment of the damaged strand and a copying of the intact complementary strand. The most fre-

Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts, EN6 3LD, UK.

References and Notes

[5] **An Adenosine Deaminase That Generates Inosine at the Wobble Position of tRNAs**
Andre P. Gerber; Walter Keller
Science, New Series, Vol. 286, No. 5442. (Nov. 5, 1999), pp. 1146-1149.
Stable URL:
http://links.jstor.org/sici?sici=0036-8075%2819991105%293%3A286%3A5442%3C1146%3AAADTGI%3E2.0.CO%3B2-6

[9] **Proofreading and Aminoacylation of tRNAs before Export from the Nucleus**
Elsebet Lund; James E. Dahlberg
Science, New Series, Vol. 282, No. 5396. (Dec. 11, 1998), pp. 2082-2085.
Stable URL:
http://links.jstor.org/sici?sici=0036-8075%2819981211%293%3A282%3A5396%3C2082%3APAAOTB%3E2.0.CO%3B2-Z

[11] **An Integrated Model of the Transcription Complex in Elongation, Termination, and Editing**
Peter H. von Hippel
Science, New Series, Vol. 281, No. 5377. (Jul. 31, 1998), pp. 660-665.
Stable URL:
http://links.jstor.org/sici?sici=0036-8075%2819980731%293%3A281%3A5377%3C660%3AAIMOTT%3E2.0.CO%3B2-H

[14] **Transfer RNA as a Cofactor Coupling Amino Acid Synthesis with that of Protein**
Michael Wilcox; Marshall Nirenberg
Proceedings of the National Academy of Sciences of the United States of America, Vol. 61, No. 1. (Sep. 15, 1968), pp. 229-236.
Stable URL:
http://links.jstor.org/sici?sici=0027-8424%2819680915%2961%3A1%3C229%3ATRAACC%3E2.0.CO%3B2-2

LINKED CITATIONS

[17] **Accuracy of in vivo Aminoacylation Requires Proper Balance of tRNA and Aminoacyl-tRNA Synthetase**

Robert Swanson; Patricia Hoben; Martin Sumner-Smith; Haruki Uemura; Lynley Watson; Dieter Soll

Science, New Series, Vol. 242, No. 4885. (Dec. 16, 1988), pp. 1548-1551.

Stable URL:

http://links.jstor.org/sici?sici=0036-8075%2819881216%293%3A242%3A4885%3C1548%3AAOIVAR%3E2.0.CO%3B2-S

[20] **Mutational Isolation of a Sieve for Editing in a Transfer RNA Synthetase**

Eric Schmidt; Paul Schimmel

Science, New Series, Vol. 264, No. 5156. (Apr. 8, 1994), pp. 265-267.

Stable URL:

http://links.jstor.org/sici?sici=0036-8075%2819940408%293%3A264%3A5156%3C265%3AMIOASF%3E2.0.CO%3B2-I

[20] **Enzyme Structure with Two Catalytic Sites for Double-Sieve Selection of Substrate**

Osamu Nureki; Dmitry G. Vassylyev; Masaru Tateno; Atsushi Shimada; Takashi Nakama; Shuya Fukai; Mitiko Konno; Tamara L. Hendrickson; Paul Schimmel; Shigeyuki Yokoyama

Science, New Series, Vol. 280, No. 5363. (Apr. 24, 1998), pp. 578-582.

Stable URL:

http://links.jstor.org/sici?sici=0036-8075%2819980424%293%3A280%3A5363%3C578%3AESWTCS%3E2.0.CO%3B2-G

[21] **Discrete Determinants in Transfer RNA for Editing and Aminoacylation**

Stephen P. Hale; Douglas S. Auld; Eric Schmidt; Paul Schimmel

Science, New Series, Vol. 276, No. 5316. (May 23, 1997), pp. 1250-1252.

Stable URL:

http://links.jstor.org/sici?sici=0036-8075%2819970523%293%3A276%3A5316%3C1250%3ADDITRF%3E2.0.CO%3B2-K

[21] **Insights into Editing from an Ile-tRNA Synthetase Structure with tRNA ^{Ile} and Mupirocin**

Laura F. Silvian; Jimin Wang; Thomas A. Steitz

Science, New Series, Vol. 285, No. 5430. (Aug. 13, 1999), pp. 1074-1077.

Stable URL:

http://links.jstor.org/sici?sici=0036-8075%2819990813%293%3A285%3A5430%3C1074%3AIIEFAI%3E2.0.CO%3B2-I

LINKED CITATIONS

[23] **Thermus thermophilus: A Link in Evolution of the tRNA-Dependent Amino Acid Amidation Pathways**

Hubert Dominique Becker; Daniel Kern

Proceedings of the National Academy of Sciences of the United States of America, Vol. 95, No. 22. (Oct. 27, 1998), pp. 12832-12837.

Stable URL:

http://links.jstor.org/sici?sici=0027-8424%2819981027%2995%3A22%3C12832%3ATTALIE%3E2.0.CO%3B2-J

[26] **Glu-tRNA ^{Gln} Amidotransferase: A Novel Heterotrimeric Enzyme Required for Correct Decoding of Glutamine Codons during Translation**

Alan W. Curnow; Kwang-Won Hong; Robert Yuan; Sung-Il Kim; Orlando Martins; Wade Winkler; Tina M. Henkin; Dieter Soll

Proceedings of the National Academy of Sciences of the United States of America, Vol. 94, No. 22. (Oct. 28, 1997), pp. 11819-11826.

Stable URL:

http://links.jstor.org/sici?sici=0027-8424%2819971028%2994%3A22%3C11819%3AGAANHE%3E2.0.CO%3B2-F

[28] **Crystal Structure of the Ternary Complex of Phe-tRNA ^{Phe} , EF-Tu, and a GTP Analog**

Poul Nissen; Morten Kjeldgaard; Soren Thirup; Galina Polekhina; Ludmila Reshetnikova; Brian F. C. Clark; Jens Nyborg

Science, New Series, Vol. 270, No. 5241. (Dec. 1, 1995), pp. 1464-1472.

Stable URL:

http://links.jstor.org/sici?sici=0036-8075%2819951201%293%3A270%3A5241%3C1464%3ACSOTTC%3E2.0.CO%3B2-%23

[34] **Recognition of the Codon-Anticodon Helix by Ribosomal RNA**

Satoko Yoshizawa; Dominique Fourmy; Joseph D. Puglisi

Science, New Series, Vol. 285, No. 5434. (Sep. 10, 1999), pp. 1722-1725.

Stable URL:

http://links.jstor.org/sici?sici=0036-8075%2819990910%293%3A285%3A5434%3C1722%3AROTCHB%3E2.0.CO%3B2-%23

[39] **Role of a Peptide Tagging System in Degradation of Proteins Synthesized from Damaged Messenger RNA**

Kenneth C. Keiler; Patrick R. H. Waller; Robert T. Sauer

Science, New Series, Vol. 271, No. 5251. (Feb. 16, 1996), pp. 990-993.

Stable URL:

http://links.jstor.org/sici?sici=0036-8075%2819960216%293%3A271%3A5251%3C990%3AROAPTS%3E2.0.CO%3B2-4

LINKED CITATIONS

[40] **Posttranslational Quality Control: Folding, Refolding, and Degrading Proteins**
Sue Wickner; Michael R. Maurizi; Susan Gottesman
Science, New Series, Vol. 286, No. 5446. (Dec. 3, 1999), pp. 1888-1893.
Stable URL:
http://links.jstor.org/sici?sici=0036-8075%2819991203%293%3A286%3A5446%3C1888%3APQCFRA%3E2.0.CO%3B2-4

[42] **Progress Toward the Evolution of an Organism with an Expanded Genetic Code**
David R. Liu; Peter G. Schultz
Proceedings of the National Academy of Sciences of the United States of America, Vol. 96, No. 9. (Apr. 27, 1999), pp. 4780-4785.
Stable URL:
http://links.jstor.org/sici?sici=0027-8424%2819990427%2996%3A9%3C4780%3APTTEOA%3E2.0.CO%3B2-P

[43] **X-Ray Crystal Structures of 70S Ribosome Functional Complexes**
Jamie H. Cate; Marat M. Yusupov; Gulnara Zh. Yusupova; Thomas N. Earnest; Harry F. Noller
Science, New Series, Vol. 285, No. 5436. (Sep. 24, 1999), pp. 2095-2104.
Stable URL:
http://links.jstor.org/sici?sici=0036-8075%2819990924%293%3A285%3A5436%3C2095%3AXCSO7R%3E2.0.CO%3B2-Y