The pathway to GTPase activation of elongation factor SelB on the ribosome

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In all domains of life, selenocysteine (Sec) is delivered to the ribosome by selenocysteine-specific tRNA (tRNAsec) with the help of a specialized translation factor, SelB in bacteria. Sec–tRNAsec recodes a UGA stop codon next to a downstream mRNA stem–loop. Here we present the structures of six intermediates on the pathway of UGA recoding in Escherichia coli by single-particle cryo–electron microscopy. The structures explain the specificity of Sec–tRNAsec binding by SelB and show large–scale rearrangements of Sec–tRNAsec. Upon initial binding of SelB–Sec–tRNAsec to the ribosome and codon reading, the 3OS subunit adopts an open conformation with Sec–tRNAsec covering the sarcin–ricin loop (SRL) on the 3OS subunit. Subsequent codon recognition results in a local closure of the decoding site, which moves Sec–tRNAsec away from the SRL and triggers a global closure of the 3OS subunit shoulder domain. As a consequence, SelB docks on the SRL, activating the GTPase of SelB. These results reveal how codon recognition triggers GTPase activation in translational GTPases.

Visualizing intermediates of UGA recoding

We reconstituted recoding complexes in vitro with fMet–tRNAMet in the peptidyl site and SelB–Sec–tRNAsec stalled by the guanosine triphosphate analogue GDPNP on the aminoacyl site of the ribosome. As a model mRNA directing Sec incorporation, we used a construct with a UGA stop codon next to a downstream mRNA stem–loop. Here we use single-particle cryo-electron microscopy (cryo-EM) and extensive sorting of cryo-EM images to obtain snapshots of intermediates along the pathway of Sec–tRNAsec delivery to the ribosome. The cryo-EM structures, in combination with ensemble rapid kinetics and all-atom explicit-solvent molecular dynamics simulations, provide detailed insights into the mechanism of UGA recoding by Sec and suggest a universal mechanism for GTPase activation on the ribosome.

Recognition of Sec–tRNAsec

SelB binds Sec–tRNAsec with extraordinary high affinity and discriminates against Ser–tRNAsec, which is an on-pathway intermediate of Sec–tRNAsec biosynthesis4,17, and all other aa-tRNAs. Conversely, EF-Tu, which binds all elongator aa-tRNAs with uniform affinity, has very low affinity for Sec–tRNAsec (ref. 17). Comparison of the present SelB–...
The SECIS recruits domain 4 of SelB\(^ {10} \) and may guide the ternary complex towards the ribosome. We constructed a model for an early recruitment complex by docking SelB–Sec-tRNA\(^ {\text{Sec}} \) onto the SECIS of the initial complex (Fig. 2a). In the model, SelB and Sec-tRNA\(^ {\text{Sec}} \) do not interact with the ribosome, but a rotation would move SelB–Sec-tRNA\(^ {\text{Sec}} \) into its position in the initial binding state. SECIS-dependent tethering via domain 4 is SelB-specific, as EF-Tu is recruited by the L7/L12 stalk of the 50S ribosomal subunit\(^ {22} \). In the subsequent states domain 4 serves as a flexible anchor for SelB–Sec-tRNA\(^ {\text{Sec}} \) (Fig. 2b, Extended Data Fig. 4a, b). When SelB moves towards the GTPase-activated state, domain 4 maintains its interactions with the SECIS and the adjacent 30S elements (helix h16 of 16S rRNA (rRNA) and protein S4) and follows mainly the movements of the 30S subunit. These rigid-body motions of domain 4 do not affect the remaining part of SelB, because the highly flexible linker connecting domains 3 and 4 uncouples the two parts.

**Requirements for SelB GTPase activation**

GTP hydrolysis by EF-Tu is a major checkpoint that controls the rate and fidelity of decoding\(^ {23} \). Rapid GTP hydrolysis by EF-Tu is triggered by the correct codon–anticodon interaction\(^ {24} \); the requirements for the GTPase activation in SelB are unknown. In the presence of the correct SECIS and the UGA codon, the rate of GTP hydrolysis in SelB–[\( \gamma \)-\( 32 \)P]GTP–Sec-tRNA\(^ {\text{Sec}} \) on the ribosome is about 3 s\(^ {\text{s}} \), which is four orders of magnitude higher than the intrinsic GTPase activity of the ternary complex in the absence of ribosomes (Fig. 2c, d, Extended Data Fig. 4c–f). Mutation of three key recognition bases in the SECIS\(^ {10,25} \) results in a 600-fold reduction of the GTPase rate, in line with the role of the SECIS as a tether for SelB (see above and refs 10, 26). With a correct SECIS element, but a non-cognate UUC codon in the aminoacyl site, the rate of GTP hydrolysis is also reduced by 600-fold compared to the cognate complex (Fig. 2d), suggesting that recognition of the cognate UGA codon by Sec-tRNA\(^ {\text{Sec}} \) is essential for GTPase activation. Thus, the requirement for the correct codon–anticodon interaction as a trigger for GTP hydrolysis is similar for SelB and EF-Tu. For EF-Tu, the existence of several pre-hydrolysis steps, including initial binding and codon reading, has been demonstrated by ensemble kinetics and single molecule FRET experiments\(^ {27,28} \). The similarities between SelB and EF-Tu as to the reaction pathway and codon-dependence of the GTPase activation suggest a conserved mechanism of decoding and aa-tRNA selection, with GTP hydrolysis being a crucial step between initial selection and proofreading\(^ {24,25,30} \).

**Structural dynamics of the 30S subunit**

Pioneering crystallographic studies on complexes of isolated 30S subunits demonstrated that cognate codon–anticodon complex formation triggers local rearrangements of three universally conserved bases of 16S rRNA in the decoding centre (G530, A1492, and A1493) and a global domain closure in the 30S subunit31,32. Here we describe the rearrangements of the 30S subunit upon UGA codon recognition by Sec-tRNA\(^ {\text{Sec}} \) in the context of the 70S ribosome (Fig. 3, Extended Data Fig. 5).

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**Figure 1 | Structures of intermediates along the pathway of UGA recoding by SelB–Sec-tRNA\(^ {\text{Sec}} \).** a, Overview of the structures. Top row: Schematics of complexes with rRNA binding sites of the ribosome (A, P, E), UGA stop codon; sh, 30S subunit shoulder. Bottom row, cryo-EM reconstructions of distinct intermediates with the resolution (in Å) and the number of particles for each state. IC, initial complex; IB, initial binding; CR, codon reading; GA, GTPase-activated; C, classical tRNA; and H, hybrid tRNA states. b, Structure of SelB–GDPNP–Sec-tRNA\(^ {\text{Sec}} \) and the GDPNP–Sec-tRNA\(^ {\text{Sec}} \) structures with the known crystal structure of EF-Tu–GTP–Cys-tRNA\(^ {\text{Cys}} \) (ref. 11) explains the specificity of tRNA\(^ {\text{Sec}} \) recognition (Fig. 1g, Extended Data Fig. 3). The linker (Leu340–Gln363; *Escherichia coli* numbering used throughout) between domains 3 and 4 of SelB, which is absent in EF-Tu, binds and distorts the extra-long variable arm of tRNA\(^ {\text{Sec}} \) (Extended Data Fig. 3a–c, l). Furthermore, an extended loop in SelB domain 3 (Ile326–Asn330) facilitates interactions with the acceptor- and T-stems of tRNA\(^ {\text{Sec}} \) at the anticodon box that distinguishes tRNA\(^ {\text{Sec}} \)\(^ {\text{Sec}} \) from canonical tRNAs\(^ {18} \). The corresponding loop in EF-Tu adopts a different conformation that allows sequence-specific interactions with canonical tRNAs\(^ {19,20} \), but impedes interactions with tRNA\(^ {\text{Sec}} \)\(^ {\text{Sec}} \) (Extended Data Fig. 3e, f). Finally, the amino-acid-binding pocket of SelB is lined with positively charged residues (Extended Data Fig. 3g–i), allowing SelB to specifically recognize the negatively charged selenol group and to discriminate against Ser-tRNA\(^ {\text{Sec}} \). In EF-Tu the amino-acid-binding pocket is negatively charged; this, together with the low affinity of EF-Tu for the tRNA\(^ {\text{Sec}} \) body\(^ {21} \), explains why EF-Tu does not bind Sec-tRNA\(^ {\text{Sec}} \). Notably, the variable arm of tRNA\(^ {\text{Sec}} \) undergoes a large-scale rotation of about 500° upon release of Sec-tRNA\(^ {\text{Sec}} \) from SelB and accommodation in the aminoacyl site (Extended Data Fig. 3l). This rotation is essential to allow binding of the next aa-tRNA to the aminoacyl site after peptidyl-tRNA\(^ {\text{Sec}} \) translocation to the peptidyl site.

**SelB–specific domain 4**

The SECIS recruits domain 4 of SelB\(^ {10} \) and may guide the ternary complex towards the ribosome. We constructed a model for an early recruitment complex by docking SelB–Sec-tRNA\(^ {\text{Sec}} \) onto the SECIS of the initial complex (Fig. 2a). In the model, SelB and Sec-tRNA\(^ {\text{Sec}} \) do not interact with the ribosome, but a rotation would move SelB–Sec-tRNA\(^ {\text{Sec}} \) into its position in the initial binding state. SECIS-dependent tethering via domain 4 is SelB-specific, as EF-Tu is recruited by the L7/L12 stalk of the 50S ribosomal subunit\(^ {22} \). In the subsequent states domain 4 serves as a flexible anchor for SelB–Sec-tRNA\(^ {\text{Sec}} \) (Fig. 2b, Extended Data Fig. 4a, b). When SelB moves towards the GTPase-activated state, domain 4 maintains its interactions with the SECIS and the adjacent 30S elements (helix h16 of 16S ribosomal RNA (rRNA) and protein S4) and follows mainly the movements of the 30S subunit. These rigid-body motions of domain 4 do not affect the remaining part of SelB, because the highly flexible linker connecting domains 3 and 4 uncouples the two parts.

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and correct SECIS (UGA); error bars, s.e.m. of the exponential fitting with hydrolysis rates as compared to complexes carrying the cognate codon GTP–Sec-tRNASec (ternary complex; TC).

Figure 2 | Roles of SelB domain 4 and the GTPase activation on the ribosome. a, A model for the ribosome-independent recruitment of SelB onto the SECIS guiding SelB–Sec-tRNA Sec to its position in the initial binding state (arrow). b, Interactions of SelB and the SECIS at the mRNA entry site (entry) in the GTPase-activated state marked by dashed contours; the interactions are maintained in the initial binding and codon reading states (data not shown). h16, helix of 16S rRNA; S3–S5, proteins S3 to S5. c, Time courses of GTP hydrolysis by SelB on ribosomes programmed with mRNA comprising a correct SECIS and a cognate UGA codon (UGA), or an incorrect SECIS and a cognate UGA codon (iSECIS), or a correct SECIS, but a non-cognate codon (UUC), and by the free SelB–GTP–Sec-tRNA Sec (ternary complex; TC). d, The reduction in the GTP hydrolysis rates as compared to complexes carrying the cognate codon and correct SECIS (UGA); error bars, s.e.m. of the exponential fitting with \( n = 2 \) for UGA and \( n = 3 \) for iSECIS, UUC and ternary complex.

and show how these changes contribute to the mechanism of GTPase activation of SelB (Fig. 4).

In the initial complex before SelB–GDPNP–Sec-tRNA Sec binding, the 30S subunit is in a non-rotated classical state\(^3\),\(^\text{34}\) (Fig. 3a, Extended Data Fig. 5d). The A1492 and A1493 bases appear to be in dynamic equilibrium between ‘flipped-in’ and ‘flipped-out’ states with respect to h44 of 16S rRNA. G530 is in the anti-conformation which is maintained in all subsequent states. Initial binding of SelB–GDPNP–Sec-tRNA Sec is codon-independent, as evident from the large distance between tRNA Sec anticodon and UGA codon (10 Å, Fig. 3a, Extended Data Fig. 5b). In the initial binding state, the flipped-in conformation of the A1492 and A1493 bases is stabilized and the aminoacyl site is open (Fig. 3a). The 30S shoulder rotates outwards, away from the 50S subunit. This 30S domain opening widens the intersubunit space, which may be important to facilitate initial binding of SelB domain 2 to the 30S shoulder or to prevent premature docking of SelB on the 50S subunit (see below). Further progression of tRNA Sec towards the mRNA codon in the codon reading state may allow the interaction between the third codon base and the anticodon of tRNA Sec, but this state is still incompatible with full codon–anticodon complex formation (Fig. 3a, Extended Data Fig. 5b).

In the GTPase-activated state, the codon is recognized, the codon–anticodon helix adopts the Watson–Crick geometry, and A1492 and A1493 are stabilized in the flipped-out position, which results in a local closure of the aminoacyl site. Together with G530, the adenosines interact with the minor groove of the codon–anticodon duplex at the first and second codon position. At the same time, the 30S subunit undergoes a large-scale domain closure, which mainly involves an inward rotation of the 30S shoulder and decreases the distance spanned by the SelB–GDPNP–Sec-tRNA Sec complex. During all these steps, the universally conserved A1913 in helix 69 (H69) of 23S rRNA interacts with A1492 and A1493 in h44 in a state-specific fashion and acts to stabilize the flipped-in position of the two adenosines in the initial binding and codon reading states (Extended Data Fig. 5a). The closed conformation of the 30S subunit in the GTPase-activated state is practically identical to that observed for the canonical EF-Tu–GDP–Trp-tRNA Trp ternary complex on the ribosome\(^2\). However, compared to the states preceding codon recognition, the rearrangement is more extensive in the present complexes than in those inferred from the structures of isolated 30S subunit complexes\(^3\) (Fig. 3a).

Conformational changes of tRNA Sec

The aa-tRNA has an essential role in signalling rearrangements at the decoding site to the 50S subunit, leading to GTPase activation of EF-Tu\(^3\),\(^\text{38}\). Our cryo-EM data show marked structural changes in tRNA Sec on the ribosome, which affect three main areas of the tRNA Sec molecule: the variable arm, the elbow region consisting of the D- and T-loops, and the D-stem–anticodon–stem region (Fig. 3b). The D- and T-loops move closer to each other in the initial binding state and change only slightly in other states. By contrast, the anticodon- and D-stems undergo large-scale distortions in each transition. In the GTPase-activated state tRNA Sec adopts a conformation similar to that of the canonical aa-tRNAs in the EF-Tu–ribosome complex\(^3,\text{35,38}\). In principle, the observed large-scale conformational rearrangements of the tRNA could present a kinetic barrier that controls the GTPase activation in
Docking of SelB onto the SRL

The SRL constitutes the key activator of the GTPase activity for all translational GTPases. Notably, docking of the SelB GTP-binding domain on the SRL occurs only upon formation of the GTPase-activated state (Fig. 4a). Whereas the tRNA Sec anticodon moves towards the codon in the 30S decoding centre, the elbow region changes its interactions from the initial contact with the SRL and protein L11 (initial binding state) to SRL and H43 of 23S rRNA (codon reading state) before docking onto H89 of 23S rRNA (Fig. 4a). The movements of the tRNA Sec anticodon towards the codon and of the RNA elbow towards H89, as well as the formation of the GTPase-activated state (monitored by the position of the key GTPase residue His61 in SelB) are coupled (Fig. 4b). These motions control the progression of SelB towards the GTPase-activated state, in line with the major role of tRNA in signalling codon recognition.

Stepwise tRNA Sec docking is facilitated by several elements of the ribosome, including the L11–rRNA arm (consisting of protein L11 and helices H43 and H44 of 23S rRNA), the SRL (together with protein L6), protein S12, and H69 of 23S rRNA that guide tRNA Sec to the decoding centre. The L11–rRNA arm acts as a relay that hands over the tRNA during transitions from state to state (Extended Data Fig. 7a). Binding of tRNA Sec results in a >10 Å displacement of the entire L11–rRNA in the initial binding state. In subsequent states, the L11–rRNA arm relaxes stepwise into its initial position. Notably, in the initial binding and codon reading states, the SRL interacts with tRNA Sec, rather than with SelB (Fig. 4c). A2660 acts as a platform for docking of the T-stem residues of tRNA Sec, but the contacts with tRNA Sec change between the initial binding and codon reading states. Further movement of tRNA Sec in the GTPase-activated state disrupts these contacts and enables docking of SelB onto the SRL. On the 30S subunit, protein S12 engages the acceptor stem of tRNA Sec in the codon reading state and serves as a pivot for tRNA Sec rotation into the GTPase-activated state (Extended Data Fig. 7b). A1913 of H69, in turn, reaches into the decoding centre and may help to guide the anticodon towards the codon in the aminocyl site during the transition from the codon reading to the GTPase-activated state by interacting with the functionally important modified residue isopentenyl-A37 in tRNA Sec (ref. 40; Extended Data Fig. 5a).

In the initial binding, codon reading and GTPase-activated states, SelB maintains its interactions with tRNA Sec and follows its movements (Fig. 4a, e). SelB domain 2 remains bound to the shoulder of 16S rRNA, while adapting to the changes in the position of tRNA Sec. Upon transition from the initial binding to the codon reading state, this adaptation requires a rotation of domain 2 relative to the 30S subunit shoulder, which changes the interactions and moves SelB slightly towards the 50S subunit (Fig. 4e, upper panel, and Extended Data Fig. 7c). In the GTPase-activated state, as a consequence of codon recognition and the global 30S domain closure, SelB rotates together with tRNA Sec and the 30S shoulder. Domain 1 of SelB—comprising several charged residues (Arg34, Glu62 and Arg116)—moves into close proximity to the complex. To understand the dynamics of those rearrangements, we carried out 24–125 all-atom explicit-solvent molecular dynamics simulations of SelB–GTP–Sec–tRNA Sec (Fig. 3c, Extended Data Fig. 6a, b; Methods). We mapped the free-energy conformational landscape on the ribosome and identified the lowest-energy structural model of the ternary complex free in solution. All tRNA Sec conformations observed on the ribosome are sampled spontaneously within less than a microsecond in solution (Fig. 3c). Given that the GTPase activation takes milliseconds, the tRNA Sec network.© 2016 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

Docking of SelB onto the SRL. Distances are measured at Cα of Ala120.

Figure 4 | Sequential docking of SelB–Sec–tRNA Sec on the SRL.

a, Movements of the tRNA Sec anticodon (Rd) and elbow (Relb) and of SelB His61 (Ria) of SelB H11 (Ria) of SelB H43 and H89, helices of 23S rRNA. b, State-specific distance changes. Ria (N3 of C35 in tRNA Sec to N1 of G in UGA); Relb (tRNA Sec elbow, C5 of U35 in tRNA Sec to O2 of A2473 in H89); Ria (ND1 of His61 in SelB to O2 of G2661 in SRL). c, SRL interactions with Sec–tRNA Sec and SelB His61. d, Overview of the ribosome–SelB complex in the GTPase-activated state indicating the areas for close-ups in c, e, f. e, Docking of SelB onto the SRL. Distances are measured at Cα of Ala120.
the SRL (Fig. 4e, bottom panel). These residues may help to promote the small interdomain motion of SelB that is required to completely dock onto the SRL (Extended Data Fig. 7d). The molecular dynamics simulations of the free SelB–GTP–Sec-tRNA Sec complex indicate that this interdomain motion in SelB is rapidly sampled in solution (Extended Data Fig. 7e) and largely independent of tRNA dynamics (Extended Data Fig. 6c).

**GTPase activation**

The active site of all translational GTPases is highly conserved. The invariant histidine (His61 in SelB, His84 in EF-Tu) is essential for rapid GTP hydrolysis in all translational GTPases.41–43 The molecular dynamics simulations of SelB–GTP–Sec-tRNA Sec in solution suggest that His61 can rapidly fluctuate between a flipped-out (pointing away from the γ-phosphate) and a flipped-in (reaching towards the γ-phosphate) conformation (Fig. 4f, Extended Data Fig. 7f, g), similarly to His84 in EF-Tu44,45. In the codon reading state the conformational equilibrium of His61 is shifted towards a partially flipped-in conformation (Fig. 4f). The bulky residue Val9 of SelB appears to stabilize this inactive conformation, thereby impeding further movement of His61 towards the nucleotide (Fig. 4f), which explains the low rate of spontaneous GTP hydrolysis before codon recognition (Fig. 2c).

In the GTPase-activated state, SelB adopts a conformation that is similar to that of the pre-hydrolysis state of EF-Tu,53,54 (Fig. 4f, Extended Data Fig. 7h). The universally conserved residue G2661 of the SRL stabilizes His61 of SelB in the flipped-in conformation, pointing towards the water molecule aligned for the attack on the γ-phosphate. Val9 stacks onto the His61 imidazole ring, providing additional stabilization of the active conformer. The phosphate of A2662 coordinates a Mg2+ ion, which may be important in positioning Asp10 in SelB (homologous to Asp21 in EF-Tu, which is crucial for GTP hydrolysis43); a similar γ-phosphate coordination is seen with the corresponding residue Asp22 of elongation factor G in the pre-hydrolysis state on the ribosome46. Further SelB residues (His8, Arg34, Glu62, Arg116) stabilize the interactions with the SRL (Extended Data Fig. 7d, right panel). Given the evolutionary conservation of the residues constituting the GTPase centre, GTP hydrolysis is likely to follow the same universal pathway for all translational GTPases.

**Conclusions**

The present data show how SelB delivers Sec-tRNA Sec to the ribosome, provide insights into the local and global conformations of the ribosome upon codon recognition by Sec-tRNA Sec and suggest the mechanism for GTPase activation of SelB (Fig. 5). Sec-tRNA Sec and SelB in solution can sample many different conformations. The interactions with the ribosome rectify these spontaneous fluctuations and guide the progression towards GTPase activation. In the initial complex, the SECIS element in the mRNA is exposed for SelB binding and the universally conserved bases A1492 and A1493 in the decoding centre fluctuate between flipped-in and flipped-out conformations (Fig. 5, step 1). Recruitment to SECIS tethers SelB to the vicinity of the ribosome; this contact between SelB domain 4 and the SECIS is maintained in all subsequent steps (Fig. 5, step 2). Initial binding of SelB–Sec-tRNA Sec to the ribosome induces an open conformation of the 30S subunit and stabilizes A1492 and A1493 in a flipped-in conformation, thereby facilitating codon reading (Fig. 5, step 3). Subsequent codon recognition triggers local closure of the decoding site, with A1492 and A1493 flipped out, and a global domain closure of the 30S subunit. The closed conformation facilitates re-positioning of the tRNA and docking of SelB on the SRL. Docking at the SRL aligns key residues at the nucleotide binding pocket of SelB and results in GTPase activation (Fig. 5, step 4). The structure of the GTPase-activated state is likely to be universal for translational GTPases in all domains of life. The docking of GTPases onto the SRL as a result of correct codon–anticodon complex formation and 30S domain closure may represent a common mechanism by which the ribosome ensures the pre-hydrolysis selection of the cognate aa-tRNA delivered by SelB, EF-Tu, or their eukaryotic homologues. This work emphasizes the power of the combination of high-resolution cryo-EM, rapid kinetics and molecular dynamics simulations for the reconstruction of multistep reaction pathways and understanding the function of dynamic molecular machines.

**Online Content**

Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions N.F. conceived the project, performed cryo-EM experiments and data analysis and drafted the paper. P.N. built atomic models and performed pseudo-crystallographic refinement. L.V.B. performed and analyzed molecular dynamics simulations. C.M. performed the kinetic analysis. A.F. and A.L.K prepared ribosome complexes for cryo-EM. Z.W. and G.F.S. performed homology modelling. N.F., M.V.R. and H.G. performed pseudo-crystallographic refinement. L.V.B. performed and drafted the paper. P.N. built atomic models and performed pseudo-crystallographic refinement. L.V.B. performed and analyzed molecular dynamics simulations. C.M. performed the kinetic analysis. A.F. and A.L.K prepared ribosome complexes for cryo-EM. Z.W. and G.F.S. performed homology modelling. N.F., M.V.R. and H.S. finalized the paper with inputs from all authors.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to N.F. (niels.fischer@mpibpc.mpg.de) and H.S. (hstark1@gwdg.de).

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Ribosomes, factors and RNAs. Ribosomes from *E. coli* MRE 600, initiation factors (IF1, IF2, IF3), and 16S-tRNA^Sec^ were prepared as described47-49. *E. coli* SelB and SelD (gift from M. Wahl, Free University of Berlin) and SelB^H^ containing a hexahistidine tag (gift from A. Böck, LMU Munich) were expressed in BL21 (DE3) cells and purified according to published protocols48,49. Biochemical analysis showed that SelB carrying the His-tag is fully functional in the interactions with guanine nucleotides, SECIS elements, and Sec-tRNA^Sec^ in vitro and in vivo50-52 and as seen expressed in *E. coli* BL21 transformed with the plasmid pCR2013 (gift from A. Böck, LMU Munich)53 and purified and aminoacylated as described43-54. The mRNA UGA was a derivative of mlP75s55 (Extended Data Fig. 4d). The templates for mRNAs UUC and iSECIS (Extended Data Fig. 4e, f) were prepared by PCR mutagenesis. Transcription and refolding of the mRNAs were performed as described43,50.

**Complex preparation for cryo-EM.** 70S ribosomes (3 μM) were incubated with IF1, IF2, IF3 (4.5 μM), mRNA (15 μM), and (γ^32P^)GTP (60 μM) in buffer A (50 mM Tris-HCl, pH 7.5, 70 mM NH4Cl, 30 mM KCl, 7 mM MgCl2, 2 mM DTT) with 1 mM GTP for 30 min at 37 °C. Initiation efficiency was close to 100% as verified by nitrocellulose binding. Initiation complexes were purified by gel filtration on a Biosprint 450 HR 5 μm column (Waters). To prepare the ribosome–SelB complexes, ternary complexes SelB–GDPNP–Sec-tRNA^Sec^ were prepared in buffer B (50 mM Hepes-KOH, pH 7.5, 70 mM NaCl, 30 mM KCl, 7 mM MgCl2, 2 mM DTT) by incubating SelB (1 μM) with GDPNP (2 mM) for 4 min at 37 °C, adding Sec-tRNA^Sec^ (1 μM) and incubating for 2 min at 23°C. Ternary complex (0.5 μM) was incubated with initiation complex (0.06 μM) at 0 °C in buffer B supplemented with 0.6 M spermine and 0.4 M spermidine before application onto EM grids.

**GTPase activity.** Initiation complexes were prepared as described42. Ternary complex SelB–γ^32P^GTP–Sec-tRNA^Sec^ was prepared in buffer A, by incubating SelB (9 μM) with γ^32P^GTP (60 μM) for 20 min at room temperature (RT), followed by addition of Sec-tRNA^Sec^ (9 μM) and incubation for 2 min at room temperature. Unbound γ^32P^GTP was removed by gel filtration56. The GTPase activity of SelB was determined at single round conditions, by mixing purified ternary complex (0.05 μM) with UGA, iSECIS and UUC mRNA-programmed initiation complexes at the indicated concentrations. Intrinsic GTP hydrolysis was measured in the absence of ribosomes. When necessary, quench-flow experiments were performed in a KinTek apparatus. Reactions were quenched with formic acid (25% v/v) and the extent of GTP cleavage was determined by thin layer chromatography and phosphor imaging57. The rate of GTP hydrolysis was determined by exponential fitting of the time courses using GraphPad Prism software (GraphPad Software, Inc.); time courses were normalized to the respective reaction end levels.

**Cryo-EM analysis.** Cryo-EM grids were prepared by applying 5 μl of initiation complex–SelB–GDPNP–Sec-tRNA^Sec^ complexes onto EM grids (Quantifoil 3.5/1 μm, Jena) covered with pre-floated continuous carbon and subsequently vitrified using a Vitrobot Mark IV (FEI Company, Eindhoven) operated at 4 °C. Cryo-EM analysis of ribosome complexes was performed as described58-60. The mRNA UGA was a derivative of mlP75s55 (Extended Data Fig. 4d). The templates for mRNAs UUC and iSECIS (Extended Data Fig. 4e, f) were prepared by PCR mutagenesis. Transcription and refolding of the mRNAs were performed as described43,50.

**RiboAnalysis.** Reaction end levels were determined by measuring in the absence of ribosomes. When necessary, quench-flow experiments were performed as described58-60. The mRNA UGA was a derivative of mlP75s55 (Extended Data Fig. 4d). The templates for mRNAs UUC and iSECIS (Extended Data Fig. 4e, f) were prepared by PCR mutagenesis. Transcription and refolding of the mRNAs were performed as described43,50.

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refinement steps in phenix.real_space_refine Ramachandran plot restraints were enabled. For parts of the model exhibiting larger conformational differences and/or lower local map resolution, additional cycles of real space refinement and manual fitting were performed against experimental map filtered to lower resolution, which was gradually increased between subsequent refinement steps until convergence. To maintain the intermolecular interactions of selected model fragments within local environment, all residues within at least 1.5 Å radius were included. For final refinement steps the cryo-EM map was sharpened and masked using a smoothed model-based envelope generated within 3.0 Å radius around atoms of the ribosome model of the GTPase-activated state before conversion to reciprocal space structure factors; for these steps and improved visualization the cryo-EM maps resolved at ≤ 3.6 Å resolution (initial complex, classical and GTPase-activated states) were resampled to a pixel size of 0.789 Å. The final model of the GTPase-activated state consisting of 154,136 individual atoms was refined to 24.90% and 0.936 for Rwork and CCwork (definition is given below), respectively. The model of the GTPase-activated state exhibits a good stereochemistry with 90.87% of residues in the most favoured region and 0.88% residues in the disallowed region of the Ramachandran plot, protein side chain outliers of 2.83% and all atom clash score 15.09.

The remaining states (initial complex, initial binding, codon reading, classical and hybrid states) were modelled based on the final model of GTPase-activated state in an analogous way. For cryo-EM reconstructions resolved at a resolution lower than 3.6 Å, a 3.8 Å radius for generating a smooth model-based envelope was chosen before generating reciprocal space structure factors used for the final refinement steps. FSCwork and CCwork/overall were calculated in a resolution-dependent manner using SPAlign program (CCP4 suite). Real space correlation coefficients (RSCC) were calculated using RESOLVE. All atomic models fit well the experimental cryo-EM map as judged based on the following criteria. (1) The Pearson correlation coefficient (CCwork) calculated between model and map structure factors (Fmodel and Fmap) used for refinement is greater than 0.2 for the highest resolution shell and the overall correlation coefficient (CCoverall) is not lower than 0.9. (2) The calculated FSCwork Value between model map coefficients (Fmodel - phase_model) and structure factors derived from the cryo-EM map (FEM - phase_EM) used for refine-ment are not lower than 0.5 for the highest resolution shell and overall greater than 0.8. (3) The cumulative RSfCC values are greater than 0.8, 0.6 and 0.4 for 53–75%, 88–94% and 95–97% of the residues, respectively. Detailed refinement statistics are presented in Extended Data Table 1, FSC and CCsCCwork Curves in Extended Data Fig. 2a–c.

**Modelling of the recruitment complex.** To build the initial recruitment complex, SelB domain D4 from the structure of the isolated SECIS-domain D4 complex (wh2 to wh4; PDB ID: 2PLY) was docked onto the initial complex by superimposing the SECIS. Then, wh2 was used to model our structure of SelB–GDPNP–Sec-tRNAseco onto the initial complex-domain 4 complex.

**Refinement against half maps.** Refinements of final models against datasets obtained from two half maps were performed at 2.5 Å resolution in PHENIX using several cycles of real space refinement followed by reciprocal space refinement employing reference model restraints. To remove possible model bias a random shift of 0.3 Å was applied to all atomic positions before real space refinement. The FSC and CC were calculated between the models and the masked half-maps used for refinement, as well as between the model and the other half-map for cross-validation. The individual half-maps were masked using a smoothed mask derived from the respective refined model using a radius of 3.0 or 3.8 Å depending on the estimated highest resolution limit.

**Molecular dynamics simulations. Simulation setup.** To obtain the energetics and dynamics of the free ternary complex, molecular dynamics simulations were started from the ribosome-bound conformations (initial binding, codon reading, and GTPase-activated states). Coordinates of Sec-tRNA, SelB (amino acids 1–401), and GTP were extracted from the cryo-EM structures along with resolved water molecules and ions in the vicinity (≤ 5 Å). The system was protonated, solvated, and ions were added as described earlier.

All simulations were carried out with Gromacs (ref. 82) using the amberff12sb force field and the SPC/E water model. Parameters for potassium and chloride ions were taken from ref. 85 and for modified nucleotides from Aduri et al. Atom types for selenocysteine were obtained with ANTECHAMBER and partial charges were determined using DFT-B3LYP with a 6–31+G basis set. Bond and virtual-site constraints, temperature and pressure coupling were applied as described.

For each of the three starting structures, the system was pre-equilibrated as described with potential-energy minimization and 50-ns molecular dynamics simulations with position restraints followed by release of position restraints during 20 ns. Production runs started at 70 ns. At times 170, 270, and 370 ns, coordinates were extracted from the trajectory, new velocities were assigned and new simulations were started (12 simulations; total production run simulation time of 24 μs).

**Conformational dynamics of the ternary complex.** To address the question of whether intrinsic conformational changes are rate-limiting for the transitions between the free ternary complex and the ribosome-bound states, we carried out a principal component analysis (PCA) on three subsets of atoms: ‘v-arm’ (P-atoms of variable arm, ‘no-v-arm’ (remaining peptidyl-atoms of tRNA), and ‘D1’ (Cor-atoms of SelB domain D1). The trajectories were rigid-body fitted using T-stem and acceptor-stem peptidyl-atoms for the ‘v-arm’ and ‘no-v-arm’ subsets and D2 Cor-atoms for the ‘D1’ subsets (Δt = 10 ps, discarding first 200 ns). The trajectories were concatenated and the atomic displacement covariance matrix was calculated. The first eigenvectors, sorted according to their eigenvalues, represent the most dominant conformational modes. For ‘no-v-arm’, the first two eigenvectors largely consist of a bending motion of the anticodon arm. To estimate the free-energy landscape of this motion, the projections of all frames onto these eigenvectors were binned. The relative free energy of each bin was calculated via ΔGbin = -k_B T ln(ρbin/ρmax), where k_B is the Boltzmann constant, T the temperature, ρbin the number of frames in the bin and ρmax is the maximum of all ρbin (Fig. 4c, Extended Data Fig. 6a, 7e).

For ‘no-v-arm’ conformational modes 1 and 2, the bending angles (θ1 and θ2) as a function of the projection onto the respective mode were calculated. The vector between the centres of mass of U34–A36 peptidyl-atoms and of T-stem peptidyl-atoms was calculated. For each projection, the angle between the vector and the vector relative to the bin with ΔG = 0 was calculated.

To monitor the local geometry of the active site in the free ternary complex, the minimal distances between His61 imidazole atoms and GTP atoms (RGTG) as well as the van-der-Waals distance between Va9 and Met36 (Rva9) were calculated (Δt = 10 ps, discarding first 200 ns). The resulting free-energy landscape is shown in Extended Data Fig. 7f.

**Transition rates for RNA movements.** To estimate the magnitudes of rates for transitions of tRNAseco in the free ternary complex between the conformations corresponding to the ribosome-bound states, the projections onto the ‘no-v-arm’ conformational modes 1 and 2 were calculated, thus obtaining 2-dimensional trajectories. For each simulation the number of transitions n between the region around the free-energy minimum (all bins with 0 ≤ ΔG ≤ 0.5 k_BT) and the ribosome-bound conformations was counted. For each ribosome-bound tRNA conformation a region was defined by an ensemble of 10,000 structures generated from the cryo-EM coordinates and b-factors. For each peptidyl-atom and ensemble structure, the coordinate obtained from cryo-EM was shifted in a random direction by distance δ drawn from a normal distribution p(δ). The normal distribution p(δ) with μ = 0 and σ = rms (calculated from the b-factor) was set to zero for d < σ and d > σ. The resulting structures were projected onto the ‘no-v-arm’ conformational modes. The region was defined as all bins with entries from these structures. The transition rates were calculated for each simulation by dividing n by the simulation time τsim. The mean rates and standard deviations were obtained by weighting the individual transition rates by the corresponding τsim−1.

**Coupling of RNA and SelB conformational modes.** To address a possible coupling of ‘v-arm’ and ‘no-v-arm’ conformational modes, we investigated whether the projections onto these modes are correlated as would be expected for coupled motions. For all four pairs of eigenvectors υ and ω, where υ is either ‘no-v-arm’ mode 1 or 2 and ω is either ‘v-arm’ mode 1 or 2, the correlation coefficient of the projections was calculated. To estimate the statistical error, a bootstrapping method was applied. First, the autocorrelation function τ was defined by f_autocorr(τ) = exp(-1), where f_autocorr(Δτ) is the autocorrelation function of the projection. For a given pair of eigenvectors υ and ω, the maximum of the two corresponding τ values (τmax) was used as an interval to resample the projections, resulting in sets of V = τmax/τmax projections. This resampling was repeated 1,000 times and mean and standard deviations of the correlation coefficients were calculated for the simulations started from the individual ribosome-bound conformations and for all simulations computed with different seeds. To check whether the resulting correlation coefficients are statistically significant, the standard deviations expected from uncorrelated projections were estimated by randomly drawing N projections (1,000 repetitions; Extended Data Fig. 6b, grey points).

To address coupling between tRNA dynamics and motions of SelB domain 1 (D1), the same analysis was carried out for ‘no-v-arm’ and ‘D1’ conformational modes (Extended Data Fig. 6c).

**Data availability statement.** Cryo-EM maps/coordinates of the atomic models for each state have been deposited in the Electron Microscopy Data Bank (EMDB) (http://www.emdbank.org/) with the following accession codes: initial complex (D1) (EMD-4122/SZLC, GTPase-activated state, EMD-4124/SZLC; classical and hybrid state, EMD-4123/5ZLE; hybrid state, EMD-4126/5ZLF). Cryo-EM micrographs and particle images have been deposited in the EMPIAR database (https://www.ebi. ac.uk/pdbe/emdb/empiar/) with accession code EMPIAR-10077.
Extended Data Figure 1 | Computational sorting of ribosome particle images. a, Hierarchical sorting scheme. Numbers refer to classification steps. SVC, supervised classification by projection matching; ‘bad’ 70S, the particles of low quality and/or those showing mixtures of different compositions and conformations; initial complex (hybrid) (IC (hybrid)), ratcheted state of the ribosome with one tRNA in the hybrid state. b, Mask used for focused classification in step 4. c, Cryo-EM reconstruction from 100,000 random unsorted particle images. Left, initial refinement stage at 10 Å resolution showing only scattered density (red) for SelB, Sec-tRNA Sec and SECIS. Right, final cryo-EM map at 3.7 Å resolution. d, Structural changes resolved by computational sorting as quantified by the r.m.s.d. and the changes in mass due to ligand binding and dissociation.
Extended Data Figure 2 | See next page for caption.
Extended Data Figure 2 | Cryo-EM map and model quality. a, Fourier shell correlation (FSC) curves and model validation. FSC curves are shown for each state. Black, the FSC curve computed between the masked independent half-maps from cryo-EM refinement (half1 and half2); blue, the FSC curve between the final cryo-EM map (map) and the final model (model); red, the FSC curve between half-map 1 (half1) and the model obtained by refinement only against half-map 2 (model2). The vertical black dashed line indicates the resolution according to the 0.143 criterion (grey line) and the maximum resolution at which the full atomic models were refined. b, FSC curves (FSCwork) computed for each state between reflections from solvent-flattened cryo-EM maps and model maps generated from refined atomic coordinates. Differences to d largely result from solvent-flattening (Methods). c, CCwork curves as obtained by refinement for each state. For reliable resolution estimates CCwork is expected to be >0.2 in the highest resolution shell. d, Cryo-EM densities and models of the quaternary complex, SelB–GDPNP–Sec–tRNA^{Sec}–SECIS-mRNA for each SelB-bound ribosome state. Densities are coloured as in Fig. 1b and rendered at ~2σ, except wh3, wh4 and SECIS of the GTPase-activated state, which are rendered at ~1.5σ. Top right: Sec-tRNA^{Sec} density (purple) in the initial binding state at 5.3 Å resolution; the conformation of the invariant histidine 61 is not discernible (question mark). Middle right: Density for histidine 61 (blue) in the codon reading state indicating a partially flipped-in conformation. e, Densities for rRNA modifications as seen in the GTPase-activated state at 3.4 Å resolution. Densities are rendered at ~3σ. Arrows denote the characteristic distortion of the nucleobase of D2449 and methyl groups for the other modified nucleotides. f, g, Cryo-EM reconstructions of the individual states (as indicated) coloured according to local resolution. Left: Surface view; right: Cut-away view. Heat maps are adjusted to the respective resolutions ranges of the cryo-EM maps in f and g. The arrow denotes the substantially lower local resolution of SelB residues 485 to 614 in the GTPase-activated state; atomic models for such regions were correspondingly refined at lower resolution (Methods).
Extended Data Figure 3 | See next page for caption.
Extended Data Figure 3 | Structural basis for the specificity of tRNA^{sec} recognition by SelB. All superpositions are based on domains 1 and 2 of SelB and EF-Tu, respectively. a, Overview of SelB–GDPNP–Sec-tRNA^{sec} bound to the ribosome in the GTPase-activated state. Numbers refer to the close-ups in b, e and g, respectively, which depict specific SelB–Sec-tRNA^{sec} interactions. SelB d1–3, domains 1 to 3 of SelB; VA, variable arm. b, Interactions between SelB (domain 3, red, and the linker, residues 340–363 in khaki) and the backbone of the extra-long variable arm of tRNA^{sec}. These interactions do not form in the canonical EF-Tu–aa-tRNA complexes. c, The structure of tRNA^{sec} in contrast to canonical tRNAs, tRNA^{sec} entails an additional base-pair in the acceptor stem (A5a–U67a, red), a unique sequence in the T-stem, the so-called antideterminant box (C7–G66, G49–U65 and C50–G64, orange), and a very long variable arm (yellow). d, Superposition of tRNA^{sec} (purple) and the canonical tRNA^{Cys} (dark grey) in the complex with SelB–GDPNP and EF-Tu–GDPNP, respectively. The structure of the canonical EF-Tu–GDPNP–Cys-tRNA^{Cys} is from PDB structure 1B23 (ref. 11). Note the distortion in the acceptor stem of tRNA^{sec} (residues G1–U6/G72–A67) as compared to tRNA^{Cys} that compensates for the additional base pair. As a result of the distortion, the main factor-binding sites of both tRNAs, the CCA end and the T-stem, overlap and the bases are arranged in register (‘in’). In the T-stem, the bases are shifted by exactly one base pair (Δ1) between the two tRNAs, which shifts the bases out of register (‘out’) in the acceptor stems of the two tRNAs. e, Specific interaction between the extended loop (blue) in SelB domain 3 and the antideterminant box in the T-stem of tRNA^{sec}. f, Sequence-specific interaction between the loop (blue) in EF-Tu domain 3 and the T-stem of tRNA^{Cys} (PDB ID 1B23 ref. 11). Note the different structure of the hairpin-loop as compared to SelB, contributing to the particularly low affinity of EF-Tu for tRNA^{sec} (ref. 89) (see also k, middle panel). g, Charge distribution in the amino-acid-binding pockets of SelB (left) and EF-Tu (right). The colour bar on the right denotes the electrostatic potential in kT e−1. Note the similar geometry of tRNA binding in the two complexes. In SelB, the pocket is positively charged, facilitating the interaction with the negatively charged selenogroup of Sec-tRNA^{sec} and the discrimination against the precursors of Sec-tRNA^{sec} biosynthesis, deacylated tRNA^{sec} and Ser-tRNA^{sec}, as well as against most canonical tRNAs. In EF-Tu, the amino-acid-binding pocket is negatively charged, thereby discriminating against Sec-tRNA^{sec}. h, Recognition of the selenogroup in Sec-tRNA^{sec} by SelB. The positively charged, highly conserved Arg181 and Arg236 in SelB contact the negatively charged selenogroup (Se, orange), whereas the aromatic ring of Tyr42 stacks onto the selenogroup. The importance of these residues was demonstrated by mutational analysis (ref. 89). The universally conserved Asp180, which is also important for Sec-tRNA^{sec} binding (ref. 6), forms a secondary binding shell stabilizing Arg236. i, Solvent-exposed amino-acid-binding site as found in the crystal structure of isolated Aquifex aeolicus (aq) SelB–GDPNP co-crystallized with l-cysteine as Sec mimic (PDB ID 4ZU9) (9). The position of Cys deviates by 5 Å from the amino acid position in the present SelB–GDPNP–Sec-tRNA^{sec} structure (grey), resulting in a distinct interaction pattern and a more solvent-exposed binding site of the Cys. j, Superpositions of SelB–GDPNP–Sec-tRNA^{sec} and EF-Tu–GDPNP–Cys-tRNA^{Cys} revealing differences in T-stem recognition by the loop in SelB domain 3 versus EF-Tu. Top: The loop structure in SelB is compatible with binding of canonical tRNA^{Cys}. Other tRNAs with different T stem sequences (particularly at position 63, red arrow) may be unfavourable for the interaction with the conserved Ser327 in SelB. Middle: The loop structure in EF-Tu is incompatible with the conformation of the antideterminant box (orange) in tRNA^{sec}. Bottom: In isolated aqSelB (PDB ID 4ZU9), the loop adopts a similar conformation as in the SelB ternary complex, suggesting a conserved loop structure that facilitates recognition of tRNA^{sec} by SelB. The acceptor and T stem region of tRNA^{sec} in the GTPase-activated state (rendered at 2σ), which depicts an alternative conformation of Arg329; green mesh, density rendered at 1σ. I, Scheme depicting the functional importance of rearrangements of the tRNA^{sec} variable arm for translation elongation after Sec incorporation. (1) Upon release from SelB, tRNA^{sec} accommodates in the aminoacyl site in the classical state. The variable arm undergoes a large reorientation from a SelB-bound distorted conformation in the classical state (left close-up); the latter ground state conformation is similar to the conformation of the variable arm found in complexes of tRNA^{sec} with its conversion enzymes, seryl-tRNA synthetase and SelA (9). (2) After peptide bond formation (resulting in the hybrid state, not shown), peptidyl-tRNA^{sec} is translocated to the classical peptidyl site. (3) Binding of the next aa-tRNA (orange) is only compatible with the variable arm adopting the classical state conformation; the GTPase-activated state-conformation of the variable arm would sterically interfere with binding of the next aa-tRNA (right close-up). The long variable arm of canonical class 2 aa-tRNA is not distorted upon EF-Tu binding (9) and, consequently, does not require any changes during translation elongation.

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Extended Data Figure 4 | The SelB–SECIS interactions on the ribosome and kinetic analysis of GTP hydrolysis. **a**, Close-up of the SECIS–ribosome interactions in the GTPase-activated state. Ribosomal proteins S3, S4, and S5 contribute to the helicase activity of the ribosome. Arg130 and Arg131 of S3 and Lys44 and Arg46 of S4 are important for mRNA unwinding. Other potential helicase elements are Arg71 and Arg125 in S3, which interact with the base of the SECIS hairpin and may thereby facilitate unwinding of the mRNA secondary structure elements. The mode of SECIS recognition by SelB on the ribosome is similar to that in isolated SelB domain 4–SECIS complexes, including three essential bulged nucleotides in the SECIS (U21, G26 and U27; present data and refs 9, 25 and 72). **b**, Interaction of SelB domain 4 with the shoulder of the 30S subunit. Winged-helix motif 4 (wh4) of SelB forms salt bridges with protein S4, while wh2 and wh3 embrace helix 16 of 16S rRNA by interacting mainly with the rRNA backbone. **c**, Concentration dependence of the apparent rate of GTP hydrolysis by SelB measured with increasing concentrations of ribosomes programmed with mRNAs ‘UGA’ (black), ‘iSECIS’ (blue) and ‘UUC’ (red). **d**, mRNA construct ‘UGA’ used for structural and kinetic analyses containing the cognate UGA codon coding for Sec and the functional minimal fdhF–SECIS. The Sec codon and the essential bases of the SECIS are indicated by boxes; SD, Shine–Dalgarno sequence. **e, f**, mRNA constructs ‘iSECIS’ and ‘UUC’ used for kinetic analysis. Changes in comparison to the ‘UGA’ mRNA are indicated in blue (iSECIS) and red (UUC).
Extended Data Figure 5 | See next page for caption.
Extended Data Figure 5 | Local and global conformational changes of the 30S subunit. a, Rearrangements of A1913 in H69 of 23S rRNA. A1913 stabilizes A1492 and A1493 and guides Sec-tRNA\textsubscript{Sec} to the mRNA codon by interaction with isopentenyl-A37 (i\textsubscript{6}A37) of tRNA\textsubscript{Sec}. The respective conformation (syn or anti) is denoted; dashed lines indicate potential interactions with distances ≤4 Å. b, Close-up of the distance R\textsubscript{ASL} between the UGA (N1 of G) and anticodon of tRNA\textsubscript{Sec} (N3 of C35). Note the potential interaction between the third codon position and the tRNA anticodon in the codon reading state. c, Global conformational changes of the 30S subunit upon accommodation (GTPase-activated to classical) and hybrid state formation (classical to hybrid). tRNA accommodation in the aminoacyl site does not cause major 30S subunit rearrangements, in contrast to the eukaryotic system, where tRNA accommodation correlates with a large-scale conformational change\textsuperscript{94}. Subsequent tRNA hybrid state formation is coupled to the well-known rotational movement of the 30S subunit\textsuperscript{95}. The typical rotational changes all over the 30S subunit are clearly distinct from the changes observed upon domain opening and closure, which are mainly found at the shoulder region (Fig. 3a). The heat map quantifies the movements of 16S rRNA backbone phosphate atoms as obtained by superposition on 23S rRNA of the initial complex state (Fig. 3a). d, Deviations of the 16S rRNA backbone phosphates in the initial binding, codon reading, or GTPase-activated states from the initial complex superimposed on 23S rRNA (top) or 16S rRNA (bottom). Negative values correspond to the 30S domain opening, positive values to domain closure. Landmarks of the 30S subunit are indicated. e, f, Experimental densities of the decoding centre region rendered at 2–2.5σ, if not indicated otherwise. For better visibility, densities for tRNA\textsubscript{Sec} were omitted for the GTPase-activated state. e, Anti-conformation of G530 and stacking interaction with C518 of 16S rRNA as seen in the different states. Bottom left: Note the density of tRNA\textsubscript{Sec} (purple) suggesting a partial interaction of U34 with the mRNA codon in the codon reading state. f, Different conformational states of A1492, A1493 (red) of 16S rRNA and A1913 (slate blue) of 23S rRNA. Top left: In the initial complex the reduced densities for the three nucleotides indicate a dynamic equilibrium between two states: (1) A1492, A1493 flipped out (‘out’, red) with A1913 in anti-configuration (‘anti’, slate blue) and (2) A1492, A1493 flipped in (‘in’, dark red) with A1913 in syn-configuration (‘syn’, dark slate blue). Top middle: The dynamic nucleotides in the initial complex are discernible at lower threshold (red mesh, density at ~1σ). Top right and bottom left: Density in the initial binding and codon reading states is compatible with the bases of A1492 and A1493 flipped-in and A1913 in the anti-configuration. Bottom right: In the GTPase-activated state, A1492 and A1493 are flipped-out and A1913 remains in the anti-conformation. g, Density for the codon–anticodon interaction in the GTPase-activated state rendered at ~3σ.
Extended Data Figure 6 | Molecular dynamics simulations of the free ternary complex. a, \(\text{tRNA}^\text{Sec}\) dynamics obtained by molecular dynamics simulations of the free SelB–GTP–Sec–tRNA^\text{Sec} ternary complex. The free energy landscape for the conformational dynamics of the \(\text{tRNA}^\text{Sec}\) body, excluding the variable arm, is shown as a heat map. The free energy was estimated from separate simulations starting from the structures of the ternary complex bound to the ribosome in initial binding, codon reading, and GTPase-activated states (upper and lower left panels), as well as from all simulations combined (lower right panel). The results of different simulations have a large overlap, especially in regions of low free energy. The crosses denote the tRNA conformations in the ribosome-bound cryo-EM states and the free energy minimum of the free ternary complex found in the molecular dynamics simulations. b, Weak coupling between the conformational dynamics of \(\text{tRNA}^\text{Sec}\) excluding the variable arm (no-v-arm) and of the variable arm (v-arm). Each panel shows the correlation coefficients for a pair of conformational modes (red points, with standard deviation) for the simulations starting from each state (initial binding, codon reading, and GTPase-activated), as well as for all simulations combined. The grey bars denote the standard deviation of the correlation coefficient expected from random drawing of projections. All pairs of modes show a small but significant correlation, indicating a weak coupling. c, Coupling between tRNA dynamics (no-v-arm modes) and SelB motions (SelB domain 1, D1, relative to domain 2, D2). Only a weak overall correlation is seen between no-v-arm mode 1 and D1 mode 2. The three ribosome-bound conformations do not change much with regards to no-v-arm mode 1 (Fig. 3b), which suggests that there is no direct coupling between tRNA dynamics and SelB motions on the ribosome.
Extended Data Figure 7 | Role of L11–rRNA arm, protein S12 and the dynamics of SelB domains. a, Role of L11–rRNA arm in guiding Sec-tRNA\textsubscript{Sec} towards H89. Residues of protein L11, H43 and H89 of 23S rRNA are shown in mauve; dashed lines mark interactions with the D loop (DL) and T loop (TL) of tRNA\textsubscript{Sec} (purple); arrows denote changes with respect to the preceding state. 
b, Protein S12 as a pivot for tRNA\textsubscript{Sec} movement. S12 His76 interacts with the backbone of tRNA\textsubscript{Sec} (residue U69) guiding the tRNA from its position in the codon reading state (grey) to the GTPase-activated state (coloured).

c, Inter-subunit rearrangement in SelB upon transition from initial binding to codon reading. Shown is the small movement observed in addition to the rotation of SelB relative to the 30S shoulder as depicted in Fig. 4f, upper panel. 
d, SelB residues facilitating SRL docking. Left and middle: Spontaneous fluctuations within SelB that are required—in addition to the rotation upon 30S domain closure (shown in Fig. 4, lower panel)—for transition from codon reading (grey) to the GTPase-activated state (coloured). e, Inter-subunit rearrangement in SelB upon transition from initial binding to codon reading. Shown is the small movement observed in addition to the rotation of SelB relative to the 30S shoulder as depicted in Fig. 4f, upper panel. 
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Extended Data Table 1 | Data collection and model refinement

<table>
<thead>
<tr>
<th>Ribosomal state</th>
<th>IC</th>
<th>IB state</th>
<th>CR state</th>
<th>GA state</th>
<th>C state</th>
<th>H state</th>
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<td>4.8</td>
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<td>α, β, γ (°)</td>
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<td>9/0</td>
<td>7/0</td>
<td>0/0</td>
<td>0/1</td>
<td>3/0</td>
</tr>
</tbody>
</table>

*| **RSCC** | 0.12** | 0.12** | 0.011 | 0.011 | 0.011 | 0.012 | 0.009 |
| **Ramachandran plot statistics** | 1.220 | 1.350 | 1.285 | 1.301 | 1.238 | 1.236 |
| Most favored (%) | 90.29 | 92.12 | 92.15 | 90.83 | 90.05 | 90.81 |
| Disallowed (%) | 1.56 | 1.93 | 1.83 | 1.30 | 1.97 | 1.93 |
| Avg. RSCC for sRNASEC/SECIS | 0.72/0.70 | 0.58/0.57 | 0.72/0.41 | 0.61/0.20 | 0.63/0.35 | 0.70/0.39 |
| Avg. RSCC for SelB d1-3/wh1-2 | 0.68/0.73/0.66 | 0.68/0.70/0.52 | 0.81/0.78/0.39 | n.a. | n.a. | n.a. |

*For refinement, maps at ≥ 4.6 Å resolution were cropped to 280 x 280 x 280 pixels; maps at ≤ 3.6 Å resolution were resampled to 400 x 400 x 400 pixels, corresponding to a pixel size of 0.789 Å.
†Refinement target: MLHL maximum likelihood with experimental phase probability distribution.
‡Highest resolution shell is shown in parenthesis.
§Rwork: Σ | | FEM − | | FMODEL | | / | | Σ | | FEM | | , where FEM are structure factors calculated on the basis of solvent flattened EM map and FMODEL are structure factors calculated from the refined model. The structure factors belonged to the working set which was used for reciprocal space refinement.
¶FSCwork is averaged over all FSC shells (FSCoverall) calculated between FEM and FMODEL belonging to the working set. The value for the highest resolution shell is shown in parenthesis and was calculated using the form: FSCwork(shell) = Σ(FEM × FMODEL cos(Δphase))/((v(Σ | | FEM | | 2) × (v(Σ | | FMODEL | | 2)))).
#Residue-averaged real space local correlation coefficient in region of model (RSCC) to EM map calculated with RESOLVE.
*Calculated with RESOLVE*
**For the initial complex, the average RSCC for the SECIS was computed against the map filtered to lower resolution; the resulting low RSCC value indicates substantial conformation variability of the SECIS.
††Modelled based on cryo-EM map filtered to lower resolution.