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Supporting Information

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for

Importance of Translation--Replication Balance for Efficient Replication by the Self-Encoded Replicase

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Plasmid construction. pUC-MDV(–)β(+) and pUC-MDV(+)β(–) were constructed previously.[1] pUC-MDV(+)β(+) and pUC-MDV(–)β(–) were constructed by inserting the β-subunit sequence of Qβ replicase into the *Bgl*II restriction sites of the plasmid pUC-MDV-LR and pUC-MDV(–),[2] respectively. A DNA fragment containing the β-subunit was prepared by PCR by using primer 1, primer 2, and plasmid pETβ[2] as the template. pUC-MDV(–)TR-β(+) was constructed by insertion of a *Kpn*I site just upstream of the β-subunit sequence in pUC-MDV(–)β(+) by PCR using primers 3 and 4, and pUC-MDV(–)β(+) as the template, followed by digestion with *Kpn*I and ligation with the annealed oligo DNA 1 and DNA 2 and the plasmid with oligo DNA 1 on the sense strand was selected. pUC-MDV(+)TR-β(–), which was used for preparation of the complementary strand of MDV(–)TR-β(+), was constructed in the same way as pUC-MDV(–)TR-β(+) except using pUC-MDV(+)β(–) as the PCR template. The primer sequences are shown in Table S1.

Calculation of standard deviation. Standard deviations of translation rates (Fig. 2, gray lines), antisense strand rates (Fig. 3A, gray lines), and the coefficients of t^2 (Fig. 3C, gray lines) were calculated according to following equation.

$$s_X^2 = \left(\frac{\partial f(A,B,C)}{\partial A} s_A\right)^2 + \left(\frac{\partial f(A,B,C)}{\partial B} s_B\right)^2 + \left(\frac{\partial f(A,B,C)}{\partial C} s_C\right)^2 + \cdots$$

where X is a function of parameters: A, B, and C (*i.e.*, X= f(A,B,C, …)); and σ is the standard deviation of each parameters. In the case of translation rate, X, A, B, and C correspond to V^{rep}, k_{cat}^{rib}, K_M^{rib}, and α^{rib}, respectively.

Modification of kinetic model

We modified the kinetic model of the infection process of Qβ phage reported previously[3] (Fig. 5) as follows: (1) Reactions related to coat protein synthesis and plus strand synthesis were omitted. (2) We introduced the replicase-ribosome-RNA complex, and the dissociation process of the ribosome-RNA and the replicase-RNA complexes. The newly introduced processes are neglected in the previous study, but could have some effect under our experimental conditions.

Derivatin of equations

From the assumption that the binding steps of sense strand RNA to the ribosome or the replicase are in equilibrium, the relationships among their concentrations are given by:

$$K_M^{rep} = \frac{[S]\cdot[Rep]}{[Rep\text{-}S]},\quad K_M^{rib} = \frac{[S]\cdot[Rib]}{[Rib\text{-}S]},\quad K_M^{rib} = \frac{[Rep\text{-}S]\cdot[Rib]}{[Rep\text{-}Rib\text{-}S]}, \qquad \textbf{S1}$$

where [S], [Rep], and [Rib] are the free sense strand RNA, free replicase, and free ribosome concentration, respectively; [Rib-S] is the ribosome-sense strand complex concentration; [Rep-S] is the replicase-sense strand complex concentration; and [Rep-Rib-S] is the replicase-sense strand-ribosome complex concentration.

The relationships among the total ribosome (Rib_t), replicase (Rep_t), sense strand (S_t) concentration, and their complexes are given by:

$$[Rep_t] = [Rep] + [Rep\text{-}S] + [Rep\text{-}Rib\text{-}S],$$

$$[Rib_t] = [Rib] + [Rib\text{-}S] + [Rep\text{-}Rib\text{-}S],$$

$$[S_t] = [S] + [Rep\text{-}S] + [Rib\text{-}S] + [Rep\text{-}Rib\text{-}S]. \qquad \textbf{S2}$$

From the assumption that both $[S_t]$ and $[Rib_t]$ are in excess relative to $[Rep_t]$, eq. S2 were approximated as follows:

$$[Rep_t] = [Rep] + [Rep\text{-}S] + [Rep\text{-}Rib\text{-}S],$$

$$[Rib_t] = [Rib] + [Rib\text{-}S],$$

$$[S_t] = [S] + [Rib\text{-}S]. \qquad \textbf{S3}$$

Substitution of eq. S1 with eq. S3 gives:

$$K_{\mathrm{M}}^{\mathrm{rep}} = \frac{([\mathrm{S_t}]-[\mathrm{Rib\text{-}S}])([\mathrm{Rep_t}]-[\mathrm{Rep\text{-}S}]-[\mathrm{Rep\text{-}Rib\text{-}S}])}{[\mathrm{Rep\text{-}S}]},$$

$$K_{\mathrm{M}}^{\mathrm{rib}} = \frac{([\mathrm{S_t}]-[\mathrm{Rib\text{-}S}])([\mathrm{Rib_t}]-[\mathrm{Rib\text{-}S}])}{[\mathrm{Rib\text{-}S}]},$$

$$K_{\mathrm{M}}^{\mathrm{rib}} = \frac{[\mathrm{Rep\text{-}S}]([\mathrm{Rib_t}]-[\mathrm{Rib\text{-}S}])}{[\mathrm{Rep\text{-}Rib\text{-}S}]}. \qquad \textbf{S5}$$

Solving eq. S5 gives the following equations:

$$[\mathrm{Rib\text{-}S}] = \frac{1}{2}\left(K_{\mathrm{M}}^{\mathrm{rib}} + [\mathrm{S_t}] + [\mathrm{Rib_t}] - \sqrt{(K_{\mathrm{M}}^{\mathrm{rib}} + [\mathrm{S_t}] + [\mathrm{Rib_t}])^2 - 4[\mathrm{S_t}][\mathrm{Rib_t}]}\right). \qquad \textbf{S6}$$

$$[\mathrm{Rep\text{-}S}] = [\mathrm{Rep_t}] \cdot g\ . \qquad [\mathrm{S7}]$$

$$[\mathrm{Rep\text{-}Rib\text{-}S}] = \frac{[\mathrm{Rep_t}] \cdot g \cdot ([\mathrm{Rib_t}]-[\mathrm{Rib\text{-}S}])}{K_{\mathrm{M}}^{\mathrm{rib}}}, \qquad \textbf{S8}$$

where $g = \dfrac{1}{\dfrac{K_{\mathrm{M}}^{\mathrm{rep}}}{([\mathrm{S_t}]-[\mathrm{Rib\text{-}S}])} + 1 + \dfrac{([\mathrm{Rib_t}]-[\mathrm{Rib\text{-}S}])}{K_{\mathrm{M}}^{\mathrm{rib}}}}$. **S9**

In the experiments, not all of the ribosomes and replicase are active. Thus, we introduced the active fraction ratio of ribosome (α^{rib}) and replicase (α^{rep}) into eq. S6, S8, and S9, yielding:

$$[\mathrm{Rib\text{-}S}] = \frac{1}{2}\left(K_{\mathrm{M}}^{\mathrm{rib}} + [\mathrm{S_t}] + a^{\mathrm{rib}}[\mathrm{Rib_t}] - \sqrt{(K_{\mathrm{M}}^{\mathrm{rib}} + [\mathrm{S_t}] + a^{\mathrm{rib}}[\mathrm{Rib_t}])^2 - 4[\mathrm{S_t}]a^{\mathrm{rib}}[\mathrm{Rib_t}]}\right), \qquad \textbf{3}$$

$$[\mathrm{Rep\text{-}S}] = a^{\mathrm{rep}}[\mathrm{Rep_t}] \cdot g\ , \qquad \textbf{4}$$

$$g = \frac{1}{\dfrac{K_{\mathrm{M}}^{\mathrm{rep}}}{([\mathrm{S_t}]-[\mathrm{Rib\text{-}S}])} + 1 + \dfrac{(a^{\mathrm{rib}}[\mathrm{Rib_t}]-[\mathrm{Rib\text{-}S}])}{K_{\mathrm{M}}^{\mathrm{rib}}}}. \qquad \textbf{5}$$

From the kinetic model, the rate of β-subunit translation (V^{rep}) and antisense synthesis ($V^{\mathrm{antisense}}$) were written as:

$$V^{\mathrm{rep}} = k_{\mathrm{cat}}^{\mathrm{rib}} \cdot [\mathrm{Rib\text{-}S}], \qquad \textbf{1}$$

$$\begin{aligned} V^{\mathrm{antisense}} &= k_{\mathrm{cat}}^{\mathrm{rep}} \cdot [\mathrm{Rep\text{-}S}] \\ &= k_{\mathrm{cat}}^{\mathrm{rep}} \cdot a^{\mathrm{rep}}[\mathrm{Rep_t}] \cdot g \end{aligned}. \qquad \textbf{2}$$

Under the coupling condition, where replicase translation and antisense strand synthesis by the translated replicase occur, considering V^{rep} and $V^{\mathrm{antisense}}$ were constant over time under these experimental conditions, the concentration of replicase and antisense strand are given by:

$$[\mathrm{Rep_t}] = V^{\mathrm{rep}} \cdot t, \qquad \textbf{6}$$

$$[\mathrm{As}_t] = \frac{1}{2} k_{\mathrm{cat}}^{\mathrm{rep}} \cdot g \cdot a^{\mathrm{rep}} V^{\mathrm{rep}} \cdot t^2 . \qquad \mathbf{7}$$

Derivation of optimum ribosome concentration.

When ribosomes are present in excess relative to the sense strand RNA, the middle equation of eq. S5 can be converted to:

$$K_{\mathrm{S}}^{\mathrm{rib}} = \frac{([\mathrm{S}_t] - [\mathrm{Rib}\text{-}\mathrm{S}])[\mathrm{Rib}_t]}{[\mathrm{Rib}\text{-}\mathrm{S}]} .$$

By arranging the equation, we obtained:

$$[\mathrm{Rib}\text{-}\mathrm{S}] = \frac{[\mathrm{S}_t][\mathrm{Rib}_t]}{K_{\mathrm{M}}^{\mathrm{rib}} + [\mathrm{Rib}_t]} .$$

By using this equation, eq. 1, eq. 5, and eq. 7, the total antisense strand concentration can be written as a function of total active ribosome concentration (α^{rib} [Rib_t]):

$$[\mathrm{As}_t] = \frac{1}{2} k_{\mathrm{cat}}^{\mathrm{rep}} \cdot g \cdot a^{\mathrm{rep}} V^{\mathrm{rep}} \cdot t^2$$

$$= \frac{1}{2} k_{\mathrm{cat}}^{\mathrm{rep}} \cdot k_{\mathrm{cat}}^{\mathrm{rib}} \cdot a^{\mathrm{rep}} \frac{[\mathrm{Rib}\text{-}\mathrm{S}]}{\frac{K_{\mathrm{M}}^{\mathrm{rep}}}{[\mathrm{S}_t] - [\mathrm{Rib}\text{-}\mathrm{S}]} + 1 + \frac{a^{\mathrm{rib}}[\mathrm{Rib}_t] - [\mathrm{Rib}\text{-}\mathrm{S}]}{K_{\mathrm{M}}^{\mathrm{rib}}}} \cdot t^2$$

where $[\mathrm{Rib}\text{-}\mathrm{S}] = \frac{[\mathrm{S}_t] a^{\mathrm{rib}} [\mathrm{Rib}_t]}{K_{\mathrm{M}}^{\mathrm{rib}} + a^{\mathrm{rib}} [\mathrm{Rib}_t]}$.

The coefficient of t^2 of this equation is convex upward, which takes a maximum value when the total active ribosome concentration is equal to $K_{\mathrm{M}}^{\mathrm{rib}}$, assuming that α^{rep} is constant over the ribosome concentration for simplicity.

Measurement of MDV-1 synthesis.

At indicated times, aliquots of reaction mixture were subjected to polyacrylamide gel (5%) electrophoresis. The gels were fixed in acetic acid (5%) for 5 min, dried, and subjected to autoradiography. The band intensity corresponding to MDV-1 was quantified by using ImageQuant software (FujiFilm, Tokyo, Japan). By comparing the results to the intensities of known UTP concentration, incorporated UTP into MDV-1, and subsequently synthesized MDV-1 amounts were calculated.

Derivation of equations used in a^{rep} determination.

As MDV-1 was present in excess relative to total active replicase, the synthesis rate of MDV-1 was proportional to total active replicase concentration with k_{MDV} as a coefficient.

$$\frac{d[\mathrm{MDV}\text{-}1]}{dt} = k_{\mathrm{MDV}} \cdot a^{\mathrm{rep}} \cdot [\mathrm{Rep}_t] . \qquad \mathbf{S16}$$

The *de novo* translated replicase increase over time as described in eq. 6, so eq. S16 was converted to:

$$\frac{d[\text{MDV -1}]}{dt} = k_{\text{MDV}} \cdot a^{\text{rep}} \cdot V^{\text{rep}} \cdot t \,. \quad \textbf{S17}$$

Integration of eq. S17 gives the newly synthesized MDV-1 concentration as:

$$[\text{MDV -1}] = \frac{1}{2} k_{\text{MDV}} \cdot a^{\text{rep}} \cdot V^{\text{rep}} \cdot t^2 \,. \quad \textbf{S18}$$

As it should take some time (2.7 min on average according to Fig. 3B) for replicase to be translated and be the active form, we introduced a lag time:

$$[\text{MDV -1}] = \frac{1}{2} k_{\text{MDV}} \cdot a^{\text{rep}} \cdot V^{\text{rep}} \cdot (t - 2.7)^2 \quad \textbf{S19}$$

Table S1. Sequences of the primers in this study.

Primer 1	5'-GGAGAGATCTCCTCTAGAAATAATTTTGTT
Primer 2	5'-GGAGAGATCTCTCGAGTGCGGCCGCAAGCT
Primer 3	5'-GATTGGTACCGAGGCCTGCTAGAGCACG
Primer 4	5'-GCCCGGTACCCTCCTCTAGAAATAATTTTG
Oligo DNA 1	5'-CTTTCTTTGTTTCTTTGTTTGGTAC
Oligo DNA 2	5'-CAAACAAAGAAACAAAGAAAGGTAC

References

[1] K. Hosoda, T. Matsuura, H. Kita, N. Ichihashi, K. Tsukada, T. Yomo, *J Biol Chem* 2007, *282*, 15516-15527.

[2] H. Kita, J. Cho, T. Matsuura, T. Nakaishi, I. Taniguchi, T. Ichikawa, Y. Shima, I. Urabe, T. Yomo, *J Biosci Bioeng* 2006, *101*, 421-426.

[3] M. Eigen, C. K. Biebricher, M. Gebinoga, W. C. Gardiner, *Biochemistry* 1991, *30*, 11005-11018.

[4] P. J. Henderson, *Biochem J* 1973, *135*, 101-107.

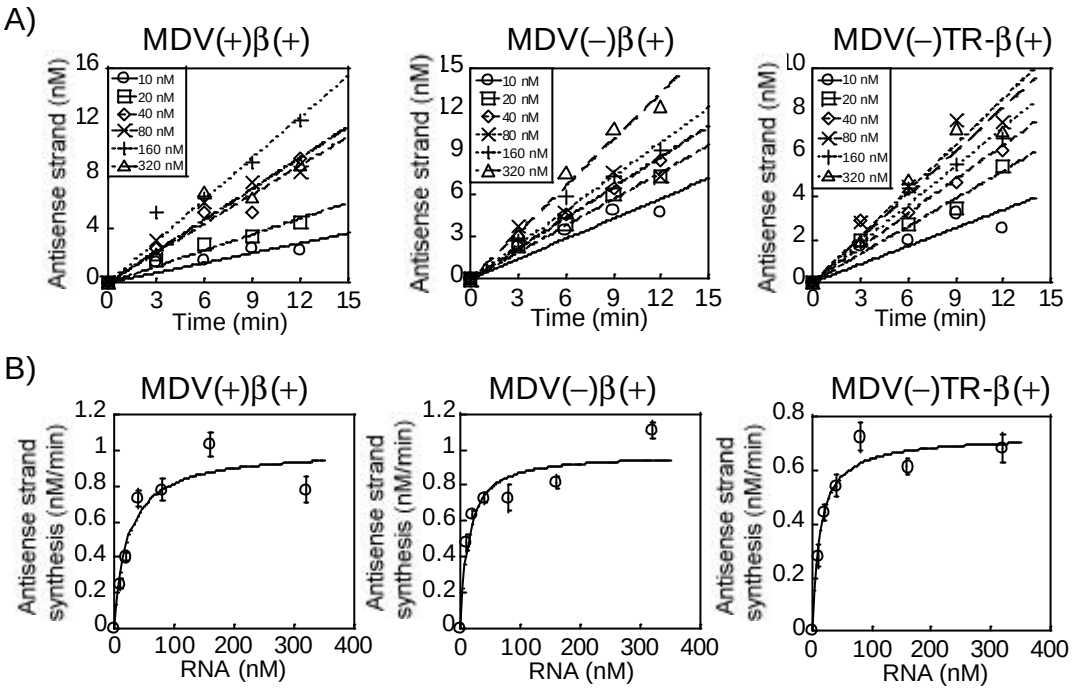

Figure S1. K_M^{rep} and k_{cat}^{rep} determination. To determine K_M^{rep} and k_{cat}^{rep}, antisense strand synthesis was performed at various concentrations of sense strand RNAs. The standard reaction mixture without ribosomes and purified Qβ replicase (40 nM) was used. Under these experimental conditions, eq. 2 and eq. 4 were simplified to eq. S13 described below because [Rib_t] and [Rib-S] were zero. (A) Time course curves of changes in antisense strand concentration. The insets show the total sense RNA concentrations ([S_t]). The results were subjected to linear regression analysis and the slopes ($V^{antisense}$) are plotted in (B). (B) Antisense strand synthesis rates. By fitting the curve with eq. S13, we determined K_M^{rep} and k_{cat}^{rep} because other parameters were known (α^{rep} was 0.2 (1), [Rep_t] was 40 nM). The error bar indicates standard error. Here, we assumed Michalelis-Menten-like kinetics, which is known to explain well the result of RNA replication by Qbeta replicase.[1] A small disagreement between fittings and results could due to experimental error because of short sampling time (0-12 min) and RT-QPCR methods. However, the parameter obtained (K_M^{rep}, k_{cat}^{rep}) will be affected at most 1.5-fold judged from the experimental errors. and these changes do not affect the conclusion significantly.

$$V^{\text{antisense}} = \frac{k_{\text{cat}}^{\text{rep}}[\text{S}_\text{t}] \cdot a^{\text{rep}}[\text{Rep}_\text{t}]}{K_\text{M}^{\text{rep}} + [\text{S}_\text{t}]} \quad \text{[S13]}$$

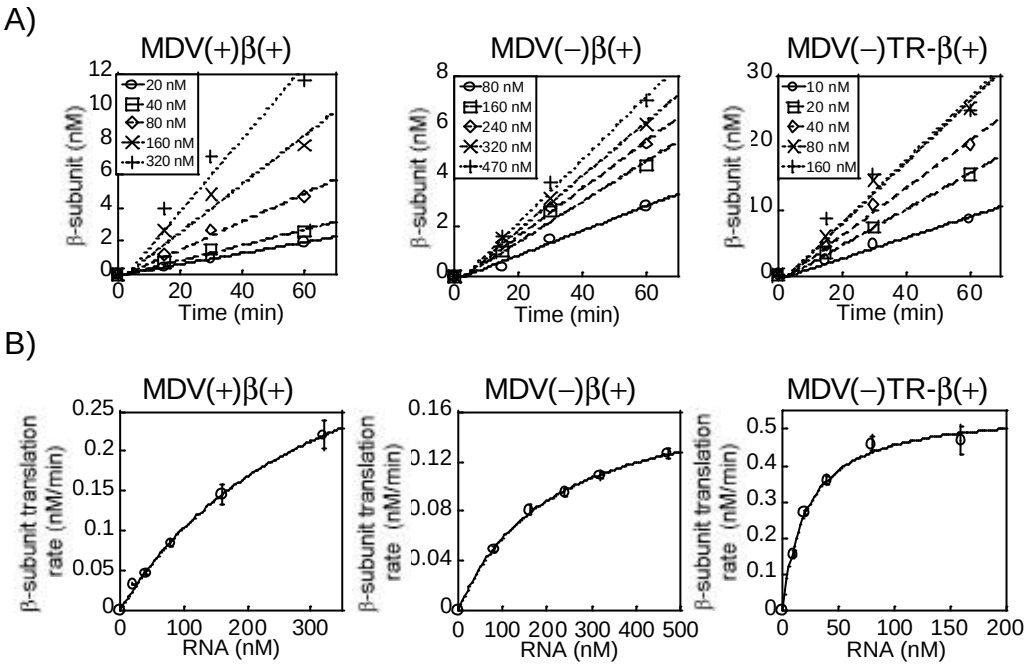

Figure S2. K_M^{rib} and k_{cat}^{rib} determination. To determine K_M^{rib} and k_{cat}^{rib}, the translated replicase β-subunit was quantified at various sense strand RNA concentrations. The standard reaction mixture containing ribosomes (75 nM), including [^{35}S]-methionine, and without UTP was used. Under these experimental conditions, as total sense RNA (S_t) was present in excess relative to the total active ribosomes ($\alpha^{rib}[Rib_t]$), eq. 3 was approximated to $[Rib\text{-}S]=[S_t]\,\alpha^{rib}[Rib_t]/(K_M^{rib}+[S_t])$. Using this equation, eq. 1 was converted to eq. S14 as described below. The equation was the same as that of the Michaelis-Menten curve. (A) Time course curves of replicase β-subunit translation. The insets show the total sense RNA concentrations ($[S_t]$). The results were subjected to linear regression analysis and the slopes (V^{rep}) are plotted in (B). (B) β-Subunit translation rates. By fitting the curve with eq. S14, we determined the K_M^{rib} and k_{cat}^{rib} because other parameters were known (α^{rib} was estimated to be 0.17 in Fig. S3, $[Rib_t]$ was 75 nM). The error bar indicates standard error.

$$V^{rep}=\frac{k_{cat}^{rib}[S_t]\cdot a^{rib}[Rib_t]}{K_M^{rib}+[S_t]} \quad [S14]$$

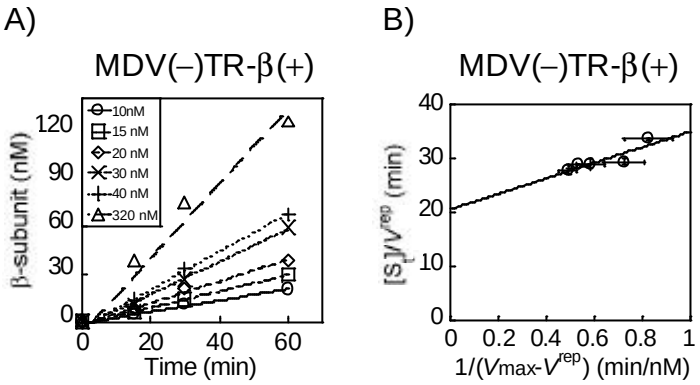

Figure S3. Determination of α^{rib}. To determine the active ribosome fraction ratio (α^{rib}), an RNA titration experiment was performed according to the method of Henderson.[4] The translated replicase β-subunit was quantified at various concentrations of sense strand RNA, MDV(–)TR-β(+). The standard reaction mixture with 300 nM total ribosomes ([Rib_t]) and [^{35}S]-methionine was used. (A) Time course of replicase β-subunit translation. The insets show the total sense RNA concentrations ([S_t]). The results were subjected to linear regression analysis and the slopes (V^{rep}) were determined. The translation rate at 320 nM RNA was used as V_{max} (2.4 nM/min). Then, [S_t]/V^{rep} and 1/(V_{max}–V^{rep}) were calculated and plotted in (B). The resultant plots were fitted with eq. S15 described below and we estimated α^{rib} as 0.17. K_M^{rib} was also estimated to be 15 nM by this fitting, consistent with the previously determined value shown in supporting Fig. S2 (22 nM). The error bar indicates standard error.

$$\frac{[S_t]}{V^{rep}} = K_M^{rib}\frac{1}{V_{max}-V^{rep}} + \frac{a^{rib}\cdot[Rib_t]}{V_{max}} \quad [S15]$$

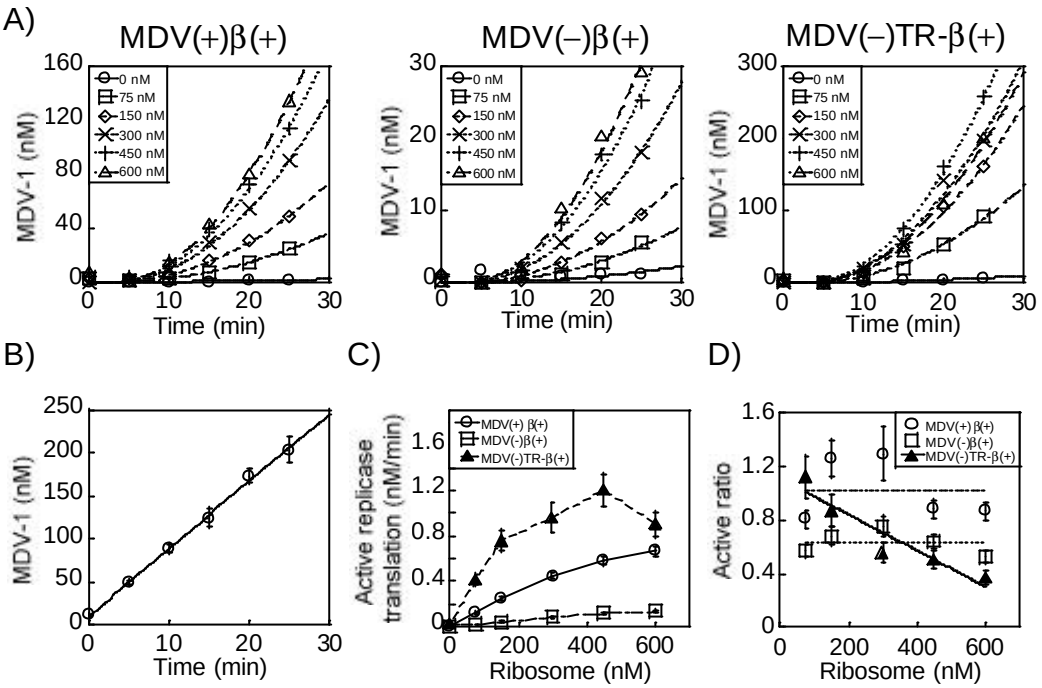

Figure S4. Determination of α^{rep}. To estimate the active ratio of *de novo* translated replicase (α^{rep}), replication activity of the translated replicase was compared with that of the purified replicase the active ratio of which had already been estimated [1]. The replicase activities of the *de novo* translated replicase were measured by adding excess MDV-1 when the reaction started, and measuring the amount of newly synthesized MDV-1. The excess MDV-1 had no significant effect on translation (data not shown). (A) MDV-1 synthesis by *de novo* translated replicase. The standard reaction mixture with various ribosome concentrations, [^{32}P]-UTP (7.4 kBq/µl), 70 nM sense RNAs, and 500 nM MDV-1 was used. The synthesized MDV-1 concentration was measured as described in SI text. The insets show the ribosome concentrations. The results were fitted by eq. S19 (see the SI text for derivation) and we determined the coefficients of t^2, $k_{MDV}\cdot\alpha^{rep}\cdot V^{rep}$. (B) MDV-1 synthesis by the purified replicase. We used the standard reaction mixture with various ribosome concentrations, [^{32}P]-UTP (7.4 kBq/µL), sense RNAs (70 nM), MDV-1 (500 nM), the purified replicase (37.5 nM), the active ratio of which was estimated to be 20% (1), and without serine and lysine to inhibit translation. As the results were similar for all sense strand RNAs and all ribosomal concentrations, representative result is shown. By fitting with eq. S16, we determined k_{MDV} as 1.04 (/min) because here all parameters were known (α^{rep} was 0.2 and [Rep_t] was 37.5 nM). (C) Active replicase translation rates. By dividing $k_{MDV}\alpha^{rep}\cdot V^{rep}$ determined in (A) by k_{MDV} determined in (B), we calculated the active replicase translational rate, $\alpha^{rep}\cdot V^{rep}$, of each template RNA. (D) Active ratio (α^{rep}) of the *de novo* translated replicase. α^{rep} was calculated by dividing $\alpha^{rep}\cdot V^{rep}$ calculated in (C) by V^{rep} in Fig. 2 (UTP–). Here, we used V^{rep} value in the absence of sense

RNA replication (*i.e.*, UTP–) because in this experiment, excess MDV-1 inhibited the replication of sense strand RNA. Actually, the translational rate with excess MDV-1 was similar to that without UTP (data not shown). For MDV(+)β(+) and MDV(–)β(+), the α^{rep} seemed not to depend on the ribosome concentration, and therefore we used the average value of α^{rep} at all ribosome concentrations, whereas for MDV(–)TR-β(+), α^{rep} seemed to depend on ribosome concentration, and we used linear regression (see legend of Table 1). The decrease in α^{rep} by increasing ribosome concentration was due to the almost saturated replicase activity at 150 nM ribosomes (Fig. S4C), while total β-subunit concentration increase linearly in the ribosome concentration range of 0–450 nM. The reason for the saturation of replicase activity is not clear, but one possible explanation is that at high ribosome concentration (*i.e.*, at high translational rate) heterotetramer replicase formation may be the rate-limiting step.

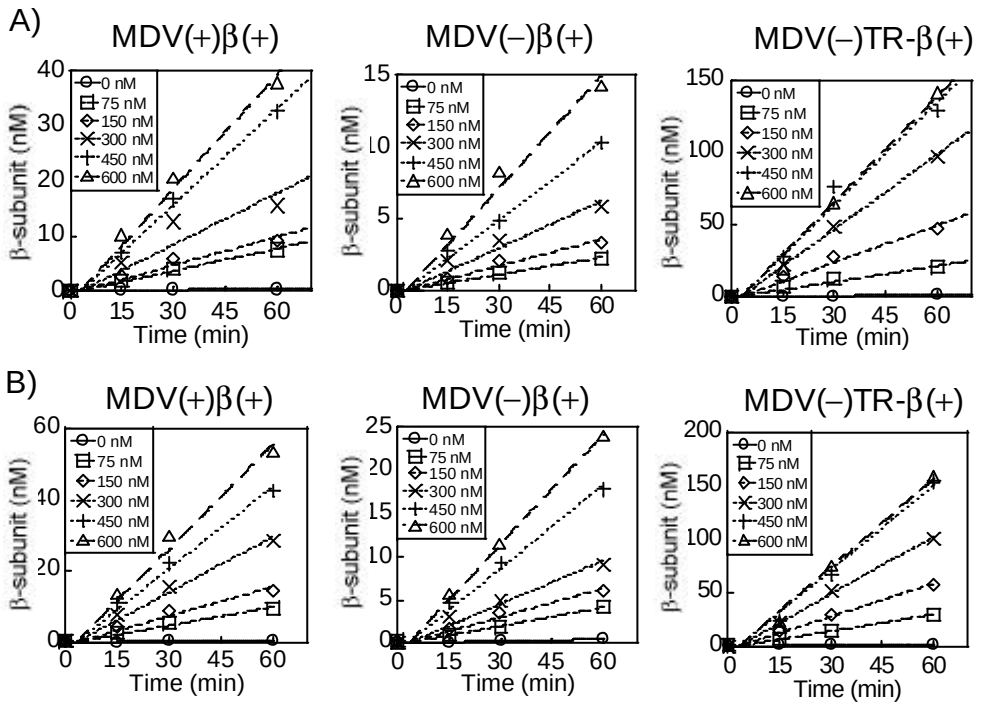

Figure S5: Time course data of Fig. 2. (A) The time courses of translation of replicase β-subunit without replication (UTP–) and (B) translation with replication (UTP+) were measured as described in the legend of Fig. 2. The slopes (V^{rep}) are plotted in Fig. 2. The insets show the total ribosome concentration.

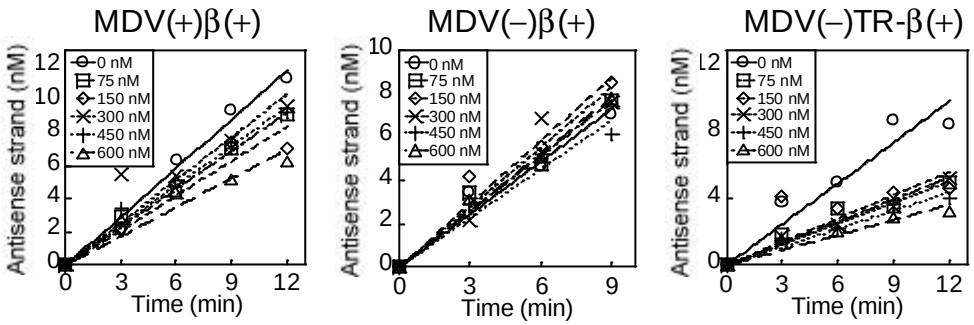

Figure S6: Time course data of Fig. 3A. Antisense strand synthesis by the purified replicase was measured as described in the legend of Fig. 3. The slopes ($V^{antisense}$) are plotted in Fig. 3A. The insets show ribosome concentration.

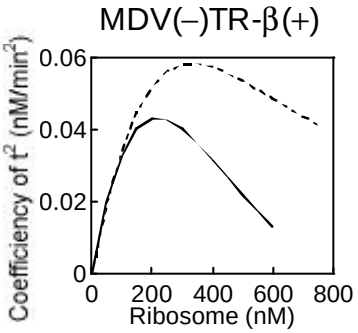

Figure S7: The calculated a^{exp} values when we assumed constant replicase active ratio for MDV(–)TR-β(+). We calculated the coefficient of t^2 of antisense strand synthesis by the *de novo* translated replicase when the replicase active ratio was assumed to be constant (*i.e.* α^{rep} is 1, dotted line), and compare it with the value when the replicase active ratio was decreasing linearly over ribosome concentration (black line, the same data as black line in Fig. 3C). The calculated coefficienct of t^2 showed a bell-shaped curve even at the constant active ratio.