Published in final edited form as:

Science. 2013 June 28; 340(6140): . doi:10.1126/science.1235490.

Elongation factor G bound to the ribosome in an intermediate state of translocation

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Abstract

A key step of translation by the ribosome is translocation, which involves the movement of mRNA and tRNA with respect to the ribosome. This allows a new round of protein chain elongation by placing the next mRNA codon in the A site of the 30S subunit. Translocation proceeds via an intermediate state in which the acceptor ends of the tRNAs have moved with respect to the 50S subunit but not the 30S subunit, to form hybrid states. The GTPase elongation factor G (EF-G) catalyzes the subsequent movement of mRNA and tRNA with respect to the 30S subunit. Here we present a crystal structure at 3 Å resolution of the *Thermus thermophilus* ribosome with a tRNA in the hybrid P/E state bound to EF-G with a GTP analog. The structure provides insights into structural changes that facilitate translocation and suggests a common GTPase mechanism for EF-G and elongation factor Tu.

Following peptidyl transfer, translocation of tRNAs on the ribosome was shown to occur spontaneously with respect to the 50S subunit, while the anticodon ends and mRNA remain anchored in their original sites in the 30S subunit, resulting in the formation of A/P and P/E tRNA hybrid states (1). The hybrid tRNA states are accompanied by a rotation of the ribosomal subunits relative to one another, together with a series of conformational changes in the L1 stalk and main body of the ribosome (2, 3).

In the second step of translocation, the GTPase elongation factor G (EF-G) catalyzes the movement of mRNA and tRNAs with respect to the 30S subunit, thereby placing the next codon of mRNA in the A site and restoring the ribosome to the canonical, unrotated state. Various experiments suggest that EF-G with GTP stabilizes a rotated state of the ribosome (4) with hybrid tRNAs (5-7). EF-G is structurally similar to the ternary complex of elongation factor Tu (EF-Tu), tRNA and GTP, with its domain IV mimicking the anticodon stem-loop of tRNA (8-10). The structures of EF-G bound to the ribosome in both the canonical and rotated states have been observed by cryoEM (11-13). Whereas these studies have greatly advanced our understanding of the changes in the ribosome induced by EF-G binding, a high-resolution structure can provide greater details of the interactions of EF-G with the rotated state of the ribosome and insights into the molecular mechanisms that lead to translocation.

It was originally assumed that EF-G simply lowers the free-energy barrier of the spontaneous reaction and that GTP hydrolysis is required to release EF-G from the post-translocated ribosome (14, 15). The currently prevailing view, based on kinetic experiments suggesting that GTP hydrolysis precedes and accelerates translocation (16-18), is that the rotated-state ribosome plays the role of a GTPase activator for EF-G. Rapid GTP hydrolysis upon ribosome binding is thought to accelerate rate-limiting conformational changes that result in an unlocking of the ribosome leading to translocation (17).

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How GTP hydrolysis is activated by the ribosome remains somewhat controversial. Mutation of the highly conserved His⁸⁴ of EF-Tu to alanine resulted in a 10⁶-fold reduction in catalytic activity (19). The structure of the ternary complex bound to the ribosome showed that His⁸⁴ in the switch II region was involved in hydrogen-bonding interactions both with A2662 of the sarcin-ricin loop (SRL) of 23S rRNA and a water molecule positioned for hydrolysis of the γ -phosphate of the GTP analog γ -methylenguanosine 5'-triphosphate (GDPCP) (20).

Suggestions that the histidine might play a role as a catalytic base were later questioned (21-23). Subsequently, a structure of RF3 bound to a rotated-state ribosome (24) placed the histidine in a very different position, suggesting that it was unlikely to play a direct role in catalysis and that any mechanism of GTP hydrolysis is not general, as was first proposed (20).

A breakthrough in determining high-resolution structures of the ribosome bound to EF-G in various states was made when the crystal structure of GDP-bound EF-G stalled on a post-translocated ribosome was solved (25). Here, we report a crystal structure refined using data to 2.9 Å, of the ribosome bound to EF-G with GDPCP. In addition, the structure consists of an mRNA with a phenylalanine codon in the P site, and a tRNA^{Phe} in the P/E hybrid state. As was done in previous cryoEM structures (11, 12), the A-site tRNA was left out in order to obtain the intermediate rotated state of the ribosome, because in its presence with wild-type EF-G, the ribosome proceeds within seconds to the post-translocational canonical state even without GTP hydrolysis (16, 26). This structure with EF-G bound to the rotated state of the ribosome prior to GTP hydrolysis lacks an A-site tRNA, but otherwise represents a key hitherto missing high-resolution structure in the elongation cycle.

Results

Overall structure

Crystallographic data are shown in Table 1. After molecular replacement using the 50S and 30S subunits as search models, the P/E tRNA, mRNA, EF-G and GDPCP were clearly visible in difference Fourier maps (Fig. 1; mRNA not shown), and the entire structure was built and refined (Fig. 2A). The main body of the 30S subunit is rotated ~7° counterclockwise with respect to the 50S (as viewed from the solvent side) (Fig. 2B; Movie S1). Although the precise rotation angles differ, the inter-subunit interactions and central bridges are similar to those previously seen in the hybrid state with RRF (27) or RF3 (24, 28), suggesting a ratcheting motion that is conserved across the translational pathway. The head of the 30S is swiveled by ~5° as compared to the canonical state (Fig. 2C; Movie S2). Two separate ratcheted states that differ in the degree of head swiveling have been identified by cryoEM of an EF-G-ribosome complex (13). As displayed in Fig. 2C, the 30S head of this structure has a conformation close to that of the Tl^{PRE} state in that cryoEM study (r.m.s.d. of 1.7 Å as opposed to 11.1 Å when compared to Tl^{POST} state). Recently it was shown that the Tl^{PRE} state also closely resembles cryoEM reconstructions of ribosomes containing both P/E and A/P hybrid tRNAs after peptidyl transfer (29), which is further evidence that our structure represents a valid model for the main intermediate state of translocation. The head swivel is thought to widen a constriction in the 30S to allow translocation of the P-site tRNA to the E site (13, 30, 31). In the rotated state seen here, this constriction is widened by ~2.7 Å compared to the canonical state, suggesting that further widening must occur at some point to allow translocation of the anticodon stem-loop of tRNA from the P to the E site. It has been proposed that inter-subunit ratcheting and 30S head swiveling are sequential events that provide directionality to mRNA and tRNA translocation (32).

Interaction of the L1 stalk with the P/E hybrid tRNA

The tRNA^{Phe} in the P/E conformation is distorted, with a twist in the D-stem of the main body enabling the acceptor arm to swing $\sim 35^\circ$ towards the E site of the 50S subunit, similar to that seen in the hybrid states with RRF (27) or RF3 (28) (r.m.s.d. of 0.81 Å and 1.55 Å respectively). The elbow of the P/E tRNA is cradled by the L1 stalk of the 50S ribosomal subunit, which has pivoted about the base of helix H76 (Fig. 3A) and swung into the fully closed conformation seen in lower resolution studies (11, 12). In structures with a canonical E-site tRNA in the post-translocational state, the L1 stalk is in a “half-closed” conformation (25). Relative to that conformation, the distal part of the L1 stalk has moved inward by ~ 25 Å to interact with the P/E tRNA (Fig. 3B), resulting in an angle of $\sim 17.4^\circ$ between these two positions. Moreover, there is a distance of ~ 37 Å between the closed conformation seen here and the fully open conformation observed in structures of the ribosome with a vacant E site (30). This dynamical nature of the L1 stalk has been studied using two kinds of smFRET experiments and demonstrated to have a mechanistic role during translocation (33, 34). In the absence of any factor, the L1 stalk fluctuates between half-closed and closed conformations corresponding to nonratcheted and ratcheted states of the ribosome; binding of EF-G shifts this equilibrium towards the closed conformation of the ratcheted state. Our structure supports the notion that the L1 stalk-tRNA interaction persists throughout translocation (33). However, a separate study suggests that hybrid state formation and L1 stalk closure are not tightly coupled (35).

A detailed description of the interactions between the L1 protein and tRNA is made possible by the stabilization of the stalk in the closed conformation, resulting in excellent maps that show side-chain conformations (Fig. 3C). The majority of these interactions are electrostatic, such as Arg⁵⁹, Arg¹²⁹ and Arg¹⁶⁴ forming salt bridges with the negatively charged phosphate backbone of the tRNA, but there is also a stacking interaction between base C56 and the imino ring of Pro¹³³. Such contacts are probably maintained as the L1 stalk chaperones the P/E tRNA to the E/E conformation during translocation (33), since superposing the current structure with that docked into the post-translocated ribosome structure reveals that the backbone of the L1 protein does not change upon the transition.

Interactions of EF-G with L11, L12 and L6

On the other side of the 50S subunit from the L1 stalk, the interaction of EF-G with L6, L11 and the L12 stalk are indistinguishable from those previously described for the post-translocational state (25) (Fig. 4). In particular, the C-terminal domain of one of the L12 molecules is seen interacting with EF-G and the N-terminal domain of L11, and on the opposite side, L6, at the base of the L12 stalk also interacts with EF-G through a flexible C-terminal domain extension.

Changes in the conformation of domain IV of EF-G

Details of the structural changes in EF-G during translocation can be discerned by superposing domains I and II of EF-G in this structure with those of the isolated factor (36) or in the post-translocational state (25). In this superposition, the isolated structure of EF-G would have a conformation of domain IV that would largely avoid a steric clash with A-site tRNA (Fig. 5A). Presumably this orientation of domain IV resembles the transient state immediately after EF-G binds to the rotated state and just before translocation occurs in the 30S. In the structure described here, domain IV has moved partly into the A site and would clash with A-site tRNA (Fig. 5A; Movie S1), which explains why slow translocation can occur even without GTP hydrolysis. Thus ribosome binding alone must promote a conformation of EF-G that partially facilitates translocation. However, the fragmented density and high B-factors for domain IV suggests that it has a dynamical nature, consistent with its requirement for being able to coexist transiently with A-site tRNA.

A comparison with EF-G in the post-translocational state (25) shows that the tip of domain IV has moved by another ~ 6.6 Å and more fully occupies the A site (Fig. 5B; Movie S1). This further movement is a result of the rotation of the superdomain I-II relative to domains III-V that presumably occurs following GTP hydrolysis.

Changes in the catalytic site

The catalytic site of EF-G shows distinct differences from the post-translocated GDP form (25) or the isolated EF-G with GDPNP (36) that yield insights into activation of GTP hydrolysis. The switch I region was unresolvable in previous crystal structures of both post-translocated and isolated EF-G, but is ordered in this structure from Met⁵⁵ onwards. The switch I region adopts a single turn of a 3_{10} helix that contacts helix B₃ of domain III, as in the isolated structure of the EF-G homologue EF-G-2 in the GTP form, and as also seen at lower resolution by cryoEM studies of a ribosomal complex similar to the structure described here (12). The γ -phosphate of GDPCP is surrounded by several highly conserved residues, notably His⁸⁷ of switch II, and Asp²² and Lys²⁵ in the P loop (Figs. 6A, B). His⁸⁷ and Asp²² point away from bound nucleotide in the isolated and post-translocated states of EF-G, but have moved respectively by ~ 6.4 Å and ~ 3.3 Å towards the γ -phosphate of GDPCP upon ribosome binding (Fig. 6C; Movie S3) to assume a conformation very similar to that seen before in EF-Tu (Fig. 6D) (20). As with EF-Tu, the conformation of the activated His⁸⁷ is stabilized by hydrogen bonding interactions with both A2662 of the SRL, and the catalytic water molecule poised for hydrolysis of the phosphate ester (Fig. 6B). Two Mg⁺⁺ ions uniquely positioned by the GAGA tetrad of the SRL stabilize the inward conformation of Asp²² where it coordinates a second water molecule above the γ -phosphate of GDPCP (Fig. 6B). This second water could play a further role in catalysis by donating a hydrogen bond to the γ -phosphate O2. The structure strongly suggests that the change in orientation of Asp²² and His⁸⁷ upon EF-G binding is part of GTPase activation by the ribosome, and that the mechanism of GTP hydrolysis is essentially the same for both EF-Tu and EF-G.

Although the final activated state of EF-Tu and EF-G GTP are highly similar, in EF-Tu the equivalent Asp²¹ has its activated conformation even in the isolated ternary complex. Thus, different steps may be required to reach the same activated state. Interestingly, the toxin ricin depurinates A2660 of the GAGA tetrad. It is likely that depurination of A2660 prevents the surrounding region from adopting the conformation required to bind the metal ions necessary to stabilize Asp²² and neighboring regions of EF-G in the activated form. EF-Tu does not make these interactions, explaining why ricin only affects EF-G function (37).

While a proposal was made that His⁸⁷ might be acting as a general base in EF-Tu (20), the structure is consistent with an alternate mechanism that was proposed subsequently (21). In this mechanism, the environment of the SRL may result in an elevation of the pK_a of His⁸⁷ and stabilize the protonated state of its N⁺, thus enabling His⁸⁷ to donate a hydrogen bond to the hydrolytic water. The water can in turn donate a hydrogen bond to the carbonyl oxygen of Thr⁶⁴, and to one of the three oxygen atoms on the γ -phosphate. Under these circumstances, the occurrence of a substrate-promoted catalytic mechanism whereby the γ -phosphate abstracts a proton from the water molecule to generate a hydroxide ion that in turn cleaves the phosphate ester appears feasible. It has also been suggested that the role of the histidine is not to behave as a donor or acceptor of protons at all, but to contribute to an allosteric effect that results in stabilization of the transition state by the general electrostatic effect of the P loop (23). This scenario is compatible with the observation that in EF-G-2, a ribosome-activated GTPase that can substitute for EF-G in polyU-directed protein synthesis *in vitro* (12), the histidine and aspartate have been replaced by tyrosine and glycine respectively (Fig. 6A).

Discussion

The structure sheds light on the GTPase mechanism of EF-G and on its role in translocation. Globally a striking feature is that the interactions of the L1 stalk with the P/E tRNA appear to be the same as those with the post-translocational E-site tRNA (25), implying that the interactions are preserved throughout translocation as previously suggested (33). This also suggests that the stabilization of the closed conformation of the L1 stalk through its interaction with the P/E tRNA is an essential feature of translocation through the stabilization of hybrid states.

Another large scale movement is the swiveling of the head, which is required to open a constriction that allows passage of the P-site tRNA to the E site in the 30S subunit (13, 38). It has been suggested before that spectinomycin, an antibiotic that inhibits translocation, may act by inhibiting the movement of the head by binding to a crucial hinge point (39, 40). Our structure shows that in the rotated state, the swivel angle of the head is such that it would cause a steric clash with spectinomycin, thus supporting this idea.

Remarkably, key residues in EF-Tu and EF-G change conformation in different ways upon binding to very different states of the ribosome to form a nearly identical catalytic site (Fig. 6D), suggesting a common mechanism for activation of translational GTPases by the ribosome. This mechanism also implies that the SRL plays a crucial role in stabilizing key residues of the catalytic site in their activated conformations, which would be in keeping with their very high degree of conservation.

Recently, lethal mutations in the SRL were found not to affect GTP hydrolysis (41), suggesting that the SRL does not play a direct role in stabilizing the transition state for GTP hydrolysis. However, the interactions with the SRL occurs via phosphate backbone interactions rather than specific bases, so it is possible that in these mutant ribosomes, other nucleotides of the mutated SRL play the role of key residues in the wild-type ribosome.

In contrast to EF-Tu and EF-G, the catalytic site of RF3 on the ribosome appears different; the histidine is far from the γ -phosphate of GTP and makes different interactions with the SRL (24). It is therefore possible that the GTPase mechanism for RF3 is different, or that the structure, which lacks the expected P/E tRNA, does not represent the GTPase-activated state of RF3.

The structure reported here offers some clues into how conformational changes associated with GTP hydrolysis could facilitate translocation. GTP hydrolysis results in changes in switch I, switch II and P loop regions that form an interface between the ribosome, domain III, and GTP. These changes in switch I and II may be communicated to domain III and cause the large movements of the helices that serve to bridge the I-II and III-V super-domains (Fig. 5). This would account for the relative change in the orientation of these super-domains upon GTP hydrolysis (Fig. 5). Deletion of domain III decreases EF-G activity 10^3 -fold on the ribosome (42), supporting the notion that this region may couple GTP hydrolysis to the inter-domain movements that allow domain IV to adopt the favored conformation of the post-translocational state. Such a conformation may be adopted after tRNA translocation has occurred transiently, allowing domain IV to enter the A site and prevent a reversal of translocation. Details of the mechanism of action of EF-G will require concerted studies by many complementary techniques.

In conclusion, this work provides an atomic model of EF-G bound to the ribosome in a rotated state prior to GTP hydrolysis. It has enabled a complete description of the inward movement of the L1 stalk, stabilization of the P/E tRNA, and conformational changes in EF-G that are the key steps in facilitating translocation. GTP hydrolysis leads to a series of

changes in the switch I, switch II, and P loop regions of EF-G, which result in an inter-domain reorientation about domain III that is expected to promote translocation of any tRNA bound at the ribosomal A site. Local conformational changes at the GTP-binding site of EF-G have implicated key residues Asp²², Lys²⁵ and His⁸⁷ in GTPase activation, whose precise roles can be tested by biochemical and mutagenesis experiments. Finally, despite their action on conformationally very different states of the elongating ribosome, the structure supports a common mechanism of GTP hydrolysis by both EF-G and EF-Tu.

Materials and Methods

Full length EF-G, tRNA^{Phe}, and ribosomes from *Thermus thermophilus* harboring a C-terminal truncation of protein L9 were prepared as previously described (25, 31). mRNA with the sequence 5' GGCAAGGAGGUAAAAAUGUUUCAAAA 3' was purchased from Dharmcon (Thermo Scientific), where the phenylalanine codon in the P site is underlined.

Ribosomes (4.0 μ M) and mRNA (8.0 μ M) were incubated at 55°C for 6 min before addition of tRNA^{Phe} (16.0 μ M) and a further incubation at 55°C for 20 min. Separately, EF-G (20.0 μ M) was incubated with GTPCP (6.0 mM) for 20 min at 37°C and mixed with the ribosome complex for a final incubation at 37°C for 20 min in buffer G (50 mM KCl, 10 mM NH₄Cl, 10 mM Mg-acetate, 5 mM HEPES, pH 7.5). Immediately prior to crystallization, the detergent HEGA-9 was added (46 mM). All concentrations refer to the final values in the sample. Typical total sample volumes used for crystallization experiments did not exceed 500 μ L.

Crystals were grown *via* streak seeding and vapor diffusion in sitting drop trays by mixing 3 μ L of sample with 3 μ L reservoir solution (100mM MES pH 6.3, 75mM KCl, 6.0-6.5 % (w/v) PEG 20K). Crystals of plate morphology grew to full size (~200 μ m by 100 μ m by 50 μ m) over a period of three weeks and were cryoprotected in a step-wise fashion by sequentially increasing the concentrations of PEG 20K and PEG 400 in the crystallization buffer to 6.8 % and 30 % respectively, while maintaining the concentration of other components. Crystals were plunged into liquid nitrogen and stored until data collection.

Two independently complete sets of data were collected from single crystals on beam line ID 14-4 at the European Synchrotron Light Source (43) and on beam line IO4 at the Diamond Light Source, Harwell, UK, respectively. Data were integrated, merged and scaled using XDS(44), and found to be consistent with space group $P2_1$ and unit cell dimensions $a = 201.58$ Å, $b = 241.65$ Å, $c = 305.80$ Å and $\beta = 99.48^\circ$.

Molecular replacement was performed using MOLREP (45) in two stages, first with the 50S subunit of the 70S *T. thermophilus* structure (31) as a search model, followed by inclusion of the 30S. The solution showed a single ribosome in the asymmetric unit in the fully rotated conformation. Refinement was carried out in alternating cycles of automated refinements using either PHENIX (46) or REFMAC5 (47), with manual refinement and model building in COOT (48). A summary of refinement and data collection statistics is displayed in Table 1. All figures were generated using PyMOL (49) or Jalview for sequence alignments (50).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are indebted to Paul Elliott and the beamline staff on IO4 at Diamond Light Source and ID14-4 at the European Synchrotron Light Source for help and advice with data collection, and R.M. Voorhees and C. Spahn for critical

comments. This work was supported by grants to VR from the UK Medical Research Council (U105184332), a programme grant and Senior Investigator Award from the Wellcome Trust, the Agouron Institute and the Louis-Jeantet Foundation. Coordinates and structure factors have been deposited in the PDB with codes 4JUW and 4JUX.

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Fig. 1. Unbiased difference Fourier maps

Unbiased difference Fourier maps obtained after initial refinement with an empty ribosome as a starting model, showing (A) P/E tRNA, (B) switch 1 and GDPCP in the active site, (C-E) key conserved residues in the active site with water molecules.

Fig. 2. EF-G bound to the rotated state of the ribosome

(A) Overall view of EF-G and the ribosome. EF-G is shown in red, the 50S subunit is shown in cyan, the 30S subunit in yellow, the P/E site tRNA in green, and ribosomal protein L1 is shown in orange. (B) Global conformational changes in the 70S ribosome upon GTP hydrolysis as viewed from the perspective of the 23S RNA (cyan). (C) Change in the swivel angle of the head of the 30S in various states of the ribosome, showing the rotated state with EF-G in this study (yellow), the post-translocated state with EF-G and GDP (light gray; pdb code 2WRI) (25), the “ TI^{Pre} ” state of a cryoEM structure of EF-G with GPDNP bound to a rotated state (green)(13) and the “ TI^{Post} ” state of the same study (red). For clarity, only the ribosomal RNA is shown in B and C.

Fig. 3. Dynamics of the L1 stalk during tRNA translocation

(A) Three distinct conformations of the L1 stalk, showing the open (gray; pdb code 2WA4) (30), the half-closed (pink; pdb code 2WRJ)(25) and fully closed conformations (cyan; this study). (B) The ribosomal protein L1 (orange) stabilizes the distorted P/E tRNA (green) halfway between the canonical P (red) and E (blue, see inset) site conformations. (C) Details of interactions between the L1 protein (orange) and elbow of the P/E tRNA (green). The unbiased $F_o - F_c$ difference Fourier map is contoured at 2.5 σ .

Fig. 4. Interactions of EF-G with L6, L11 and L12

Interactions of EF-G with ribosomal proteins L11, L6, the L12 CTD near the base of the L7/L12 stalk. A single C-terminal domain of L12 is seen to interact with both EF-G and the N-terminal domain of L11.

Fig. 5. Conformational changes in EF-G during translocation

(A) Comparison of isolated EF-G structure (light green; pdb code 2BV3)(36) with EF-G in this study (red). (B) Comparison of EF-G in this study (red) with that in the post-translocated state (gray; pdb code 2WRI)(25) reveals an inter-domain rotation about domain III leading to changes in the orientation of domain IV. Inset (right) shows that in the GDPCP bound EF-G (this study), switch I forms a 3_{10} helix that stabilizes helix B3 in an altered conformation.

Fig. 6. The active site of EF-G

(A) Sequence alignment of G-domains from several translational GTPases shows conservation of residues Asp22, Lys25 and His87 except in EF-G-2. (B) Details of the catalytic site around the γ -phosphate of GTPCP (blue) with relevant distances displayed as dashes. EF-G residues and waters are in red, Mg^{++} ions in green, and residues of the SRL are in cyan. (C) Comparison of the active site of isolated EF-G with GTPNP (light green; pdb code 2BV3)(36), EF-G with GTPCP in this structure (red) and EF-G in the GTP posttranslocated state (gray; pdb code 2WRI)(25) shows that His⁸⁷ and Asp²² move toward the γ -phosphate of GTPCP on ribosome binding, and away from it upon GTP hydrolysis. (D) Similarity of the activated catalytic sites of EF-G (this structure) and EF-Tu (pdb code 2XQD) (20), suggesting common mechanism of GTPase activation for the two factors.

Table 1

Summary of crystallographic data and refinement.

	Data set 1	Data set 2	70S-tRNA-EF-G-GDPCP (merged)
Data collection			
Beamline	ID14-4 (ESRF)	IO4 (DLS)	
Space group	$P2_1$	$P2_1$	$P2_1$
<i>Cell dimensions</i>			
a, b, c (Å)	203.42, 243.05, 309.97	201.58, 241.65, 305.80	201.58, 241.65, 305.80
α, β, γ (°)	90.00, 99.53, 90.00	90.00, 99.48, 90.00	90.00, 99.48, 90.00
Resolution (Å)	39.6 – 3.1 (3.2 – 3.1)	39.6 – 2.9 (3.0 – 2.9) *	39.6 – 2.9 (3.0 – 2.9) **
R_{sym} (%)	17.8 (58.7)	24.1 (138.4)	22.4 (137.8)
$I/\sigma(I)$	8.15 (2.42)	4.93 (1.08)	7.56 (1.02)
Completeness (%)	99.6 (97.1)	97.8 (98.9)	99.8 (98.9)
Refinement			
Resolution (Å)			39.6 – 2.9
No. unique reflections			635092
$R_{\text{work}}/R_{\text{free}}$			19.6/24.5
No. atoms			150122
<i>B values</i>			
RNA			37.8
Protein			51.4
Bond length r.m.s.d. (Å)			0.004
Bond angles r.m.s.d. (°)			1.488

* $I/\sigma(I) = 2.15$ at 3.1 Å (using a bin from 3.2 – 3.1 Å resolution).** $I/\sigma(I) = 3.31$ at 3.1 Å (using a bin from 3.2 – 3.1 Å resolution).