

# Insights into the decoding mechanism from recent ribosome structures<sup>☆</sup>

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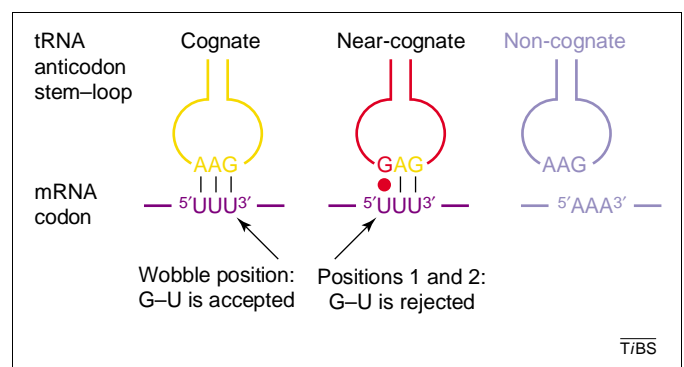
**During the decoding process, tRNA selection by the ribosome is far more accurate than expected from codon–anticodon pairing. Antibiotics such as streptomycin and paromomycin have long been known to increase the error rate of translation, and many mutations that increase or lower accuracy have been characterized. Recent crystal structures show that the specific recognition of base-pairing geometry leads to a closure of the domains of the small subunit around cognate tRNA. This domain closure is likely to trigger subsequent steps in tRNA selection. Many antibiotics and mutations act by making the domain closure more or less favourable. In conjunction with recent cryoelectron microscopy structures of the ribosome, a comprehensive structural understanding of the decoding process is beginning to emerge.**

The maintenance and retrieval of genetic information requires Watson–Crick base pairing between nucleic acids. During translation of mRNA into protein by the ribosome, accurate selection of aminoacyl-tRNA (aa-tRNA) depends upon the correct pairing of three bases between the mRNA codon and the tRNA anticodon. However, Watson–Crick base pairs are not significantly stronger than some of the mismatches that would violate the genetic code. Therefore, the actual source of specificity during tRNA selection, which must ultimately be limited by a difference in interaction energies [1], has long been unclear. This ‘decoding problem’ is most vividly illustrated by the relative stabilities of the cognate Watson–Crick pair A–U and the mismatch G–U. In free solution, replacement of A–U with G–U destabilizes the formation of double-stranded RNA less than tenfold [2,3], yet on the ribosome even those aa-tRNAs which are ‘near-cognate’ (Fig. 1) owing to G–U or other subtle codon–anticodon mismatches, are efficiently rejected with error frequencies as low as  $10^{-4}$  [4]. Consistent with earlier proposals [5,6], recent structural work shows that the ribosome itself is responsible for this specificity by recognizing Watson–Crick base-pairing geometry. These and other recent results, in conjunction with much previous biochemical and genetic data, provide an increasingly clear view of how the ribosome reads the genetic code.

## The tRNA selection pathway

Aminoacyl-tRNA is delivered to the ribosomal A site as a ternary complex with elongation factor EF-Tu and GTP. The events between the initial binding of the ternary complex and the incorporation of the amino acid into the peptide have been dissected into several kinetically distinguishable steps (reviewed in [7,8]; Fig. 2). The tRNA anticodon associates reversibly with the codon in the 30S A site, also termed decoding centre, while the aminoacyl acceptor end is still bound to EF-Tu. Cognate codon–anticodon interactions stimulate a conformational change in EF-Tu leading to GTP hydrolysis. Consequent rearrangement of the factor to a GDP-bound conformation, which dissociates from the ribosome, frees the aminoacyl end of tRNA to move into the A site of the 50S subunit, a process termed accommodation. Accommodated aa-tRNA rapidly undergoes the peptidyl transferase reaction.

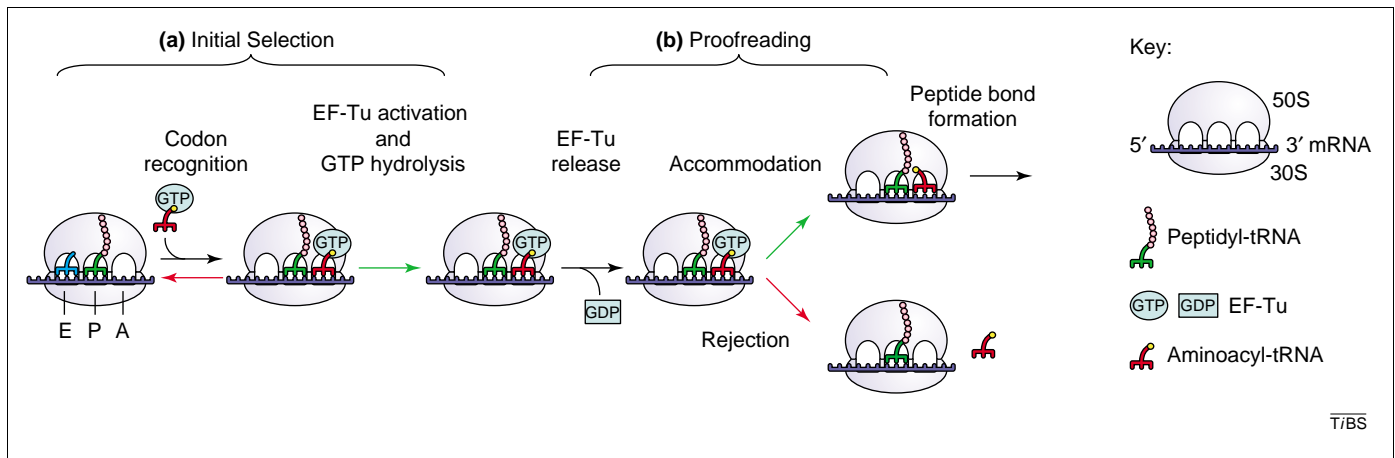
Incorrect tRNA can be rejected either before or after GTP hydrolysis: non-cognate ternary complexes with no match between the anticodon and the codon, dissociate without stimulating GTP hydrolysis, in an initial selection step. Depending on buffer conditions, near-cognate codon–anticodon pairing can stimulate GTP hydrolysis to varying degrees [9–11]. However, in a subsequent ‘proofreading’ step, near-cognate aa-tRNA dissociates rather than being accommodated into the peptidyl transferase centre on the 50S subunit. Rejection during proofreading is essentially irreversible because aa-tRNA can only efficiently enter the pathway as a ternary complex before GTP hydrolysis. This is because most of the aa-tRNA in the cell exists as a ternary complex, which also has higher affinity for an empty A site than just aa-tRNA alone. The pathway is thus



**Fig. 1.** Cognate, near-cognate and non-cognate codon–anticodon pairing. The anticodon must interact with the first two codon positions according to the Watson–Crick base pairing rules, whereas some deviation, e.g. the G–U pair, is allowed at the third, or ‘wobble’ position.

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**Fig. 2.** The aminoacyl-tRNA (aa-tRNA) selection pathway. Ternary complexes of aa-tRNA, EF-Tu and GTP bind reversibly to the ribosome. The anticodon can access mRNA codon in the 30S A site, and if the codon is recognized as correct, the EF-Tu GTPase is activated. After GTP hydrolysis, the GDP form of EF-Tu dissociates, whereupon the aminoacyl end of cognate aa-tRNA moves into the peptidyl transferase site on the 50S (accommodation). Red arrows indicate steps at which non- or near-cognate aa-tRNA are rejected, either during initial selection before GTP hydrolysis or in a proofreading step thereafter. With near-cognate ternary complex, steps represented by green arrows are slower than in the cognate case, but are accelerated by paromomycin or streptomycin [7,8,11].

driven unidirectionally by GTP hydrolysis and, ultimately, peptide-bond formation. It was recognized that in such a branched, irreversible scheme, the overall accuracy could be as much as the product of the accuracies of the two rejection steps before and after GTP hydrolysis, and thus enhance the specificity of codon–anticodon pairing [12,13].

This concept of ‘kinetic proofreading’ led to a perception of the ribosome playing a relatively passive role during aa-tRNA selection. In this view, the irreversible forward steps of GTP hydrolysis and peptide-bond formation occur at an invariant rate and act as an ‘internal clock’, so that tRNAs that pair less stably with the codon will be more likely to dissociate before the forward reactions occur [14]. Thus, the specificity of the codon–anticodon interaction alone, which was somewhat overestimated in the early literature [7,15,16], was thought to be sufficient to account for the accuracy of protein synthesis when combined with kinetic proofreading. However, the prospect of a direct involvement of the ribosome in the recognition of the codon–anticodon duplex [5,6] was revived when it was shown that the error-inducing antibiotic paromomycin changes the conformation of a 16S RNA fragment of the decoding centre [17,18]; the affected residues were strongly implicated in tRNA binding [19,20]. Furthermore, recent kinetic experiments have shown that both GTP hydrolysis and aa-tRNA accommodation (peptide bond formation) are accelerated for cognate relative to near-cognate ternary complex [10,21] (Fig. 2). This implies that the energy derived from the binding of a cognate aa-tRNA anticodon induces conformational changes in the ribosome, and is used to drive the irreversible chemical steps on the tRNA selection pathway. However, until recently, it was not clear exactly how the ribosome discriminates between cognate and near-cognate tRNAs, and how this discrimination affects tRNA selection.

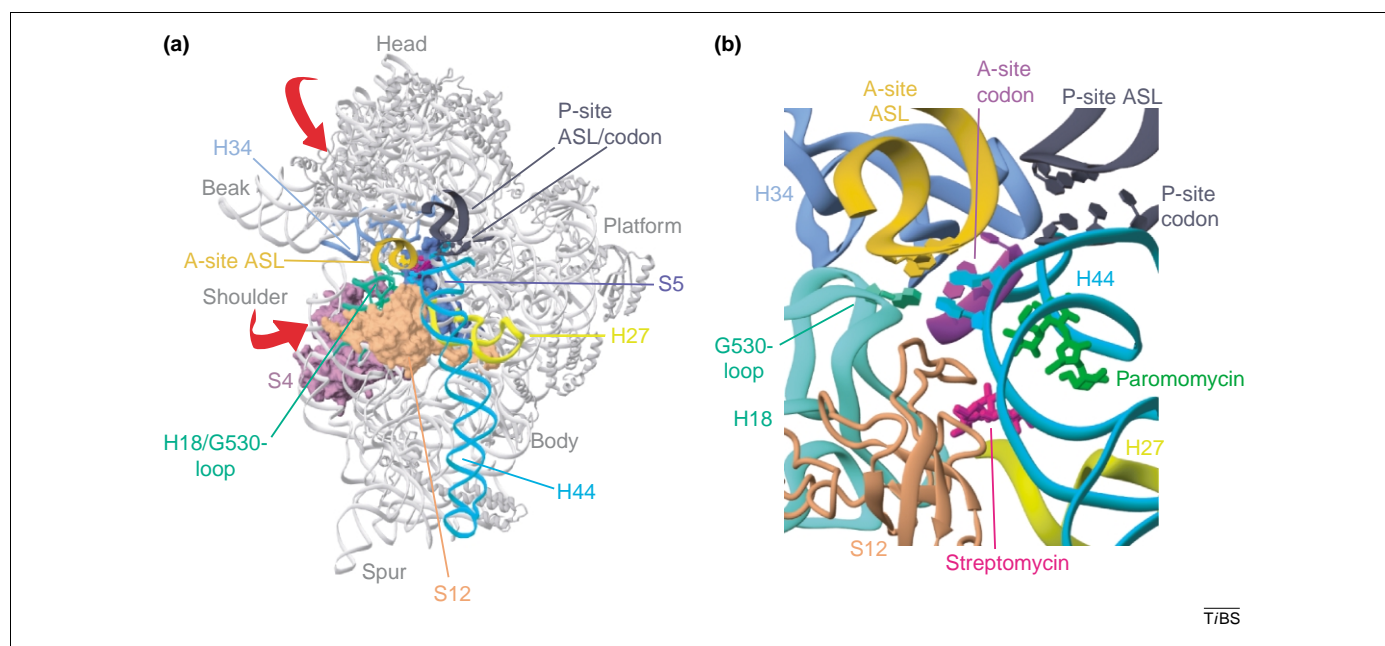
### Recognition of base-pair structure

The A site of the 30S ribosomal subunit (the decoding centre), in which the codon and anticodon pair, is made

up of four different domains: the head, shoulder, platform and helix 44. Crystal structures have revealed that 16S RNA bases of the decoding centre specifically contact the cognate codon–anticodon pair by induced fit [22,23] (Fig. 3). Nucleotides G530 from the shoulder domain, and A1492 and A1493 in helix H44 come together to span the minor groove of the codon–anticodon duplex at the first two codon positions. This results in a closed conformation of the 30S subunit, in which the shoulder and the head domains are rotated towards the subunit centre, compared to a more open structure when the A site is unoccupied [24] (arrows in Fig. 3a; see supplementary animations, [http://alf1.mrc-lmb.cam.ac.uk/~ribo/30S/supplementary\\_info/ogle\\_TIBS\\_2003/](http://alf1.mrc-lmb.cam.ac.uk/~ribo/30S/supplementary_info/ogle_TIBS_2003/)).

The close interactions of the three 16S RNA bases with the minor groove of the first two codon–anticodon base pairs (Fig. 4a,b) sense the characteristic shape [25] of Watson–Crick pairs. They enhance the specificity of codon–anticodon recognition far beyond the relative stabilities of base pairing alone by contributing a substantial amount of additional, geometry-dependent interaction energy [23]. When the ribosomal base A1493 interacts with the U–G mismatch between the first position of the phenylalanine codon and the anticodon of near-cognate tRNA<sup>Leu2</sup>, the wobble geometry displaces the U of the codon into the minor groove so that it can no longer form hydrogen bonds with A1493, but at the same time there is no room for a water molecule to solvate the polar groups [23] (Fig. 4c). This amounts to a large desolvation penalty [26], which can reduce the affinity of near-cognate tRNA anticodon stem–loop (ASL) fragments to the ribosome by more than three orders of magnitude. The principles and extent of discrimination against U–G at the second codon position are similar. Solution affinity measurements of cognate and near-cognate tRNA-ASL binding to the ribosome are in good agreement with the desolvation penalties expected from the disruption of hydrogen bonds in the near-cognate structures [23].

By contrast, certain non-Watson–Crick base pairs, including the G–U mismatch, are permissible at the



**Fig. 3.** Induced fit of the 30S subunit around the decoding centre [22,23]. (a) Overview of the 30S subunit structure [24], in complex with A-site tRNA anticodon stem-loop (ASL, gold) [22]. Red arrows indicate the movement of domains during the transition to the closed 30S conformation [23] (see supplementary animations, [http://alf1.mrc-lmb.cam.ac.uk/~ribo/30S/supplementary\\_info/ogle\\_TIBS\\_2003/](http://alf1.mrc-lmb.cam.ac.uk/~ribo/30S/supplementary_info/ogle_TIBS_2003/)). P-site codon and tRNA-ASL (mimicked by the 3' end of the 16S RNA and the 'spur' stem-loop of a symmetry-related molecule in the crystal [32]) are dark grey, helices H44 cyan, and H27 yellow. In the shoulder domain, H18 with the 530-loop is turquoise, and proteins S12 (orange), S4 (violet) and S5 (dark blue, on the back of the subunit) are highlighted in space-filling representation. In the head domain, H34 is blue. (b) Close-up of selected 30S elements around the decoding centre, showing the A-site codon (purple), 16S RNA nucleotides G530 in the 530-loop (turquoise), and A1492 and A1493 in H44 (cyan) the positions of paromomycin (green) and streptomycin (pink) [32]. Remaining colours as in panel A (proteins S4 and S5 not shown). Reprinted, with permission, from Ref. [22]. Copyright 2001 American Association for the Advancement of Science.

third codon position. This 'wobble' base pairing allows some tRNAs to recognize several different codons [27] and can occur because the contacts of the ribosome at this position do not depend upon the precise shape of the minor groove [22] (Fig. 4d). In agreement with this, thermodynamic measurements of tRNA-ASL binding to the ribosome show that affinity differences between a C–G and a U–G pair at the wobble position are modest and, in particular, no larger than in the absence of the ribosome [23]. However, some non-Watson–Crick base pairs are not allowed at the wobble position, presumably because the ribosome does impose certain geometric constraints, for example upon the overall width of the base pair. Pairing at the wobble position is also heavily influenced by tRNA nucleoside modifications [28].

#### Linking base-pair recognition, GTP hydrolysis and accommodation

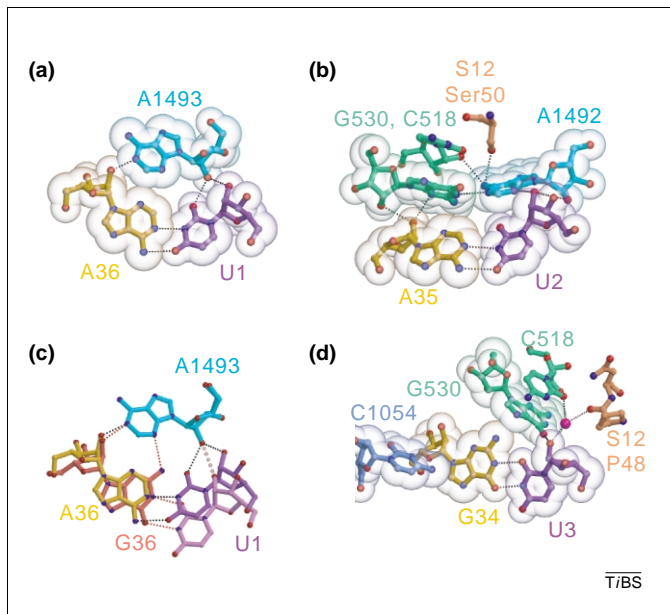
The ribosome must use the energy of interaction with the cognate codon–anticodon duplex not only to prevent aa-tRNA dissociation, but also to switch on the GTPase activity of EF-Tu and promote aa-tRNA accommodation into the peptidyl transferase site. The large distance between the sites of decoding and GTP hydrolysis suggests that the two must be coupled by conformational changes in either the ribosome or tRNA. Although several experiments suggested such long range conformational changes [17,29–32] there was no clear picture of how they could activate GTP hydrolysis. However, recent experiments suggest that cognate tRNA binding induces a domain

closure in the 30S subunit that is required for the subsequent steps in tRNA selection [23].

#### What is the role of 30S-domain closure?

When cognate tRNA-ASL binds to the ribosome, it results in a transition from an open to a closed form of the 30S subunit [22,23]. A structural and thermodynamic study of the binding of near-cognate tRNA-ASL to the ribosome [23] indicates that while the tRNA-ASL associates weakly with the A-site codon, the 30S remains in an open conformation, similar to when the decoding centre is unoccupied. However, the error-inducing antibiotic paromomycin allows the domains of the 30S to close around the near-cognate tRNA-ASL, as in the cognate case.

These results can be explained by considering the energetic cost associated with domain closure (due to, for example, breaking of tertiary interactions, rotation about bonds, decreasing entropy and solvation effects). The interactions with cognate tRNA-ASL pay this cost, stabilizing the 30S subunit in the closed form in the cognate, but not in the near-cognate case (Fig. 5a). Paromomycin fixes the flexible nucleotides A1492 and A1493 in a conformation similar to that induced by cognate tRNA-ASL [22,32], decreasing their entropy (Fig. 5a) and slightly reducing the distance between helix H44 and the 30S shoulder (protein S12, see supplementary animation). This eliminates part of the cost of tRNA-dependent domain closure (Fig. 5a). With cognate tRNA-ASL, this cost reduction results in increased affinity of tRNA-ASL binding, whereas with near-cognate tRNA-ASL it makes domain closure energetically favourable.

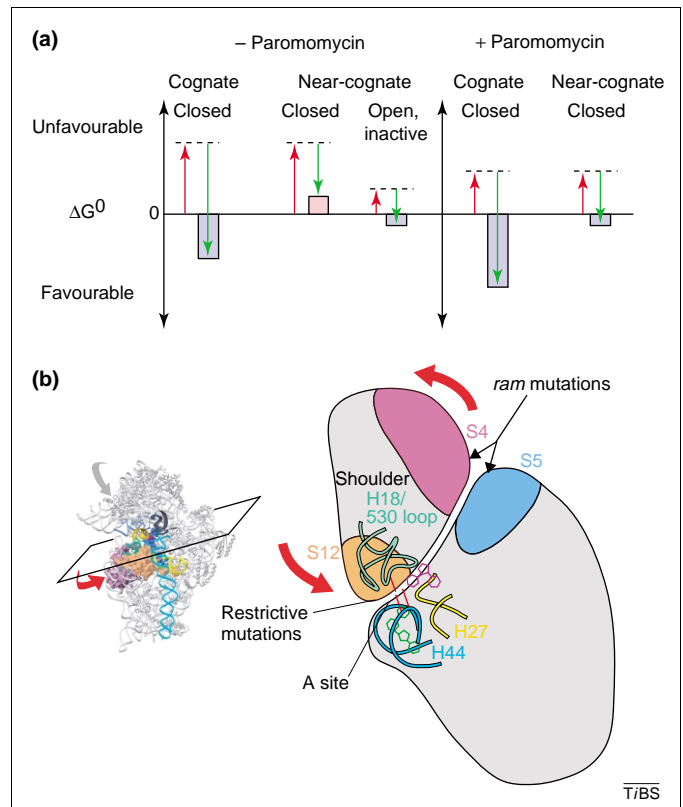


**Fig. 4.** Recognition of minor groove geometry by 16S RNA versus 'wobble'. RNA and protein residues are coloured as in Fig. 3, according to the structural elements they lie in. (a) Interaction of A1493 (cyan) with the first codon-anticodon base pair (anticodon, gold; codon, purple). (b) Interaction of A1492 (cyan), G530 (turquoise) and the second codon-anticodon base pair (anticodon, gold; codon, purple). A1492 from H44 thus directly connects to the shoulder domain. C518 is shown in turquoise, residue Ser50 from protein S12 in orange. (c) Discrimination at the first codon position. When paired to the near-cognate anticodon guanosine (red), the codon uridine (violet) is displaced into the minor groove and away from A1493. This leads to the loss of interactions (indicated by the larger broken line) between A1493 and the codon uridine in the closed form of the 30S. (d) The ribosome does not as stringently monitor minor groove geometry at the third codon position. However, some contacts are made, which would impose certain constraints upon codon-anticodon base pairing geometry in the closed form. S12 residues are shown in orange, C1054 from helix H34 in blue, G530 and C518 from helix H18 in turquoise, and the codon and anticodon in purple and gold, respectively. (a,b,d) Reprinted, with permission, from Ref. [22]. Copyright 2001 American Association for the advancement of Science. (c) Reprinted, with permission, from Ref. [23].

Paromomycin affects the rates of almost every step during ribosomal aa-tRNA selection and, in particular, accelerates both GTP hydrolysis and accommodation [31]. This and various other lines of evidence indicate that the influence of domain closure on tRNA selection begins before GTP hydrolysis, and hence plays a role in the activation of EF-Tu.

The orientation of the A-site tRNA-ASL in the closed form of the 30S subunit corresponds to its orientation after accommodation into the A site [22,33] rather than the initial tRNA orientation presented by EF-Tu. Thus, originally, it was not clear whether the initial codon recognition before GTP hydrolysis would involve the same minor groove contacts. Kinetic data suggest that kirromycin stalls the ribosome in the tRNA selection pathway just after GTP hydrolysis but before release of EF-Tu and accommodation [34]. This suggests that the kirromycin stalled complex has tRNA in an orientation close to its initial interaction with the A site. The ribosomal bases G530, A1492 and A1493 are protected from chemical modification agents in the presence of a kirromycin-stalled ternary complex as they are with accommodated tRNA [19]. This implies that these bases contact the codon-anticodon minor groove and lead to 30S-domain closure by the time GTPase is activated. In support of this, recent cryo-EM maps of a kirromycin-stalled ternary complex

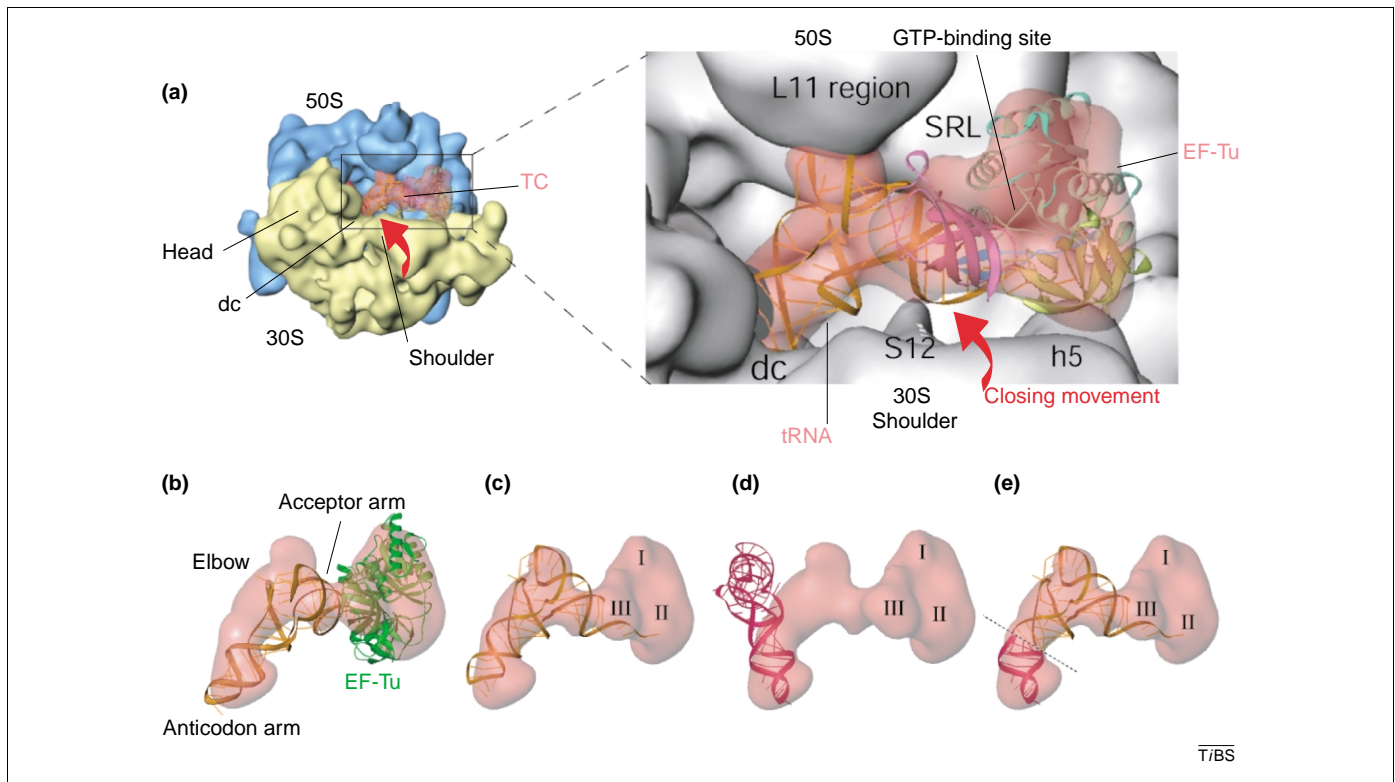
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**Fig. 5.** The cost of domain closure and effects of antibiotics or accuracy mutations [23]. (a) Schematic diagram summarizing the relative changes of free energy upon association of the 30S subunit and A-site codon with cognate or near-cognate tRNA anticodon stem-loop (ASL), and the influence of paromomycin. When cognate tRNA-ASL associates with the 30S decoding centre and the codon, the cost of 30S rearrangement into the closed form is compensated for by the total free energy of interactions made in the closed 30S complex (between the 30S, the codon and tRNA-ASL, as well as within the 30S subunit). As near-cognate tRNA-ASL does not interact optimally with the decoding centre, it cannot induce subunit closure. Rather, it associates weakly with the A-site codon in an open form of the 30S, avoiding both the cost of rearranging the subunit and the associated interactions with the decoding centre. Paromomycin reduces the cost of domain closure by pre-organizing part of the decoding centre in a tRNA-ASL-binding conformation. This increases the affinity of cognate tRNA-ASL, whereas near-cognate tRNA-ASL associates with the closed rather than the open form 30S, despite reduced or sub-optimal interactions with the decoding centre. (b) Schematic cross section of the 30S subunit (cf. inset), in the region of the decoding centre and proteins S4 (violet), S5 (blue) and S12 (orange). G530 and A1492/3 are represented by red bars; helices H44, H27 and H18 (with the G530-loop) are cyan, yellow and turquoise, respectively. The rotation of the shoulder domain (red arrows) during the transition to the closed 30S conformation disrupts an interface between S4 and S5, while the H18/530-loop/S12 region forms new contacts to H27 and H44. This emphasizes the importance of the energetics of this movement for the accuracy of tRNA selection, because mutations in these regions either increase or decrease mRNA misreading. Paromomycin (green rings) and streptomycin (pink rings) induce translational errors by facilitating domain closure. See the supplementary animations for molecular views of the interactions of proteins S4, S5 and S12 during the closing movement, and the structural effects of the antibiotics ([http://alf1.mrc-lmb.cam.ac.uk/~ribo/30S/supplementary\\_info/ogle\\_TIBS\\_2003/](http://alf1.mrc-lmb.cam.ac.uk/~ribo/30S/supplementary_info/ogle_TIBS_2003/)).

bound to the ribosome [35,36] (Fig. 6), suggest that the tRNA bends into the decoding site, placing the anticodon in an orientation closer to that observed in the domain-closed 30S-tRNA-ASL complexes in which the minor groove contacts were observed [22,23].

Domain closure as a major 30S rearrangement necessary for tRNA selection can rationalize the effects of not only paromomycin, but also another antibiotic, streptomycin, as well as many mutations that affect the fidelity of tRNA selection. Mutations in ribosomal proteins S4 and S5 that cause misreading [4] (ribosomal ambiguity or *ram*



**Fig. 6.** Cryo-EM reconstruction of the 70S ribosome bound to kirromycin-stalled ternary complex [35,36]. (a) Overview and close-up of the ternary complex on the ribosome. Red arrows approximately indicate the rotation of the shoulder during 30S domain closure. Abbreviations: dc, decoding centre; TC, ternary complex; SRL, sarcin-ricin loop; L11-region refers to ribosomal protein L11 and associated 23S RNA. (b) The crystal structure of the isolated ternary complex [50] does not fit the overall shape of the cryo-EM map without adjustments within the protein, the aminoacyl-tRNA (aa-tRNA) and their relative orientation. (c) After rigid alignment of the aa-tRNA, the anticodon stem-loop (ASL) portion does not fit the map. (d) The lower portion of the map better fits the ASL in an orientation as seen in A-site accommodated tRNA [33] or the ASL fragment in the domain-closed 30S [22]. (e) Combination of the accommodated ASL orientation and the best fit of the remaining aa-tRNA implies a bend or kink in the anticodon arm. Figure kindly provided by J. Frank (Howard Hughes Medical Institute, Wadsworth Center, Albany, USA). Reproduced, with permission, from Ref. [35].

mutations) occur at an interface between the two proteins that is disrupted by the rotation of the shoulder domain in the transition of the 30S subunit to the closed form (Fig. 5b). The mutations would be expected to decrease the number of bonds that must be broken during domain closure. In analogy to the effect of paromomycin, reducing the cost of domain closure will facilitate acceptance of near-cognate tRNA by the ribosome. Although the detailed tRNA-selection kinetics of *ram* ribosomes are not known, this structural explanation is consistent with the increased A-site affinity of cognate tRNA to these mutants [37].

In contrast to *ram* mutations, protein S12 on the opposite side of the shoulder from the S4/S5 interface (Fig. 5b) is the site of 'restrictive' mutations that increase the fidelity of protein synthesis. Many of these mutations change residues that contact helices H44 and H27 only after domain closure (see supplementary animations) and are therefore expected to destabilize the closed form of the 30S subunit. The interactions of these residues in S12 could progressively compensate for energetically costly events during domain closure, such as the disruption of the S4/S5 interface. Mutation of these residues would therefore increase the activation barrier to the 30S rearrangement. Indeed, such S12 mutations decrease GTP hydrolysis by EF-Tu and increase accuracy during both initial selection and proofreading [38]. Their

opposing effects on the transition to the closed form rationalize earlier findings that restrictive mutations can be suppressed by *ram* mutations [4]. Mutations within helix H27, close to where S12 forms contacts in the closed 30S, can either increase or decrease accuracy [39]. It was originally suggested that these mutations modulate a base-pairing switch in H27 during tRNA selection, but it now seems more likely that these mutations influence accuracy by subtly affecting the interaction energy at the interface between the shoulder and the H44/H27 regions.

Streptomycin, which decreases the accuracy of translation, alleviates the effects of restrictive S12 mutations [4], but severely affects *ram*-mutant ribosomes [40,41]. This can now be understood in structural terms because the antibiotic directly connects the shoulder (S12 and G530 loop) to the central part of the subunit (H27/H44) [32]. By increasing the number of interactions between these regions, streptomycin moves the shoulder even in the absence of tRNA (see supplementary animation). Although the shoulder does not rotate into precisely the same position as that resulting from cognate codon-anticodon recognition, this movement explains why *ram* mutations facilitate the binding of streptomycin [42]. Most importantly however, a dependence of GTP hydrolysis and aa-tRNA accommodation on domain closure would rationalize in structural terms how

streptomycin accelerates both these steps (Fig. 2) with near-cognate tRNA, but slows them with cognate tRNA, thus diminishing discrimination overall [11].

Finally, it is conceivable that the cost of the closing movement could be increased by the presence of tRNA at the E site (between the 'head' and the 'platform' domains), which could explain observations of reduced A-site affinity when the E site is occupied by tRNA [43].

### How does anticodon recognition activate the EF-Tu GTPase?

Hints at how GTP hydrolysis is induced come from combining this view of 30S-domain closure with cryo-EM reconstructions of a kirromycin-stalled ternary complex bound to the ribosome [35,36]. These cryo-EM maps are particularly informative because it has been possible to model individual components from crystal structures into them (Fig. 6).

EF-Tu directly contacts the sarcin-ricin loop (SRL) on the 50S, and helix H5 on the 30S subunit (Fig. 6a). The tRNA 'elbow' contacts the L11 region on the 50S subunit, which has previously been termed the 'GTPase activating centre'. This term now seems somewhat misleading, because it is the SRL loop that lies closest to the EF-Tu GTP-binding pocket. Although the details differ, cryo-EM maps show that the tRNA also contacts helix H69 (50S) and protein S12 (30S). Functional studies [4,11] suggest that almost all these sites play a role in accurate selection of aa-tRNA. The significance of the L11 region might be to ensure the ternary complex is bound in the correct orientation for GTPase activation. Surprisingly, the 50S L7/L12 stalk – which is known to be important for EF-Tu GTPase activity [44] – is quite distant from the ternary complex. This suggests that it might act indirectly, possibly by modulating 23S RNA conformation. More direct alternative mechanisms for the action of the L7/L12 stalk region have also been proposed [44].

An important, but currently unresolved, issue is the composition and structure of the EF-Tu GTPase active site. Although there is currently no indication that the ribosome directly inserts a catalytic residue, there are also no convincing candidates in EF-Tu itself as judged by crystal structures and mutational studies [45,46]. The crystal structure of EF-Tu-GDP bound to a kirromycin analogue (aurodox) suggests that a histidine residue (His84) might contribute to transition-state stabilization [47]. Interestingly, the cryo-EM models of the ribosome-bound ternary complex place both His84 and the GTP-binding site of EF-Tu close to the SRL. The relative orientation of the three EF-Tu domains are also altered compared with the EF-Tu forms seen with GTP, GDP, aurodox-GDP or the ternary complex alone (Fig. 6a,b). The catalytically active conformation of EF-Tu, which is only accessible on the ribosome, might thus be most directly influenced by its interaction with the SRL.

The fit of codon-anticodon geometry to the ribosome will be reflected in the rates of GTPase activation [10] if the activation barrier to the conformational reorganization of EF-Tu is lowered using energy derived from cognate interactions at the decoding site. At least part of this energy appears to be channelled through the most direct

connection to the EF-Tu GTP-binding site, the tRNA. This would explain why tRNA consisting of two separate fragments is unable to activate GTP hydrolysis [48]. As previously mentioned, in the cryo-EM maps, the relative positions of the EF-Tu domains and the aa-tRNA appear to be somewhat altered compared with the structure of the ternary complex alone, and the tRNA anticodon arm is distorted (Fig. 6b-e). This distortion is stabilized as a result of constraining interactions on the different parts of the tRNA, in particular the anticodon region, the elbow and the aminoacyl end of the aa-tRNA, which contacts EF-Tu near the GTP-binding site. Such bending of tRNA during GTPase activation has previously been predicted on the basis of tRNA mutations, which cause misreading [30]. Flexibility of the tRNA within the anticodon arm was originally postulated on the basis of the tRNA architecture alone [49] and is consistent with the variability of various known free and protein-bound tRNA structures. Thus, the interaction energy of cognate codon-anticodon duplex recognition must stabilize both 30S-domain closure and the deformation of the tRNA, which is in contact with EF-Tu. The rotation of the 30S shoulder during domain closure (Fig. 6a) is expected to directly influence the structure and position of EF-Tu via the contacts of S12 to the aa-tRNA, and 16S RNA-helix H5 to domain II of EF-Tu. During these movements, interaction with the SRL and the distorted tRNA could stabilize a conformation of EF-Tu that is active in GTP hydrolysis.

### Accommodation and rejection of aa-tRNA

The 30S crystal structures and the 70S cryo-EM-maps with ternary complex indicate that anticodon recognition by the decoding centre favours an accommodated orientation of the ASL portion of tRNA. The bent tRNA conformation observed in the ternary complex in the ribosome must be stabilized because of the extensive interactions it makes with the decoding centre and EF-Tu at either end. However, after dissociation of the GDP-form of EF-Tu relieves one of these constraints, this 'bent' conformation is no longer energetically favourable because the fully A-site-accommodated tRNA [33] assumes the 'classical' L-shape also found in various isolated tRNA structures. Thus, it is conceivable that one of the forces driving accommodation is a thermodynamically downhill transition of the tRNA towards this 'ground state' conformation. During this relaxation, strong interactions in the decoding centre can constrain the orientation of the anticodon arm. The probability of the tRNA relaxing into the accommodated state without significant reorientation of the ASL would thus be correlated with anticodon recognition by the decoding centre. This can be understood by considering that if the interactions between the anticodon and the decoding centre are weak, tRNA can also relax into orientations relative to the ribosome that are not determined by the ASL region and the decoding centre, but rather by other, non-specific contacts that can still be formed after dissociation of EF-Tu (e.g. to the L11 region or S12). Thus, the more restricted conformational space accessible to cognate aa-tRNA increases the probability (and hence the rate) of it reaching the accommodated state compared with near-cognate aa-tRNA, which

has more degrees of freedom. This influence of interactions at the decoding centre on accommodation explains why cognate tRNA has a faster accommodation rate as well as a faster GTPase-activation rate, and why paromomycin and streptomycin also increase both rates for near-cognate tRNA [10,11,31]. At the same time, compared with the cognate case, complete dissociation from the ribosome (proofreading) is favoured in the absence both of the interaction via EF-Tu and of specific contacts to the closed form of the 30S subunit.

### Concluding remarks

Recent structural work has shed light on many areas of the decoding problem. Crystal structures have clarified how the 30S subunit recognizes cognate aa-tRNA and how this leads to a domain closure. Antibiotics such as paromomycin promote domain closure by near-cognate tRNAs, rather than merely increase their binding affinity. The domain closure drives subsequent steps in tRNA selection, and can explain much previously unconnected biochemical and genetic data on translational accuracy. In conjunction with recent cryo-EM results and previous kinetic data, it is possible now to think about how domain closure can accelerate GTP hydrolysis and accommodation. However, a complete mechanistic description of tRNA selection will require additional data. For example, many accuracy-modulating mutations (reviewed in [4,11]), notably in the L7/12 stalk and around the peptidyl transferase centre of the 50S subunit, remain unexplained. High-resolution crystal structures of the ribosome, both in the kirromycin-stalled state and after accommodation, will probably be required to satisfactorily address these issues.

### Acknowledgements

We thank J. Frank for kindly providing Fig. 6.

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