Modelling reveals kinetic advantages of co-transcriptional splicing - Supplementary Text 2

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Contents

Kinetics of multi-step processes

The degradation of Ribo1 pre-mRNA was measured in an "OFF strain" of 5'SSRibo1 where transcription can be halted by doxycycline (Alexander *et al.*, 2010), see Figure 1A. Similarly, the degradation of lariat-exon2 was measured in an OFF strain of 3'SSRibo1 (Alexander *et al.*, 2010), see Figure 1B.

Degradation can be modelled as a single reaction that depletes the population of species C from an initially constant level (model A). Alternatively, depletion can be modelled as occurring in multiple steps that produce a series of intermediates I_1 .. I_n (model B). In both cases, we can consider the corresponding accumulation of species D: the degraded molecules derived from C. These models are defined and solved below.

(A) single step of depletion

$$\begin{aligned} dC/dt &= -\alpha C && (1)\\ dD_1/dt &= \alpha C && (2)\\ C &= C_0 e^{-\alpha t} && (3)\\ D_1 &= C_0(1-e^{-\alpha t}) && (4) \end{aligned}$$

(B) multiple steps of depletion

$$\begin{aligned} dC/dt &= -\alpha C && (5)\\ dI_1/dt &= \alpha C - \alpha I_1 && (6)\\ dI_i/dt &= \alpha I_{i-1} - \alpha I_i && (7)\\ dD_{n+1}/dt &= \alpha I_n && (8)\\ C &= C_0 e^{-\alpha t} && (9)\\ I_n &= C_0 \alpha^n t^n e^{-\alpha t} && (10)\\ D_{n+1} &= C_0 - C_0 e^{-\alpha t} - \sum_{k=1}^{n} C_0 \alpha^n t^n e^{-\alpha t}/n! && (11) \end{aligned}$$

In model B, we might choose to define all intermediate species I_i as still being instances of C as they have not yet completed the process of becoming D. This is relevant to the degradation of mRNA as partially-deadenylated

mRNA remains competent for translation (if at a reduced rate of transcription initiation (Goldstrohm and Wickens, 2008)).

Based on the ODE models of degradation as a 1 step (model A), or a 2 or 3 step process (model B), the following functions were optimised to the data using a nonlinear least squares method implemented in R (R Foundation). We assume the intermediate species contribute to the precursor C ($C_{n+1} = C_0 - D_{n+1}$). In addition to the rate parameter α, optimal values for the scaling parameter β and offset γ were also identified.

$$C_1 = \gamma + \beta e^{-\alpha t} \tag{12}$$

$$C_2 = \gamma + \beta(e^{-\alpha t} + \alpha t e^{-\alpha t}) \tag{13}$$

$$C_3 = \gamma + \beta(e^{-\alpha t} + \alpha t e^{-\alpha t} + \alpha^2 t^2 e^{-\alpha t}/2) \tag{14}$$

The alternative model predictions are shown in Figure 1, and the AIC scores and Akaike weights for each model are listed in Table 1. The Akaike weights, w_i, can be interpreted as the probability of model i, given the set of three candidate models under consideration. For lariat-exon2, the 2 step model is the most probable (P=0.59), but the simple exponential decay model (1 step) is also a candidate (P=0.24). For pre-mRNA, the 3 step model has probability 0.92, leaving only a probability of 0.08 that one of the other models applies. Therefore, there is considerable evidence for multiple steps in pre-mRNA degradation. Degradation is known to be a multi-step process, and has been modelled in detail (Cao and Parker, 2003). The optimal model parameters are listed in Table 2.

References

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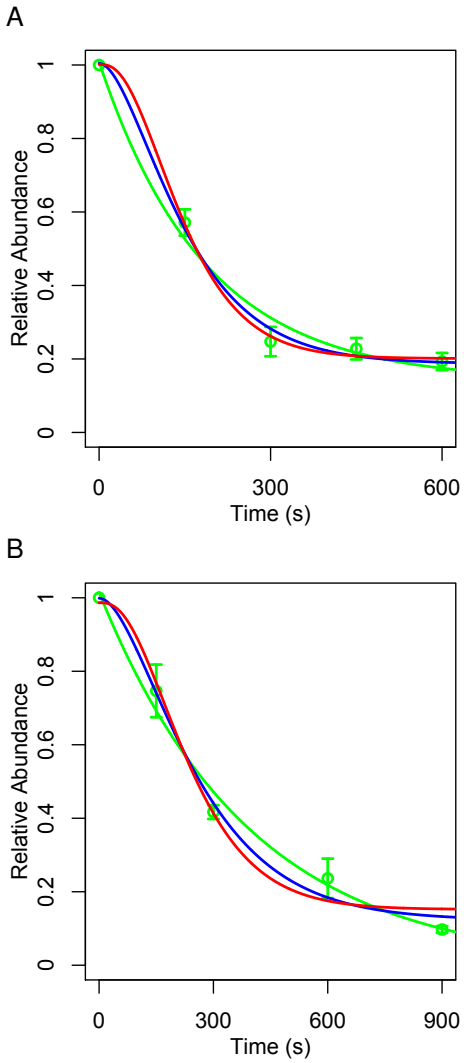

Figure 1. The degradation of 5'SSRibo1 and 3'SSRibo1 products. (**A**) Degradation of unspliced pre-mRNA (5'SSRibo1). (**B**) Degradation of lariat-exon2 (3'SSRibo1). Symbols indicate data. Error bars show the standard error of three biological replicates. Solid lines are model predictions: 1 step model (green); 2 step model (blue); 3 step model (red).

Model	pre-mRNA		lariat-exon2	
	AIC	Akaike wt.	AIC	Akaike wt.
1 step	-10.8	0.003	-11.0	0.24
2 step	-17.0	0.074	-12.8	0.59
3 step	-22.1	0.923	-10.3	0.17

Table 1. Comparison of degradation models for 5'SSRibo1 and 3'SSRibo1 products. Akaike weights represent the normalised likelihood of each of the three models (see Materials and Methods).

Data	Model	γ	β	α (half-life min.)
pre-mRNA	1 step	0.141266	0.867559	0.005415 (2.1)
pre-mRNA	2 step	0.18691	0.81739	0.01231 (1.9)
pre-mRNA	3 step	0.20105	0.80033	0.01904 (1.8)
lariat-exon2	1 step	-0.004757	1.019712	0.002534 (4.6)
lariat-exon2	2 step	0.122608	0.876035	0.007215 (3.2)
lariat-exon2	3 step	0.15184	0.83459	0.01180 (2.9)

Table 2. Optimal parameter values for 5'SSRibo1 and 3'SSRibo1 degradation models. Half lives are given for the reaction as a whole where the reaction has multiple steps.