

SI Appendix

Derivation 1. A prediction of our model is that mutation of C2394 will confer a greater fold-effect on $K_{1/2}$ than on k_{trans} , given certain assumptions.

$K_{1/2}$ and k_{trans} of translocation are differentially affected by mutations on C2394 of 23S rRNA. In order to better understand these differential effects, we simplified our model by combining the initial binding and GTP hydrolysis steps (Scheme 1).

Scheme 1.



$K_{1/2}$ and k_{trans} of translocation can be approximated as follows, using the concept of net rate constants (Gromadski & Rodnina, 2004; Cleland, 1975):

$$K_{1/2} = \frac{k_{-1}k_{-3} + k_{-1}k_4 + k_3k_4}{k_1(k_3 + k_{-3} + k_4)} \approx \frac{k_{-1}(k_{-3} + k_4)}{k_1(k_3 + k_{-3} + k_4)} \quad (\text{assuming } k_{-1} \gg k_3) \quad (\text{eqn. 1})$$

$$k_{\text{trans}} = \frac{k_3k_4}{k_3 + k_{-3} + k_4} \quad (\text{eqn. 2})$$

Here, the ratio (R) of fold effects of each mutation on $K_{1/2}$ to fold-effects on k_{trans} is defined as follows:

$$R = \frac{K_{1/2}'/K_{1/2}}{k_{\text{trans}}'/k_{\text{trans}}} \quad (\text{eqn. 3})$$

where $K_{1/2}$ and k_{trans} are those of wild-type complexes, and $K_{1/2}'$ and k_{trans}' are those of mutant complexes. When $R > 1$ (or $R - 1 > 0$), the fold effect of a given mutation on $K_{1/2}$ is greater than the fold-effect on k_{trans} , and when $R < 1$ (or $R - 1 < 0$), the opposite is true. Assuming that the C2394 mutations affect the P/P \rightleftharpoons P/E equilibrium (k_3 and k_{-3}), $K_{1/2}'$ and k_{trans}' are

defined as follows:

$$K_{1/2}' \approx \frac{k_{-1}(k_{-3}'+k_4)}{k_1(k_3'+k_{-3}'+k_4)} \quad (\text{eqn. 4})$$

$$k_{\text{trans}}' = \frac{k_3'k_4}{k_3'+k_{-3}'+k_4} \quad (\text{eqn. 5})$$

where k_3' and k_{-3}' are forward and reverse rate constants of the P/P \rightleftharpoons P/E equilibrium in the mutant complexes, and the other rate constants are equivalent to corresponding rates of wild-type complexes. Using eqn. 1, 2, 4, and 5, $R - 1$ is expressed as a function of individual rate constants:

$$R - 1 = A \cdot \left(\left(\frac{1}{x} + x \right) - \left(\frac{1}{x'} + x' \right) \right) \quad (\text{eqn. 6})$$

where

$$A = \frac{k_3'(k_{-3}'+k_4)}{(k_3'+k_{-3}'+k_4)^2} \quad (> 0)$$

$$x = \frac{k_{-3} + k_4}{k_3}$$

$$x' = \frac{k_{-3}'+k_4}{k_3'}$$

Thus, according to eqn. 6, when $\frac{1}{x} + x > \frac{1}{x'} + x'$, then $R > 1$, or $\frac{K_{1/2}'}{K_{1/2}} > \frac{k_{\text{trans}}}{k_{\text{trans}}'}$, and *vice versa*.

Examples of individual rate constants that satisfy the above relationships are listed below.

Example 1: $k_3 = 50$, $k_{-3} = 1$, $k_4 = 5$, $k_3' = 2$, $k_{-3}' = 1$ (k_3' decreased)

$$\frac{1}{x} + x = 8.5 > \frac{1}{x'} + x' = 3.3, \text{ and } \frac{K_{1/2}'}{K_{1/2}} = 7.0 > \frac{k_{\text{trans}}}{k_{\text{trans}}'} = 3.6 \quad (R = 2.0)$$

Example 2: $k_3 = 50$, $k_{-3} = 1$, $k_4 = 5$, $k_3' = 50$, $k_{-3}' = 200$ (k_{-3}' increased)

$$\frac{1}{x} + x = 5.2 > \frac{1}{x'} + x' = 2.0, \text{ and } \frac{K_{1/2}'}{K_{1/2}} = 7.5 > \frac{k_{\text{trans}}}{k_{\text{trans}}'} = 4.6 \quad (R = 1.6)$$

Example 3: $k_3 = 100$, $k_{-3} = 1$, $k_4 = 5$, $k_3' = 5$, $k_{-3}' = 30$ (k_3' decreased, k_{-3}' increased)

$$\frac{1}{x} + x = 16 > \frac{1}{x'} + x' = 7.1, \text{ and } \frac{K_{1/2}'}{K_{1/2}} = 15 > \frac{k_{\text{trans}}}{k_{\text{trans}}'} = 7.5 \quad (R = 2.0)$$

Thus, according to our model, mutations that shift the P/P \leftrightarrow P/E equilibrium to the left can confer a larger fold-effect on $K_{1/2}$ than on k_{trans} .

Derivation 2. Inhibition of A/P formation is predicted to confer a greater fold-effect on k_{trans} than on $K_{1/2}$.

Inhibition of A/P formation results in decreased k_4 in Scheme 1. Here, we define $K_{1/2}'$ and k_{trans}' as follows:

$$K_{1/2}' \approx \frac{k_{-1}(k_{-3} + k_4')}{k_1(k_3 + k_{-3} + k_4')} \quad (\text{assuming } k_{-1} \gg k_3) \quad (\text{eqn. 7})$$

$$k_{\text{trans}}' = \frac{k_3 k_4'}{k_3 + k_{-3} + k_4'} \quad (\text{eqn. 8})$$

where $K_{1/2}'$, k_{trans}' , and k_4' are $K_{1/2}$, k_{trans} , and k_4 , respectively, when A/P formation is inhibited. By using eqn. 7 and 8, $R - 1$ is calculated as follows:

$$R - 1 = \frac{K_{1/2}'/K_{1/2}}{k_{\text{trans}}/k_{\text{trans}}'} - 1 = B \cdot y \quad (\text{eqn. 9})$$

where

$$B = \frac{k_4'(k_{-3} + k_4')}{(k_3 + k_{-3} + k_4')^2} \quad (> 0)$$

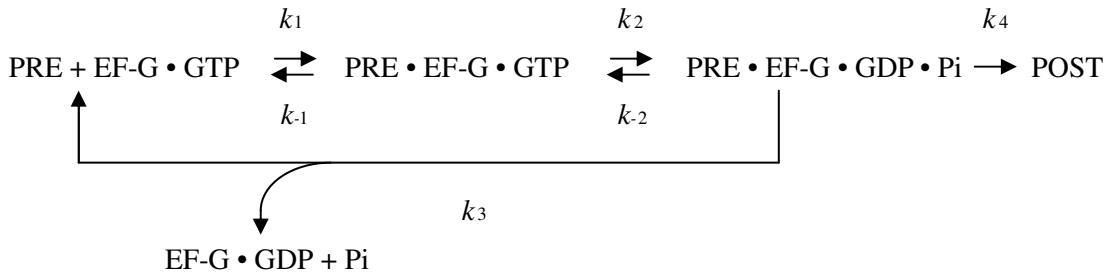
$$y = \left(\frac{k_3 + k_{-3}}{k_4} + 1 \right) \left(\frac{k_3}{k_{-3} + k_4} + 1 \right) - \left(\frac{k_3 + k_{-3}}{k_4'} + 1 \right) \left(\frac{k_3}{k_{-3} + k_4'} + 1 \right) \quad (< 0, \text{ because } k_4 > k_4')$$

Thus, $R < 1$, or $\frac{K_{1/2}'}{K_{1/2}} < \frac{k_{\text{trans}}}{k_{\text{trans}}'}$. This indicates that according to our model, at least if $k_{-1} \gg k_3$,

inhibition of A/P formation always confers a larger fold-effect on k_{trans} than on $K_{1/2}$, which was the trend observed experimentally.

Derivation 3. Consideration of an alternative model in which EF-G • GDP can dissociate from the PRE complex

Scheme 2.



In this scheme, non-productive GTP hydrolysis events precede the tRNA/mRNA movement, and the C2394 mutations change the efficiency of catalysis by simply increasing the number of turnover events before translocation occurs. Multiple turnover of GTP hydrolysis is possible because excess EF-G and GTP are present in our experiments. When a rapid equilibrium of initial turnover is assumed, a fraction of each component of the equilibrium at time t is calculated by using the graphical method of King and Altman (King & Altman, 1956).

$$\frac{[\text{PRE} \cdot \text{EF-G} \cdot \text{GTP}]}{[\text{PRE}]_t} = \frac{(k_{-2} + k_3)k_1[\text{EF-G} \cdot \text{GTP}]}{((k_2 + k_{-2} + k_3)k_1[\text{EF-G} \cdot \text{GTP}] + k_{-1}k_{-2} + k_{-1}k_3 + k_2k_3)} \quad (\text{eqn. 10})$$

$$\frac{[\text{PRE} \cdot \text{EF-G} \cdot \text{GDP} \cdot \text{Pi}]}{[\text{PRE}]_t} = \frac{k_1k_2[\text{EF-G} \cdot \text{GTP}]}{((k_2 + k_{-2} + k_3)k_1[\text{EF-G} \cdot \text{GTP}] + k_{-1}k_{-2} + k_{-1}k_3 + k_2k_3)} \quad (\text{eqn. 11})$$

where $[\text{PRE}]_t = [\text{PRE}] + [\text{PRE} \cdot \text{EF-G} \cdot \text{GTP}] + [\text{PRE} \cdot \text{EF-G} \cdot \text{GDP} \cdot \text{Pi}]$. Time-dependent change of [GDP] can be calculated as follows:

$$\frac{d[\text{GDP}]}{dt} = \frac{d[\text{EF-G} \cdot \text{GDP}]}{dt} + \frac{d[\text{PRE} \cdot \text{EF-G} \cdot \text{GDP} \cdot \text{Pi}]}{dt} + \frac{d[\text{POST} \cdot \text{EF-G} \cdot \text{GDP}]}{dt} \quad (\text{eqn. 12})$$

where

$$\frac{d[\text{EF-G} \cdot \text{GDP}]}{dt} = k_3[\text{PRE} \cdot \text{EF-G} \cdot \text{GDP} \cdot \text{Pi}]$$

$$\frac{d[\text{PRE} \cdot \text{EF-G} \cdot \text{GDP} \cdot \text{Pi}]}{dt} = k_2[\text{PRE} \cdot \text{EF-G} \cdot \text{GTP}] - (k_{-2} + k_3 + k_4)[\text{PRE} \cdot \text{EF-G} \cdot \text{GDP} \cdot \text{Pi}]$$

$$\frac{d[\text{POST} \cdot \text{EF-G} \cdot \text{GDP}]}{dt} = k_4[\text{PRE} \cdot \text{EF-G} \cdot \text{GDP} \cdot \text{Pi}]$$

Substitution and rearrangement of eqn. 12 yields:

$$\frac{d[\text{GDP}]}{dt} = k_2[\text{PRE} \cdot \text{EF-G} \cdot \text{GTP}] - k_{-2}[\text{PRE} \cdot \text{EF-G} \cdot \text{GDP} \cdot \text{Pi}] \quad (\text{eqn. 13})$$

Using eqn. 10 and 11,

$$\frac{d[\text{GDP}]}{dt} = \frac{k_1 k_2 k_3 [\text{EF-G} \cdot \text{GTP}]}{((k_2 + k_{-2} + k_3) k_1 [\text{EF-G} \cdot \text{GTP}] + k_{-1} k_{-2} + k_{-1} k_3 + k_2 k_3)} [\text{PRE}]_t \quad (\text{eqn. 14})$$

Simplifying eqn. 14 yields:

$$\frac{d[\text{GDP}]}{dt} = f([\text{EF-G} \cdot \text{GTP}]) [\text{PRE}]_t \quad (\text{eqn. 15})$$

where

$$f([\text{EF-G} \cdot \text{GTP}]) = \frac{k_1 k_2 k_3 [\text{EF-G} \cdot \text{GTP}]}{((k_2 + k_{-2} + k_3) k_1 [\text{EF-G} \cdot \text{GTP}] + k_{-1} k_{-2} + k_{-1} k_3 + k_2 k_3)}$$

Here, $[\text{PRE}]_t = [\text{PRE}]_0 \cdot \exp(-k_{\text{app}} \cdot t)$, where k_{app} is the apparent rate of translocation, and $[\text{PRE}]_0$ is $[\text{PRE}]$ at $t = 0$. Eqn. 15 is converted to:

$$\frac{d[\text{GDP}]}{dt} = f([\text{EF-G} \cdot \text{GTP}] \cdot [\text{PRE}]_0 \cdot \exp(-k_{\text{app}} \cdot t)) \quad (\text{eqn. 16})$$

At a given concentration of EF-G · GTP, k_{app} and $f([\text{EF-G} \cdot \text{GTP}] \cdot [\text{PRE}]_0)$ are constant. Therefore, assuming $[\text{GDP}] = 0$ at $t = 0$, integration of eqn. 16 yields:

$$[\text{GDP}] = \frac{f([\text{EF-G} \cdot \text{GTP}] \cdot [\text{PRE}]_0)}{k_{\text{app}}} (1 - \exp(-k_{\text{app}} \cdot t)) \quad (\text{eqn. 17})$$

This indicates that, according to Scheme 2, the apparent rate of GTP hydrolysis is equivalent to that of translocation, which contradicts our results and previous reports (Rodnina *et al.*, 1997; Savelsbergh *et al.*, 2000; Savelsbergh *et al.*, 2003; Seo *et al.*, 2006). Thus, it is likely that the GDP form of EF-G does not dissociate from the complex until translocation occurs.

References

- Cleland, W. W. (2005) *Biochemistry* 14:3220-3224.
- Gromadski, K. B. & Rodnina, M. V. (2004) *Mol Cell* 13:191-200.
- King, E. L. & Altman, C. (1956) *J Phys Chem* 60:1375-1378.
- Rodnina, M. V., Savelsbergh, A., Katunin, V. I., Wintermeyer, W. (1997) *Nature* 385:37-41.
- Savelsbergh, A., Matassova, N. B., Rodnina, M. V., Wintermeyer, W. (2000) *J Mol Biol* 300:951-961
- Savelsbergh, A., Katunin, V. I., Mohr, D., Peske, F., Rodnina, M. V., Wintermeyer, W. (2003) *Mol Cell* 11:1517-1523.
- Seo, H. S., Abedin, S., Kamp, D., Wilson, D. N., Nierhaus, K. H., Cooperman, B. S. (2006) *Biochemistry* 45:2504-2514.