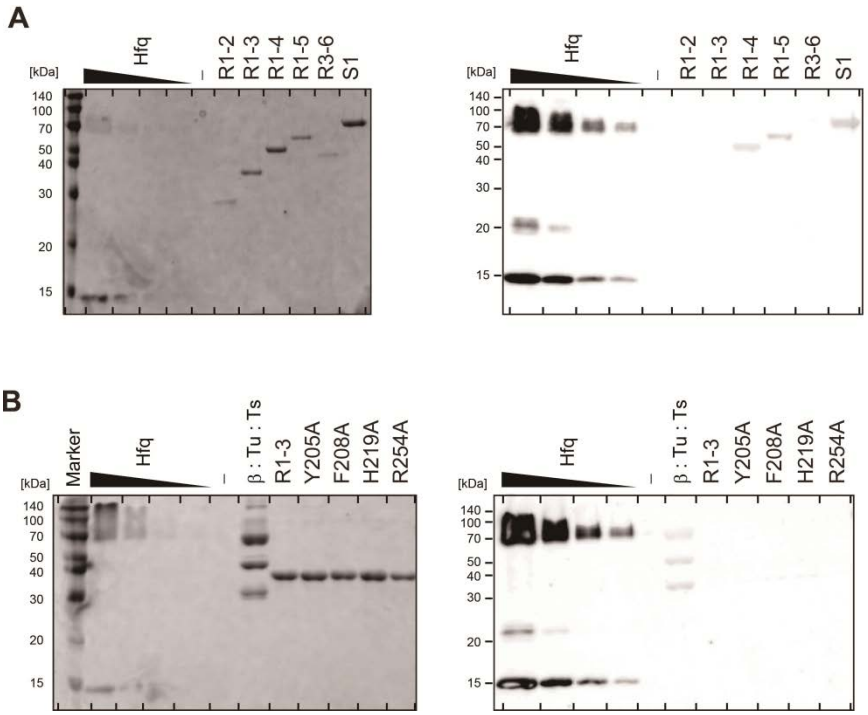
SUPPLEMENTARY DATA

Molecular insights into replication initiation by Qβ replicase using ribosomal protein S1

Daijiro Takeshita, Seisuke Yamashita and Kozo Tomita

SUPPLEMENTARY FIGURES

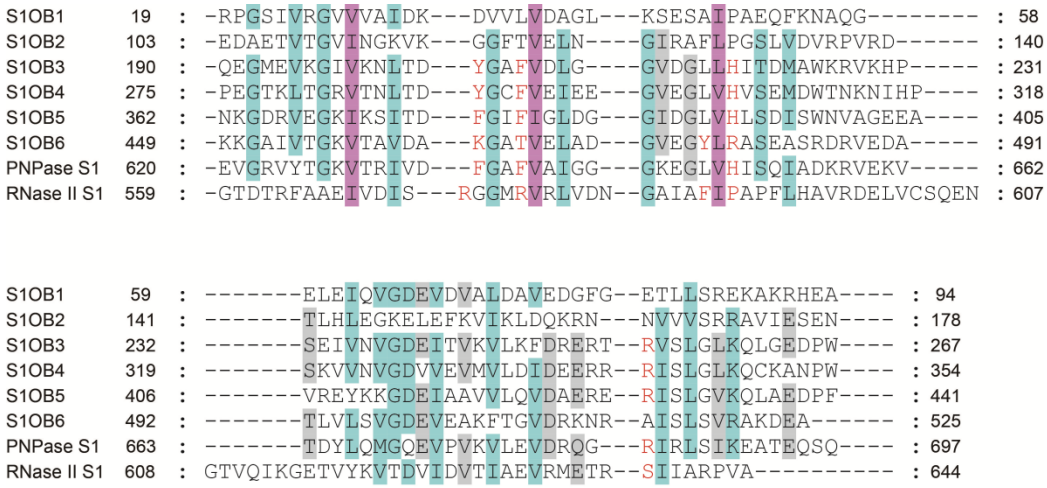
Figure S1



Supplementary Figure S1: Western-blotting of preparations of S1 and its variants used in this work.

(**A**) S1 variants (R1-2, R1-3, R1-4, R1-5, R3-6), the full-length S1 used in the present study (Figure 5C), and various amounts of purified Hfq protein (0.04, 0.07, 0.21 and 0.7 μg) were separated by SDS PAGE, and the gel was stained by CBB (left panel). The gel was western-blotted using an anti-Hfq antibody (right panel). (**B**) R1-3, its mutant proteins R1-3 (Y205A), R1-3 (F208A), R1-3 (H219A), R1-3 (R254) used in this study (Figure 6B), core Qβ replicase (β:Tu: Ts) and various amounts of purified Hfq protein (0.07, 0.13, 0.43 and 1.3 μg) were separated by SDS PAGE, and the gel was stained by CBB (left panel). The gel was western-blotted using an anti-Hfq antibody (right panel).

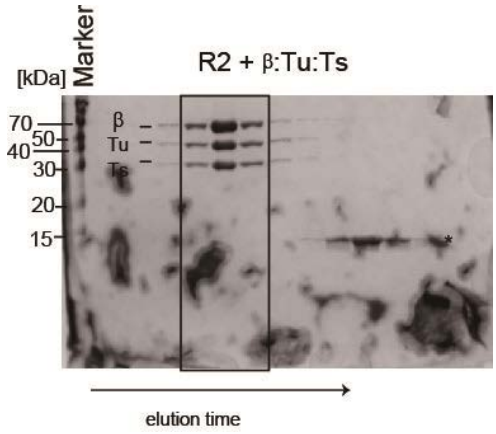
Figure S2



Supplemental Figure S2: Amino acid alignment of the OB-folds of ribosomal protein S1 and related proteins.

S1OB1~OB6 are the amino acid sequences of the six OB-folds from *E. coli* ribosomal protein S1. PNPase S1 and RNase II S1 are the amino acid sequences of the S1 domains of *E. coli* polynucleotide phosphorylase (1) and ribonuclease (RNase) II (2), respectively. Residues putatively involved in RNA-binding are colored red.

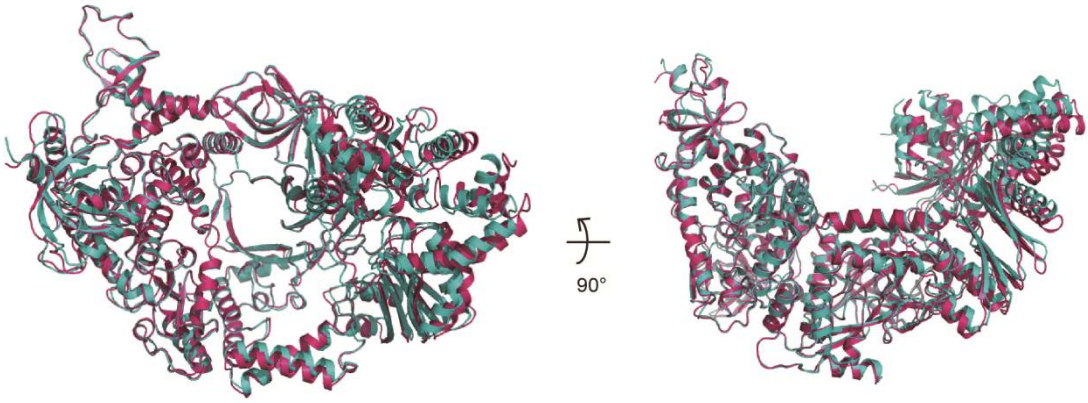
Figure S3



Supplementary Figure S3: Interaction between core Qβ replicase and R2 of S1.

Analysis of the interaction of core Qβ replicase with R2 by size-exclusion chromatography. R2 alone does not interact with the β-subunit. The fractions were separated by 15% (v/v) SDS-PAGE and stained with CBB as in Figure 1C. An Asterisk (*) in the gel indicates the band of R2.

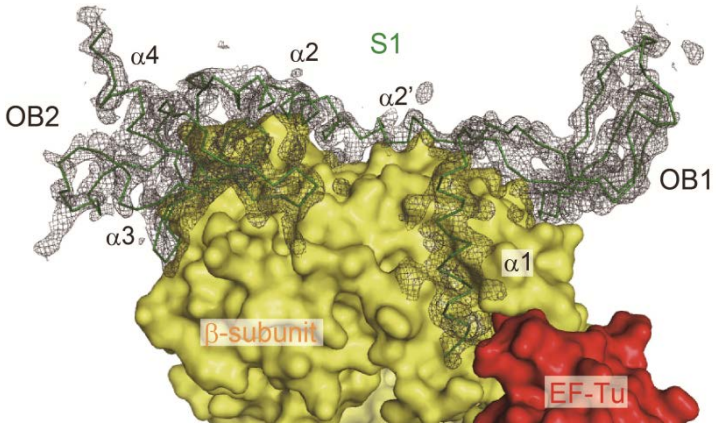
Figure S4



Supplementary Figure S4: Structures of $Q\beta_{SN}$.

Superimposition of the structures of Mol-A (colored cyan) and Mol-B (colored magenta) in the asymmetric unit. RMSD of Mol-A and Mol-B is 1.39 Å.

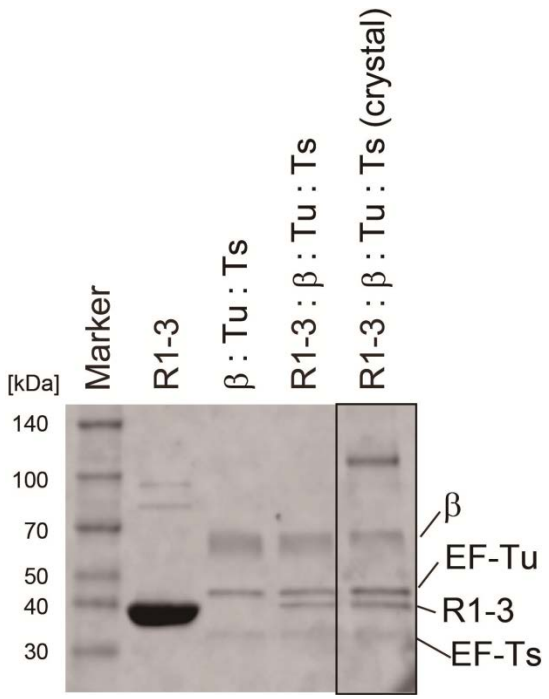
Figure S5



Supplementary Figure S5: Electron density map of S1.

2*Fo-Fc* map of the N-terminal half of S1 (R1-3; colored green), contoured at 1.0 σ (colored gray). The electron density corresponding to R3 was not visible. The β-subunit and EF-Tu are colored yellow and red, respectively, and depicted by surface models.

Figure S6



Supplementary Figure S6: Composition of the $Q\beta_{SN}$ crystal.

The $Q\beta_{SN}$ crystal was dissolved and fractionated (right lane) on a 4–20% (v/v) TGX gel (Bio-Rad), which was stained with CBB. The $Q\beta_{SN}$ crystal contains intact R1-3.

Figure S7

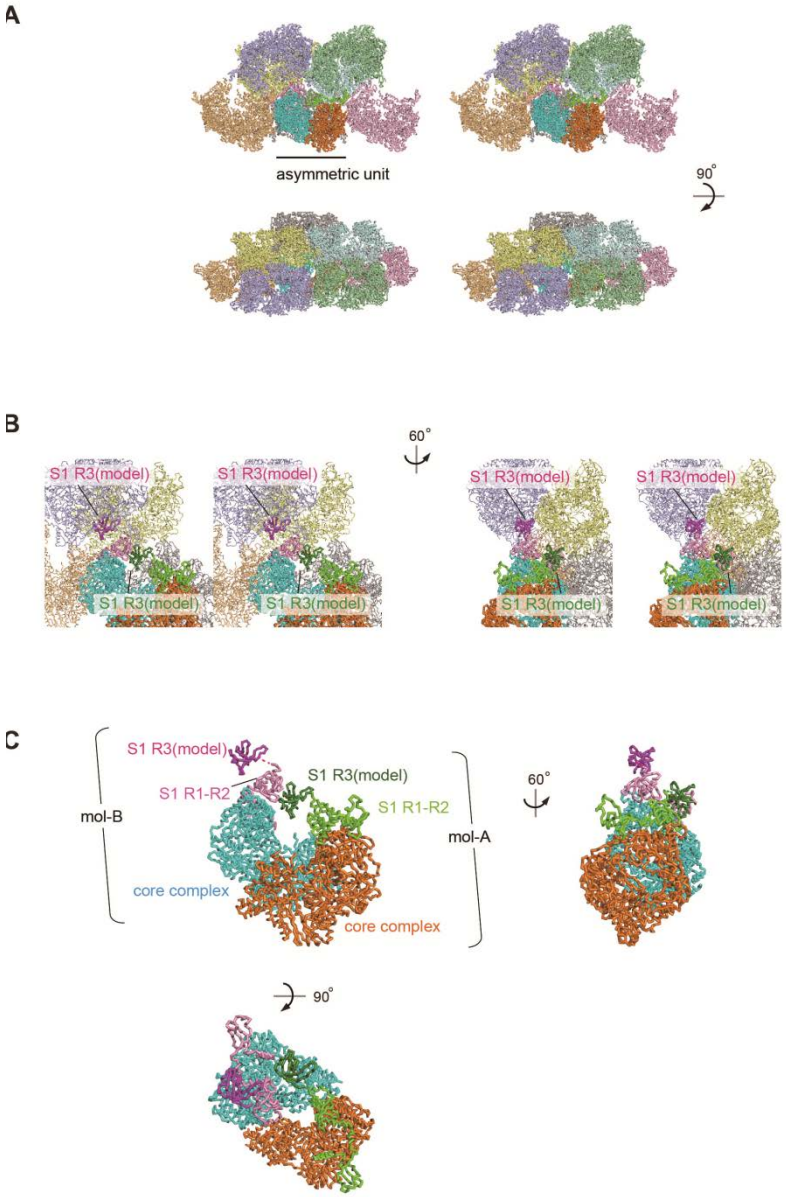
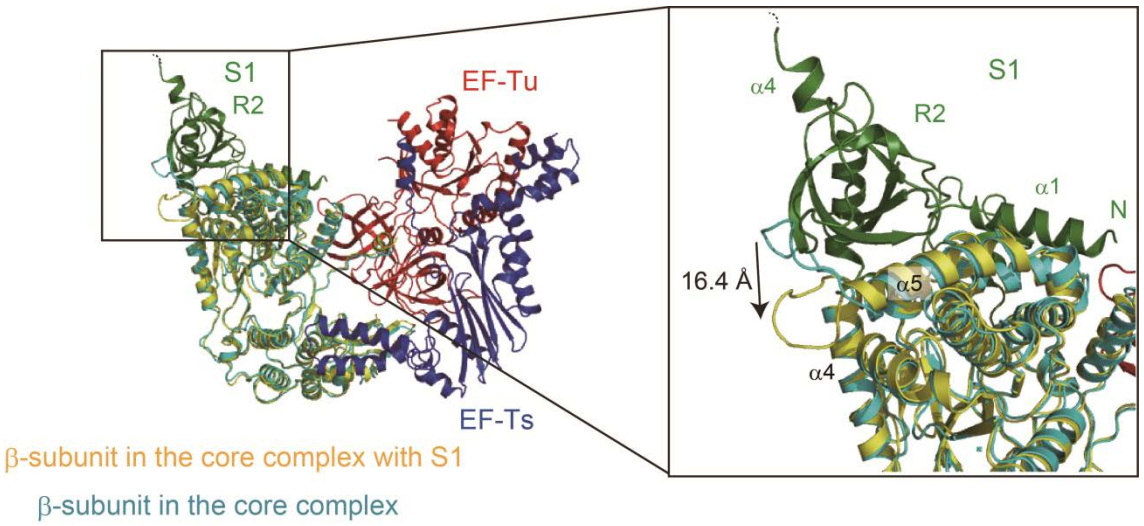


Figure S7: Crystal packing of the Qβ$_{SN}$ crystal.

There is sufficient space beyond the surface of the β-subunit, where the R3 of R1-3 of S1 is expected to be located. **(A)** Crystal packing of Qβ$_{SN}$. The crystal belongs to the space group $P2_12_12_1$, and contains two Qβ$_{SN}$ in the asymmetric unit (Mol-A and Mol-B). Mol-A and Mol-B are colored cyan and orange, respectively. **(B)** R1-2 in Mol-A and Mol-B are colored pink and green, respectively. R3 (magenta) is modeled in Mol-A, and R3 (dark green) is modeled in Mol-B. **(C)** Detailed views of (B).

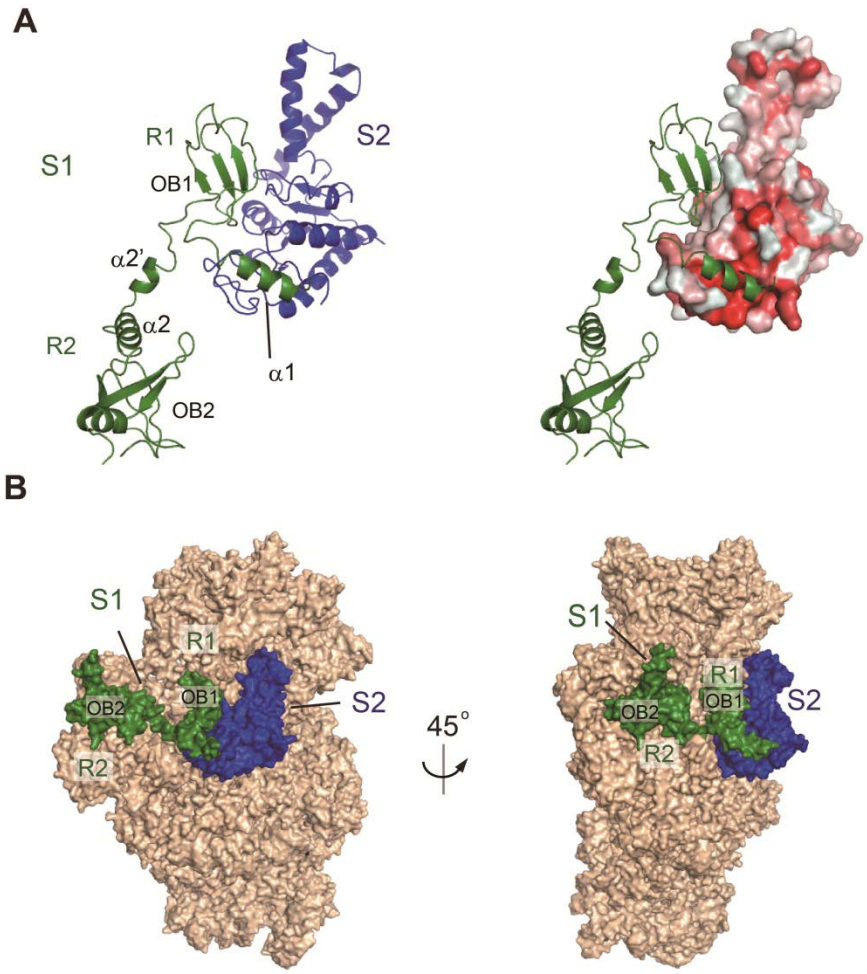
Figure S8



Supplementary Figure S8: Structural change of the β-subunit.

Superimposition of the structures of the core Qβ replicase (3; colored cyan) and Qβ replicase containing the N-terminal half of S1 (colored yellow, with the N-terminal half of S1 colored green).

Figure S9



Supplementary Figure S9: Model of the interaction between S1 and the 30S ribosome.

(A) Docking of the N-terminal α-helix (α1) of S1 (green) with the ribosomal protein S2 (blue). Hydrophobic residues in S2 are colored red in the surface model (right). **(B)** The N-terminal domains of S1 (R1-2) were docked on the 30S ribosome. The N-terminal domain of S1 is colored green and S2 is colored blue.

Table S1

	$Q\beta_{SN}$
Data collection	
Space group	$P2_12_12_1$
Cell dimensions	
a, *b*, *c* (Å)	132.19, 150.83, 189.78
Wavelength (Å)	0.9800
Resolution (Å)*	50 – 2. 90
	(2.95 – 2.90)
R_{sym}*	0.118(0.442)
$I / \sigma (I)$ *	17.3(3.3)
Completeness (%)*	99.0(96.3)
Redundancy*	8.7(4.0)
Refinement	
Resolution (Å)	20.0 – 2.90
No. reflections	83,532
R_{work}/ R_{free}	26.0/31.4
No. atoms	
Protein	21,098
Ion	20
Water	253
B factors (Å^2)	
Protein	64.2
Ion	77.2
Water	43.1
R.m.s. deviations	
Bond lengths (Å)	0.003
Bond angles (°)	0.749

*Highest resolution shells are shown in parentheses.

Supplementary Table S1: Data collection and refinement statistics.

SUPPLEMENTARY TEXT

Model of S1 docking onto the 30S ribosome

The N-terminal region of ribosomal protein S1 interacts with ribosomal protein S2 in the 30S ribosome (4, 5). We searched for the S1-docking surface on S2, using the ZDOCK server (6). For the receptor and ligand, the structures of S2 in the 30S ribosome (7; PDB ID: 2AVY) and of S1 (R1-2) in Qβ replicase (Figure 3B) were used, respectively. Among the top ten calculated solutions, four have similar binding manners, in which R1 of S1 binds to S2. The structure with the highest rank is shown in Supplementary Figure S5A. The N-terminal helix, α1, is placed on the hydrophobic patch of S2, as seen in the Qβ replicase complex (Figure 4A, B). This binding manner is also consistent with the results obtained from cross-linking between S1 and S2 in ribosomes (8). We superimposed the model complex of R1-2 of S1 and S2 onto the 30S ribosome (8). In the superimposition, the OB1 fold of S1 is placed next to the 30S ribosomal RNA, and the surface of OB1 of S1 snugly fits on the surface of the 30S ribosome. On the other hand, in the simple superimposition, R2 of S1 clashes with the ribosomal RNA. R2 of S1 would be placed at a different location on the 30S ribosome and/or in a different orientation, using the flexible linker between OB1 and OB2 (α2'-α2) (Figure 3B). In the model presented here, R2 was manually docked onto the 30S ribosome, considering the results obtained from cross-linking between amino acid residues in R2 and ribosomal proteins in the 30S ribosome (Supplementary Figure S5B).

SUPPLEMENTARY REFERENCES

1. Bycroft, M., Hubbard, T. J., Proctor, M., Freund, S. M. and Murzin, A. G. (1997) The solution structure of the S1 RNA binding domain: a member of an ancient nucleic acid-binding fold. Cell 88: 235-242.

2. Frazão, C., McVey, C. E., Amblar, M., Barbas, A., Vonrhein, C., Arraiano, C. M., and Carrondo, M. A. (2006) Unravelling the dynamics of RNA degradation by ribonuclease II and its RNA-bound complex. Nature 443: 110-114.

3. Takeshita, D. and Tomita, K. (2012) Molecular basis for RNA polymerization by Qβ replicase. Nat. Struct. Mol. Biol. 19, 229-237.

4. Sengupta, J., Agrawal, R. K. and Frank, J. (2001) Visualization of protein S1 within the 30S ribosomal subunit and its interaction with messenger RNA. Proc. Natl. Acad. Sci. U S A. 98, 11991-11996.

5. Byrgazov, K., Manoharadas, S., Kaberdina, A. C., Vesper, O. and Moll, I. Direct interaction of the N-terminal domain of ribosomal protein S1 with protein S2 in Escherichia coli. (2012) PLoS One 7: e32702. doi: 10.1371/journal.pone.0032702.

6. Pierce, B. G., Wiehe, K., Hwang, H., Kim, B. H., Vreven, T. and Weng, Z. (2014) ZDOCK server: interactive docking prediction of protein-protein complexes and symmetric multimers. Bioinformatics doi: 10.1093/bioinformatics/btu097.

7. Schuwirth, B. S., Borovinskaya, M. A., Hau, C. W., Zhang, W., Vila-Sanjurjo, A., Holton, J. M. and Cate, J. H. (2005) Structures of the bacterial ribosome at 3.5 Å resolution. Science 310, 827-834.

8. Lauber, M. A., Rappsilber, J. and Reilly, J. P. (2012) Dynamics of ribosomal protein S1 on a bacterial ribosome with cross-linking and mass spectrometry. Mol. Cell. Proteomics 11, 1965-1976.