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Review

The E-site story: the importance of maintaining two tRNAs on the ribosome during protein synthesis

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Abstract. In the sixties James Watson suggested a two-site model for the ribosome comprising the P site for the peptidyl transfer RNA (tRNA) before peptide-bond formation and the A site, where decoding takes place according to the codon exposed there. In the eighties a third tRNA binding site was detected, the E site, which was specific for deacylated tRNA and turned out to be a universal feature of ribosomes. However, despite having three tRNA binding sites, only two tRNAs occupy the ribosome at a time during protein synthesis: at the A and P sites before translocation (PRE state) and at the P and E sites after translocation (POST state). The importance of having two tRNAs in the POST state has been revealed during the last 25 years, showing that the E site contributes two fundamental features: (i) the fact that incorporation of a wrong amino acid is not harmful for the cell (only 1 in about 400 misincorporations destroys the function of a protein) stems from the presence of an E-tRNA; (ii) maintenance of the reading frame is one of the most remarkable achievements of the ribosome, essential for faithful translation of the genetic information. The presence of the POST state E-tRNA prevents loss of the reading frame.

Keywords. Protein synthesis, tRNA binding sites, A site, E site, accuracy of protein synthesis, frameshift.

Introduction

In 1965, James Watson published the book ‘Molecular Biology of the Gene’, which inspired a whole generation of scientists working in the field of molecular biology and genetics [1]. In the chapter on protein synthesis he outlined ribosomal activity in the frame of a model containing two binding sites for transfer RNAs (tRNAs), the principles of which he had published 1 year prior in a obscure journal that was not so easily accessible to the wider scientific community (Fig. 1; [2]). The three basic steps of peptide-chain elongation are depicted in this illustration:

1) Occupation of the decoding site (today called A site) by an aminoacyl (aa)-tRNA occurs according to the codon of the messenger (mRNA) exposed at this site. Adjacent to the A site is a second tRNA binding site, the P site, where the peptidyl-tRNA carrying the synthesized polypeptide (before peptide-bond formation) is located.
2) Peptide-bond formation occurs: the peptidyl residue is cleaved off and transferred to the aa-tRNA with the result that now the peptidyl-tRNA is located at the A site, extended by one amino acid.
3) A translocation takes place on the ribosome, where the tRNA•mRNA complex moves by a codon length. In doing so, the peptidyl-tRNA enters the P site, and the uncharged tRNA exits the ribosome leaving only one tRNA behind. Translocation also brings a new codon into the A site, and with re-selection of an aa-tRNA, the ribosome enters into the next elongation cycle.

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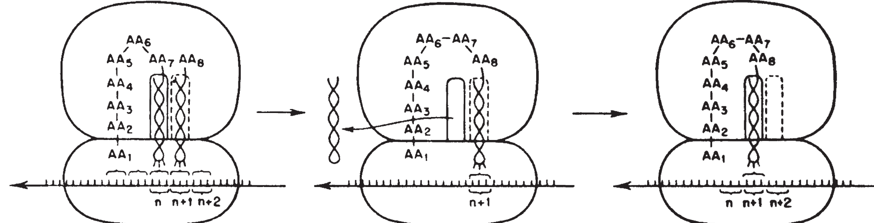

Figure 1. Two-site model for the elongation cycle with A-site occupation by aa-tRNA (left), peptide-bond formation and release of the deacylated tRNA (middle) and finally translocation of the peptidyl-tRNA (right). Taken from [2].

The two-site model with a donor site (P site) and an acceptor site (A site) was not only satisfying for theoretical reasons, but also agreed with the operational definition of these sites based on the so-called puromycin reaction [3]. Puromycin is an antibiotic that mimics the 3′ end of an aa-tRNA, namely the ultimate adenosine A76 (here a dimethyl A), attached to a methylated tyrosyl residue. The latter moiety is linked to the ribose of the adenosine, not via the canonical ester bond, but by an amide bond. This is an important feature of puromycin since the amide linkage cannot be cleaved by the peptidyl-transferase (PTF) center of the ribosome (Fig. 2a). Puromycin binds to the A-site region of the PTF center and will receive the peptidyl-residue from a peptidyl-tRNA located at the P site analogous to a canonical peptidyl transfer (compare Fig. 2b and c). However, since puromycin represents only a small part of the acceptor arm of a tRNA, it is not stably bound to the ribosome and thus falls off as peptidyl-puromycin and stops protein synthesis.

Even if the ribosome should by chance bind a peptidyl-puromycin to a free P site, the peptidyl residue will not be transferred to an incoming aa-tRNA, since the amide, rather than ester, linkage between the ribose and the tyrosyl moiety in puromycin cannot be broken. The end result is that a puromycin reaction will definitely lead to an abrupt halt in protein synthesis. With respect to the operational definitions of tRNA binding sites, a positive puromycin reaction indicates that the P site of the ribosome is occupied by a peptidyl-tRNA and that the A site is free. However, if the peptidyl-tRNA is located at the A site, then puromycin cannot bind and thus the puromycin reaction is negative. It is obvious that a puromycin reaction traces exclusively acylated (charged) tRNAs and cannot detect the presence of deacylated (uncharged) tRNAs.

This concept of the operational definition of A and P sites did not change when a puromycin reaction was also detected from a peptidyl-tRNA located at the A site [4], since this reaction is about 200–300 times slower then the puromycin reaction with a peptidyl-tRNA at the P site under near *in vivo* buffer conditions [C. M. T. Spahn and K. H. Nierhaus, unpublished]. However, the A-site reaction was taken as strong evidence for the existence of a 'hybrid site' A/P (or P/E), where the tRNAs are still located at the A site on the 30S subunit but covered already the P site on the 50S subunit. This state seems to be a transient tRNA position during the translocation reaction, but lowly populated in the pre-translocational (PRE) state (see below).

An early saturation experiment with [^{32}P]labeled deacylated tRNAs to polysomes did not yield unequivocal results, being compatible with two or three tRNA binding sites [5]. The authors suggested that if there are indeed three sites, then one should be specific for deacylated tRNA and represent a position where the tRNA resides before leaving the ribosome. On this basis they proposed the name 'E site' for this putative site, where 'E' is for exit.

Solid evidence for a third tRNA binding site was only presented 15 years later [6, 7] and confirmed by others [8, 9]. This evidence suggested that the two-site model for translocation was too simplistic and that tRNAs pass through three binding sites on the ribosome during translation. Subsequently, the E site was also observed in ribosomes from archaea [10], lower (yeast; [11]) and higher eukaryotes (rabbit; [12]). The universal presence of this site implied an important function.

The next sections will consider the structural and biochemical features of the E site, including the involvement of the E site in translocation, followed by a description of two important functions of the E site, namely (i) reducing the effects of misincorporation and (ii) maintenance of the translational reading frame.

The most frequent errors in ribosomes result from mistakes in selecting the aa-tRNA at the A site and therefore misincorporation of the incorrect aminoacyl residue. Misincorporation into the nascent peptide chain occurs once in about 3000 amino acid incorporations [13], but such events are not harmful to the cell since only one in 400 misincorporations destroys the structure and/or function of the protein [14]. The fact is that only misincorporation of amino acids that are benign for the cell is attributable to the presence of an E-site tRNA (E-tRNA).

Loss of the reading frame is a disaster, since it means immediate loss of the genetic information for the corresponding ribosome. An essential role of the E-tRNA in maintaining the reading frame has been discovered by analyzing the frameshifting mechanism within the gene encoding the termination release factor RF2 [15].

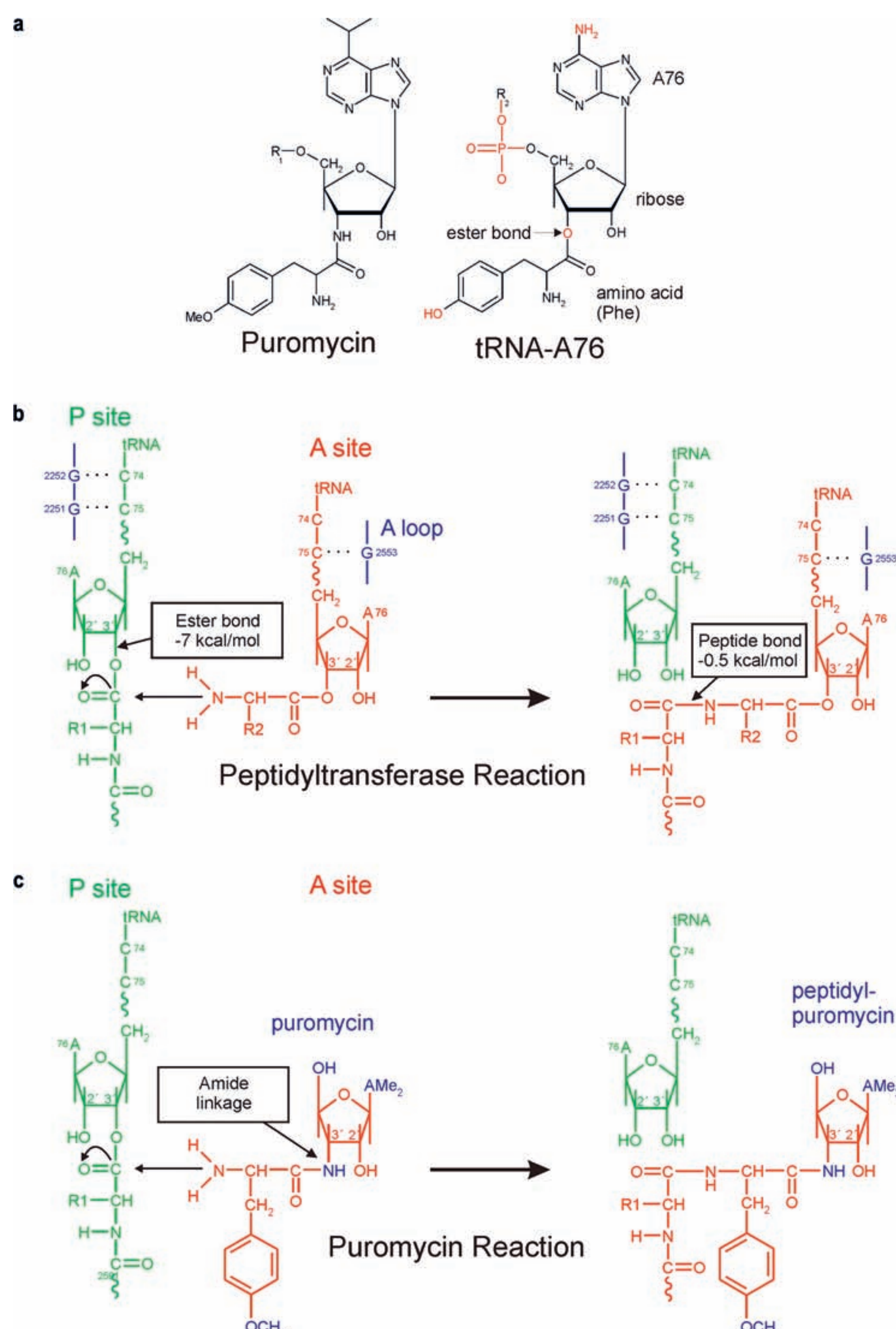

Figure 2. The puromycin reaction. (*a*) Comparison of puromycin (left) and A76 region of a Phe-tRNA (right) with the differences highlighted (red) on the tRNA (right). (*b*) The peptidyltransferase reaction with the A- and P-site tRNA ligands shown in red and green, respectively. The A- and P-loop nucleotides of the 23S rRNA interact with the acceptor stem of the A- and P-tRNA ligands, respectively. (*c*) The puromycin reaction, where puromycin (A-site ligand in red, with differences to tRNA ligand shown in blue) accepts the peptide moiety to become peptidyl-puromycin.

Visualizing the E site

Direct visualization of the E-site tRNA on the ribosome was first seen in the crystal structure of a complex of 70S ribosomes bearing tRNAs at A, P and E sites from the bacteria *Thermus thermophilus* [16] as well as in medium-resolution cryo-electron-microscopic (cryo-EM) and single-particle reconstructions of post-translocational (POST)-state ribosomes with tRNAs at P and E sites from *Escherichia coli* (see, for example, [17]). Subsequently,

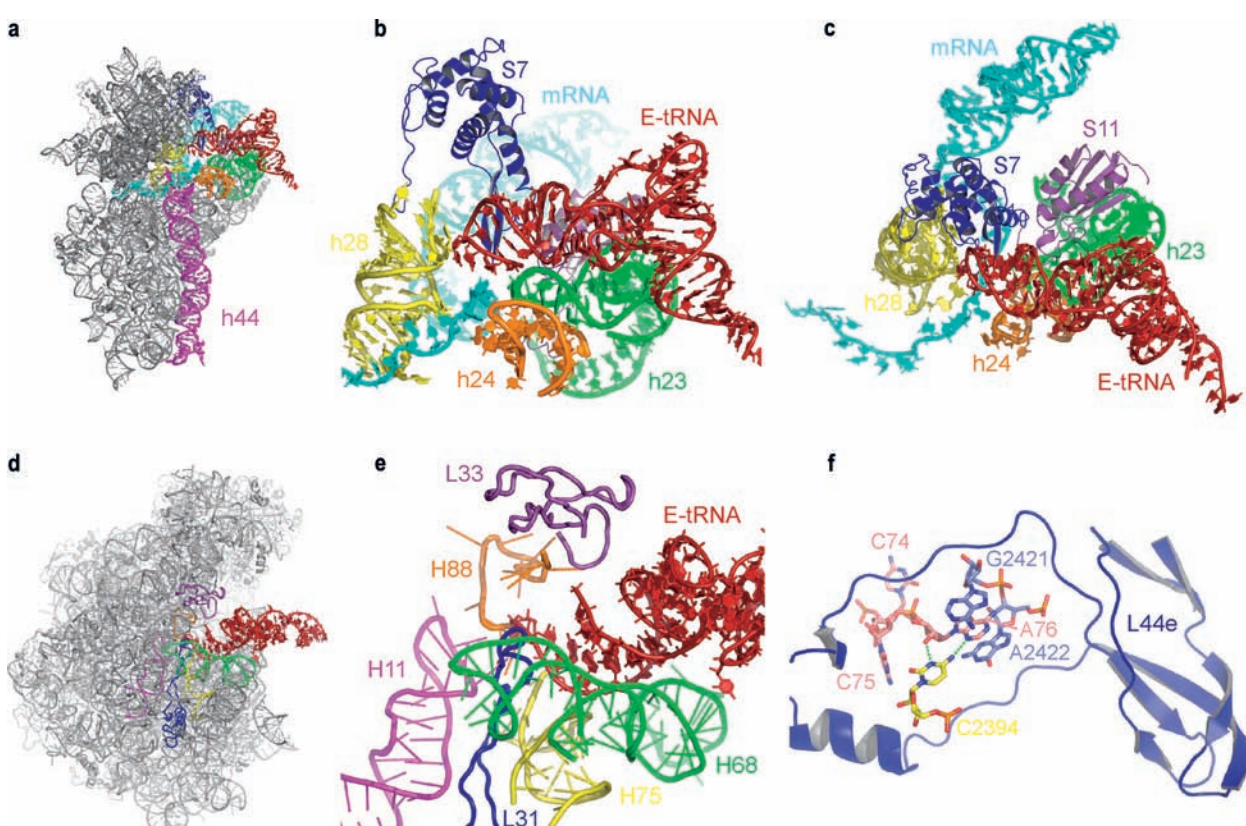

Figure 3. Interaction between the ribosomal subunits and the E-tRNA. (*a*) Intersubunit overview of the 30S subunit, with the components of the 30S subunit highlighted (see labels in *b* and *c*) that interact with the E-tRNA (red). Helix 44 is also coloured (magenta) for reference, whereas all other rRNA and ribosomal proteins are coloured grey. (*b*) Closeup of *a* showing that the anticodon stem-loop of E-tRNA (red) interacts with 16S rRNA components of h23 (green), h24 (orange) and h28 (yellow) and with ribosomal protein S7 (blue). The path of the mRNA is shown using the thrS-mRNA (cyan). (*c*) Close up, bird's-eye view of *a*, showing interaction of ribosomal proteins S7 (blue) and S11 (purple) with ASL of E-tRNA. Panels *a–c* are a composite of E-tRNA [18], mRNA [57] and the 30S subunit [65] from PDB1GIX/Y, PDB1YL3/4 and PDB1IBL, respectively. (*d*), 50S subunit viewed from L1 stalk side, with components of the ribosome that interact with the E-tRNA highlighted (see labels in *e*). (*e*) Closeup of *d* showing that the acceptor stem of E-tRNA (red) interacts with 23S rRNA components of H11 (magenta), H75 (yellow), H68 (green) and H88 (orange) and with ribosomal proteins L31 (blue) and L33 (purple). Panels *d* and *e* are a composite of E-tRNA (PDB1GIX/Y; [18]) with the 50S subunit [66]. (*f*) Interaction of CCA (pink) with the *H. marismortui* 50S subunit 23S rRNA and ribosomal protein L44e (blue). Note that the C74, C75 and A76 are splayed. The terminal A76 is inserted and stacked between G2421 and A2422 (pale blue) and forms hydrogen bonds (green dashes) with the highly conserved C2394 (yellow). All numbering is *E. coli*, and this figure was made from PDB1QVG [21]. All figures were made with PyMol (www.pymol.org).

higher-resolution crystal structures have provided the most accurate description of the tRNA binding sites so far [18]. Unlike the A- and P site tRNA binding positions, which are predominantly composed of ribosomal RNA (rRNA), the E-site tRNA binding position is composed of both rRNA and ribosomal proteins. On the small ribosomal subunit (30S), the anticodon-stem loop (ASL) of the E-tRNA is positioned between the head and platform of the 30S (Fig. 3a), where it interacts with nucleotides within helices 28 (h28), h23 (690 loop), h24 (790 loop) of the 16S rRNA as well as ribosomal proteins S7 and S11 (Fig. 3b, c). Specifically, the ASL of the E-tRNA is contacted on one side by the β-hairpin of S7 and the α-helix (h6) of S11, on another by the loop regions of h23 (693) and h24 (788–789) and the tip of the ASL contacts h28 (1382) [18]. This placement is consistent with the cross-linking of the E-tRNA to ribosomal protein S7 and the 30 nucleotides at the 3′ end of the 16S rRNA [19].

On the bacterial large 50S subunit, the acceptor stem inserts into a pocket formed from 23S rRNA nucleotides, namely helices 11 (H11), H68, H74–75 and H88, and is approached on each side by ribosomal proteins L31 and L33 (Fig. 3d, e). Ribosomal protein L33 has been cross-linked to tRNA probes modified at or near the 3′ end [19]. A large proportion of this contact is formed through minor-groove interactions of the acceptor stem with H68, and the 3′ end interacts with the stem of H88 (Fig. 3d, e), in agreement with hydroxyl radical cleavage of H88 and surrounding rRNA by reactive moieties tethered to the 3′ end of E-site-bound tRNA [20]. The structural detail of the interaction of the acceptor stem, in particular, the terminal CCA-3′ end of the E-tRNA with the ribosome

were revealed by crystallography. High-resolution crystal structures of RNA oligonucleotides mimicking either the deacylated acceptor and TΨC arm, or the CCA end, of a tRNA in complex with the *Haloarcula marismortui* 50S subunit were determined [21]. Interestingly, unlike in the A and P sites, where the bases of C74 and C75 stack upon each other, in the E site, the bases of the CCA end are splayed apart. The terminal A76 inserts and stacks between two bases, G2421 and A2422, and forms hydrogen bonds from the N3 and the 2′ OH with N4 and N3 of the highly conserved C2394 of the 23S rRNA (Fig. 3f). These interactions are consistent with the protection of C2394 from chemical probing by binding of deacylated tRNA to the ribosome [22] and mutations at this site that destabilize E-tRNA binding [23]. Unlike bacteria, eukaryotes and archaea (such as *H. marismortui*) contain a single ribosomal protein L44e (and L15e) within the E site, instead of bacterial proteins L31 and L33. In the aforementioned crystal structure, the CCA end of the E-tRNA inserts through a loop in L44e, whereas on a bacterial ribosome an extension of L31 approaches this region. The divergence of the ribosomal proteins and conservation of the rRNA within the E site suggests that the common ancestor to bacterial and archaeal/eukaryotic ribosomes may have had an 'all-RNA' E site, at least on the 50S subunit [21].

An additional interaction observed between the ribosome and the E-tRNA, by cryo-EM and crystallography studies, is that of the L1 stalk with the elbow of the tRNA. In the 70S•$tRNA_3$ crystal structure, the L1 stalk is in a closed form [18], and therefore would block tRNA release from the E site, whereas in the *Deinococcus radiodurans* 50S structure the L1 stalk is in a more open conformation [24], which would allow release of the E-tRNA. Thus, the movement of the L1 stalk may control removal of deacylated tRNA from the E site of the ribosome. This mechanism may be universal since in yeast there is a huge 70 Å difference in the position of the L1 stalk when comparing a P-tRNA-bound 80S ribosome (pseudo-PRE, state) with a stalled translating ribosome (artificially induced POST state) [25, 26]. Furthermore, yeast have an additional elongation factor eEF3 that hydrolyzes ATP to open the L1 stalk, enabling release of the E-tRNA upon binding of the ternary complex to the A site [11].

Features of the E site

Three features characterize the E site: (i) Deacylated tRNA present at the E site (E-tRNA) is bound in a stable fashion. (ii) The E-tRNA is connected to the mRNA via

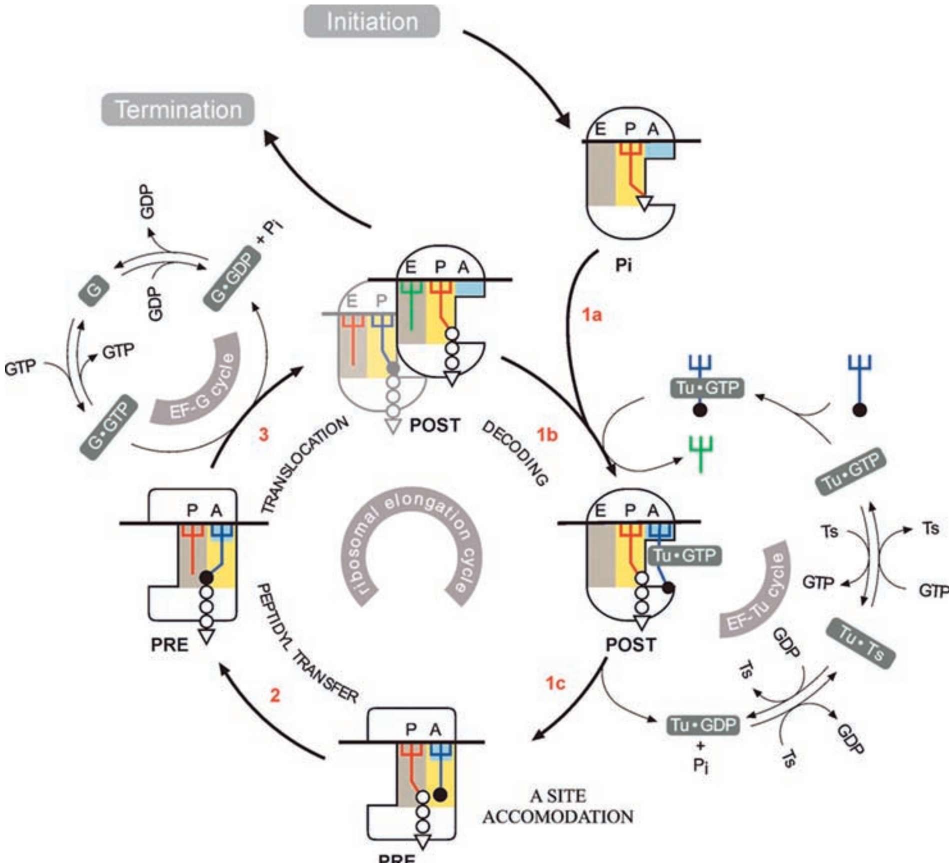

Figure 4. The elongation cycle in the frame of the allosteric three-site model. Modified from [67, 68].

codon-anticodon interaction. (iii) The E site is linked to the A site in a reciprocal manner (for review see [27]).

The fact that the E-tRNA is bound to this site in a stable fashion has been shown both *in vivo* and *in vitro*. The *in vivo* evidence is seen in the following observation: Most of the ribosomes (50–100%) are in the POST state, i.e. after translocation the tRNAs from the A and P sites to the P and E sites (see Fig. 4). An analysis of formed polysomes *in vivo* revealed that at least 70% of the POST ribosomes carry an E-tRNA after an isolation procedure that lasts 15–20 h [28]. Similarly, POST ribosomes prepared *in vitro* can be pelleted through a sucrose cushion for 12 h without losing significant amounts of E-tRNA [29]. The important factor here is the use of *in vivo* near buffer conditions with low Mg^{2+} (4.5–6 mM) and the presence of polyamines, particularly spermidine (2 mM). In contrast, the E-tRNA can be easily lost with half-lives of seconds up to 3 min in standard buffers with 10 mM Mg^{2+} and without polyamines (Figs. 1 and 2 in [30], but see also [31]).

Codon-anticodon interaction of the E-tRNA has been shown in two ways. [^{32}P]-labeled E-tRNA could only be efficiently chased from a programmed ribosome when a large excess of cognate deacylated tRNA was added, namely tRNA with an anticodon complementary to the codon at the E site, but not with non-cognate tRNA [11, 32]. Further evidence was provided by both cryo-EM analysis of POST-state ribosomes and ribosome crystals containing three tRNAs [17, 18], revealing that the distance between tRNA nucleotides 37 (3′-adjacent to the anticodon) of A and P-tRNAs is 23 Å. The corresponding distance of P- and E-tRNAs is even shorter (21 Å), suggesting that the tRNAs at the P and E sites can undergo simultaneous codon-anticodon interaction as they do at A and P sites (Fig. 5a, b). Although the E-tRNA in the ribosome crystal was non-cognate, namely the anticodon was not complementary to the respective codon, the middle bases of both codon (mRNA) and anticodon (tRNA) formed a base pair. This indicates that cognate codon and anticodon pairs present at the E site during protein synthesis would – for energetic reasons – form three base pairs.

The feature with the most significant consequence for translation is the presence of a reciprocal linkage between A and E site (allosteric three-site model of the elongating ribosome [33, 34]; for review see [35]). This linkage dictates that occupation of the A site with an aa-tRNA reduces the E-site affinity for tRNA, with the result that the E-tRNA is released from the ribosome. The opposite is also true, namely an occupation of the E site induces a low-affinity A site for binding an aa-tRNA. Recently, the precise step of A-site occupation triggering the release of the E-tRNA has been identified. A-site occupation occurs in two steps: First, decoding takes place, where the ternary complex aa-tRNA•EF-Tu•GTP predominantly establishes contacts with the codon of the mRNA. Fol-

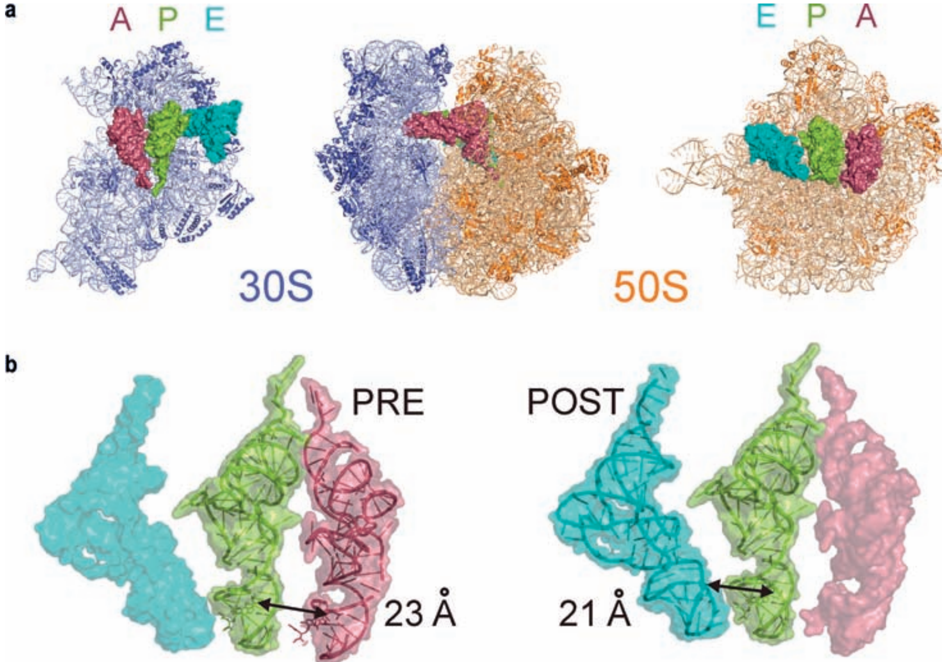

Figure 5. The tRNA binding sites on the ribosome. (*a*), The bacterial ribosome is composed of two subunits, a small 30S (blue) and large 50S (orange) subunit. Each subunit is composed of RNA and protein coloured light and dark, respectively. The 70S ribosome has three tRNA binding sites, termed the A (for aa-tRNA, shown in magenta), P (for peptidyl-tRNA before peptide bond formation, shown in olive) and E (for exit site, which binds deacylated tRNA, shown in cyan). (*b*), During the elongation cycle (see Fig. 3), the ribosome oscillates between PRE and POST states. The distances between ribose (ring oxygen) of residue 37 of A- and P-tRNA and P- and E-tRNA, respectively, were measured from the crystal structure [18].

lowing the decoding step, GTP is cleaved by EF-Tu, EF-Tu•GDP leaves the ribosome and the aa-tRNA moves into the A site (accommodation). The release of the E-tRNA is triggered after the decoding step, but before EF-Tu-dependent GTP cleavage and accommodation of the aa-tRNA into the A site [36].

Another consequence of the reciprocal linkage is the fact that during the elongation cycle there are always two, but never three, tRNAs found on the ribosome, either in the PRE state, with tRNAs located at the A and P sites, or in the POST state, with P and E sites occupied [28]. Only during the initial decoding process, where the aa-tRNA is still bound to EF-Tu at the A site, are three tRNAs found on the ribosome. The three tRNAs seen in the crystals of *Thermus thermophilus* ribosomes [18] are probably the result of a superposition of PRE and POST states in the crystal, explaining the relatively moderate resolution of 5–7 Å.

The PRE and POST states are the two main states of the elongation phase and are separated by high activation energy barriers of about 120 kJ/mol [37]. One important function of the elongation factors is to reduce the energy barriers, thus allowing the transition from one state to the other. In the absence of elongation factors and GTP, the ribosomes are frozen in either of the two main elongating states. A welcome consequence is that both states can be prepared with good homogeneity *in vitro*, without fear that a purified PRE state will re-establish equilibrium between PRE and POST states. Such homogeneity is a prerequisite for structural studies such as cryo-EM and X-ray crystallography.

One remark on the G-protein features of the elongation factors EF-Tu and EF-G: Reduction of energy barriers is also a standard function of an enzyme. An enzyme accelerates its respective reaction rate by factors often in the range of 10^8–10^{11}; however the reaction is catalyzed until equilibrium is reached. Therefore, an enzyme can catalyze a reaction in both the forward and backward directions depending on the starting conditions in the test tube. This is in striking contrast to G-proteins, such as elongation factors, since these factors not only reduce the activation energy barrier but also determine the direction of reaction, driving the reaction in one direction to completion, namely into the PRE or POST states. For this reason, we have two universal elongation factors, one for the transition POST to PRE (EF-Tu in bacteria or EF1 in archaea and eukaryotes) and another for the PRE to POST transition (EF-G or EF2, respectively). Determining the direction of reaction is a specialty of G-proteins, and they perform this function by adopting two different conformers depending on whether they bind GTP or GDP [38]. In the GTP form, a G-protein adopts an 'on' conformation, in which it binds the substrate and triggers a reaction (e.g. EF-Tu in the ternary complex catalyzes the transition of the POST to the PRE state). In this reaction the product (for EF-Tu the PRE state) probably binds even more strongly to the ternary complex, and this gain in energy might determine the direction of reaction. At the same time, the PRE state sends a signal to aa-tRNA•EF-Tu•GTP triggering GTP hydrolysis, and EF-Tu adopts the GDP conformer, loses its affinity for the PRE state and dissociates as EF-Tu•GDP from the ribosome, whereas the liberated aa-tRNA accommodates into the A site and now, with its aminoacyl residue, occupies the PTF center.

Figure 4 describes the elongation phase of protein synthesis in the frame of the allosteric three-site model [33, 34], highlighting the steps catalyzed by the elongation factors EF-Tu and EF-G. The PRE and POST states are shown with rectangular and round ribosomes, respectively, to indicate the two main states of elongation, and the gap in either the A or E site indicates the low-affinity state.

The sequential movement of tRNAs through the ribosome

The E site is specific for deacylated tRNA [7]. Modification of the 3′ end of a tRNA decreases or even abolishes the affinity of tRNA for the E site, whereas binding to A and P sites remains unaffected [39]. Biochemical data first suggested a direct contact of A76 and C2394 at the E site [40], which was borne out by crystal structures of ribosomes containing tRNA fragments with an intact 3′ end [21]. As mentioned, the terminal A76 is stacked between G2421 and A2422, and shows H bonds from the base and 2′OH to C2394, shielding this residue and explaining its specificity for deacylated tRNA (Fig. 3f). An intriguing observation is that this specific interaction between the 3′ end of a deacylated tRNA with the E site is required for an efficient translocation reaction [41]. In particular, the 2′ OH group of A76, as well as position 71 of a P-tRNA, were demonstrated to be essential for translocation [42]. Various cryo-EM structures of PRE and POST ribosomes have resolved the tRNA movement from the P to the E site and the participation of its 3′ end. The ribosomal subunits perform a ratchet-like movement, where the 30S subunit turns counterclockwise ~6° relative to the 50S subunit on EF-G•GTP binding, as seen from the cytoplasmic side of the small subunit, and returns upon EF-G-dependent GTP cleavage [43] (see Fig. 6a–c). This observation has led to the hypothesis that tRNAs move sequentially from A and P sites to P and E sites, respectively. The binding of EF-G•GTP to a PRE state moves the tRNAs from A and P sites into the hybrid sites A/P and P/E, and translocation from A/P and P/E hybrid sites to P and E sites is completed upon EF-G-dependent GTP cleavage (Fig. 6b, c).

An alternative view was forwarded with the hybrid-site model [44], according to which the tRNAs switch into hy-

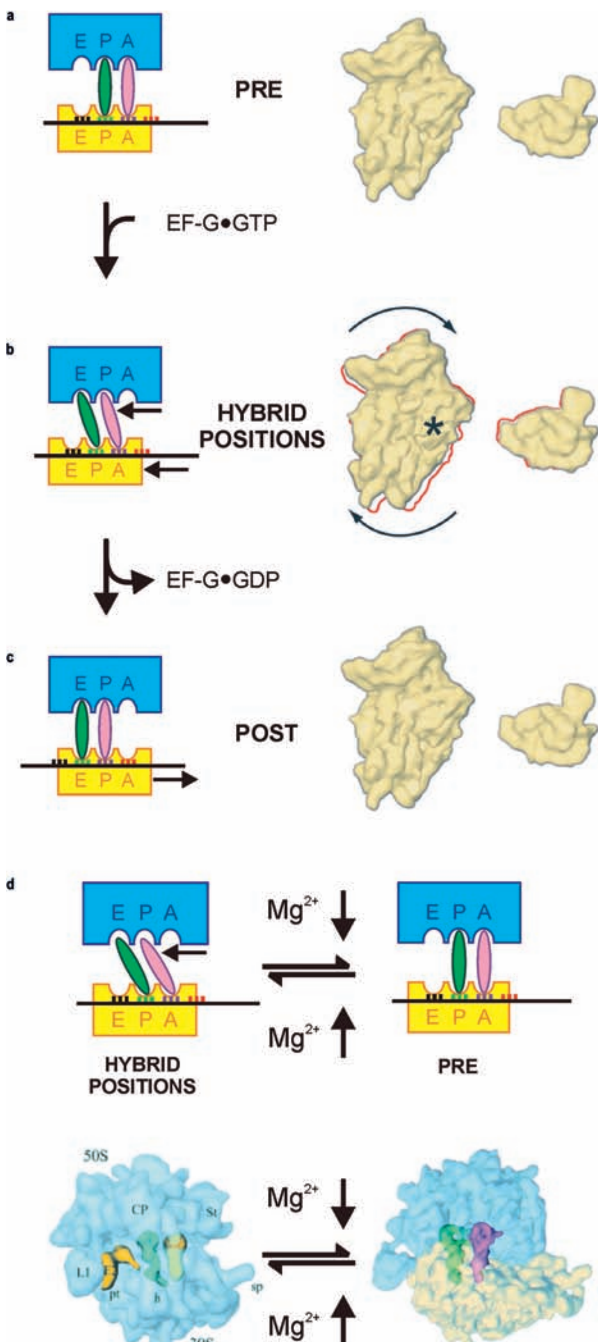

Figure 6. Translocation of tRNAs through the ribosome. (*a*) Left, schematic representation of the large 50S (blue) and small 30S (yellow) subunits as a PRE-state 70S ribosome with tRNAs bound at A (pink) and P sites (green). Right, cryo-EM reconstruction of small subunit (yellow) viewed from interface and top side. (*b*) Left, binding of EF-G•GTP leads to ratchet motion of 30S subunit relative to 50S (indicated by arrow) and movement of tRNAs in hybrid states. Right, ratchet movement of small subunit as seen by cryo-EM upon binding elongation factor [69]. (*c*) Left, hydrolysis of GTP to GDP leads to ratchet back and translocation of tRNAs to form the POST state. The schematic was inspired by [43] and the cryo-EM images were modified from [69]. (*d*) Equilibrium between PRE (right) and HYBRID states (left) is modulated by magnesium concentration (Mg^{2+}), such that high Mg^{2+} leads to formation of hybrid sites, and low Mg^{2+} leads to the canonical PRE state. Respective cryo-EM images of PRE and HYBRID states taken from [17].

brid states just after peptide-bond formation, but before EF-G interacts with the ribosome to trigger the translocation reaction. Recently the Green lab published supportive evidence demonstrating that a significant population of ribosomes after peptide bond formation adopt a hybrid state that is a favourable substrate for subsequent EF-G-dependent translocation [45, 46]. An important consideration with regard to these analyses is that the PRE states and the subsequent translocations were performed in a buffer containing 15 and 20 mM Mg^{2+}, conditions far from *in vivo* (4–5 mM Mg^{2+}) that only allow slow and error-prone protein synthesis and are characterized by weak E-site occupation (see above). Indeed, even at 10 mM Mg^{2+}, deacylated tRNAs at the P site adopt hybrid P/E positions, in striking contrast to canonical P-site positions observed by cryo-EM under *in vivo* near conditions (low Mg^{2+}, polyamines, [47]; see Fig. 5d).

Applying near-*in vivo* conditions, footprinting studies have identified a significant, albeit weak E-site signal in a PRE-state ribosome, indicative of hybrid-site formation [23], whereas cryo-EM analysis with preparations from two different groups have identified tRNAs in the canonical A and P sites [17, 48]. This apparent discrepancy can be reconciled by taking into account that the footprinting technique can identify even minor populations of functional complexes, whereas cryo-EM focuses on the major fraction of a mixture of functional populations. An equilibrium between a PRE state with tRNA in the canonical A and P sites and a PRE state with hybrid sites has been postulated (measurements at 15 mM Mg^{2+}; [49]). Then at least under low Mg^{2+} conditions the equilibrium lies on the side with the canonical A and P sites (Fig. 6d). Only during translocation do tRNAs transiently pass through hybrid positions to form POST states containing canonical P and E tRNAs.

Misincorporations are not harmful for the cell, due to the E site

As mentioned in the introduction, misincorporations occur with a frequency of 1 in about 3000 amino acid incorporations [13], and only one misincorporation in 400 is actually fatal for the structure and/or function of a protein. The reason for this is that when a near-cognate aa-tRNA is selected, the amino acid that is incorporated is either identical to the cognate or at least the chemical characteristic of the amino acid is similar to the correct one. This arises because a near-cognate tRNA has an anticodon that is similar to the correct or cognate tRNA, and the arrangement of the genetic code is such that near-cognate and cognate codons correlate with the same or similar amino acids. Indeed, a selection error leading to the incorporation of the identical amino acid occurs with all amino acids coded for by a ‘family box’ of four codons.

For example, the four GCN codons that code for Ala are decoded by two different 'isoaccepting' tRNAs: $tRNA^{Ala1}$ recognizes the codons GCA, GCG and GCU, whereas $tRNA^{Ala2}$ is specific for GCC [50]. For each cognate aa-tRNA, between two and four near-cognate aa-tRNAs can exist. This still leaves unanswered the question of what mechanism prevents the acceptance of one or the other of the 90% of non-cognate aa-tRNAs that will have a more fatal consequence for the structure/function of a protein if misincorporated.

The answer to this problem is related to the aforementioned reciprocal relationship between A and E sites [11, 34]. A conspicuous consequence of this reciprocal relationship is a striking dependence of the activation energy on the presence of an E-tRNA for A-site occupation. With an occupied E site, the activation energy was found to be three times larger than that determined with a free E site, namely 120 kJ/mol versus 40 kJ/mol (AcPhe-tRNA binding at 3 mM Mg^{2+} in the presence of 2 mM spermidine; [37]).

If we assume for a moment that the A site is always in a high-affinity state for a ternary complex aa-tRNA•EF-Tu•GTP independent of the E-site state, then the binding energy of this A-site ligand would be strongly dominated by interactions outside of the anticodon and therefore cannot add to the discrimination potential. Note that EF-Tu within the ternary complex is identical in all ternary complexes and thus contributes to the non-discriminatory fraction of the binding energy. EF-Tu increases the affinity of tRNA to the A site by two orders of magnitude (enzymatic binding) compared with aa-tRNA binding alone (non-enzymatic binding) [51]. The inevitable consequence is that every non-cognate ternary complex interferes with the selection process and should therefore occasionally be incorporated into protein synthesis before the equilibrium between cognate aa-tRNAs and near- plus non-cognate aa-tRNAs is reached. Establishing equilibrium is a requirement for exploiting the discrimination potential of codon-anticodon interactions (for review see [27]).

There is evidence that the E-tRNA-induced low-affinity state of the A site reduces or even abolishes the non-discriminatory interactions of the ternary complex by limiting it to monitoring codon-anticodon interaction. The essential experiment is shown in Table 1, where AcPhe-tRNA is bound to the P site of poly(U)-programmed ribosomes, and a mixture of ternary complexes containing cognate Phe-tRNA (A-site codon UUU) or non-cognate Asp-tRNA (requires a GAU/C codon) is offered to the A site. The decoding error is determined by a high-performance liquid chromatography (HPLC) analysis of the dipeptides formed [52]: a high-affinity A site resulting from a free E site allows incorporation of the non-cognate Asp residues, as indicated by a large error of 0.72%, whereas with an occupied E site, only background values of Asp incorporation are seen (0.05%; experiment 1). Another detail shown in experiment 2 is that binding a near-cognate $tRNA^{Leu}$ (codon UUG/A) to the E site does not prevent Asp incorporation. In other words, codon-anticodon interaction at the E site seems to be the trigger for the ribosome to flip into the POST state and to establish a low-affinity A site for proper selection of the ternary complexes.

Table 1. Accuracy of aa-tRNA selection at the A site depends on E-site occupation.

Conditions					Results	
Exp. no.	Initial complex	E site	Addition of ternary complex		Error (%) [AcPheAsp/(AcPhePhe + AcPheAsp)]×100]	Ratio error (E site free) to error (E site occupied)
			[^{14}C]Phe-tRNA	[^{3}H]Asp-tRNA		
1	P; 4 x cognate; E P	free	0.2×	4×	0.72	14
		occ.			0.05	
2	P; 4 x near cognate; E P	free	0.2×	4×	1.2	0.75
		occ			1.6	

A cognate deacylated $tRNA^{Phe}$ must be at the E site in order to avoid the incorporation of non-cognate Asp in poly(U)-programmed 70S ribosomes (experiment 1). A near-cognate deacylated $tRNA^{Leu}$ (codon UUG/A) is not sufficient (experiment 2), implying that a tRNA at the E site must undergo codon-anticodon interaction in order to induce the low-affinity A site that is prerequisite for non-interference of non-cognate aa-tRNAs at the A site. Data from [52].

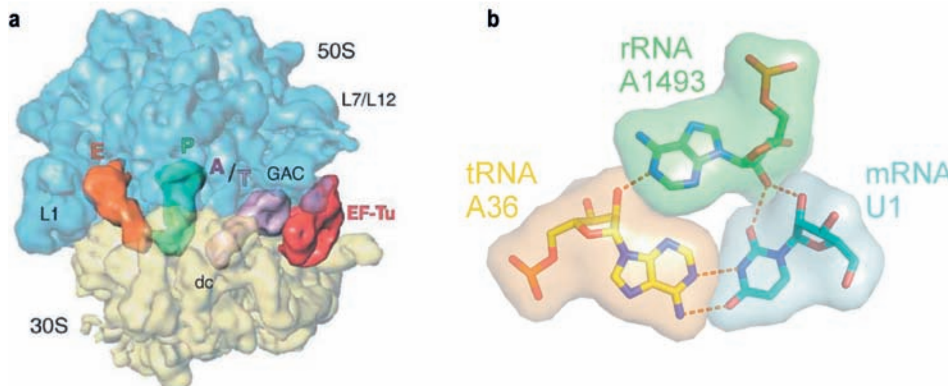

Figure 7. (*a*) Cryo-EM and single-particle reconstruction of the 70S *E. coli* pre-translocation-state ribosome in the process of binding the ternary complex EF-Tu-aa-tRNA-GTP. This complex was stalled with the antibiotic kirromycin, which allows hydrolysis of GTP to GDP but not the associated conformational changes in EF-Tu that allow it to dissociate. The P- and E-site tRNAs are indicated in green and orange, respectively. Taken from [70]. (*b*) The principles of decoding, at the first position, a codon of the mRNA at the A site of the ribosome. A1493 (green) of the 16S rRNA binds within the minor groove of the base pair between A36 (yellow) and U1 (cyan) of the tRNA and mRNA, respectively. A1493 monitors the stereochemical correctness of the A36-U1 base pair via hydrogen-bond interactions (dashed). Based on [65].

There are many studies supporting allosteric linkage between the A and E sites. For example, (i) the antibiotic edeine, which binds in the E site on the 30S subunit, induces translational misreading at the A site [53]; (ii) weakening of the E-tRNA via mutations at the S7-S11 interface, which binds the anticodon loop of an E-tRNA, induces dramatic selection problems such as misincorporation and readthrough [54]; and (iii) loss of codon-anticodon interaction at the E site provokes high-efficiency frameshifting [15, 55]. (iv) A tRNA with an altered CCA-3′ end does not bind well to the E site [39], which may explain the increase in error rate at the A site, when $tRNA^{Val}$ bearing a mutation at the CCA end is present at the E site [56]. Furthermore, allosteric effects between the A and E sites have been observed in the crystal structure of 70S ribosomes from *Thermus thermophilus* at 5.5 Å, where components of the E site were well ordered when an E-tRNA was present. In contrast, the elements were disordered when the A site instead of the E site was occupied [57]. Cryo-EM structures of ribosomes with a bound ternary complex in the presence of non-cleavable GTP analogs demonstrate that the decoding step occurs in a state where most of the tRNA interactions with the A site are avoided before the aa-tRNA is accommodated [58], thus enabling decoding at a low-affinity state of the A site (Fig. 7a).

The important consequence of the reciprocal relationship between A and E sites is apparent: in the low-affinity state the A site practically does not exist for the non-cognate aa-tRNAs, thus reducing the selection problem by an order of magnitude. Instead of 1 out of 42 different aa-tRNA species (a tRNA species is defined as a tRNA with a distinct anticodon), the selection is reduced to only 1 out of up to 4 tRNAs. In this respect the selection task is now similar to that of a transcriptase that selects one out of four nucleotides during RNA synthesis and does so with a precision of one error in more than 50,000 incorporations without even taking a proofreading mechanism into account [59, 60]. Thus the reciprocal A site – E site mechanism explains why the non-cognate ternary complexes do not show any ribosome-triggered GTPase activity, why the respective amino acids are never incorporated into proteins, and why even an excess of more than 100-fold non-cognate aa-tRNAs does not effect the rate or accuracy of protein synthesis ([61], A. Batertzko and K. H. Nierhaus, unpublished observation).

A final remark on the mechanism that the ribosome uses to select the cognate ternary complex out of pool of cognate plus near-cognate complexes: X-ray analyses have revealed that the decoding center recognizes the stereochemical correctness of the Watson-Crick base pairing of the codon-anticodon complex at the A site rather than the stability of base pairing. In the latter case one would expect that G:C-rich codons would be more precise than A:U-rich codons; however, this not the case [62]. Instead, the decoding center predominantly senses the correct arrangement of the 2′OH positions of the codon-anticodon interaction (Fig. 7b; for review and references see [63]).

The E site maintains the reading frame

Maintenance of the reading frame is of utmost importance for translation of genetic information and is one of the most remarkable features of the ribosome; a spontaneous frameshift occurs only once in at least 30,000 amino acid incorporations [64]. Recently, by studying the translation of mRNA coding for the termination release factor RF2, the underlying ribosomal mechanism of read-

ing frame maintenance was unravelled. The RF2 mRNA has a UGA stop codon at the 26th codon position that is recognized by RF2. This is the basis for a simple negative feedback regulation system: when sufficient amounts of RF2 are present in the cell, the synthesis of RF2 is terminated after the 25th codon, and the 25-mer peptide is released and rapidly degraded. If, however, there is a shortage of RF2 in the cell, a +1 frameshift occurs with up to 100% efficiency, i.e. four orders of magnitude more often than a spontaneous frameshift. This means that a mechanism for maintenance of the reading frame must exist, which is switched off during the synthesis of the RF2 factor.

A special feature of the RF2 frameshift site is a Shine-Dalgarno-like (SD) sequence, which has an unusually short spacer of two nucleotides to the last sense codon in the initial frame. Generally, the SD sequence is found in front of the initiation codon AUG with a spacer of 5–9 nucleotides.

The outcome from a systematic analysis of the RF2-frameshifting phenomenon was that the structure of the RF2-mRNA (in particular the SD-like sequence) provokes a premature release of the E-tRNA, and that the presence of the E-tRNA prevents slippage of the mRNA via codon-anticodon interaction. It follows that the premature release of the E-tRNA paves the way for the highly efficient frameshift [15]. An *in vivo* analysis of another highly efficient frameshift confirmed the essential role of codon-anticodon interaction at the E site for preventing frameshift [55]. Additionally, the mutation of C2394G, the highly conserved nucleotide within the 23S rRNA that base-pairs with A76 of deacylated tRNA at the E site, has, as previously mentioned, been shown *in vitro* to lead to translocation defects, but also promotes frameshifting and misreading at stop codons *in vivo*, particularly at UAA [23].

Marquez et al. [15] also demonstrated that in the absence of an SD-like sequence and without an occupied E site, a +1 frameshift can occur in up to 25% of cases, provided that the peptidyl-tRNA at the P site can slip in the +1 direction. If we assume the –1 frameshifts would be equally likely in the absence of an E site, and taking into account the wobbling potential of the third base pair in a codon-anticodon complex, one can estimate that without the E-tRNA, only polypeptides of a chain length of less than 50 amino acids could be synthesized before a frameshift would destroy the genetic information. Facing the average length of a protein of 300–400 amino acids in prokaryotes and 500–600 in eukaryotes, life on this planet as we know it today would not be possible without a functional E site.

1 Watson, J. D. (1965) Molecular Biology of the Gene. Biology Teaching Monograph Series, W. A. Benjamin, New York.
2 Watson, J. D. (1964) The synthesis of proteins upon ribosomes. Bull. Soc. Chim. Biol. 46, 1399–1425.
3 Traut, R. R. and Monro, R. E. (1964) The puromiycin reaction and its relation to protein synthesis. J. Mol. Biol. 10, 63–72.
4 Semenkov, Y., Shapkina, T., Makhno, V. and Kirillov, S. (1992) Puromycin reaction for the A-site-bound peptidyl-transfer RNA. FEBS Lett. 296, 207–210.
5 Wettstein, F. O. and Noll, H. (1965) Binding of transfer ribonucleic acid to ribosomes engaged in protein synthesis: number and properties of ribosomal binding sites. J. Mol. Biol. 11, 35–53.
6 Rheinberger, H.-J. and Nierhaus, K. H. (1980) Simultaneous binding of the 3 tRNA molecules by the ribosome of E coli. Biochem. Internatl. 1, 297–303.
7 Rheinberger, H.-J., Sternbach, H. and Nierhaus, K. H. (1981) Three tRNA binding sites on *Escherichia coli* ribosomes. Proc. Natl. Acad. Sci. USA 78, 5310–5314.
8 Grajevskaja, R. A., Ivanov, Y. V. and Saminsky, E. M. (1982) 70-S ribosomes of *Escherichia coli* have an additional site for deacylated tRNA binding. Eur. J. Biochem. 128, 47–52.
9 Lill, R., Robertson, J. M. and Wintermeyer, W. (1984) tRNA binding sites of ribosomes from *Escherichia coli*. Biochemistry 23, 6710–6717.
10 Saruyama, H. and Nierhaus, K. H. (1986) Evidence that the three-site model for ribosomal elongation cycle is also valid in the archaebacterium Halobacterium halobium. Mol. Gen. Genet. 204, 221–228.
11 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.
12 Elskaya, 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.
13 Bouadloun, F., Donner, D. and Kurland, C. G. (1983) Codon-specific missense errors *in vivo*. EMBO J. 2, 1351–1356.
14 Kurland, C. G., Jørgensen, F., Richter, A., Ehrenberg, M., Bilgin, N. and Rojas, A.-M. (1990) In: The Ribosome – Structure, Function, and Evolution, pp. 513–526, Dahlberg, A., Hill, W. E., Garrett, R. A., Moore, P. B., Schlessinger, D. and Warner, J. R. (eds.), Amer. Soc. Microbiol., Washington, DC.
15 Marquez, V., Wilson, D. N., Tate, W. P., Triana-Alonso, F. and Nierhaus, K. H. (2004) Maintaining the ribosomal reading frame: the influence of the E site during translational regulation of release factor 2. Cell 118, 45–55.
16 Cate, J. H., Yusupov, M. M., Yusupova, G. Z., Earnest, T. N. and Noller, H. F. (1999) X-ray crystal structures of 70S ribosome functional complexes. Science 285, 2095–2104.
17 Agrawal, R. K., Spahn, C. M. T., Penczek, P., Grassucci, R. A., Nierhaus, K. H. and Frank, J. (2000) Visualization of tRNA movements on the *Escherichia coli* 70S ribosome during the elongation cycle. J. Cell. Biol. 150, 447–459.
18 Yusupov, M. M., Yusupova, G. Z., Baucom, A., Lieberman, K., Earnest, T. N., Cate, J. H. and Noller, H. F. (2001) Crystal structure of the ribosome at 5.5 Å resolution. Science 292, 883–896.
19 Wower, J., Scheffer, P., Sylvers, L. A., Wintermeyer, W. and Zimmermann, R. A. (1993) Topography of the E-site on the *Escherichia coli* ribosome. EMBO J. 12, 617–623.
20 Joseph, S. and Noller, H. F. (1996) Mapping the rRNA neighborhood of the acceptor end of tRNA in the ribosome. EMBO J. 15, 910–916.
21 Schmeing, T. M., Moore, P. B. and Steitz, T. A. (2003) Structures of deacylated tRNA mimics bound to the E site of the large ribosomal subunit. RNA 9, 1345–1352.
22 Moazed, D. and Noller, H. F. (1989) Interaction of tRNA with 23S rRNA in the ribosomal A, P, and E sites. Cell 57, 585–597.

23 Sergiev, P., Lesnyak, D., Kiparisov, S., Burakovsky, D., Leonov, A., Bogdanov, A., Brimacombe, R. and Dontsova, O. (2005) Function of the ribosomal E-site: a mutagenesis study. Nucleic Acids Res. 33, 6048–6056.
24 Harms, J., Schluenzen, F., Zarivach, R., Bashan, A., Gat, S., Agmon, I., Bartels, H., Franceschi, F. and Yonath, A. (2001) High resolution structure of the large ribosomal subunit from a mesophilic eubacterium. Cell 107, 679–688.
25 Beckmann, R., Spahn, C. M., Eswar, N., Helmers, J., Penczek, P. A., Sali, A., Frank, J. and Blobel, G. (2001) Architecture of the protein-conducting channel associated with the translating 80S ribosome. Cell 107, 361–372.
26 Spahn, C. M., Beckmann, R., Eswar, N., Penczek, P. A., Sali, A., Blobel, G. and Frank, J. (2001) Structure of the 80S ribosome from Saccharomyces cerevisiae-tRNA-ribosome and subunit-subunit interactions. Cell 107, 373–386.
27 Blaha, G. and Nierhaus, K. H. (2001) Features and functions of the ribosomal E site. Cold Spring Harbor Symp. Quant. Biol. 65, 135–145.
28 Remme, J., Margus, T., Villems, R. and Nierhaus, K. H. (1989) The third ribosomal tRNA-binding site, the E site, is occupied in native polysomes. Eur. J. Biochem. 183, 281–284.
29 Wadzack, J., Burkhardt, N., Jünemann, R., Diedrich, G., Nierhaus, K. H., Frank, J., Penczek, P., Meerwinck, W., Schmitt, M., Willumeit, R. and Stuhrmann, H. B. (1997) Direct localization of the tRNAs within the elongating ribosome by means of neutron scattering (Proton-spin contrast-variation). J. Mol. Biol. 266, 343–356.
30 Semenkov, Y. P., Rodnina, M. V. and Wintermeyer, W. (1996) The 'allosteric three-site model' of elongation cannot be confirmed in a well-defined ribosome system from *Escherichia coli*. Proc. Natl. Acad. Sci. USA 93, 12183–12188.
31 Nierhaus, K. H., Jünemann, R. and Spahn, C. M. T. (1997) Are the current three-site models valid descriptions of the ribosomal elongation cycle? Proc. Natl. Acad. Sci. USA 94, 10499–10500.
32 Rheinberger, H.-J., Sternbach, H. and Nierhaus, K. H. (1986) Codon-anticodon interaction at the ribosomal E site. J. Biol. Chem. 261, 9140–9143.
33 Rheinberger, H.-J. and Nierhaus, K. H. (1983) Testing an alternative model for the ribosomal peptide elongation cycle. Proc. Natl. Acad. Sci. USA 80, 4213–4217.
34 Rheinberger, H.-J. and Nierhaus, K. H. (1986) Allosteric interactions between the ribosomal transfer RNA-binding sites A and E. J. Biol. Chem. 261, 9133–9139.
35 Nierhaus, K. H. (1990) The allosteric three-site model for the ribosomal elongation cycle: features and future. Biochemistry 29, 4997–5008.
36 Dinos, G., Kalpaxis, D. L., Wilson, D. N. and Nierhaus, K. H. (2005) Deacylated tRNA is released from the E site upon A site occupation but before GTP is hydrolyzed by EF-Tu. Nucleic Acids Res. 33, 5291–5296.
37 Schilling-Bartetzko, S., Bartetzko, A. and Nierhaus, K. H. (1992) Kinetic and thermodynamic parameters for transfer RNA binding to the ribosome and for the translocation reaction. J. Biol. Chem. 267, 4703–4712.
38 Bourne, H. R., Sanders, D. A. and McCormick, F. (1991) The GTPase superfamily: conserverd structure and molecular mechanism. Nature 349, 117–127.
39 Lill, R., Lepier, A., Schwägele, F., Sprinzl, M., Vogt, H. and Wintermeyer, W. (1988) Specific recognition of the 3′-terminal adenosine of tRNAPhe in the exit site of *Escherichia coli* ribosomes. J. Mol. Biol. 203, 699–705.
40 Bocchetta, M., Xiong, L., Shah, S. and Mankin, A. S. (2001) Interactions between 23S rRNA and tRNA in the ribosomal E site. RNA 7, 54–63.
41 Lill, R., Robertson, J. M. and Wintermeyer, W. (1989) Binding of the 3′ terminus of tRNA to 23S rRNA in the ribosomal exit site actively promotes translocation. EMBO J. 8, 3933–3938.
42 Feinberg, J. S. and Joseph, S. (2001) Identification of molecular interactions between P site tRNA and the ribosome essential for translocation. Proc. Natl. Acad. Sci. USA 98, 11120–11125.
43 Frank, J. and Agrawal, R. K. (2000) A ratchet-like inter-subunit reorganization of the ribosome during translocation. Nature 406, 318–322.
44 Moazed, D. and Noller, H. F. (1989) Intermediate states in the movement of transfer RNA in the ribosome. Nature 342, 142–148.
45 Sharma, D., Southworth, D. R. and Green, R. (2004) EF-G-independent reactivity of a pre-translocation-state ribosome complex with the aminoacyl tRNA substrate puromycin supports an intermediate (hybrid) state of tRNA binding. RNA 10, 102–113.
46 Dorner, S., Brunelle, J. L., Sharma, D. and Green, R. (2006) The hybrid state of tRNA binding is an authentic translation elongation intermediate. Nat. Struct. Mol. Biol. 13, 234–241.
47 Agrawal, R. K., Penczek, P., Grassucci, R. A., Burkhardt, N., Nierhaus, K. H. and Frank, J. (1999) Effect of buffer conditions on the position of tRNA on the 70S ribosome as visualized by cryo-electron microscopy. J. Biol. Chem. 274, 8723–8729.
48 Valle, M., Zavialov, A., Sengupta, J., Rawat, U., Ehrenberg, M. and Frank, J. (2003) Locking and unlocking of ribosomal motions. Cell 114, 123–134.
49 Blanchard, S. C., Kim, H. D., Gonzalez, R. L. Jr, Puglisi, J. D. and Chu, S. (2004) tRNA dynamics on the ribosome during translation. Proc. Natl. Acad. Sci. USA 101, 12893–12898.
50 Dong, H. J., Nilsson, L. and Kurland, C. G. (1996) Co-variation of tRNA abundance and codon usage in *Escherichia coli* at different growth rates. J. Mol. Biol. 260, 649–663.
51 Schilling-Bartetzko, S., Franceschi, F., Sternbach, H. and Nierhaus, K. H. (1992) Apparent association constants of transfer RNAs for the ribosomal A-site, P-site, and E-site. J. Biol. Chem. 267, 4693–4702.
52 Geigenmüller, U. and Nierhaus, K. H. (1990) Significance of the third tRNA binding site, the E site, on E. coli ribosomes for the accuracy of translation: an occupied E site prevents the binding of non-cognate aminoacyl-transfer RNA to the A site. EMBO J. 9, 4527–4533.
53 Dinos, G., Wilson, D. N., Teraoka, Y., Szaflarski, W., Fucini, P., Kalpaxis, D. and Nierhaus, K. H. (2004) Dissecting the ribosomal inhibition mechanisms of edeine and pactamycin: the universally conserved residues G693 and C795 regulate P site tRNA binding. Mol. Cell 13, 113–124.
54 Robert, F. and Brakier-Gingras, L. (2003) A functional interaction between ribosomal proteins S7 and S11 within the bacterial ribosome. J. Biol. Chem. 278, 44913–44920.
55 Trimble, M. J., Minnicus, A. and Williams, K. P. (2004) tRNA slippage at the tmRNA resume codon. RNA 10, 805–812.
56 O'Connor, M., Willis, N. M., Bossi, L., Gesteland, R. F. and Atkins, J. F. (1993) Functional tRNAs with altered 3′ ends. EMBO J. 12, 2559–2566.
57 Jenner, L., Romby, P., Rees, B., Schulze-Briese, C., Springer, M., Ehresmann, C., Ehresmann, B., Moras, D., Yusupova, G. and Yusupov, M. (2005) Translational operator of mRNA on the ribosome: how repressor proteins exclude ribosome binding. Science 308, 120–123.
58 Valle, M., Sengupta, J., Swami, N. K., Grassucci, R. A., Burkhardt, N., Nierhaus, K. H., Agrawal, R. K. and Frank, J. (2002) Cryo-EM reveals an active role for aa-tRNA in the accommodation process. EMBO J. 21, 3557–3567.
59 Blank, A., Gallant, J. A., Burgess, R. R. and Loeb, L. A. (1986) An RNA polymerase mutant with reduced accuracy of chain elongation. Biochemistry 25, 5920–5928.
60 Libby, R. T., Nelson, J. L., Calvo, J. M. and Gallant, J. A. (1989) Transcriptional proofreading in *Escherichia coli*. EMBO J. 8, 3153–3158.

61 Bilgin, N., Ehrenberg, M. and Kurland, C. (1988) Is translation inhibited by noncognate ternary complexes? FEBS Lett. 233, 95–99.
62 Andersson, S. G. E., Buckingham, R. H. and Kurland, C. G. (1984) Does codon composition influence ribosome function? EMBO J. 3, 91–94.
63 Ogle, J., Carter, A. and Ramakrishnan, V. (2003) Insights into the decoding mechanism from recent ribosome structures. TIBS 28, 259–266.
64 Jorgensen, F. and Kurland, C. G. (1990) Processivity errors of gene expression in *Escherichia coli*. J. Mol. Biol. 215, 511–521.
65 Ogle, J. M., Brodersen, D. E., Clemons Jr, W. M., Tarry, M. J., Carter, A. P. and Ramakrishnan, V. (2001) Recognition of cognate transfer RNA by the 30S ribosomal subunit. Science 292, 897–902.
66 Wilson, D., Harms, J., Nierhaus, K., Schlünzen, F. and Fucini, P. (2005) Species-specific antibiotic-ribosome interactions: Implications for drug development. Biol. Chem. 386, 1239–1252.
67 Nierhaus, K. H. (1996) Protein synthesis – an elongation factor turn-on. Nature 379, 491–492.
68 Nierhaus, K. H. (1996) The tricks of ribosomal elongation factors. Angew. Chem. Int. Ed. Engl. 35, 2198–2201.
69 Spahn, C. M., Gomez-Lorenzo, M. G., Grassucci, R. A., Jorgensen, R., Andersen, G. R., Beckmann, R., Penczek, P. A., Ballesta, J. P. and Frank, J. (2004) Domain movements of elongation factor eEF2 and the eukaryotic 80S ribosome facilitate tRNA translocation. EMBO J. 23, 1008–1019.
70 Valle, M., Zavialov, A., Li, W., Stagg, S. M., Sengupta, J., Nielsen, R. C., Nissen, P., Harvey, S. C., Ehrenberg, M. and Frank, J. (2003) Incorporation of aa-tRNA into the ribosome as seen by cryo-electron microscopy. Nat. Struct. Biol. 10, 899–906.

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