

## Supporting Text

### Seven-Variable Model of NF- $\kappa$ B Signaling

We use the following abbreviations:  $N_n$  &  $N$ , free nuclear and cytoplasmic NF- $\kappa$ B;  $I_m$ , I $\kappa$ B mRNA;  $I_n$  &  $I$ , free nuclear and cytoplasmic I $\kappa$ B;  $(NI)_n$  &  $(NI)$ , nuclear and cytoplasmic NF- $\kappa$ B-I $\kappa$ B complex; IKK, I $\kappa$ B kinase. The seven-variable model is defined by the equations

$$\begin{aligned}\frac{dN_n}{dt} &= k_{Nin}N - k_{fn}N_nI_n + k_{bn}(NI)_n, \\ \frac{dI_m}{dt} &= k_tN_n^2 - \gamma_mI_m, \\ \frac{dI}{dt} &= k_{tl}I_m - k_fNI + k_b(NI) - k_{Iin}I + k_{Iout}I_n, \\ \frac{dN}{dt} &= -k_fNI + (k_b + \alpha)(NI) - k_{Nin}N, \\ \frac{d(NI)}{dt} &= k_fNI - (k_b + \alpha)(NI) + k_{NIout}(NI)_n, \\ \frac{dI_n}{dt} &= k_{Iin}I - k_{Iout}I_n - k_{fn}N_nI_n + k_{bn}(NI)_n, \\ \frac{d(NI)_n}{dt} &= k_{fn}N_nI_n - (k_{bn} + k_{NIout})(NI)_n.\end{aligned}$$

Fig. 9 shows a plot of nuclear NF- $\kappa$ B concentration obtained by integrating these equations using the following parameter values (1):  $k_{Nin} = 5.4 \text{ min}^{-1}$ ,  $k_{Iin} = 0.018 \text{ min}^{-1}$ ,  $k_{Iout} = 0.012 \text{ min}^{-1}$ ,  $k_{NIout} = 0.83 \text{ min}^{-1}$ ,  $k_t = 1.03 \mu\text{M}^{-1} \cdot \text{min}^{-1}$ ,  $k_{tl} = 0.24 \text{ min}^{-1}$ ,  $k_f = k_{fn} = 30 \mu\text{M}^{-1} \cdot \text{min}^{-1}$ ,  $k_b = k_{bn} = 0.03 \text{ min}^{-1}$ ,  $\alpha = 1.05 \times \text{IKK} \text{ min}^{-1}$ ,  $\gamma_m = 0.017 \text{ min}^{-1}$ . The initial conditions were  $N = 1 \mu\text{M}$ , IKK =  $0.5 \mu\text{M}$ , and all other concentrations zero.

### Reduction from Seven Variables to Three Variables

First, taking note of the fact that  $k_f$  and  $k_{fn}$  are large, we assume that all complexes are in equilibrium, i.e.

$$\begin{aligned}k_fNI &\approx (k_b + \alpha)(NI), \\ k_{fn}N_nI_n &\approx (k_{bn} + k_{NIout})(NI)_n.\end{aligned}$$

Simulations show that these are good approximations. In terms of  $I_n^{tot} \equiv I_n + (NI)_n$  and  $N_c^{tot} \equiv N + (NI) = N_{tot} - N_n$ , which are slowly varying, we can rewrite the above equations as follows:

$$\begin{aligned}(NI) &= (N_{tot} - N_n) \frac{I}{K_I + I}, \\ N &= (N_{tot} - N_n) \frac{K_I}{K_I + I}, \\ (NI)_n &= I_n^{tot} \frac{N_n}{K_N + N_n}, \\ I_n &= I_n^{tot} \frac{K_N}{K_N + N_n},\end{aligned}$$

where  $K_I \equiv (k_b + \alpha)/k_f = 0.035 \mu\text{M}$  and  $K_N \equiv (k_{bn} + k_{NIout})/k_{fn} = 0.029 \mu\text{M}$ , using the parameter values above.

Using these expressions, the equations of the seven-variable model reduce to the following four (Fig. 9):

$$\begin{aligned}\frac{dN_n}{dt} &= k_{Nin}K_I \frac{(N_{tot} - N_n)}{K_I + I} - k_{NIout} \frac{I_n^{tot} N_n}{K_N + N_n}, \\ \frac{dI_m}{dt} &= k_tN_n^2 - \gamma_mI_m, \\ \frac{dI}{dt} &= k_{tl}I_m - \alpha \frac{(N_{tot} - N_n)I}{K_I + I} - k_{Iin}I + k_{Iout}K_N \frac{I_n^{tot}}{K_N + N_n},\end{aligned}$$

$$\frac{dI_n^{tot}}{dt} = k_{Iin}I - k_{Iout}K_N \frac{I_n^{tot}}{K_N + N_n} - k_{NIout} \frac{I_n^{tot} N_n}{K_N + N_n}.$$

First, we note that the terms  $-k_{Iin}I$  and  $k_{Iout}K_N \frac{I_n^{tot}}{K_N + N_n}$  in the  $dI/dt$  equation are much smaller than  $-\alpha \frac{(N_{tot} - N_n)I}{K_I + I}$  and can be neglected as long as  $IKK$  is nonzero. Second, simulations reveal that the term  $k_{NIout} \frac{I_n^{tot} N_n}{K_N + N_n}$ , in the  $dI_n^{tot}/dt$  equation, also shows sharp spikes as a function of time which coincide with the spikes of  $N_n$ . The value of this term is substantial only when  $N_n \gg K_N$ , i.e., during the spikes of  $N_n$ , and at those times  $I_n^{tot}$  dips to its minimum. We therefore make the approximation that  $I_n^{tot}$  can be replaced by its minimum value,  $I_{n,min}^{tot}$ , which satisfies the equation

$$k_{Iin}I = k_{Iout}K_N \frac{I_{n,min}^{tot}}{K_N + N_n} + k_{NIout} \frac{I_{n,min}^{tot} N_n}{K_N + N_n}.$$

In the regime where  $N_n \gg K_n$  this gives

$$I_{n,min}^{tot} \approx \frac{k_{Iin}}{k_{NIout}} I.$$

Using this we can reduce to a three-variable model

$$\begin{aligned} \frac{dN_n}{dt} &= k_{Nin}K_I \frac{(N_{tot} - N_n)}{K_I + I} - k_{Iin} \frac{IN_n}{\delta + N_n}, \\ \frac{dI_m}{dt} &= k_t N_n^2 - \gamma_m I_m, \\ \frac{dI}{dt} &= k_{tl} I_m - \alpha \frac{(N_{tot} - N_n)I}{K_I + I}. \end{aligned}$$

Fig. 10 *Upper* shows a plot of the oscillations of nuclear NF- $\kappa$ B for the parameter values given above. When the transcription rate is increased, the frequency of oscillation goes down (Fig. 10 *Lower*).

## Rescaling the Three-Variable Model

For ease of analysis, we reduce the number of parameters in the model by rescaling all variables to become dimensionless. We use the following transformations on the above equations:

$$\begin{aligned} t &\rightarrow (1/\gamma_m)t, \\ N_n &\rightarrow N_{tot}N_n, \\ I_m &\rightarrow (k_t N_{tot}^2 / \gamma_m)I_m, \\ I &\rightarrow (k_t k_{tl} N_{tot}^2 / \gamma_m^2)I, \end{aligned}$$

which gives

$$\begin{aligned} \frac{dN_n}{dt} &= A \frac{(1 - N_n)}{\epsilon + I} - B \frac{IN_n}{\delta + N_n}, \\ \frac{dI_m}{dt} &= N_n^2 - I_m \\ \frac{dI}{dt} &= I_m - C \frac{(1 - N_n)I}{\epsilon + I}, \end{aligned}$$

with

$$\begin{aligned} A &= \frac{k_{Nin}K_I\gamma_m}{k_t k_{tl} N_{tot}^2} \approx 0.007, \\ B &= \frac{k_{Iin}k_t k_{tl} N_{tot}}{\gamma_m^3} \approx 954.5, \\ C &= \frac{\alpha \gamma_m}{k_t k_{tl} N_{tot}} \approx 0.035, \\ \delta &= \frac{K_N}{N_{tot}} \approx 0.029, \\ \epsilon &= \frac{K_I \gamma_m^2}{k_t k_{tl} N_{tot}^2} \approx 2 \times 10^{-5}. \end{aligned}$$

## Steady-State Solution of the Three-Variable Model

The steady-state values of  $N_n$ ,  $I_m$  and  $I$  are solutions to

$$A \frac{(1 - N_n)}{\epsilon + I} - B \frac{IN_n}{\delta + N_n} = 0,$$

$$N_n^2 - I_m = 0,$$

$$I_m - C \frac{(1 - N_n)I}{\epsilon + I} = 0.$$

$I_m$  and  $I$  can be eliminated using

$$I_m = N_n^2,$$

$$I = \frac{N_n^2 \epsilon}{C - CN_n - N_n^2}.$$

From this we find that the steady-state value of  $N_n$  is a solution of the equation

$$(C - CN_n - N_n^2)^2 = \frac{BC\epsilon^2}{A} \frac{N_n^3}{\delta + N_n},$$

or equivalently,

$$N_n^5 + (\delta + 2C)N_n^4 + C \left[ 2(\delta - 1) + C - \frac{B}{A}\epsilon^2 \right] N_n^3 + C[(C - 2)\delta - 2C]N_n^2 + C^2(1 - 2\delta)N_n + C^2\delta = 0.$$

In general, this has two real solutions, one with  $C - CN_n - N_n^2 > 0$  and the other with  $C - CN_n - N_n^2 < 0$ . The latter results in a negative value for  $I$  and therefore is not an acceptable solution. Thus, we are left with only one fixed point.

## Linear Stability of the Fixed Point

We linearize the equations around the fixed point, which gives the Jacobian

$$J = \begin{pmatrix} -\frac{A}{\epsilon+I} - \frac{\delta BI}{(\delta+N_n)^2} & 0 & -\frac{A(1-N_n)}{(\epsilon+I)^2} - \frac{BN_n}{\delta+N_n} \\ 2N_n & -1 & 0 \\ \frac{CI}{\epsilon+I} & 1 & -\frac{C\epsilon(1-N_n)}{(\epsilon+I)^2} \end{pmatrix}.$$

This matrix can be used to examine the stability of the fixed point. If  $\lambda_i$  are the (possibly complex) eigenvalues of this matrix, then the fixed point is unstable if

$$\max_i [\text{Re}(\lambda_i)] > 0$$

and stable if

$$\max_i [\text{Re}(\lambda_i)] < 0.$$

For small values of  $\epsilon$ , corresponding to strong saturation of the degradation, the fixed point is unstable, and the system goes into a periodic cycle. As  $\epsilon$  is increased the fixed point becomes stable and the oscillations disappear (Fig. 11). This happens when the value of  $\epsilon$  becomes comparable with the steady-state value of  $I$ , which is precisely when the degradation rate stops being saturated.

## Including the $\beta, \epsilon$ Isoforms of $I\kappa B$

Unlike the  $\alpha$  isoform, the  $\beta, \epsilon$  isoforms of  $I\kappa B$  are produced independent of  $NF-\kappa B$ . Their effect can be included simply by adding a constant term,  $c$ , to the production of  $I_m$

$$\frac{dN_n}{dt} = k_{Nin} K_I \frac{(N_{tot} - N_n)}{K_I + I} - k_{Iin} \frac{IN_n}{\delta + N_n},$$

$$\frac{dI_m}{dt} = c + k_t N_n^2 - \gamma_m I_m,$$

$$\frac{dI}{dt} = k_{tl} I_m - \alpha \frac{(N_{tot} - N_n)I}{K_I + I}.$$

When this term is large enough the oscillations are damped (Fig. 12). As expected if the  $N_n^2$  term is deleted there are no oscillations and the system converges to a stable steady state.

## Linear Production of $I_m$

In this section we consider the effect of taking the production of  $I_m$  to be linear in  $N_n$ , instead of  $N_n^2$ :

$$\begin{aligned}\frac{dN_n}{dt} &= k_{Nin}K_I \frac{(N_{tot} - N_n)}{K_I + I} - k_{Iin} \frac{IN_n}{\delta + N_n}, \\ \frac{dI_m}{dt} &= k_t N_n - \gamma_m I_m, \\ \frac{dI}{dt} &= k_{tl} I_m - \alpha \frac{(N_{tot} - N_n)I}{K_I + I}.\end{aligned}$$

Using very similar transformations, we rescale the variables

$$\begin{aligned}t &\rightarrow (1/\gamma_m)t, \\ N_n &\rightarrow N_{tot}N_n, \\ I_m &\rightarrow (k_t N_{tot}/\gamma_m)I_m, \\ I &\rightarrow (k_t k_{tl} N_{tot}/\gamma_m^2)I,\end{aligned}$$

which gives

$$\begin{aligned}\frac{dN_n}{dt} &= A \frac{(1 - N_n)}{\epsilon + I} - B \frac{IN_n}{\delta + N_n}, \\ \frac{dI_m}{dt} &= N_n - I_m, \\ \frac{dI}{dt} &= I_m - C \frac{(1 - N_n)I}{\epsilon + I},\end{aligned}$$

with

$$\begin{aligned}A &= \frac{k_{Nin}K_I\gamma_m}{k_t k_{tl} N_{tot}}, \\ B &= \frac{k_{Iin} k_t k_{tl}}{\gamma_m^3}, \\ C &= \frac{\alpha \gamma_m}{k_t k_{tl}}, \\ \delta &= \frac{K_N}{N_{tot}}, \\ \epsilon &= \frac{K_I \gamma_m^2}{k_t k_{tl} N_{tot}}.\end{aligned}$$

If we use the same parameter values as before we do not get sustained oscillations. Not surprisingly, the region in parameter space where we get sustained oscillations has shifted. Simply taking  $A = 0.001$ , we get spiky oscillations, as in the original model (Fig. 13A). Fig. 13B shows the response to changes in  $IKK$ .

The steady-state value of  $N_n$  is now a solution to

$$(C - CN_n - N_n)^2 = \frac{BC\epsilon^2}{A} \frac{N_n^2}{\delta + N_n}.$$

The steady-state value of  $I$  can be calculated from the value of  $N_n$  using

$$I = \frac{N_n \epsilon}{C - CN_n - N_n}.$$

In general, there are again two real solutions, one with  $C - CN_n - N_n > 0$  and the other with  $C - CN_n - N_n < 0$ . The latter results in a negative value for  $I$  and therefore is not an acceptable solution. Thus, we are left with only one fixed point. Linearizing the equations around the fixed point gives almost the same Jacobian. The only difference is in one matrix element:  $J_{2,1} = 1$  instead of  $2N_n$ . Fig. 14 shows the stability of the fixed point as a function of  $\epsilon$ .

## Equilibrium Binding of NF- $\kappa$ B to a Gene

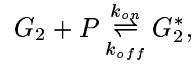
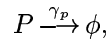
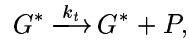
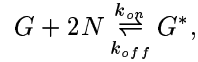
When the binding of NF- $\kappa$ B to an operator site is in equilibrium the gene activity is

$$G^* = \frac{N_n^2}{k_{off}/k_{on} + N_n^2}.$$

This leads to a high sensitivity of the peak gene activity to changes in IKK concentration as discussed in the main text. Fig. 15 and Table 1 show that this sensitivity is robust to changes in parameter values. The only significant change in the effective Hill coefficient occurs with changes in  $B$ .

## Strongly Nonequilibrium Binding of NF- $\kappa$ B to a Gene

In the extreme case where  $k_{off}$  is negligible, each spike of nuclear NF- $\kappa$ B contributes to increase the gene activity,  $G^*$ , until it saturates to unity as shown by the solid line in Fig. 16. If this gene controls the activity of a second one in a cascade



then the latter will turn on later (dashed line in Fig. 16) with a time delay that depends on the timescales of transcription, translation and promoter activation. This is reminiscent of the experiments of Hoffman *et al.* (1) which show the gene IP-10 turning on quickly after the introduction of IKK, while the gene RANTES turns on after a delay.

## References

- [1] Hoffmann, A., Levchenko, A., Scott, M. L. & Baltimore, D. (2002) *Science* **298**, 1241–1245.