Interactions among oscillatory pathways in NF-κB signaling network

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May 19, 2010
The oscillations are thought to arise from a negative feedback loop whose main players are:

**NF-κB** nuclear factor κB a dimeric transcription factor;

**IK-Bα** an inhibitor of κB which binds to NF-κB sequestering it in the cytoplasm;

**IKK** an enzyme that acts on IKBα causing it to release the NF-κB. IKK exists in several forms: neutral (IKKn), active (IKK) and inactive (IKKi).

**TNFα** tumour necrosis factor α, an extracellular signal;

 Proteins are shown in ovals, mRNAs in rounded boxes.
The movie above\(^1\) shows cultured human cells (SK-N-AS) that have been modified to produce red fluorescently labelled NF-\(\kappa\)B dimers and green IKB\(\alpha\). They were stimulated continuously with TNF\(\alpha\) and photographed every 3 minutes for 10 hours.

\(^1\)It’s part of the supplemental materials from D.E. Nelson et al. (2004), Oscillations in NF-\(\kappa\)B signaling control the dynamics of gene expression, *Science*, **306**.
The first part of the talk concerns a reduced model illustrated below.

It differs from the core oscillator model in two main ways:

- The various forms of IKK are not resolved separately.
- The dynamics of TNF\(\alpha\) stimulation are not modelled in detail: instead, a pulse of stimulation appears as an abrupt jump in active IKK concentration.
An important feature of the system sketched above, which is essentially the model from Caroline Horton’s thesis, is that it includes two conserved, or nearly-conserved quantities.

- There is no mechanism for the synthesis or destruction of NF-κB, so that the total number of dimers—given by a linear combination of the nuclear and cytoplasmic concentrations—is conserved.

- A consequence of the simplified treatment of IKK is that only active IKK appears, and its unbound form decays slowly (half-life long compared to the period of oscillations) with linear kinetics.

Hoffmann reported damped oscillations in the level of nuclear NF-κB. This suggests the existence of an attracting fixed point with complex eigenvalues, but no such fixed point exists for biologically relevant parameter values:

The asymptotic state of Hoffmann’s model has $[\text{Ikk}] = 0$, total NF-κB = 0.1 and nuclear NF-κB = 0.
The diagram at left illustrates the bifurcation structure of the reduced model in the limit that total IKK is conserved. Black curves indicate stable equilibria, the dashed red curve a line of unstable equilibria while the blue-green curve shows the extreme values of a branch of stable limit cycles.

In all cases, total NF-$\kappa$B = 0.1, as in Hoffman's simulations.

A pulse of TNF$\alpha$ stimulation appears here as an abrupt jump in the free IKK concentration. If the decay of free IKK is slow compared to the oscillations, one would expect the subsequent evolution to be organized by the structures illustrated above.
Traces of nuclear NF-κB concentration as a function of the total IKK.

- When the post-stimulus concentration of free IKK jumps into the range $0.01 < IKK_0 < 0.05$, the envelope of the stable periodic orbit makes good predictions of the peak amplitudes and the flat minima.
- The initial pulse is always larger than expected: more careful analysis shows this to be a consequence of the process of relaxation onto the slow manifold.
A more rigorous treatment of the slow-fast dynamics illustrated above involves an appeal to Tikhonov’s Theorem\(^2\), the main idea being that if the relaxation to \([\text{IKK}] = 0\) is sufficiently slow, we can imagine that the dynamics are close to the stable limit cycle oscillations found when total IKK is held to some constant value \(c\).

Defining \(\theta(t)\) to be a phase coordinate on the limit cycles, we could count the number of pulses we expect to see by computing \((\Delta\theta/2\pi)\) where

\[
\Delta\theta \approx \int_{t_0}^{t_1} \frac{2\pi}{T(c(t))} \, dt.
\]

Here \(t_0\) is the time at which the total IKK level first enters the range for which stable limit cycles exist, \(t_1\) is the time at which it leaves and \(T(c)\) is the period of limit cycle when total IKK \(= c\).

This formula is only approximate as it excludes certain rapidly varying terms having to do with variation in \(d\theta/dt\) with \(\theta\).

\(^2\)See, for example, I Dvořák and Šiška (1989), Bulletin of Mathematical Biology, 51, 255–274.
Counting pulses, continued

Changing the variable of integration to $c$, the level of total IKK, yields

$$\Delta \theta \approx \frac{2\pi}{k} \int_{c(t_0)}^{c(t_1)} \frac{dc}{T(c)(-k[IKK])} = \frac{2\pi}{k} \int_{c(t_0)}^{c(t_1)} \frac{dc}{T(c)\{IKK\}}$$

where $[IKK]$ is the concentration of free IKK and $d[IKK]/dt = -k[IKK]$ is the rate at which it (and hence, in this model, total IKK) degrade.

The panel at left shows the period of the limit cycle as a function of total IKK. The central region, with $0.01 < IKK < 0.05$, corresponds to stable limit cycles and is the part that plays the role of $T(c)$ in the expression above.
The panels above show the time course of free vs. total IKK for various initial conditions at each of four different levels of NF-κB and suggest that

$$[IKK] \approx \gamma \text{(Total IKK)} + \text{oscillatory terms.}$$

The panel at lower right is the relevant one, with total NF-κB = 0.1, and there \(\gamma \approx 0.37\).
Replacing \([Ikk]\) with \(\gamma c\) in our expression for \(\Delta \theta\) yields

\[
\Delta \theta \approx \frac{2\pi}{\gamma k} \int_{c(t_0)}^{c(t_1)} \frac{dc}{cT(c)}
\]

which is now fairly easy to compute, at least numerically.

The panel at left shows, as a solid blue curve, the result of integrating the formula above numerically, while the dashed line with boxes shows a count of the number pulses observed in simulations.

The close agreement seems interesting as it offers the prospect of comparison with easily measured experimental quantities.
Our next project concerned the possibility of probing interactions between oscillatory subsystems. As a start, she analysed the power spectra of responses to trains of three pulses. Note that the power is concentrated at multiples of the pulse frequency: the cells’ responses appear to be completely entrained by the forcing pulse trains.
Two feedback loops with TNF$\alpha$ modulation

The next series of numerical studies involve the model illustrated at right, which is more recent\textsuperscript{3} than the one used in the pulse-counting project.

- TNF$\alpha$ stimulation now appears as a time-dependent coefficient

\[ 0 \leq T_R(t) \leq 1 \]

that modulates the rate of the reaction: IKKn $\rightarrow$ IKK.

- We do not need to assume a linear relation between concentration of applied TNF$\alpha$ and $T_R(t)$: only that $T_r = 0$ means no stimulation and $T_r = 1$ means saturating stimulation.

The introduction of $T_R(t)$ allows us to explore (at least in silico) the consequences of weaker (lower-dose) stimulation, as well as those of more sustained periodic forcing.

The bifurcation diagram at left illustrates the way in which oscillations in the localization of NF-κB depend on $T_R$. The solid black curve in the region $0 \leq T_R \leq T_*$ corresponds to steady, non-oscillating behaviour, while the dashed black curve for $T_R > T_* \approx 0.366$ indicates a branch of oscillatory solutions. The red curves show the limits—the peak and trough values—of the stable oscillatory responses that exists for these values of $T_R$. The panel at right shows periods of the oscillations as a function of $T_R$. 
The panel at left shows the response to sustained periodic stimulation in which strong ($T_R = 1$) pulses of 5 minute duration alternate with stimulus-free intervals of 55 minute duration. The panel at right shows the power spectral density of the response as a function of pulsing frequency for a wide range of pulse frequencies. Each member of the fan of lines has integer slope.
Weaker stimulation reveals the system’s natural frequency.

The panels above show the results of stimulation with trains of weak pulses ($T_R = 0.1$) and give evidence of subharmonic resonance.
To explore further the possibility of coupling between oscillatory pathways we applied a sinusoidally modulated stimulus

\[ T_R(t) = \epsilon(1 + \eta \sin(2\pi \nu t)). \]

The panel at right shows \( T_R(t) \) with \( \nu = 1, \eta = 0.5 \) and \( \epsilon = 0.6 \).

The point of this somewhat fiddly parameterization is that it allows us to control, separately, the average strength of stimulation and the relative strength of the modulation:

- The time average of \( T_R(t) \) is \( \epsilon \).
- \( 0 \leq \eta \leq 1 \) and \( \nu > 0 \) are, respectively, the relative amplitude and the frequency of the sinusoidal modulation.
- \( T_R(t) \) has period \( \tau = 1/\nu \) and range

\[ 0 \leq \epsilon(1 - \eta) \leq T_R(t) \leq \epsilon(1 + \eta) \leq 1. \]
Complex nonlinear resonances

The panel at right shows power spectral densities for responses to relatively strong stimuli ($\epsilon = \eta = 0.5$).

The bright lines represent power at frequencies $f$ of the form

$$f = p\nu + q\nu_0$$

with $p, q \in \mathbb{Z}$.

The tracework of lines above correspond to various forms of resonance

- In the strip above $\nu = \nu_0$ the response is as in the original experiments, resonant with the forcing.
- Strong horizontal features show power at the system’s natural frequency.
- The network of lines with slope of $\pm 1$ indicates a sort of “beating” phenomenon, with power at frequencies of the form $f = q\nu_0 \pm \nu$. 
In the left panel above the forcing is so strong ($\epsilon = 0.5$ and $\eta = 1$) that the system is, mainly, simply entrained by the forcing, while in the panel at right the response to a much weaker modulation ($\epsilon = 0.5$, but $\eta = 0.1$) is dominated by the horizontal bands indicating oscillation at the system’s natural frequency.
When the modulation frequency and amplitude \((\nu, \eta)\) lie inside the shaded regions above the system’s response is periodic, with a period \(\tau\) that is an integer multiple of the modulation period \(1/\nu\) and that also lies close to an integer multiple of the natural period \(1/\nu_0\):

\[
\tau = \frac{p}{\nu_0} \approx \frac{q}{\nu}
\]
The figure above comes from a comprehensive survey of bifurcation structure as a function of $T_R$ and the other parameters, considered one at a time: it’s a bifurcation diagram for $T_R$ when the Hill coefficient for NF-$\kappa$B induced protein synthesis is $h = 3$ (our standard parameter sets have $h = 2$).

A manuscript from the White lab that’s currently under review explores responses to low-dose TNF$\alpha$ stimulation and finds that 100-minute oscillations still occur, though their onset can be delayed—stochastically in the case of very weak stimulation.
The figure above illustrates the main components in a model\textsuperscript{4} of the \textit{segment polarity network}, a system of genetic regulatory interactions partly responsible for determining the body plan of a developing fruit fly (\textit{Drosophila melanogaster}). Messenger RNA’s are shown as ovals, while their products appear in round-cornered boxes.

The figure at right shows the pattern of segment polarity gene expression in an embryo that is developing correctly: the boundaries of the **parasegments** are marked by single-cell wide bands of gene expression.

Within each parasegment:

- cells at anterior (head) end express *en* strongly, but not *wg*;
- cells at posterior (tail) end express *wg* strongly, but not *en*;
- remaining cells do not express either of these genes.
The figure above illustrates the way that cell-cell interactions are modelled. The drawings are really only suggestive: the model has no spatial structure save that it specifies which cells are in contact with which others.

- Each cell may contact as many as 6 others, thus cells are drawn with 6 faces.
- Membrane-bound proteins produced in a cell are assumed to be shared equally over the cell’s faces.
- The faces are numbered in such a way that face $j$ in one cell is in contact with face $(j + 3) \mod 6$ in a neighbouring cell.
The production of hedgehog protein on the $j$-th face of cell $i$ is modelled by the ODE

$$\frac{d[HH]_{i,j}}{dt} = \frac{P_{max}\sigma_{hh}[hh]_i}{6} - \frac{[HH]_{i,j}}{H_{HH}} - k_{PTCHH}[HH]_{i,j}[PTC]_{n,j+3}$$

Here

- $P_{max}$ is a maximal rate of translation that applies to all mRNAs and $0 < \sigma_{HH} \leq 1$ is a hedgehog-specific adjustment. The factor of $1/6$ appears because $HH$ is shared out over the cell’s six faces and this ODE describes $HH$ on just one face, the $j$-th one.

- $H_{HH}$ is the expected lifetime of a hedgehog protein.

- $K_{PTCHH}$ is the rate constant for the formation of patched-hedgehog complex, $PH$. The formation of this complex mediates communication between the cells. Here $n$ represents the index of a neighbouring cell and $j$ and $j + 3$ are faces that are in contact.
After suitable non-dimensionalization, the model had 48 free parameters. These could be constrained, very weakly, by considerations such as:

- Protein and mRNA lifetimes shorter than, say, a few seconds do not make any sense.
- Reactions must be sufficiently fast that steady states are achieved within hours or tens of hours (as opposed to, say, weeks).
- Hill coefficients $\nu > 35$ have not been observed in any system.

Subject to these modest constraints, they chose sets of parameters at random from within the biologically plausible subset of $\mathbb{R}^{48}$, initialized a set of 4 cells in one of the ways illustrated at above and simulated the system for a period of around 2000 minutes.
Modification of the initial model

After suitable non-dimensionalization, the model had 48 free parameters. When using only the interactions illustrated by solid arrows on the initial slide, very few (1 in 3000) sets of parameters yielded qualitatively correct behaviour, even when initialized with crisp initial conditions.

The issue is that the original network includes no interactions that can induce a sense of direction in the expression of en: if WG is the only influence on en production, then one would expect both neighbours of wg expressing cells to express en.

One needs active repression of en in cells anterior to wg-expressing cells as well as some modification that induces spatial bias in the (indirect) hedgehog-wingless interaction. The dashed arcs on the first slide indicate interactions for which there was some experimental support.
Further numerical experiments

For the modified system, randomly selected patterns very frequently (around 1 time in 200) yielded qualitatively correct, stable responses to crisp initial conditions.

Even with degraded initial conditions, randomly-chosen parameters were reasonably frequently (1 in 375 with low-level, ubiquitous initial $ci$ and $ptc$ expression) successful.

In the crisp (respectively, degraded) simulations “correct” behaviour meant that the target pattern of $en$ and $wg$ appeared within 200 (600) minutes of simulated time and remained stable for 1000 minutes. Although the results are plotted for a strip of 12 cells, there were really only 4, connected periodically so that each cell had 6 neighbours.
The figure at left above illustrates 1192 successful parameter sets for the experiments with crisp initial conditions. The panel at right shows similar results for the degraded conditions. In both cases, almost every value in each parameter’s range appeared as part of some successful combination. In both panels individual parameter values are plotted logarithmically on “spokes”. The very darkest lines indicate means and standard deviations.
Quality scoring

Rows are labelled by parameters while columns represent variations around a single successful solution. The individual panels show a quality score achieved by simulations.

Thus to make the plot at upper left, von Dassow et al. chose a successful parameter set (from those discovered by random search) and then varied $K_{WGen}$, a parameter of the activation of en production by WG.

In their scheme low scores are good (dashed lines show cutoffs) and the point is that one can vary individual parameters substantially (horizontal axes all span 3 decades of variation) and still achieve success.
Remarks, hints and conjectures

• Their model-building exercise suggested evidence for then-unknown interactions: these have now been verified experimentally.

• This work has the flavour—and appropriates some of the vocabulary—of the qualitative theory of ODEs, but is not obviously tied to the existence of various sorts of attractors. In the segment polarity network, a small-amplitude oscillation is just as effective as a constant steady state.

• Scoring functions that embody qualitative desiderata play a critical role.

The panel above, which is part of the supplementary material to Ashall et al., map the values of four scoring functions associated with qualitatively correct behaviour in four different sorts of simulation. The boundaries between regions indicating correct and incorrect behaviours look teasingly similar to bifurcation structures, so perhaps one could establish a more rigorous connection.