Supplementary material for "Conditional Cooperativity in Toxin-Antitoxin Regulation Prevents Random Toxin Activation and Promotes Fast Translational Recovery" by Ilaria Cataudella, Ala Trusina, Kim Sneppen, Kenn Gerdes, and Namiko Mitarai

Supplement A: Parameter constraint for dissociation constants to reproduce the conditional cooperativity



Figure 5: Concentration of RelB₂RelE as a function of (total RelE)/(total RelB monomer) calculated according to law of mass action. The amount of total RelB monomer is fixed to 200 nM. K_{B2E} is set to 0.3 nM, and the cases where K_{B2E2} =0.03nM, 0.3nM, 3nM are shown.

In the in-vitro experiment on the conditional cooperativity by Overgaard et al. [9], it has been shown that the formation of the operator- $(RelB_2RelE)_2$ complex depends on the RelE/RelE molar ratio. Especially, in Fig.2C in [9], the amount of RelB monomer is fixed to 200nM, and the amount of RelE is changed from (total RelB monomer):(total RelE)=16:1 to 1:4, and it has been found that the amount of operator-(RelB_2RelE)_2 complex gradually increases upto 2:1 ratio, and suddenly drops to almost zero at 1:1 ratio and beyond.

Inspired by this experiment, we calculated the the amount of $RelB_2RelE$ complex according to the law of mass action

$$[B_2 E] = \frac{[B_2][E]}{K_{B2E}},$$
(2)

$$[B_2 E_2] = \frac{[B_2 E_2][E]}{K_{B2E2}} \tag{3}$$

$$[B_{2T}] = [B_2] + [B_2E] + [B_2E_2], \qquad (4)$$

$$[E_T] = [E] + [B_2 E] + 2[B_2 E_2].$$
(5)

with keeping $[B_{2T}]=100$ nM (therefore relB monomer concentration is 200 nM).

 K_{B2E} is fixed to 0.3nM, and the cases with $K_{B2E2} = 0.03$ nM, 0.3 nM (the reference parameter value), 3nM are shown. With the reference parameter, $K_{B2E2} = 0.3$ nM, a clear peak of RelB₂RelE is found at 2:1 ratio, while at 1:1 ratio it drops lower than the level at 16:1 ratio. When $K_{B2E2} = 3$ nM, the drop at 1:1 ratio is not as strong. When $K_{B2E2} = 0.03$ nM, the peak of RelB₂RelE is not as high. Therefore, we conclude that the conditional cooperativity is the best reproduced when K_{B2E2} and K_{B2E2} are at similar value.



Figure 6: A,B,C: The repression fold of the relBE promoter for various total amount of RelB and RelE, with changing the dissociation constant of RelB₂RelE₂ formation K_{B2E2} . The white point shows the total amount of RelE and RelB in the non-starved state. For all the figure, the dissociation constant of RelB₂RelE formation is fixed to be $K_{B2E}=0.3$ nM. A: $K_{B2E2} = 0.3$ nM, which is the value used in the paper. B: $K_{B2E2} = 3$ nM. C: $K_{B2E2} = 30$ nM. The solid line in the figure shows the line where the amount of total RelE is equal to that of total RelB₂ (i.e., RelE_t: RelB_t = 1:2), while the dashed line shows the line where the amount of total RelE is equal to the double amount of total RelB₂ (i.e., RelE_t: RelB_t = 1:1).

Furthermore, Figs. 6 show the the repression fold of the relBE promoter for various total amount of RelB and RelE, keeping $K_{B2E}=0.3$ nM but changing K_{B2E2} . In 6A with $K_{B2E2}=0.3$ nM, we can see that when RelB_t: RelB_t =1:2 (here RelB_t is total concentration in monomer) the system stay repressed since there are many RelB₂RelE, while almost complete de-repression happens when total RelE exceed the RelE_t: RelB_t =1:1 line because most of the RelB₂RelE is converted to RelB₂RelE₂. However, as we increase K_{B2E2} , this sharp de-repression gets blurred.

Supplement B: Switch to high RelE require degradation of RelB in complex



Figure 7: Development of free RelE in case that there is no active degradation of RelB in complexes, thus RelB in complex the same half-life as τ_E . Free RelE is seen to remain low, in contrast to behavior of standard model (Fig2B) where RelB in complex is degraded a factor 4 times slower than in complex but still degrated much fater than RelE.

The response to starvation in Fig2 depends on the possible ways that RelB can be degraded. In particular, the starved state depends critically on our assumption of increased degradation of RelB during starvation, and also on the assumption that RelB can be degraded in the RelB₂RelE complex. Fig7 shows that the toxin dominated state is not reached when RelB is completely protected in complex, thus having the same life time as RelE in complex. In summary, the necessary feaure to obtain toxin activation is a high degradation-rate of RelB not only in the free state but also in the complex with RelE.

Supplement C: Effect of the cleavage rate of mRNA by toxin



Figure 8: Effect of changing the k_c value on evolution of free RelE and relBE mRNA. At time t = 200 minutes the system is switched to aminoacid starvation.

A: Concentration of free RelE over time. The higher the value of k_c the sooner a substantial raise in the concentration is recorded. In order for free RelE to raise above 1 nM within 20 minutes k_c needs to be higher than 1 $nM^{-1}min^{-1}$. A slower raise also results in higher accumulation of RelE on the long period. This is a direct consequence of the higher concentration of $RelB_2RelE$ complexes due to higher RelB level, that act as a reservoir for free toxin once the antitoxin starts getting degraded.

B: Concentration of relBE mRNA over time. Lower values of the cleavage rate k_c result in a higher increase in the amount of mRNA at the onset of starvation, allowing an effective production of antitoxin RelB that slows down the raise in the concentration of RelE shown above.

Fig8 shows how lower values of k_c allow a stronger increase in *relBE* mRNA at the onset of starvation, enhancing RelB's ability to fight back, and thus slowing down the raise in free RelE.

Supplement D: Stripping delays entry into high free toxin state

We now investigate the effect of only removing the possibility for $RelB_2RelE_2$ complex formation when this is bound to the operator, in other word we investigate the role of the assumed reaction where free RelE directly "strips" [18] the operator and thereby derepresses it. If RelB₂RelE and the operator as well as the complex formations by RelB's and RelE's were characterized by a fast on and off dynamics, the effect of such a stripping would be small. This is because the speed of the reaction determines the relaxation time to the thermal equilibrium, where the stripping and the reverse reaction satisfies the detailed balance and hence cancels out. However, when the unbinding rate of $(RelB_2RelE)_2$ bound to the operator is estimated to be low, stripping modifies the temporal behavior significantly. For example, it has been suggested that the stripping plays a crucial role in quickly deactivating human NF- κ B [19, 18]. In the RelBE case, with a diffusion limited on-rate of about 0.06/sec/molecule, and a repression factor of 800 in the non-starved conditions, the residence for the complex $(\text{RelB}_2\text{RelE})_2$ on the operator is estimated to be long (~ 6 min), and the effect of stripping can be substantial.



Figure 9: The model behavior without stripping. A: Time development of the probability distribution of free RelE, sampled over 1000 cells. B: Average trajectory of *relBE* mRNA and free RelE without stripping. Compared to in Fig. 2B, entry into the toxin dominated state is faster.

Fig. 9 shows the behavior of the system without stripping, demonstrating that absence of stripping results in faster transition into the RelE dominated

state, and increases fluctuations of RelE during starvation (compare it with Fig. 2). That is, without stripping, it takes more time before the operator is derepressed when RelE becomes dominant, because the system needs to wait until bound RelB₂·RelE leaves from the operator. In this scenario, the system cannot "fight back" by strong de-repression and hence strong production of RelB does not occur as fast as in the case with stripping. Thus, without stripping the toxin is much more prone to be activated.

Note that our assumption of a diffusion limited on-rate may be incorrect: On the one hand, DNA facilitated search increases the on-rate *in vitro* [20], but *in vivo* unspecific bindings of RelB₂RelE typically slow down the search [21]. If the on-rate of (RelB₂RelE)₂ is lower than assumed here, the effect of stripping becomes even more pronounced than illustrated in the figure.

Supplement E: Effect of time delay in the change of parameters at transitions between the starved and the non-starved states

In the main text the switching from one level of nutrients to another (aminoacid starvation to rich medium and vice versa) was achieved by changing some key parameters, namely, the free RelB halflife τ_B , the halflife of RelB in complexes τ_c , the translation rate for *RelB* (and consequently the translation rate for *RelE*), and the halflife of free *RelE*. The changes in the parameters were treated as happening istantaneously for simplicity of the model.

Here we investigate the effect of varying the life time of RelB and the translation rate slower (linearly over time) at the transition to understand the the role of these time scales. 3

³The change of the life time of free RelE τ_E does not have significant effect in the transition because τ_E is at shortest 43 min, much longer than the systems dynamics at the transitions.



Figure 10: Effect of changing the halflife of free RelB (τ_B) and RelB in complexes (τ_c) from fast grow conditions levels ($\tau_B = 3min$ and $tau_c = 12min$) to amino-acid starvation estimated level ($\tau_B = 0.375min$ and $\tau_c = 1.5min$) linearly over time in two different cases : over a time interval of 30 min (A) and 5 min (B)



Figure 11: Effect of changing the halflife of free RelB (tau_B) and RelB in complexes (τ_c) from amino-acid starvation estimated levels $(\tau_B = 0.375min \text{ and } \tau_c = 1.5min)$ to fast grow conditions level $(\tau_B = 3min \text{ and } \tau_c = 12min)$ linearly over time in two different cases : over a time interval of 30 min (A) and 5 min (B)

Effect of the RelB degradation. Figure 10 shows the effect of varying τ_B and τ_c over 30 min (A) and 5 min (B) at the transition from fast growth conditions to amino-acid starvation. The time scale of the change is directly reflected to the time for free RelE to rise (a2). This is expected from the fact that this change was required to have the fast enough entrance to the high-toxin state at the starvation. In order to reproduce the experimental observation that the effect of RelE seen on the protein level about 10 min after the amino acid starvation, we predict that the effect of activation of Lon on τ_B and τ_c should be significant after 10 min.

On the other hand, as can be seen in fig. 11, the dynamics of recovery from starved state is little affected the time scale of change of τ_B and τ_C . We conclude that recovery behavior is robust with respect to a slower change of τ_B and τ_c .



Figure 12: Effect of continuos variation of the translation rate for RelB and RelE continuosly over time on the dynamics of the transitions from fast growth conditions to amino-acid starvation and vice-versa. Panel A) Behaviour over time of free RelB (a1), free RelE (a2) and relBE mRNA (a3) at the transition from fast growth conditions to amino-acid starvation in three different cases : translation rate is changed abrubtly at the switching time from fast growth level (15 nM/mRNA/min) to amino-acid starvation level (1.5 nM/mRNA/min)(red line), translation rate is changed linearly over a time span of 5 minutes (blue line), translation rate is changed linearly over a time span of 5.0 minutes (blue line), translation rate is changed abrubtly at the transition from amino-acid starvation to fast growth conditions in three different cases : translation rate is changed abrubtly at the transition from amino-acid starvation to fast growth conditions in three different cases : translation rate is changed linearly over a time span of 30 minutes (blue line), translation rate is changed abrubtly at the switching time from fast growth level (15 nM/mRNA/min) to amino-acid starvation level (1.5 nM/mRNA/min) (red line), translation rate is changed linearly over a time span of 30 minutes (blue line), translation rate is changed linearly over a time span of 30 minutes (blue line), translation rate is changed linearly over a time span of 30 minutes (blue line), translation rate is changed linearly over a time span of 30 minutes (blue line), translation rate is changed linearly over a time span of 30 minutes (blue line), translation rate is changed linearly over a time span of 30 minutes (blue line), translation rate is changed linearly over a time span of 30 minutes (blue line), translation rate is changed linearly over a time span of 30 minutes (blue line), translation rate is changed linearly over a time span of 30 minutes (blue line).

Effect of the translation rate. We explore the effects of changing the value of the translation rate at the two switching point (fast growth to amino-acid starvation and vice-versa) linearly over time instead of abruptly as it was done in the main text. We took into account two extreme cases, in one case the change in translation rate happens over a time span of 5 minutes (blue lines in fig. 12) and in the other case the time span is 30 minutes.

In the case of transition to amino-acid starvation (fig. 12 A) the activation of free RelE is delayed by almost the same amount as the time interval over which

the translation rate is changed. This is natural because the high translation rate gives RelB to fight back against the rise of RelE.

In the case of transition to recovery phase (fig. 12 B), even though also in this case we see a noticeable delay in the fall of free RelE, the free RelE falls to low level much faster than the introduced time delay. This is because the system need enough number of RelBs produced to repress RelE, and this can be realized even before the translation happens at full speed.

Supplement F: Conditional cooperativity gives faster recovery from amino-acid starvation induced growth-arrest than without conditional cooperativity independent of the delay in the recovery of the translation rate

As it has been shown in supplement E, the time scale over which the translation rate increases after starvation phase affect the time scale of the fall of the free RelE. Here we confirm that the conditional cooperativity will still give faster recovery than without consistional cooperativity even if the translation rate increase slower.

Figure 13 compare without conditional cooperativity case (top) and with conditional cooperativity case (bottom), when the translation rate changes instantaneously (circles) or over 30 min. In both cases we see that the case without conditional cooperativity is much slower in recovery. We conclude that our qualitative conclusion of importance of conditional cooperativity for recovery from the high-toxin phase is robust against the detail of the time scale of parameter change.



Figure 13: Comparison between time-scale over which recovery takes place without (A) and with (B) conditional cooperativity, in the case where translation rate is changed abruptly from amino-acid starvation value to fast-growth value (blue line) and in the case where it's changed linearly with time over an interval of 30 minutes.