Peptide Chain Elongation: Models of the Elongation Cycle

Secondary article

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Three models of the ribosomal elongation cycle are used to explain the mechanism of protein synthesis on the ribosome. These are the allosteric three-site model, the hybrid site model, and the α-ε model. The α-ε model reconciles most of the experimental data currently available.

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Introduction

Three transfer RNA (tRNA)-binding sites have been found on ribosomes from bacteria, archaea and low and high eukarya and thus are probably a universal feature of ribosomes (Spahn and Nierhaus, 1998). The three sites are termed A, P and E, respectively. A tRNA enters the ribosome at the A site, moves sequentially through the sites A→P→E during protein synthesis and leaves the ribosome from the E site. Statistically, two of these sites are occupied simultaneously by tRNAs during the growth of the nascent peptide chain (Remme *et al.*, 1989), either the A and P sites or the P and E sites. The strict requirement for three tRNA-binding sites on ribosomes seems to be a consequence of the translocation mechanism (see below).

The A site contains the ribosomal decoding centre that exposes a codon of the mRNA and selects the aminoacyl-tRNA (A for aminoacyl-tRNA) with an anticodon of the tRNA complementary to the exposed codon. The decoding centre is part of the A site that is located on the small ribosomal subunit. The P site carries the peptidyl-tRNA before peptide bond formation (P for peptidyl-tRNA), and the E site is specific for deacylated tRNA (E for exit).

Three functional phases of the ribosome can be distinguished: the initiation, elongation and termination phases. The first tRNA that binds to the small ribosomal subunit during the initiation phase of protein synthesis is the initiator tRNA. This is a distinct tRNA species used as methionyl-tRNA (Met-tRN●) in eukarya and archaea and as formyl-methionyl-tRNA (fMet-tRN) in bacteria and eukaryotic organelles (chloroplasts and mitochondria). The initiator tRNA occupies the P site region of the small subunit with the anticodon stem–loop structure. When the large subunit joins, the ribosomal initiation complex is achieved: the initiator codon AUG is in codon–anticodon contact with the initiator tRNA, and the A site exposes the next downstream codon and is ready to accept the corresponding aminoacyl-tRNA. The aminoacyl-tRNA is not the direct substrate for the A site, but rather a ternary complex aminoacyl-tRNA·EF-Tu·GTP containing the elongation factor EF-Tu (the corresponding elongation factor in archaea and eukarya is termed EF-1). With the binding of such a ternary complex the ribosome enters the elongation cycle, the central part of gene expression, where the genetic information of the mRNA is translated into the sequence of amino acids. The reactions and mechanisms of the elongation cycle seem to be universally conserved, whereas those of both the initiation and termination phase are strikingly different in bacteria and eukarya/archaea, respectively.

If one of the three stop codons moves into the A site during a translocation reaction, the ribosome enters the termination phase: The synthesized peptide is released and the tRNAs and messenger RNA (mRNA) dissociate from the ribosome.

The Three Basic Reactions of the Elongation Cycle and the Role of the Elongation Factors

When the cognate aminoacyl-tRNA has been selected, leading to the occupation of the A site, two tRNAs are found on the ribosome, the peptidyl-tRNA at the P site and the newly bound aminoacyl-tRNA at the A site. This state marks the end of the first basic reaction. The second reaction is peptide bond formation, where the ester bond of the polypeptide is cleaved and the peptidyl residue is linked to the free amino group of the aminoacyl-tRNA. The result is that a peptidyl-tRNA resides now in the A site elongated by one aminoacyl residue. The peptidyl transfer is an activity of the peptidyltransferase centre, an integral part of the large ribosomal subunit. In the third reaction, the translocation, the complex mRNA–tRNAs is shifted by one codon length so that the tRNAs move from the A and P sites to the P and E sites, respectively.

The translocation reaction marks a transition between the two main conformational states of the ribosome during

peptide synthesis, viz. the state before translocation, the pre state, and that after translocation, the post state. The states are separated by high activation energies, which are the higher the more the ionic conditions approach *in vivo* values and can reach 120 kJ mol^{-1} (Schilling-Bartetzko *et al*., 1992). This means that the ribosomal pre and post states are best defined under physiological conditions.

The elongation factors are GTPases, and when complexed with guanosine triphosphate (GTP) and in the case of EF-Tu in addition with an aminoacyl-tRNA, they lower the activation energy barrier separating the two main states of the ribosome and thus dramatically accelerate protein synthesis by more than 10^4-fold. Therefore, they resemble enzymes that also lower the activation energy of a reaction and consequently achieve enormous acceleration factors on the order of 10^6 to 10^{12}. But, enzymes accelerate a reaction only until the equilibrium is reached, i.e. the enzyme does not alter the equilibrium, only its rate of attainment. An elongation factor can do more, it accelerates a reaction and determines the direction of reaction: EF-Tu•GTP catalyses the post→pre transition and EF-G•GTP the reverse reaction, the pre→post transition. For this reason there are always two elongation factors, one for each direction of reaction. Only the higher fungi such as yeast or *Candida albicans* require a third factor, EF-3, which is essential for protein synthesis and represents an E site factor. Interestingly, these effects are also seen in the presence of the noncleavable GTP analogues. This means that it is not primarily the GTP hydrolysis that drives the reaction, but the specific binding of the elongation factors to the main states of the ribosome.

EF-Tu and EF-G are both prototypes of the large superfamily known as G proteins, members of which regulate important processes of the cell (Bourne *et al*., 1991). All G proteins are GTPases and pass through a basic GTPase cycle. They bind GTP and GDP, resulting in either an 'on' or an 'off' conformation, respectively. In the 'on' conformation (GTP is bound) a G protein can bind a protein or a complex and trigger a distinct reaction. After or while triggering this reaction the GTPase centre of the G protein is activated. The terminal (γ) phosphate residue is cleaved off, GDP remains bound and the G protein adopts an 'off' conformation, in which it dissociates from the protein or complex. A new cycle will begin after the GDP has been replaced by GTP.

Figure 1 shows the GTPase cycles of EF-G and EF-Tu binding in the 'on' conformation to the ribosome. EF-G follows the simplest scheme for a G protein. Complexed with GTP, it binds to a ribosome which has to be in the 'pre' state and to carry the peptidyl-tRNA at the A region after a peptidyl transfer. EF-G lowers the activation energy barrier between the pre and post states of the ribosome. Since the post state possibly represents a lower energetic level, the ribosome falls into the post state and carries the peptidyl-tRNA at the P region and the deacylated tRNA at the E region. The lower energetic level of the post state is indicated by the fact that after peptide bond formation an incubation at 37°C for a couple of minutes is sufficient to promote a translocation from the pre to the post state in the absence of the translocation factor EF-G and GTP ('spontaneous translocation'). In contrast, a reverse translocation has never been observed with an isolated post state. During or after the translocation reaction, the ribosome activates the GTPase centre via an unknown mechanism, the resulting EF-G·GDP takes up the 'off' conformation and falls off the ribosome. The activation barrier is raised again and keeps the ribosome stable in the post state.

EF-G has a very low affinity to both GTP and GDP (2.7×10^4 L mol^{-1} and 2.5×10^5 L mol^{-1}, respectively; Baca *et al*., 1976) so that the actual concentrations of GTP and GDP in the cell ($\sim$1 mmol L^{-1} and 0.1 mmol L^{-1}, respectively) are sufficient to replace GDP by GTP without the help of an additional factor, thus enabling the next round of an EF-G cycle.

The situation with EF-Tu is more complicated since EF-Tu·GTP cannot bind to the ribosome but has to bind an aminoacyl-tRNA first, thus forming a ternary complex aminoacyl-tRNA·EF-Tu·GTP. This shows the second essential role that EF-Tu has besides catalysing the post→pre transition, namely to carry an aminoacyl-tRNA to the ribosome.

The elongation factors follow the general scheme of the G proteins in that the energy released by GTP hydrolysis is required solely for the efficient release of the corresponding factor rather than for driving the triggered reaction, since EF-G, for example, also promotes a translocation reaction in the presence of noncleavable analogues. Some groups favour the view that the energy released by EF-G-dependent GTP cleavage is also used for the translocation reaction; however, compelling evidence for this opinion of EF-G as a 'motor protein' has not yet been presented (see Spahn and Nierhaus, 1998, for further details).

When the crystal structure of the ternary complex was compared with that of EF-G·GDP, it became immediately evident that the structures are very similar (Nissen *et al*., 1995). The EF-G structure shows five domains, three of which (III–V) imitate the structure of the tRNA component of the ternary complex (**Figure 2**). This is a surprising example of molecular mimicry, where protein domains mimic the structure of a tRNA. A possible explanation for the functional importance of this mimicry is the following: after EF-G·GTP has established the post state of the ribosome the domains of EF-G mimicking a tRNA bind to the vacant decoding centre at the A site. This action prevents a reverse translocation of the ribosome into the pre state as long as the presence of EF-G on the ribosome keeps the activation energy barrier low. The localization of EF-G on the ribosome by means of cryoelectron microscopy was in accord with this interpretation: the tip of domain IV of EF-G corresponds to the anticodon region of a tRNA within a ternary complex and was found to

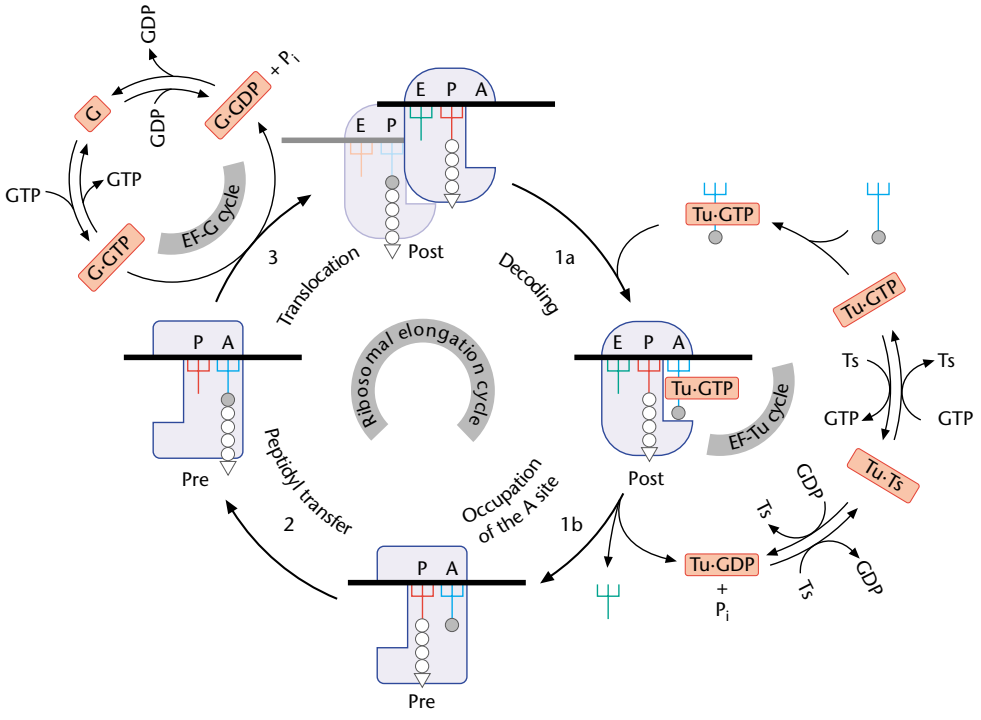

Figure 1 The allosteric three-site model with the EF-Tu and EF-G cycles. The model is characterized by two features: (1) A and E sites are coupled in reciprocal fashion, i.e. an occupied E site induces a low affinity of the A site, and vice versa an occupied A site prevents the binding of a tRNA to the E site. (2) The two tRNAs on the elongating ribosome are linked to the mRNA via codon–anticodon interaction. Ts indicates the GDP/GTP exchange factor EF-Ts.

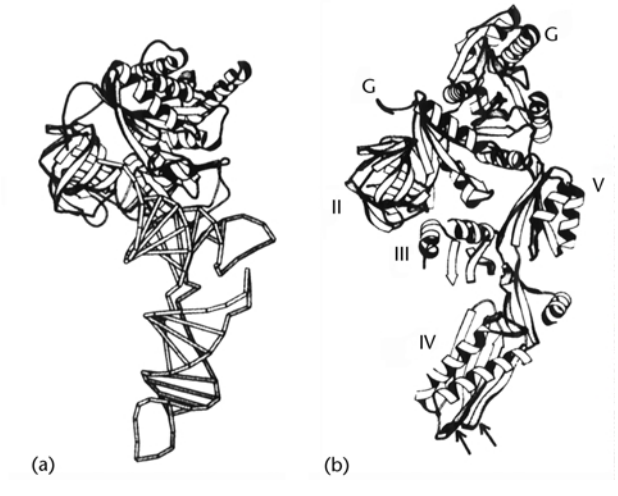

Figure 2 X-ray crystal structures of the elongation factors. (a) The ternary complex Phe-tRNA•EF-Tu•GTP. (b) EF-G from *Thermus thermophilus*. The domains III, IV and V of EF-G mimic the tRNA moiety within the ternary complex (molecular mimicry). G, G domain that contains the GTPase centre. From Nakamura Y, Ito K and Isaksson LA (1996) *Cell* **87**:147–150, with permission.

coincide with the anticodon loop of an aminoacyl-tRNA present at the A site.

Allosteric Three-site Model

Functional analyses of the ribosomal E site have led to the allosteric three-site model (Nierhaus, 1990). The central feature of this model is the assumption of a reciprocal linkage between the first and the third tRNA-binding sites, i.e. the A site and the E site. This means that an occupation of the A site induces a low-affinity state at the E site, thus coupling the occupation of the A site to the release of deacylated tRNA from the E site (**Figure 1**). Similarly, an occupied E site triggers a low-affinity state of the A site that plays an important role for the accuracy of the decoding step. The ribosome in the post state performs the decoding process at the decoding centre at the low-affinity A site (step 1a in **Figure 1**), and after a successful decoding the ribosome switches to the pre state, the GTPase centre of EF-Tu cleaves GTP, EF-Tu•GDP adopts its 'off' conformation and dissociates from the ribosome, and the aminoacyl-tRNA binds to the high-affinity A site (step 1b in **Figure 1**). The allosteric three-site model explains this

reciprocal linkage with an allosteric coupling of the A and E sites in the sense of a negative cooperativity. A consequence of this feature is that on average two tRNAs are always present on the elongating ribosome, at the A and P sites in the pre state and at the P and E sites in the post state. In fact, two tRNAs per ribosome are observed at various ratios of pre and post states *in vivo* and *in vitro*.

A wealth of data supports the assumption of a reciprocal coupling: (1) A direct demonstration of the reciprocal linkage was shown using a heteropolymeric mRNA displaying three different codons at the three sites together with three cognate tRNAs labelled with three different isotopes. (2) The activation energy for occupation of the A site depends on the occupation of the E site: when a tRNA is present at the E site the activation energy is twice as large as that observed with a free E site (80 kcal mol^{-1} instead of 40 kcal mol^{-1}; Schilling-Bartetzko *et al*., 1992). (3) Thiostrepton, viomycin and all aminoglycosides severely impair A site binding only if the E site is occupied (Hausner *et al*., 1988). (4) The reciprocal coupling of A and E sites has been also observed in the ribosomes of organisms from other evolutionary domains, i.e. with ribosomes of archaeal and eukaryotic organisms.

A second feature of this model is the simultaneous codon–anticodon interaction of both tRNAs present at A and P sites before translocation and at P and E sites after translocation. A tight interaction of both tRNAs with the mRNA before and after translocation might be important for an exact movement of the mRNA by one codon length during translocation, thus playing an essential role for sustaining the reading frame of the translating ribosome.

Hybrid Site Model

A structural analysis of the rRNAs of various functional complexes has been used to define the hybrid site model (Moazed and Noller, 1989). The crucial observation was that bases of rRNAs could be protected against chemical modifications by binding tRNAs specifically to the P, A and E sites. The peptidyl-tRNA analogue AcPhe-tRNA bound to the P site could thus yield a 'P site pattern', binding of a ternary complex Phe-tRNA·EF-Tu·GTP to the A site after prefilling the P site with a deacylated tRNA resulted in an 'A site pattern', and an 'E site pattern' – not as well defined in the poly(U)-dependent system used – was derived from the binding of deacylated tRNA requiring an intact CCA-3′ terminus.

The essential result was that a tRNA does not seem to move *in toto* from one site to the adjacent one but rather via hybrid sites. For example, consider a pre state with an aminoacyl-tRNA in the A site and a peptidyl-tRNA at the P site (**Figure 3**). After peptide bond formation the newly made peptidyl-tRNA is not at the A site but rather is shifted to an A/P hybrid site (the letter before the slash indicates the site on the 30S and that after the slash the site on the 50S subunit). Concomitantly, the deacylated tRNA is not at the P site but at the P/E hybrid site. Note that these tRNA shifts occur exclusively on the 50S subunit before the translocation reaction, i.e. the translocation reaction triggers a movement exclusively on the 30S subunit. The result is that the peptidyl-tRNA is at the P site and the deacylated tRNA at the E site the latter is thought to reside exclusively on the 50S subunit. The diagnostic features of the hybrid site model are (1) that a peptidyl-tRNA (or its simple analogue AcPhe-tRNA) is not found at the A site but rather at the A/P hybrid site, and (2) that a deacylated tRNA does not occupy the P site but rather the P/E hybrid site.

The hybrid site model is depicted in **Figure 3**. The tRNAs are passing through the ribosome with a 'creeping' movement. On the 30S subunit the picture follows the classical scheme outlined in the preceding section. On the 50S subunit, however, the movement is thought to start with peptide bond formation, before the EF-G•GTP-dependent translocation induced by EF-G. According to this concept the tRNA movement occurs independently on the two ribosomal subunits, i.e. with one extremity fixed (the anticodon tip at the 30S subunit or the acceptor end at the 50S subunit), while the other is moving. If correct, this model would provide the first hint as to why ribosomes require the universal two-subunit structure.

In functional studies, the P site is operationally defined as the site at which an acylated tRNA can react with puromycin, in contrast to an A site location. This view is not changed by the recent observation that an acylated tRNA at the A site also can undergo a puromycin reaction (Semenkov *et al*., 1992). This reaction differs qualitatively from that of the P site in that it is extremely slow: at 6 mmol L^{-1} Mg^{2+} and polyamines it is more than 200 times slower than the puromycin reaction from the P site, and is therefore not relevant for the operational definitions of the A and P sites. Note that the hybrid site model uncouples the functional definition of the P site from the structural one, since this model locates the peptidyl residue after peptide bond formation at the P site region of the peptidyltransferase centre (the peptidyl-tRNA is at the A/P site), but the peptidyl residue does not react with puromycin. Consequently, this model must distinguish two P site positions on the 50S subunit: one puromycin reactive (P/P) and one puromycin nonreactive (A/P).

At least three criticisms can be made of the hybrid site model:

1. Since the protections observed on the 16S rRNA are in accord with the classical models, the concept of hybrid sites rests on the protection patterns of the 23S rRNA. However, the protection of 16 out of 17 bases was dependent on the intact state of the last two bases of the universal CCA-3′ terminus of the tRNAs. It is conceivable that the location of the CCA end of a

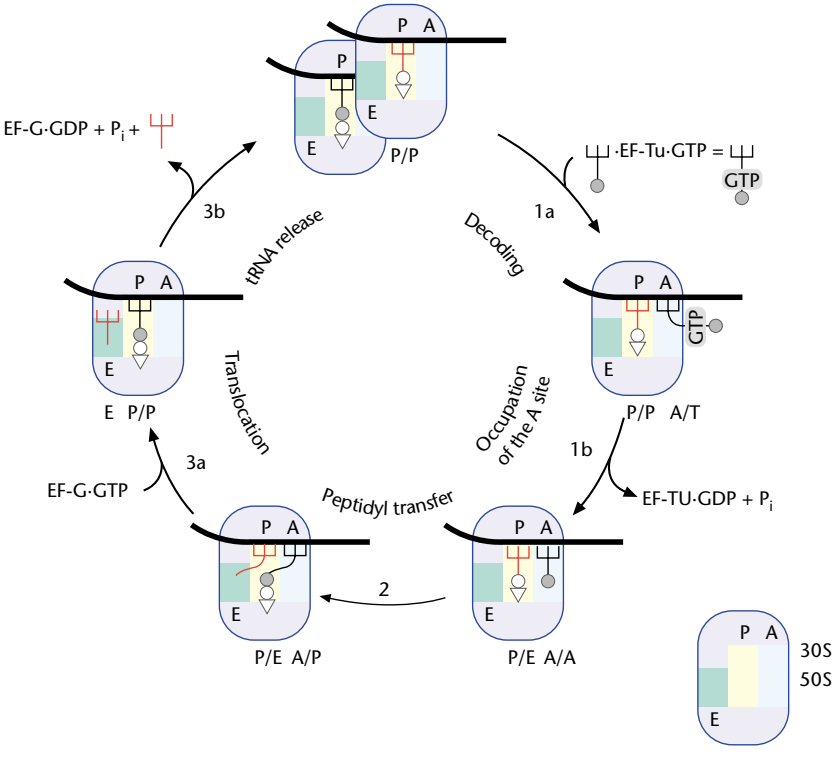

Figure 3 The hybrid site model. A tRNA passes hybrid sites during an elongation cycle. A site binding occurs in at least two steps. First, the codon is recognized on the 30S subunit and the ternary complex is bound in the A/T site. The presence of EF-Tu prevents peptide bond formation in this state as described for the allosteric three-site model. After GTP cleavage and dissociation of EF-Tu·GDP, the aminoacyl-tRNA can now interact with the 50S A site and the tRNA rearranges to the A/A site. Peptide bond formation takes place, which is immediately followed by a spontaneous movement of the tRNA region contacting the 50S subunit. As a result the newly created peptidyl-tRNA and deacylated tRNA are in the A/P and P/E hybrid sites, respectively. An EF-G-dependent translocation completes the elongation cycle. The movement of the tRNAs occurs only on the 30S side and as a consequence the deacylated tRNA is in the E site and the peptidyl-tRNA in the P/P site.

tRNA at a distinct tRNA-binding site is different depending on whether the α-amino group of the aminoacyl residue is free and positively charged before peptide-bond formation or is involved in a peptide bond after peptide-bond formation.

2. The experiments from which the model is derived use Mg^{2+} concentrations ranging from 5 to 25 mmol L^{-1}. It is well known, however, that the binding properties and interdependencies of the various sites are extremely sensitive to changes of Mg^{2+} concentration. This sensitivity probably reflects an increasing distortion of the ribosome with increasing Mg^{2+} concentrations, which one would expect to affect a fine-structure analysis such as the chemical probing of the rRNA bases.
3. The diagnostic features of the hybrid site model have been directly tested by inspecting appropriate functional complexes using cryoelectron microscopy. Since only 15% of a tRNA bound to 70S ribosomes contact the 30S subunit and 85% the 50S subunit, the position of a peptidyl-tRNA in a hybrid site A/P after peptide-bond formation is expected to be drastically different to that of an aminoacyl-tRNA at the A/A site before peptide-bond formation. However, both aminoacyl-tRNA and peptidyl-tRNA were found at corresponding topographical positions if they occupied the A site. Similarly, deacylated tRNA and peptidyl-tRNA, respectively, were found at equivalent positions if they were located at the P site.

Thus, the flexible CCA end might change its position although the rest of the tRNA molecule stays unaltered in the A or P site. The CCA end may not be a reliable reporter structure for the site location of the relatively rigid tRNA molecule.

Alpha-epsilon Model

A footprinting method was applied to map protection sites of the tRNA when bound to the ribosome. The method is based on the fact that the small and chemically neutral molecule I_2 (iodine) can cleave the sugar–phosphate

backbone of phosphorothioated RNA, i.e. RNA with a sulfur instead of a nonbridging oxygen at the phosphate groups. The cleavage works equally well with modified phosphates in single or double strands. tRNAs were transcribed *in vitro*, and during transcription a few phosphorothioated nucleotides were incorporated at A, G, U or C positions. The biological activity with respect to tRNA charging, *in vitro* translation and ribosome binding of thioated tRNAs was indistinguishable from that of *in vitro* transcribed tRNA containing no thioated nucleotides (Dabrowski *et al*., 1995). Iodine (I_2) cleaves with low efficiency at the thioated positions. No cleavage occurs if the access of iodine is prevented by a ribosomal component or by hydrogen bonds or coordinated metal ions involving the thioated phosphate residue. A comparison with the cleavages observed with a tRNA in solution yields the protection pattern of the tRNA present at a distinct binding site. Regardless of what has caused the protection at a distinct modified phosphate, the protection pattern of a tRNA essentially reflects, directly or indirectly, contacts of the tRNA with the corresponding ribosomal binding site.

A tRNA in the A site of a pretranslocational ribosome had a strikingly different pattern from a corresponding tRNA in the P site. However, after translocation to the P and E sites both tRNAs barely changed their respective patterns (Dabrowski *et al*., 1998). The conclusion was that the tRNAs bound to a structural domain of the ribosome, and that this structural domain moved from the A and P sites to the P and E sites during translocation, keeping both tRNAs tightly bound. The proposed structural domain thus contains two binding regions which were called α and ε, respectively (**Figure 4**). A tRNA bound to the α region can be displayed at the A site before translocation and at the P site after translocation. Likewise, a tRNA present at the ε region can appear at P and E sites before and after translocation, respectively. It follows that at the A site only α can appear and at the E site only ε (hence the nomenclature), whereas at the P site either α or ε can show up.

In addition, the model integrates the fact that the posttranslocational ribosome with a low-affinity A site is able to select the aminoacyl-tRNA cognate to the codon at the A site, i.e. the decoding process occurs before the α-ε domain of the ribosome flips back from the P-E sites to the A-P sites. The decoding centre, being exclusively on the 30S subunit, cannot move, is located at the A site and is called δ. It follows that δ is superimposed on α in the pre state, but is separated from the α-ε domain in the post state (**Figure 4**). This feature of the α-ε model predicts that the ribosome has two tRNA-binding sites in the pre state and three in the post state (the two high-affinity sites α-ε at the P and E sites and the low-affinity decoding centre δ at the A site).

All three features of the allosteric three-site model are valid in the α-ε model, although they are extended or reinterpreted. (1) Three sites exist, but only in the post case. (2) The A and E sites have a reciprocal relationship in that an occupied E site is accompanied by a low-affinity decoding centre δ at the A site and an occupied A site with no affinity at the E site. The new interpretation of this relationship is that there is no allostery involved: during translocation the α region moves from the A to the P sites leaving δ behind in the A site, and during occupation of the A site the α-ε domain jumps from the P-E to the A-P sites leaving the E site without a tRNA-binding region. (3) The two tRNAs do not dramatically change their mutual arrangement relative to each other and their arrangement relative to the mRNA, i.e. the codon–anticodon interactions which exist before translocation are maintained during and after translocation on the α-ε binding domain.

According to the α-ε model the critical step during the elongation cycle is not the translocation reaction but rather the binding of the new aminoacyl-tRNA to the A site. Indeed, the occupation of the A site is the rate-limiting step of elongation rather than the translocation reaction.

The Translocation Reaction

The translocation reaction is one of the most important challenges for structural research, due to the sheer size of the components which have to be moved relative to each other. Two adjacent tRNAs of 25 kDa each are linked to the mRNA via codon–anticodon interactions, and the tRNAs·mRNA complex has to glide by three nucleotides on the ribosome, which has a molecular mass of more than 2500 kDa. The tRNAs reside at the ribosomal A and P sites, respectively, before translocation and arrive at the P and E sites after translocation.

Furthermore, the movement must be extraordinarily precise at both extremities of the L-shaped tRNAs, which are about 7.5 nm apart. The step width at the codon–anticodon end has to be exactly three nucleotides in order to maintain the reading frame and to insure that the next codon enters the ribosomal decoding site, which is the A site. The opposite end, the CCA end of a peptidyl-tRNA, has to be accurately docked into the A site of the peptidyltransferase centre on the large ribosomal subunit before translocation and into the P site after translocation.

In the frame of the allosteric three-sites model a tRNA is moved *in toto* from one site to the other. If we assume that the three tRNA-binding sites correspond to different and defined topographical areas of the ribosome formed by distinct ribosomal components, a molecular mechanism for the translocation cannot be easily derived from this model, since it requires the exact movement of two large molecules on the surface of a huge complex.

The hybrid site model attacks the problem by assuming a 30S (16S rRNA) part and a 50S (23S rRNA) part of the A and P sites, which can be occupied sequentially. However, breaking down the movement of the $(tRNA)_2$–mRNA

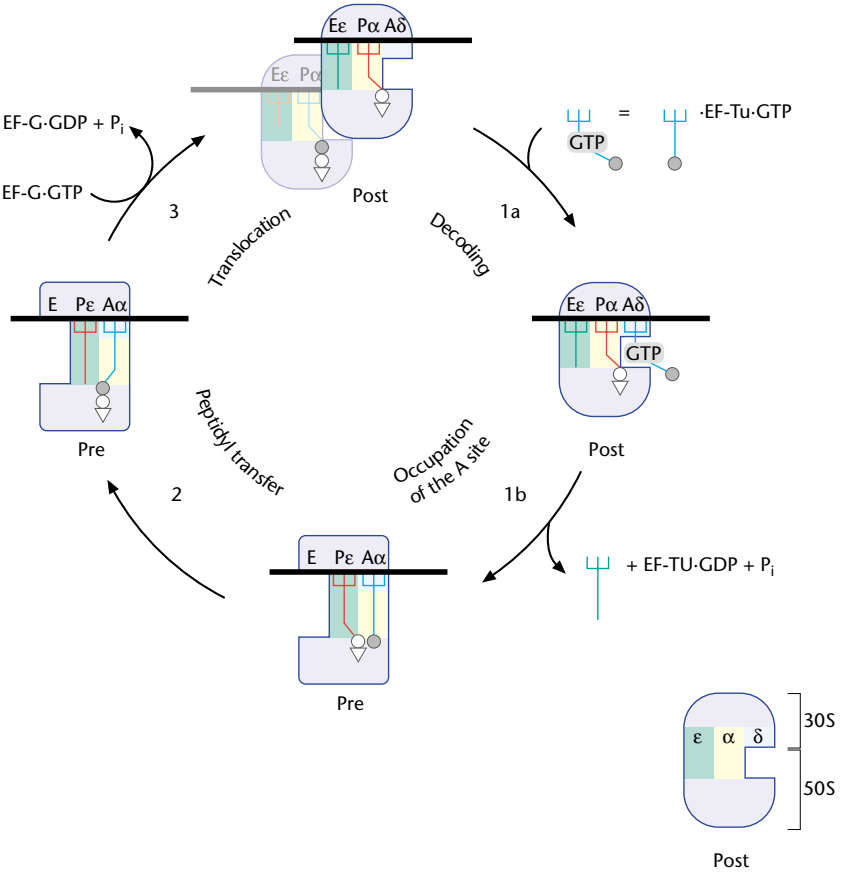

Figure 4 The α-ε model. The occupation of the A region is separated into two reactions, 1a and 1b, similar to the two preceding models. During the decoding process 1a, the anticodon region of the aminoacyl-tRNA within the ternary complex interacts with the decoding centre δ leaving the ribosome in the post state. At this time three tRNAs are bound to the ribosome. When the decoding process has recognized a cognate ternary complex, the δ centre might tightly fix the freshly established codon–anticodon interaction. This induces a conformational change of the ribosome, thus allowing the α-ε domain to come off the tRNAs at the P and E sites and shift back to the tRNAs at the A and P sites (reaction 1b). The tRNA at the E site has lost its binding site ε and falls off the ribosome, thus explaining the well-documented reciprocal relationship between A and E sites. The next basic reaction is peptide bond formation (reaction 2) followed by the EF-G-dependent translocation (reaction 3), which occurs by moving the ribosomal α-ε domain together with the tRNAs and the mRNA, which remain tightly bound before, during and after the translocation reaction to the α-ε domain.

complex into two steps does not simplify nor solve the problem and does not change the conceptual difficulties of the allosteric three-site model in explaining the translocation reaction, although the concept of the tRNA-binding site differs drastically in the two models. The models do not necessarily contradict each other, they are based on different experimental approaches. The allosteric three-site model was derived from functional studies and the hybrid site model from a structural analysis of various functional complexes.

The α-ε model introduces a new concept of translocation in that a movable domain of the ribosome is thought to bind two tRNAs tightly and to hold them before, during and after translocation. It is this domain, the α-ε domain, that transports both tRNAs from the A and P sites to the P and E sites, thus making possible a fine tuning of the movement of the $(tRNA)_2$–mRNA complex. This model predicts a reciprocal linkage between A and E sites but there is no allostery involved, as in the allosteric three-site model (see preceding section).

A distinct feature of the α-ε model is that the mutual arrangement of the two tRNAs does not change drastically during translocation. The two tRNAs move simultaneously on the two subunits in a coordinated fashion, rather than independently on the two subunits as predicted by the hybrid site model. It follows that the α-ε model cannot be reconciled with the hybrid site model.

An unchanged mutual arrangement of the two tRNAs on elongating ribosomes was inferred from a neutron-scattering analysis of the tRNA positions (Nierhaus *et al.*,

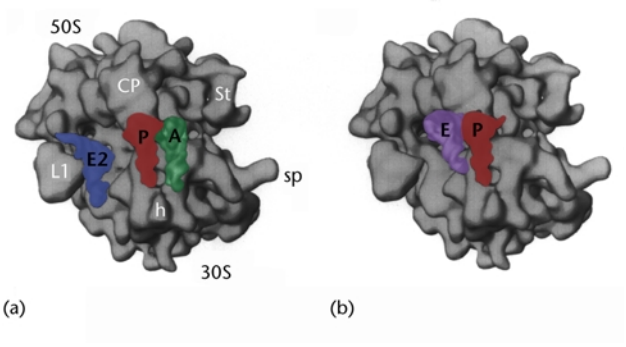

Figure 5 tRNAs within the ribosome before (a) and after translocation (b) as observed by cryoelectron microscopy. The top of a transparent 70S ribosome is viewed, with the 30S ribosomal subunit in front (h, head; s, spore) and the 50S subunit at the back (L1, L1 protuberance; CP, central protuberance; St, L7/L12 stalk). If the ribosome was not transparent, the 30S head and the central protuberance of the 50S subunit would cover the tRNAs at the A and P sites almost completely. The E2 position does not mark a stable and defined binding site, but rather represents a transient position the tRNA is passing by after it has left the E site. Design by Amy Heagle and Rajendra K Agrawal; modified according to Agrawal RK, Penczek P, Malhotra A *et al.* (1998) *Proceedings of the 14th International Congress of Electron Microscopy* **1**: 717–718.

1998). Although first studies applying cryoelectron microscopy seemed to contradict the neutron-scattering findings, a recent analysis also confirmed that both tRNAs barely change their mutual arrangement during translocation (**Figure 5**). The critical point of the preparation of the functional complexes was that a polyamine buffer was used with ion concentrations near to the corresponding values found *in vivo*. An essential outcome is that peptidyl-tRNA and aminoacyl-tRNA were found at the same position when bound to the A site and deacylated tRNA and peptidyl-tRNA resided at an equivalent position when bound to the P site. A hybrid site was not observed on elongating ribosomes.

The outlined mechanism of translocation gives also clues as to why tRNAs adopt the universal L-shape and why protein synthesis requires three tRNA-binding sites. The tight binding of the two tRNAs to the α-ε domain of the ribosome is indicated by an extensive contact pattern of both tRNAs (Dabrowski *et al.*, 1998) that contrasts to the poor contacts observed with mRNA (Alexeeva *et al.*, 1996), i.e. the mRNA is not held by the ribosome but fixed by the two adjacent codon–anticodon interactions that are essential and responsible for the movement of the mRNA during translocation by one codon. It follows that the maintenance of the adjacent codon–anticodon interactions before, during and after translocation plays a key role in keeping the reading frame during protein synthesis. Note that two adjacent codon–anticodon interactions impose a steric problem: the codons have a length of about 1 nm, whereas the diameter of a tRNA is 2 nm. The strict requirement of two adjacent codon–anticodon interactions for moving the mRNA probably represents the selection pressure for developing the universal L-shape of the tRNAs, which solves the steric problem. The L-shape enables two tRNAs to contact adjacent codons and simultaneously to be in contact at a site 7.5 nm away from the anticodon at the peptidyltransferase centre. Furthermore, the requirement to bind two tRNAs tightly to the movable domain during the translocation reaction defines the essential character of three tRNA-binding sites as found universally on all ribosomes.

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