Supporting Text

Seven-Variable Model of NF-κB Signaling

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

$$\frac{dN_n}{dt} = k_{Nin}N - k_{fn}N_nI_n + k_{bn}(NI)_n,$$

$$\frac{dI_m}{dt} = k_tN_n^2 - \gamma_mI_m,$$

$$\frac{dI}{dt} = k_{tl}I_m - k_fNI + k_b(NI) - k_{Iin}I + k_{Iout}I_n,$$

$$\frac{dN}{dt} = -k_fNI + (k_b + \alpha)(NI) - k_{Nin}N,$$

$$\frac{d(NI)}{dt} = k_fNI - (k_b + \alpha)(NI) + k_{NIout}(NI)_n,$$

$$\frac{dI_n}{dt} = k_{Iin}I - k_{Iout}I_n - k_{fn}N_nI_n + k_{bn}(NI)_n,$$

$$\frac{d(NI)_n}{dt} = k_{fn}N_nI_n - (k_{bn} + k_{NIout})(NI)_n.$$

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

$$k_fNI \approx (k_b + \alpha)(NI),$$

$$k_{fn}N_nI_n \approx (k_{bn} + k_{NIout})(NI)_n.$$

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:

$$(NI) = (N_{tot} - N_n)\frac{I}{K_I + I},$$

$$N = (N_{tot} - N_n)\frac{K_I}{K_I + I},$$

$$(NI)_n = I_n^{tot}\frac{N_n}{K_N + N_n},$$

$$I_n = I_n^{tot}\frac{K_N}{K_N + N_n},$$

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

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

$$\frac{dN_n}{dt} = k_{Nin}K_I\frac{(N_{tot} - N_n)}{K_I + I} - k_{NIout}\frac{I_n^{tot}N_n}{K_N + N_n},$$

$$\frac{dI_m}{dt} = k_tN_n^2 - \gamma_mI_m,$$

$$\frac{dI}{dt} = k_{tl}I_m - \alpha\frac{(N_{tot} - N_n)I}{K_I + I} - k_{Iin}I + k_{Iout}K_N\frac{I_n^{tot}}{K_N + N_n},$$

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

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

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

$$\frac{dI_m}{dt} = k_tN_n^2 - \gamma_mI_m,$$

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

Fig. 10 *Upper* shows a plot of the oscillations of nuclear NF-κB 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:

$$t \to (1/\gamma_m)t,$$

$$N_n \to N_{tot}N_n,$$

$$I_m \to (k_tN_{tot}^2/\gamma_m)I_m,$$

$$I \to (k_tk_{tl}N_{tot}^2/\gamma_m^2)I,$$

which gives

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

$$\frac{dI_m}{dt} = N_n^2 - I_m$$

$$\frac{dI}{dt} = I_m - C\frac{(1-N_n)I}{\epsilon+I},$$

with

$$A = \frac{k_{Nin}K_I\gamma_m}{k_tk_{tl}N_{tot}^2} \approx 0.007,$$

$$B = \frac{k_{Iin}k_tk_{tl}N_{tot}}{\gamma_m^3} \approx 954.5,$$

$$C = \frac{\alpha\gamma_m}{k_tk_{tl}N_{tot}} \approx 0.035,$$

$$\delta = \frac{K_N}{N_{tot}} \approx 0.029,$$

$$\epsilon = \frac{K_I\gamma_m^2}{k_tk_{tl}N_{tot}^2} \approx 2\times10^{-5}.$$

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 λ_i are the (possibly complex) eigenvalues of this matrix, then the fixed point is unstable if

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

and stable if

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

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

Including the β, ϵ Isoforms of IκB

Unlike the α isoform, the β, ϵ isoforms of IκB are produced independent of NF-κB. 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:

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

$$\frac{dI_m}{dt} = k_t N_n - \gamma_m I_m,$$

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

Using very similar transformations, we rescale the variables

$$t \to (1/\gamma_m)t,$$

$$N_n \to N_{tot}N_n,$$

$$I_m \to (k_t N_{tot}/\gamma_m)I_m,$$

$$I \to (k_t k_{tl} N_{tot}/\gamma_m^2)I,$$

which gives

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

$$\frac{dI_m}{dt} = N_n - I_m,$$

$$\frac{dI}{dt} = I_m - C\frac{(1-N_n)I}{\epsilon+I},$$

with

$$A = \frac{k_{Nin}K_I\gamma_m}{k_t k_{tl}N_{tot}},$$

$$B = \frac{k_{Iin}k_t k_{tl}}{\gamma_m^3},$$

$$C = \frac{\alpha\gamma_m}{k_t k_{tl}},$$

$$\delta = \frac{K_N}{N_{tot}},$$

$$\epsilon = \frac{K_I\gamma_m^2}{k_t k_{tl}N_{tot}}.$$

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. 13*A*). Fig. 13*B* 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 ϵ.

Equilibrium Binding of NF-κB to a Gene

When the binding of NF-κB 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-κB to a Gene

In the extreme case where k_{off} is negligible, each spike of nuclear NF-κB 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

$$G + 2N \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} G^*,$$

$$G^* \xrightarrow{k_t} G^* + P,$$

$$P \xrightarrow{\gamma_p} \phi,$$

$$G_2 + P \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} G_2^*,$$

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.