Chapter 5
A Ribosomal Perspective on the Mechanism of Selenocysteine Incorporation

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Abstract Selenocysteine (Sec) is cotranslationally inserted into polypeptides during the elongation phase of protein synthesis in response to specific UGA codons. As UGA normally signals translation termination, the Sec incorporation complex is required to modify the canonical translation machinery. Thus, a thorough understanding of the Sec incorporation mechanism necessitates careful consideration of the intricacies of general translation, specifically during the elongation phase. Here, we consider the current body of evidence that supports a key role for the ribosome in regulating the process of Sec incorporation.

5.1 Introduction

Selenocysteine (Sec) incorporation is accomplished by the action of at least two *trans*-acting factors: SECIS-binding protein-2 (SBP2) and the Sec-specific elongation factor (eEFSec; see Chap. 3). These two factors convert a translation termination reaction into an elongation reaction by changing the coding potential of UGA codons found upstream of SECIS elements. Interestingly, SBP2 is known to bind to the ribosome both in cells and in vitro [1–4], suggesting that it is providing a signal to the ribosomes that Sec codons should be bound by the eEFSec ternary complex (eEFSec/Sec-tRNA^{Sec}/GTP) rather than the translation termination complex. Although Sec incorporation is in direct competition with translation termination, this chapter focuses entirely on the elongation phase of translation. This is because Sec codons are fully competent for translation termination even in the presence of a full complement of Sec incorporation factors [1]. Thus, termination appears to occur as a default reaction when Sec incorporation is not possible or occurs at a reduced efficiency.

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Ribosomes contain three tRNA-binding sites formed by both the large and small subunit, referred to as the A, P, and E sites. The A site binds aminoacyl-tRNA (aa-tRNA), the P site binds peptidyl-tRNA (and a unique initiator tRNA), and the E (Exit)-site binds deacylated tRNA prior to its departure from the ribosome. In addition, protein synthesis requires numerous *cis*-elements and *trans*-acting factors that work in concert with the ribosome and tRNA molecules to efficiently and faithfully decode the mRNA in three phases: initiation, elongation, and termination.

The bulk of protein synthesis takes place during the elongation phase. Two elongation factors sequentially bind the ribosome and utilize the energy of GTP hydrolysis to catalyze two major reactions: (1) delivery of the aa-tRNA to the ribosome by elongation factor Tu (EF-Tu; eEF1A in eukaryotes), and (2) translocation of the mRNA–tRNA complex by elongation factor G (EF-G; eEF2 in eukaryotes). The latter brings the next codon to the ribosomal A site allowing the cycle to repeat until a termination signal is encountered. Here, we dissect the molecular events in the elongation phase and put them into the context of the requirements for successful incorporation of selenocysteine. Since most mechanistic studies have been performed in bacteria, we refer to the bacterial elongation factors, EF-Tu (the tRNA carrier protein whose eukaryotic analogue is eEF1A or eEFSec for Sec), EF-Ts (the guanine nucleotide exchange factor [GEF] that is required for maintaining the GTP-bound state of EF-Tu), and EF-G (the translocase whose eukaryotic analogue is eEF2).

5.2 Initial Binding

The elongation cycle begins following translation initiation with the initiator tRNA in the P site, and an empty A site. The aa-tRNA is delivered to the ribosome as a ternary complex with EF-Tu and GTP. The very first event that is thought to occur is referred to as initial binding, which is a rapid and transient interaction between the ternary complex and the ribosome. This interaction is codon-independent and may function to recruit the ternary complex to the ribosomal A site [5, 6]. Even though this event is codon-independent, it is likely that eEFSec is normally excluded from initial binding as this would likely inhibit normal ternary complex binding. Thus, this step may represent the first barrier that must be overcome when switching from canonical elongation to one that is likely mediated by the SBP2/SECIS interaction. Since SBP2 has also been shown to form a stable SBP2/SECIS/eEFSec complex, it is possible that the function of this complex is distinct from one that may regulate ribosome conformation as discussed below.

Initial binding is believed to involve a protein–protein interaction between EF-Tu and one of the L7/L12 ribosomal stalk proteins (hereafter L12). Mutagenic studies coupled with kinetic analysis of this initial binding event suggests that it involves an interaction between helix D in the G-domain of EF-Tu (Domain I) and helices 4/5 of the C-terminal domain of L12 [7]. Interestingly, EF-Tu also uses Helix D to interact with the N-terminal domain of its GEF EF-Ts, and it has been proposed that the EF-Tu/L12 interaction resembles that of the EF-Tu/EF-Ts complex [7]. Interestingly,

the Sec-specific elongation factors, eEFSec and SelB, have several deletions that correspond to regions in EF-Tu that are involved in its interaction with EF-Ts. Not only does this suggest the lack of a GEF for eEFSec and SelB, but it also raises the intriguing possibility that these deletions interfere with initial binding by disrupting the interaction between the Sec-specific elongation factors and the ribosomal stalk proteins. A potential function for SBP2 on the ribosome then would be to alter the conformation of the L12 stalk to allow recruitment of the eEFSec ternary complex to the ribosomal A site, or alternatively to alter the conformation of eEFSec so it can interact with the L12 stalk. This may, in fact, be more likely because in this way SBP2 won't interfere with eEF1A TC binding.

In eukaryotes, the L12 stalk is replaced by an analogous complex that consists of the phosphoproteins (P-proteins) P1, P2 (L12), and P0 (L10) [8]. Although the P-proteins do not share sequence homology with L12, the eukaryotic factors are functionally equivalent. The functional significance of the P-proteins in specifying the recruitment of the eukaryotic elongation factors, eEF1A and eEF2, was demonstrated by exchanging the bacterial L12 stalk proteins for the eukaryotic counterparts. This replacement conferred a functional interaction between prokaryotic ribosomes and eukaryotic elongation factors demonstrating the importance of the stalk proteins for achieving specificity across these two domains [9]. This study suggests that initial binding is conserved, but a codon-independent interaction has not been reported in eukaryotes. In addition, it should be noted that the molecular basis for the interaction between eEF1A and the eukaryotic ribosome has not been characterized; hence, there is no experimental evidence to suggest that the interaction between eEF1A and the eukaryotic stalk proteins is mediated through helix D in the G-domain of eEF1A as has been proposed for EF-Tu.

L12 is also important for GTPase activation of EF-Tu following codon recognition. Along with L11, which binds H43–44 at the base of the L12 stalk, this region is referred to as the GTPase-associated center (GAC) [10]. Indeed, ribosomes lacking the L12 stalk proteins display a ~1,000-fold decrease in the rates of ribosome-stimulated GTP hydrolysis of EF-Tu [11]. In bacteria, the SECIS element is required to stimulate the ribosome-dependent GTPase activity of SelB, supporting the idea that it is in a conformation that is unable to functionally interact with L12 in the absence of the SECIS [12]. It would be interesting to see if SBP2 could similarly stimulate the latent GTPase activity in eEFSec through its interaction with the ribosome.

5.3 Codon Recognition: Kinetic Proofreading and Induced Fit

Initial binding is followed by codon recognition, which occurs in two discriminatory steps and is driven by two distinct mechanisms: kinetic proofreading and induced fit [13]. During kinetic proofreading, the aa-tRNAs are selected on the basis of anticodon–codon complementarity as dictated by the rules of Watson–Crick base pairing. As such, cognate tRNA will bind the A site with the highest affinity, while

non-cognate tRNAs on the other side of the spectrum cannot bind and are rapidly rejected. Near-cognate tRNAs can associate with the A site as well as cognate tRNAs, but exhibit a substantial increase in their dissociation rates [14]. Codon recognition occurs in an initial selection step following initial binding of the ternary complex, and then again following GTP hydrolysis and dissociation of the aa-tRNA from EF-Tu in a separate proofreading step [15].

Aside from this thermodynamic discrimination, ribosomes also actively participate in the selection process via a defined set of conformational changes that lead to the acceleration of two rate-limiting steps in the tRNA selection pathway: (1) GTPase activation followed by rapid GTP hydrolysis, and (2) accommodation of the tRNA into the peptidyl transferase center (PTC) followed rapidly by peptide bond formation. These conformational changes on the ribosome reflect an induced fit mechanism triggered in the presence of cognate tRNA. The crystal structure of the small ribosomal subunit programmed with cognate or near-cognate tRNA fragments called anticodon stem loops (ASLs) confirmed these earlier observations that suggested an induced fit mechanism in response to cognate tRNA [16–18]. In the presence of a cognate ASL, residues A1492, A1493 in the decoding center of the ribosome were completely flipped such that they could directly engage the codon–anticodon duplex and monitor its geometry. The small subunit was also observed to undergo a global conformational change referred to as domain closure. During domain closure, the shoulder and head domains of the small subunit rotate toward the decoding center. This reconfiguration allows nucleotide G530 in helix 18 of the shoulder domain to rotate from a *syn*- to an *anti*-conformation such that it can also interact with and monitor the codon–anticodon mini-helix. While these X-ray crystal structures were performed using ASLs and the small ribosomal subunit in isolation, the most recent crystal structure of the 70S ribosome complexed with EF-Tu ternary complex has corroborated these initial findings [19]. Since most eukaryotic ribosomes evolved to support termination factor accommodation at UGA codons, the question arises whether the ribosomal conformation changes that occur during canonical codon recognition also occur during Sec incorporation. The fact that codon/anticodon pairing is not sufficient to fully explain translational fidelity suggests that there may be unique conformational changes required to change the identity of a stop codon that may not be intrinsic to the ribosome and thus relegated to the functions of SBP2 or eEFSec or even through the Sec-tRNA^{Sec} itself as discussed below.

5.4 Communication Between Functional Centers

As mentioned above, cognate codon–anticodon interactions in the decoding center lead to an increase in the rate of GTP hydrolysis. This indicates that the information in the decoding center has to be reported to the GAC of the ribosome to activate the elongation factor's latent GTPase activity. The global domain closure induced upon binding of the cognate tRNA suggested that information in the decoding center was being transmitted to the GAC through the intersubunit bridges [17]. At odds with

this hypothesis, however, was an earlier study showing that two tRNA fragments corresponding to the ASL/D-arm and acceptor end/T-arm were incapable of stimulating GTP hydrolysis even in the presence of paromomycin [20], despite the fact that paromomycin was shown to induce the domain closure of the small subunit in the presence of both cognate and near-cognate ASL fragments [17] and to stimulate GTP hydrolysis for cognate and near-cognate tRNA [21]. The requirement for an intact tRNA suggests that the signal from the decoding center is propagated through the tRNA body instead of the intersubunit bridges.

In 1971, a $tRNA^{Trp}$ mutant with a G24A substitution in the D-arm was identified and named the Hirsh suppressor [22]. The Hirsh suppressor is near-cognate with respect to the UGA codon, but is somehow capable of evading rejection during codon recognition and thus functions as a UGA suppressor. Direct evidence in favor of signal propagation through the tRNA was revealed by kinetic studies demonstrating that the Hirsh suppressor was capable of accelerating the rate of GTP hydrolysis and peptide bond formation even when ribosomes were programmed with a near-cognate codon [23]. This indicates that the Hirsh suppressor tRNA can adopt the conformation normally induced by cognate tRNA binding and stabilized by domain closure. However, in the absence of structural data, we cannot rule out that the Hirsh suppressor is somehow inducing base flipping and domain closure on its own without the need for a cognate codon–anticodon interaction. If this is true, it would suggest that you need both the conformational changes (base flipping and domain closure) that may be contributing to this signal propagation through the intersubunit bridges as well as, an intact tRNA. In support of this, it was recently demonstrated that the Hirsh suppressor was unable to enhance GTP hydrolysis and peptide bond formation when the residues in the decoding center (A1492, A1493, and G530) were mutated indicating that this suppressor has not completely bypassed the molecular events that canonically lead to transmission of this signal [24]. These studies open up the distinct possibility that the Sec-$tRNA^{Sec}$ plays an active role in determining the efficiency of the Sec incorporation reaction, perhaps ultimately providing the molecular explanation for differential utilization of the Sec-$tRNA^{Sec}$ isoforms (see Chap. 44).

5.5 Proofreading

Following GTP hydrolysis, domain rearrangements within EF-Tu result in release of the aa-tRNA from the ternary complex, thereby freeing the 3′ $^{74}CCA^{76}$ acceptor end (CCA-end) containing the amino acid [25, 26]. Upon dissociation from EF-Tu, the codon–anticodon base pair in the decoding center is interrogated once again in a second discriminatory step called proofreading [15]. Herein, near-cognate tRNAs that get past initial selection are rapidly rejected while cognate tRNAs become fully accommodated in the ribosomal A site and make stabilizing contacts with the PTC. Accommodation is followed rapidly by peptide bond formation [27]. Cognate tRNAs accelerate the rate of accommodation, and thus proofreading, like initial selection, is also believed to operate through an induced fit mechanism.

5.6 tRNA Accommodation and Peptide Bond Formation

Peptide bond formation takes place in the PTC, the catalytic active site of the ribosome [28]. The PTC is located in a cleft below the central protuberance of the ribosome where it spans across the large subunit portion of the A and P site. This region is composed almost entirely of RNA from the central loop (C-loop) of domain V and the helices that protrude from it. The boundaries of the PTC are formed by two distinct regions referred to as the A and P-loop [29]. Directly below the PTC is the entrance to the ribosomal exit tunnel where the nascent polypeptide passes as the elongating ribosome translates the mRNA. The peptidyl tRNA is stabilized by interactions with the P-loop, which include a Watson–Crick base pair between C74 and G2251, and C75 and G2252 (*Escherichia coli* numbering used throughout unless indicated). In addition, the terminal A76 forms a stacking interaction with the ribose of A2451, and hydrogen bonds with the 2′ OH of A2450. When the A site is empty, the PTC is in an "un-induced state"; in this state both sides of the peptidyl ester group are sequestered by nucleotides U2585, C2063, and A2451, thus protecting it from nucleophilic attack [30]. Binding of aa-tRNA to the PTC results in conformational changes that are required to properly align the tRNA substrates to allow the peptidyl transferase reaction. This substrate-induced fit mechanism involves a shift of U2506 to prevent a steric clash with the amino acid moiety of the aa-tRNA, and the movement of nucleotides A2602, G2583, U2584, and U2585 which swings 90° away from the P site and exposes the peptidyl ester [31].

The CCA-end of the aa-tRNA is stabilized by several interactions with the A-loop [28]. These include stacking interactions between C74 and U2555, a Watson–Crick base pair between C75 and G2553, and a type I A-minor interaction between the terminal A76 and G2583. In addition to the protections resulting from direct contacts between the tRNA and the PTC, chemical probing studies have also revealed nucleotides whose reactivity changes due to allosteric effects [32]. Interestingly, when comparing aa-tRNA to deacylated tRNA bound to the ribosomal A site, three nucleotides (A2451, A2439, and A2602) in domain V showed altered chemical reactivities. These results indicate that the amino acid moiety can affect the confirmation of the PTC. Interestingly, while C74 is critical for the orientation of the tRNA substrates and for inducing the aforementioned conformational changes that expose the peptidyl ester to nucleophilic attack, the amino acid moiety is also thought to play an important role in shifting the equilibrium toward the induced state [30].

As stated above, the inability of the eEFSec ternary complex to decode the UGA codon in the absence of SBP2 suggests that it does not have direct access to the ribosomal A site. Another putative function for SBP2 on the ribosome may involve conformational changes in the PTC so that binding of the Sec-tRNA^{Sec} is enhanced, thus allowing this unique aa-tRNA to accommodate and take part in peptide bond formation. Alternatively, SBP2 can modify the position of the peptidyl tRNA in the P site relative to the Sec-tRNA^{Sec} in the A site.

5.7 The Role of the Esterified Amino Acid in tRNA Selection

tRNAs vary with respect to their nucleotide sequence, posttranscriptional modifications, and the amino acid they are esterified with. Each amino acid contains a particular functional group that imparts unique chemical properties to their cognate tRNA. Despite this molecular diversity, tRNAs are able to uniformly bind the ribosome suggesting that they are functionally equivalent substrates [33], raising the question of how the ribosome may deal with the unique chemistry of the Sec residue. During initial selection, the amino acid is masked by the elongation factor, but following GTP hydrolysis the amino acid becomes exposed to the ribosomal A site as the aa-tRNA becomes accommodated in the PTC. At this moment, the amino acid could affect tRNA binding and peptide bond formation. Moreover, as the polypeptide chain is extended, the amino acids gradually move through the nascent peptide exit tunnel. Molecular dynamic simulations of the ribosomal exit tunnel using different amino acid side chains reveal binding crevices and suggest that the tunnel is capable of interacting differently with various amino acids [34]. Indeed, a specific peptide motif in the secretory monitor protein, SecM, has been shown to stall the ribosome through interactions with rRNA residues in the exit tunnel [35].

Early studies comparing the binding affinities of deacylated and aa-tRNAs in the absence of elongation factor suggested that the amino acid was an important contributor in achieving uniform binding to the ribosome [36]. While certain tRNAs such as Gly-tRNA^{Gly} bound equally well whether it was amino-acylated or deacylated, other tRNAs varied by as much as two orders of magnitude. When in vitro transcribed tRNAs lacking their posttranscriptional modifications were compared to their native counterparts, they displayed substantially reduced binding to both the A and P sites of the ribosome. In addition, elements within the tRNA body were recently identified as being important in tuning the tRNA [37]. These findings suggest that the various tRNA molecules have evolved with unique features that function in concert to achieve uniformity of binding.

Given the importance of codon recognition in tRNA selection by accelerating the rate-limiting steps (GTPase activation and accommodation) through induced fit mechanisms, it has been informative to assay whether misacylated tRNAs affect GTP hydrolysis and the end point of peptide bond formation. Effraim et al. [38] used smFRET to follow the dynamics of misacylated tRNAs in real time through the various stages of the tRNA selection pathway. In addition, they measured dipeptide formation in the presence or absence of competitor tRNAs. Using a recently engineered tRNA aminoacylation ribozyme capable of accepting various aminoacyl and tRNA substrates, they engineered six tRNAs by mixing tRNA^{Phe}, tRNA^{Ala}, and tRNA^{Lys} (i.e., Ala-tRNA^{Phe}, Lys-tRNA^{Phe}, etc.). Surprisingly, misacylated tRNA resulted in dipeptide yields similar to that observed with the correctly acylated native tRNA substrates. However, when assayed under more stringent conditions (in the presence of competitor native tRNA substrate), misacylated tRNAs exhibited a 2–4 fold decrease in dipeptide formation, clearly demonstrating that misacylated tRNAs are indeed selected by the ribosome at lower efficiencies. smFRET studies showed

that the rate of codon recognition/GTP hydrolysis and accommodation/peptide bond formation for the misacylated tRNA was unaffected. Instead, the decrease observed in the competition assay was attributed to an increase in A site sampling events prior to codon recognition. This result is surprising because during A site sampling, the amino acid moiety is buried in a pocket on the elongation factor.

Future studies using a wider range of misacylated and native tRNA substrates will no doubt provide a greater understanding of the role that the amino acid plays during the tRNA selection process. This is a key area of research for the selenocysteine field as it seems highly likely that special accommodation of Sec is required for efficient and processive incorporation of this highly reactive amino acid. This will, of course, require the development of a completely reconstituted Sec incorporation system as described in Chap. 3.

5.8 Pretranslocation State Ribosomes Recruit EF-G

Following tRNA delivery and peptide bond formation, the ribosome undergoes a conformational transition from the posttranslocation state (POST) to the pretranslocation state (PRE). POST state ribosomes contain peptidyl tRNA in the P site and an empty A site, while PRE state ribosomes are characterized by occupation of the A site with peptidyl-tRNA and deacylated tRNA in the P site. This transition represents the beginning of the third major catalytic event that occurs during the translation elongation cycle – translocation of the tRNA–mRNA complex (3). Although this step may seem downstream of the Sec incorporation event, in fact the event does not end until the uniquely large $tRNA^{Sec}$ is released at the E site. Indeed, reduced rates of translocation during Sec incorporation may explain the observation that selenoprotein mRNAs are associated with lighter polysomes than control mRNAs of the same length [39, 40]. Translocation results in movement of the peptidyl tRNA from the A site to the P site, and the simultaneous movement of the deacylated tRNA from the P site to the E site. This movement of the tRNAs pulls the mRNA in the 5′ direction so that the next codon is positioned in the ribosomal A site thus allowing the cycle to repeat until a termination codon is reached. Translocation is catalyzed by a second translation elongation factor, the GTP-dependent ribosomal translocase, EF-G.

Both EF-G and EF-Tu bind to the elongation factor binding site composed of the SRL on H95, and the GAC on H43–44. A fundamental mechanistic question is how the ribosome distinguishes between these two elongation factors such that they do not interfere with each other during translation? Several structural and biochemical studies have provided insight on key differences between the PRE and POST translocation states of the ribosome that may allow for the sequential recruitment of these factors at the appropriate time.

One major difference between PRE and POST state ribosomes can be seen when comparing the cryo-EM structures of ribosomes trapped in these two functional states (reviewed in [41]). While the SRL appears to be relatively immobile, the GAC switches from an open to a closed conformation. In the PRE state, the GAC is

positioned toward Helix 89 (H89) near the SRL (closed), while in the POST state it shifts away from H89 (open). Consistent with these observations, the insertion of an additional base pair in the stem of H42 below the GAC predicted to constitutively mimic the POST state, reduced the binding, GTPase activity, and translocation activity of EF-G in vitro, whereas EF-Tu binding and function were unaffected [41]. Thus, the conformation of the GAC seems to be an important regulator of this selective binding event.

Yet another piece of the puzzle was uncovered by Zavialov and Ehrenberg when they demonstrated that EF-G binding and activity was controlled by the status of the tRNA in the P site [42]. PRE state ribosomes contain deacylated tRNA in the P site, while POST state ribosomes contain peptidyl-tRNA in the P site. Interestingly, treatment of POST state ribosomes with puromycin was sufficient to restore EF-G binding and ribosome-dependent GTPase activity. Puromycin is an aa-tRNA mimetic and thus functions as a substrate in peptide bond formation. However, unlike the situation in vivo where the peptide is transferred to the aa-tRNA and remains in the A site, when the peptide is transferred to puromycin it dissociates from the ribosome leaving a deacylated tRNA in the P site and an empty A site.

Cryo-EM analysis of these puromycin-treated POST state ribosomes showed that not only were these ribosomes competent for EF-G binding, but they also exhibited the same conformational flexibility exhibited by PRE state ribosomes [43]. Thus, the presence of peptidyl-tRNA in the P site locks the ribosome such that it is conformationally constrained. The mechanism that leads to this locked state at present remains unknown, but the enhanced stability of the POST state ribosome may be required to facilitate delivery of the aa-tRNA by EF-Tu. Despite the nonphysiological nature of this puromycin-treated POST state ribosome, these results imply that the removal of the peptide from the P site tRNA during peptide bond formation unlocks the ribosome, and this unlocking is an apparent prerequisite for stable EF-G binding and function. Thus the key question for Sec incorporation is whether the heretofore unexplored interplay between eEFSec and eEF2 is sufficient to promote the PRE/POST transition or does this require the function of an additional factor.

5.9 Intermediate States During Translocation

Removal of the peptide from the P site tRNA during peptidyl transfer is required to unlock the ribosome into a flexible conformation that confers EF-G binding. Early evidence for a conformational change on the ribosome following peptidyl transfer was reported by the Noller group [32]. Chemical probing of PRE state ribosomes in the absence of EF-G indicated that deacylated tRNA in the P site, and peptidyl-tRNA in the A site could spontaneously sample hybrid states. In the classical configuration, the tRNA remains completely bound to the P site (P/P site) or the A site (A/A site). In the hybrid state, the ASL of the P and A site tRNAs remains bound to the small subunit, while the CCA-ends shift and interact with the adjacent E and P sites, respectively. Single molecule studies using fluorescence-labeled tRNA molecules added to surface immobilized ribosomes support the idea that the tRNA molecules

are dynamic and fluctuate between a classical and a hybrid state [44]. Kinetic studies using mutant tRNAs and mutant rRNAs shown to destabilize the classic state, and therefore favor hybrid state formation, result in increased rates of EF-G stimulated translocation indicating that hybrid state formation is functionally relevant [45].

Cryo-EM analysis of PRE state ribosomal complexes using near physiological concentrations of magnesium (3.5 mM) suggests that PRE state ribosomes populate two macro states in the absence of EF-G: macro state I (MSI) and macro state II (MSII) [46]. In addition to the transition from the classical (A/A and P/P) to the hybrid (A/P, P/E) tRNA-binding states, MSII is also characterized by a counter-clockwise rotation (ratcheting) of the small subunit relative to the large subunit when visualized from the solvent side, and a conformational rearrangement of the dynamic L1 stalk.

The L1 stalk is located ~100Å from the PTC in *E. coli* ribosomes, and is composed of ribosomal protein L1 and its rRNA-binding site formed by H76–78 on the large subunit. Ribosomes devoid of L1 become trapped in the classic tRNA-binding state and exhibit a reduced rate of protein synthesis [47, 48]. During the MSI to MSII transition, the L1 stalk exchanges between an open position extended away from the subunit interface, to a closed position where it folds inward toward the E site and makes contacts with the deacylated tRNA in the hybrid P/E state. Following translocation, the L1 stalk maintains its contacts with the deacylated tRNA in the E/E site in what has been described as a half-open conformation [49, 50]. These results implicate the L1 stalk in the translocation mechanism and additionally in the removal of deacylated tRNA from the E site. The modulation of the L1 stalk induced upon peptide bond formation or unlocking of the ribosome exemplifies the capacity of the ribosome to communicate across large distances using allosteric networks. This fact is even more remarkable when considering that EF-G, which binds to the factor-binding site at the base of the L12 stalk nearly 170 Å away, can allosterically regulate the L1 stalk. In POST state ribosomes, the L1 stalk is in an open conformation making it accessible to *trans*-factors that can bind and potentially modulate the translation elongation cycle [51]. This example of allostery is an attractive model for the potential function of SBP2 on the ribosome. Although its binding site has not been determined, it is likely not to be involved in stable interactions at the factor-binding site or GAC as this would interfere with canonical translation. Thus in a fashion similar to the communication between the L1 stalk and EF-G, it is possible that SBP2 signals to the functional centers of the ribosome from a distant-binding site.

5.10 Conclusion

The molecular mechanisms that drive the Sec incorporation reaction remain undetermined. In order to fully understand how a unique set of Sec-specific factors are able to modify something as complex and efficient as the molecular machine responsible for protein synthesis, future experiments should be designed in the context of the tremendous body of work that has deciphered the molecular events leading to peptide bond formation during the elongation phase of translation.

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References

1. Copeland PR, Stepanik VA, Driscoll DM (2001) Mol Cell Biol 21:1491
2. Caban K, Kinzy SA, Copeland PR (2007) Mol Cell Biol 27:6350
3. Takeuchi A, Schmitt D, Chapple C et al (2009) Nucleic Acids Res 37:2126
4. Papp LV, Lu J, Striebel F et al (2006) Mol Cell Biol 26:4895
5. Rodnina MV, Fricke R, Wintermeyer W (1994) Biochemistry 33:12267
6. Rodnina MV, Pape T, Fricke R, Kuhn L, Wintermeyer W (1996) J Biol Chem 271:646
7. Kothe U, Wieden HJ, Mohr D et al (2004) J Mol Biol 336:1011
8. Gonzalo P, Reboud JP (2003) Biol Cell 95:179
9. Uchiumi T, Hori K, Nomura T, Hachimori A (1999) J Biol Chem 274:27578
10. Kavran JM, Steitz TA (2007) J Mol Biol 371:1047
11. Diaconu M, Kothe U, Schlünzen F et al (2005) Cell 121:991
12. Hüttenhofer A, Böck A (1998) Biochemistry 37:885
13. Pape T, Wintermeyer W, Rodnina M (1999) EMBO J 18:3800
14. Gromadski KB, Rodnina MV (2004) Mol Cell 13:191
15. Thompson RC, Stone PJ (1977) Proc Natl Acad Sci USA 74:198
16. Ogle JM, Brodersen DE, Clemons WM et al (2001) Science 292:897
17. Ogle JM, Murphy FV, Tarry MJ et al (2002) Cell 111:721
18. Ogle JM, Carter AP, Ramakrishnan V (2003) Trends Biochem Sci 28:259
19. Schmeing TM, Voorhees RM, Kelley AC et al (2009) Science 326:688
20. Piepenburg O, Pape T, Pleiss JA et al (2000) Biochemistry 39:1734
21. Pape T, Wintermeyer W, Rodnina MV (2000) Nat Struct Biol 7:104
22. Hirsh D, Gold L (1971) J Mol Biol 58:459
23. Cochella L, Green R (2005) Science 308:1178
24. Youngman EM, He SL, Nikstad LJ et al (2007) Mol Cell 28:533
25. Abel K, Yoder MD, Hilgenfeld R et al (1996) Structure 4:1153
26. Polekhina G, Thirup S, Kjeldgaard M et al (1996) Structure 4:1141
27. Pape T, Wintermeyer W, Rodnina MV (1998) EMBO J 17:7490
28. Nissen P, Hansen J, Ban N et al (2000) Science 289:920
29. Green R, Switzer C, Noller HF (1998) Science 280:286
30. Schmeing TM, Huang KS, Strobel SA et al (2005) Nature 438:520
31. Voorhees RM, Weixlbaumer A, Loakes D et al (2009) Nat Struct Mol Biol 16:528
32. Moazed D, Noller HF (1989) Nature 342:142
33. Ledoux S, Uhlenbeck OC (2008) Mol Cell 31:114
34. Petrone PM, Snow CD, Lucent D et al (2008) Proc Natl Acad Sci USA 105:16549
35. Nakatogawa H, Ito K (2002) Cell 108:629
36. Fahlman RP, Dale T, Uhlenbeck OC (2004) Mol Cell 16:799
37. Olejniczak M, Dale T, Fahlman RP et al (2005) Nat Struct Mol Biol 12:788
38. Effraim PR, Wang J, Englander MT et al (2009) Nat Chem Biol 5:947
39. Fletcher JE, Copeland PR, Driscoll DM (2000) RNA 6:1573
40. Martin GW, Berry MJ (2001) Genes Cells 6:121
41. Sergiev PV, Kiparisov SV, Burakovsky DE et al (2005) J Mol Biol 353:116
42. Zavialov AV, Ehrenberg M (2003) Cell 114:113
43. Valle M, Zavialov A, Li W et al (2003) Nat Struct Biol 10:899
44. Blanchard SC, Kim HD, Gonzalez RL et al (2004) Proc Natl Acad Sci USA 101:12893
45. Dorner S, Brunelle JL, Sharma D et al (2006) Nat Struct Mol Biol 13:234
46. Agirrezabala X, Lei J, Brunelle JL et al (2008) Mol Cell 32:190

47. Munro JB, Altman RB, O'Connor N et al (2007) Mol Cell 25:505
48. Subramanian AR, Dabbs ER (1980) Eur J Biochem 112:425
49. Fei J, Kosuri P, MacDougall DD, Gonzalez RL (2008) Mol Cell 30:348
50. Cornish PV, Ermolenko DN, Staple DW et al (2009) Proc Natl Acad Sci USA 106:2571
51. Munro JB, Altman RB, Tung CS et al (2010) EMBO J 29:770