A discrete model of the nuclear factor NF-kappaB regulatory network

Mercedes S. Pérez Millán
Universidad de Buenos Aires

July 8, 2010

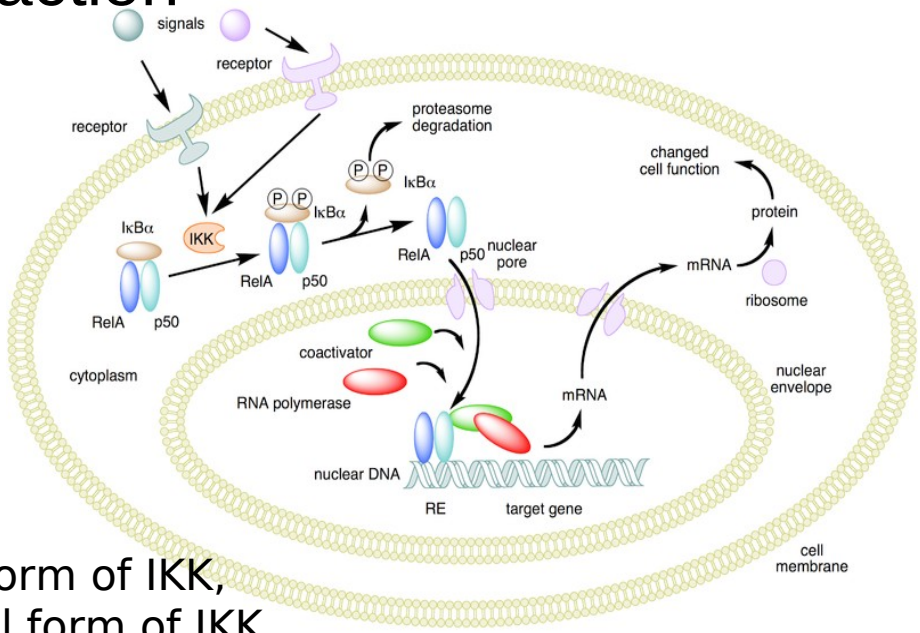
Joint work with:

- Abdul Salam Jarrah, American University of Sharjah
- Juan Ignacio Fuxman Bass, Universidad de Buenos Aires

Who is NF-κB?

The proteins of the NF-κB group are a family of transcription factors involved in important cellular processes that include inflammatory responses as well as regulation of apoptosis.

NF-κB mechanism of action



S—stimulus,
IKKa—cytoplasmic level of active form of IKK,
IKKne—cytoplasmic level of neutral form of IKK,
IKKi—cytoplasmic level of inactive form of IKK,
IκBα|NF-κB—cytoplasmic level of IκBα|NF-κB complexes,
IκBα—cytoplasmic level of IκBα,
NF-κBn—nuclear level of NF-κB,
IκBαt—IκBα mRNA transcript level,
A20t—A20 mRNA transcript level,
A20—cytoplasmic level of A20 protein,
IκBαn—nuclear level of IκBα.

Which are the existing data?

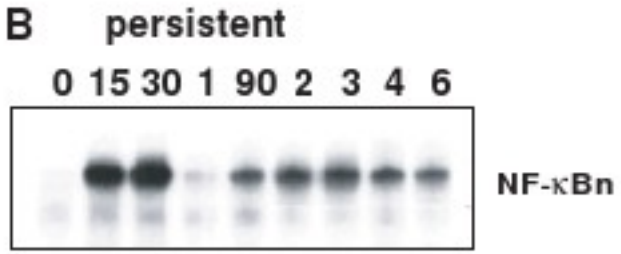


Fig. 4. Temporal control of NF-κB has qualitative effects on gene regulation. (**A**) Modeling NF-κBn concentrations in response to transient (15 min, blue) and persistent (red) stimulation in wild-type (dashed lines) and IκBα$^{-/-}$ (solid lines) cells. (**B** and **C**) Transcriptional NF-κB responses to persistent or transient stimulation with TNF-α. EMSAs (top) monitor NF-κBn after the onset of persistent (left) or transient (right) stimulation of wild-type (B) or

The IκB–NF-κB Signaling Module: Temporal Control and Selective Gene Activation
Alexander Hoffmann, Andre Levchenko, Martin L. Scott, David Baltimore

p.1244, 8 NOVEMBER 2002 VOL 298 SCIENCE www.sciencemag.org

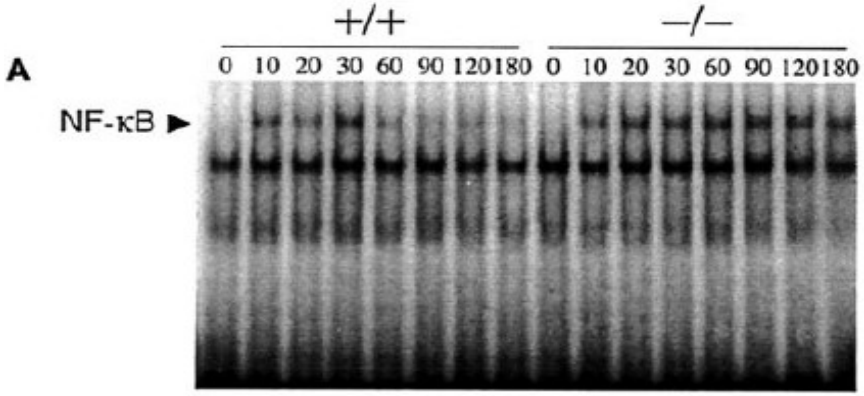


Fig. 3. Prolonged NF-κB responses to TNF in A20$^{-/-}$ MEFs. Electrophoretic mobility-shift assay (EMSA), Western, and Northern blot analyses of A20$^{+/+}$ and A20$^{-/-}$ MEFs treated repeatedly with TNF and harvested at the indicated time points. (**A**) EMSA analyses of NF-κB activity, using an NF-κB consensus oligonucleotide (SCB). (**B**)

Failure to Regulate TNF-Induced NF-κB and Cell Death Responses in A20-Deficient Mice
Eric G. Lee, David L. Boone, Sophia Chai, Shon L. Libby, Marcia Chien, James P. Lodolce, Averil Ma

www.sciencemag.org SCIENCE VOL 289 29 SEPTEMBER 2000, p.2353

Continuous model for NF-κB signaling pathway

$$\frac{d}{dt} IKKn(t) = k_{prod} - k_{deg}\, IKKn(t) - T_R k_1\, IKKn(t)$$

$$\frac{d}{dt} IKKa(t) = T_R k_1\, IKKn(t) - k_3\, IKKa(t) - T_R k_2\, IKKa(t)\, A20(t) - k_{deg}\, IKKa(t) - a_2\, IKKa(t)\, I\kappa B\alpha(t) + t_1(IKKa|I\kappa B\alpha)(t) - a_3\, IKKa(t)(I\kappa B\alpha|NF\kappa B)(t) + t_2(IKKa|I\kappa B\alpha|NF\kappa B)(t).$$

$$\frac{d}{dt} IKKi(t) = k_3\, IKKa(t) + T_R k_2\, IKKa(t)\, A20(t) - k_{deg}\, IKKi(t).$$

$$\frac{d}{dt} NF\kappa B_n(t) = i_1 k_v\, NF\kappa B(t) - a_1\, I\kappa B\alpha_n(t)\, NF\kappa B_n(t).$$

$$\frac{d}{dt} A20(t) = c_4\, A20_t(t) - c_5\, A20(t).$$

$$\frac{d}{dt} A20_t(t) = c_2 + c_1\, NF\kappa B_n(t) - c_3\, A20_t(t).$$

$$\frac{d}{dt} I\kappa B\alpha(t) = - a_2\, IKKa(t)\, I\kappa B\alpha(t) - a_1\, I\kappa B\alpha(t)\, NF\kappa B(t) + c_{4a}\, I\kappa B\alpha t(t) - c_{5a}\, I\kappa B\alpha(t) - i_{1a}\, I\kappa B\alpha(t) + e_{1a}\, I\kappa B\alpha_n(t). \quad (18)$$

$$\frac{d}{dt} I\kappa B\alpha_n(t) = - a_1\, I\kappa B\alpha_n(t)\, NF\kappa B_n(t) + i_{1a} k_v\, I\kappa B\alpha(t) - e_{1a} k_v\, I\kappa B\alpha_n(t). \quad ($$

$$\frac{d}{dt} I\kappa B\alpha_t(t) = c_{2a} + c_{1a}\, NF\kappa B_n(t) - c_{3a}\, I\kappa B\alpha_t(t).$$

$$\frac{d}{dt} (I\kappa B\alpha|NF\kappa B)(t) = a_1\, I\kappa B\alpha(t) NF\kappa B(t) - c_{6a}(I\kappa B\alpha|NF\kappa B)(t) - a_3 IKKa(t)(I\kappa B\alpha|NF\kappa B)(t) + e_{2a}(I\kappa B\alpha_n|NF\kappa B_n)(t).$$

T. Lipniacki et al. / Journal of Theoretical Biology 228 (2004) 195–215

Table 1
Two- and three-component interactions between IκBα, NF-κB and IKKa

Symbol	Values	Units	Description	Comments
a_1	0.5	$\mu M^{-1}\ s^{-1}$	IκBα−NFκB association	Hoffmann et al. (2002)
a_2	0.2	$\mu M^{-1}\ s^{-1}$	IKKa–IκBα association	Assumption
t_1	0.1	s^{-1}	IKKa|IκBα catalysis	Any large
a_3	1	$\mu M^{-1}\ s^{-1}$	IKKa-(IκBα|NF-κB) association	Assumption
t_2	0.1	s^{-1}	(IKK|IκBα|NF-κB) catalysis	Any large

Table 2
A20 and IκBα synthesis and degradation, IKK dynamics and total amount of free and complexed NF-κB

Symbol	Values	Units	Description	Comments
c_{1a}	5×10^{-7}	s^{-1}	IκBα-inducible mRNA synthesis	Assumption
c_{2a}	0.0	$\mu M\ s^{-1}$	IκBα-constitutive mRNA synthesis	Assumption
c_{3a}	0.0004	s^{-1}	IκBα mRNA degradation	Fitted, Blattner et al.
c_{4a}	0.5	s^{-1}	IκBα translation rate	Fitted
c_{5a}	0.0001	s^{-1}	Spontaneous, free IκBα protein degradation	Pando and Verma (2000)
c_{6a}	0.00002	s^{-1}	IκBα degradation (complexed to NF-κB)	Pando and Verma (2000)
c_1	5×10^{-7}	s^{-1}	A20-inducible mRNA synthesis	Assumption
c_2	0.0	$\mu M\ s^{-1}$	A20-constitutive mRNA synthesis	Assumption
c_3	0.0004	s^{-1}	A20 mRNA degradation	Assumption
c_4	0.5	s^{-1}	A20 translation rate	Assumption
c_5	0.0003	s^{-1}	A20 protein degradation	Fitted
k_1	0.0025	s^{-1}	IKK activation rate caused by TNF	Fitted
k_2	0.1	s^{-1}	IKK inactivation rate caused by A20	Fitted
k_3	0.0015	s^{-1}	IKK spontaneous inactivation rate	Fitted
k_{prod}	0.000025	$\mu M\ s^{-1}$	IKKn production rate	Fitted
k_{deg}	0.000125	s^{-1}	IKKa, IKKn and IKKi degradation	Fitted
N_F	$0.06\ V$	μM V	Total amount of free and complexed NF-κB	Assumption, Carlotti

Table 3
Transport between compartments, and assumed $k_v = V/U$, ratio of cytoplasmic and nuclear volumes

Symbol	Value	Units	Description	Comments
$k_v = V/U$	5		Cytoplasmic to nuclear volume	Assumption
i_1	0.0025	s^{-1}	NF-κB nuclear import	Fitted
e_{2a}	0.01	s^{-1}	(IκBα|NF-κB) nuclear export	Fitted
i_{1a}	0.001	s^{-1}	IκBα nuclear import	Fitted
e_{1a}	0.0005	s^{-1}	IκBα nuclear export	Assumption

Table 4
Assumed *cgen* parameters

Symbol	Value	Units	Description	Comments
c_{1c}	5×10^{-7}	s^{-1}	cgen inducible mRNA synthesis	Assumption
c_{2c}	0.0	$\mu M\ s^{-1}$	cgen constitutive mRNA synthesis	Assumption
c_{3c}	0.0004	s^{-1}	cgen mRNA degradation	Assumption

Mathematical model of NF-κB regulatory module. Tomasz Lipniacki, Pawel Paszek, Allan R. Brasier, Bruce Luxon, Marek Kimmel.

Journal of Theoretical Biology 228 (2004) 195–215

Table 2
A20 and IκBα synthesis and degradation, IKK dynamics and total amount of free and complexed NF-κB

Symbol	Values	Units	Description	Comments
c_{1a}	5×10^{-7}	s^{-1}	IκBα-inducible mRNA synthesis	Assumption
c_{2a}	0.0	$\mu M\ s^{-1}$	IκBα-constitutive mRNA synthesis	Assumption
c_{3a}	0.0004	s^{-1}	IκBα mRNA degradation	Fitted, Blattner et al.
c_{4a}	0.5	s^{-1}	IκBα translation rate	Fitted
c_{5a}	0.0001	s^{-1}	Spontaneous, free IκBα protein degradation	Pando and Verma (2000)
c_{6a}	0.00002	s^{-1}	IκBα degradation (complexed to NF-κB)	Pando and Verma (2000)
c_1	5×10^{-7}	s^{-1}	A20-inducible mRNA synthesis	Assumption
c_2	0.0	$\mu M\ s^{-1}$	A20-constitutive mRNA synthesis	Assumption
c_3	0.0004	s^{-1}	A20 mRNA degradation	Assumption
c_4	0.5	s^{-1}	A20 translation rate	Assumption
c_5	0.0003	s^{-1}	A20 protein degradation	Fitted
k_1	0.0025	s^{-1}	IKK activation rate caused by TNF	Fitted
k_2	0.1	s^{-1}	IKK inactivation rate caused by A20	Fitted
k_3	0.0015	s^{-1}	IKK spontaneous inactivation rate	Fitted
k_{prod}	0.000025	$\mu M\ s^{-1}$	IKKn production rate	Fitted
k_{deg}	0.000125	s^{-1}	IKKa, IKKn and IKKi degradation	Fitted
N_F	$0.06\ V$	μM V	Total amount of free and complexed NF-κB	Assumption, Carlotti

Integrating Computational and Biochemical Studies to Explore Mechanisms in NF-κB Signaling*

Published, JBC Papers in Press, October 20, 2008, DOI 10.1074/jbc.R800008200

Jeffrey D. Kearns and Alexander Hoffmann[1]

From the Signaling Systems Laboratory, Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California 92093

A balance must be achieved during the parameterization process to avoid *under-* or *over-constraining* the model. If too few values are experimentally determined, there may be several possible parameter sets that recapitulate network behavior, but conversely, when too many experiments are done, the model may be incapable of doing so. In these cases, the data that cannot be accounted for by the model may motivate subsequent studies and in turn result in a revised version of the model.

In the case of the NF-κB signaling module, a rich literature of biochemical rate constants derived from *in vitro* measurements and quantitative cell biology meant that one-third of the 73 parameters were known with a high degree of confidence, one-third were significantly constrained by literature data, and only the remaining third had to be derived from parameter fitting.

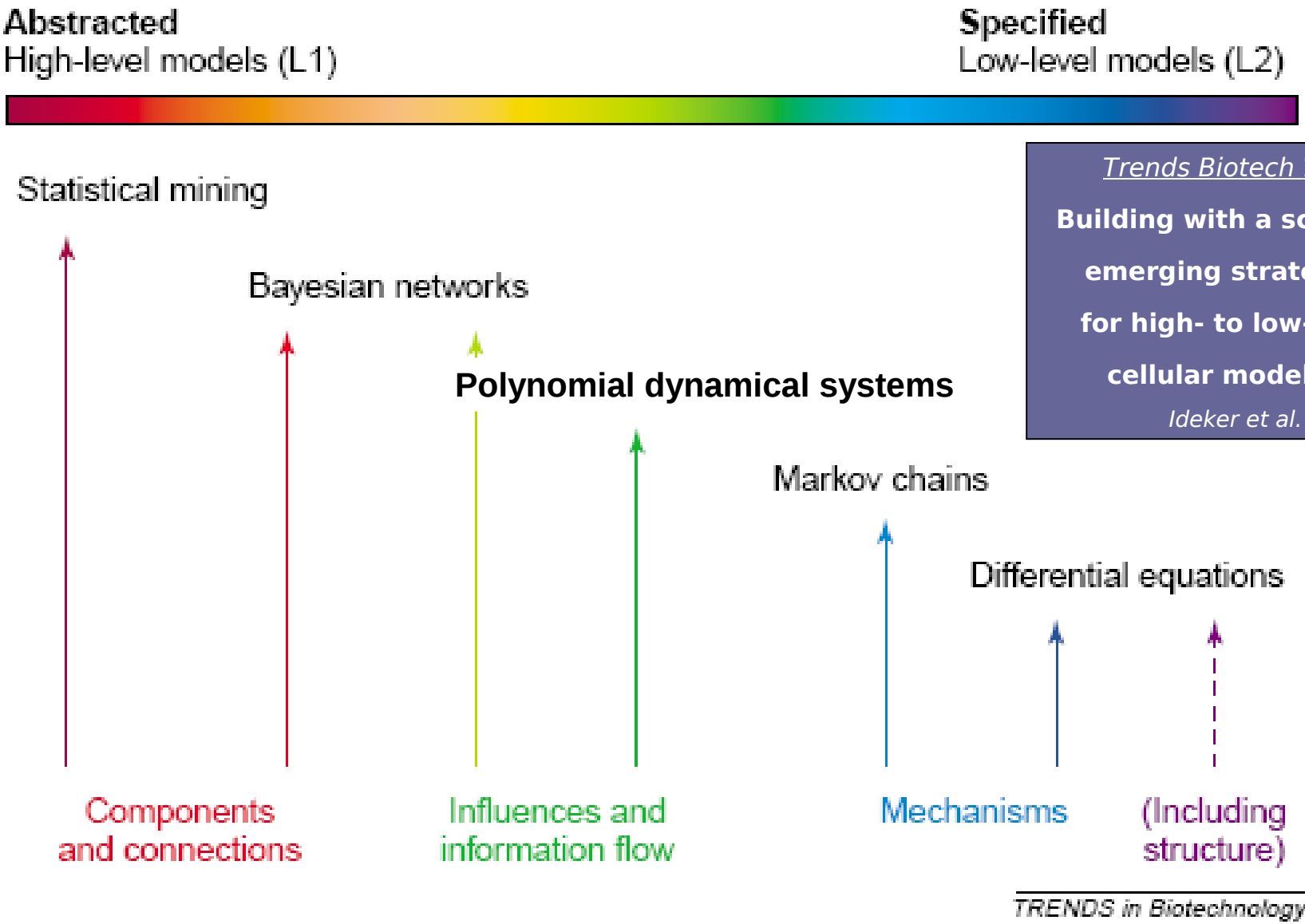
Will our model avoid fitting parameters?

No.

We will also fit parameters, but in a different context.

Which context?

Mathematical methods for modeling



Trends Biotech 2003

Building with a scaffold: emerging strategies for high- to low-level cellular modeling

Ideker et al.

More precisely: Polynomial dynamical systems over finite fields.

Which are the advantages?

Well... we also fit parameters, but over a finite set.

The model:

The model:

We discretized the data into two or three states.

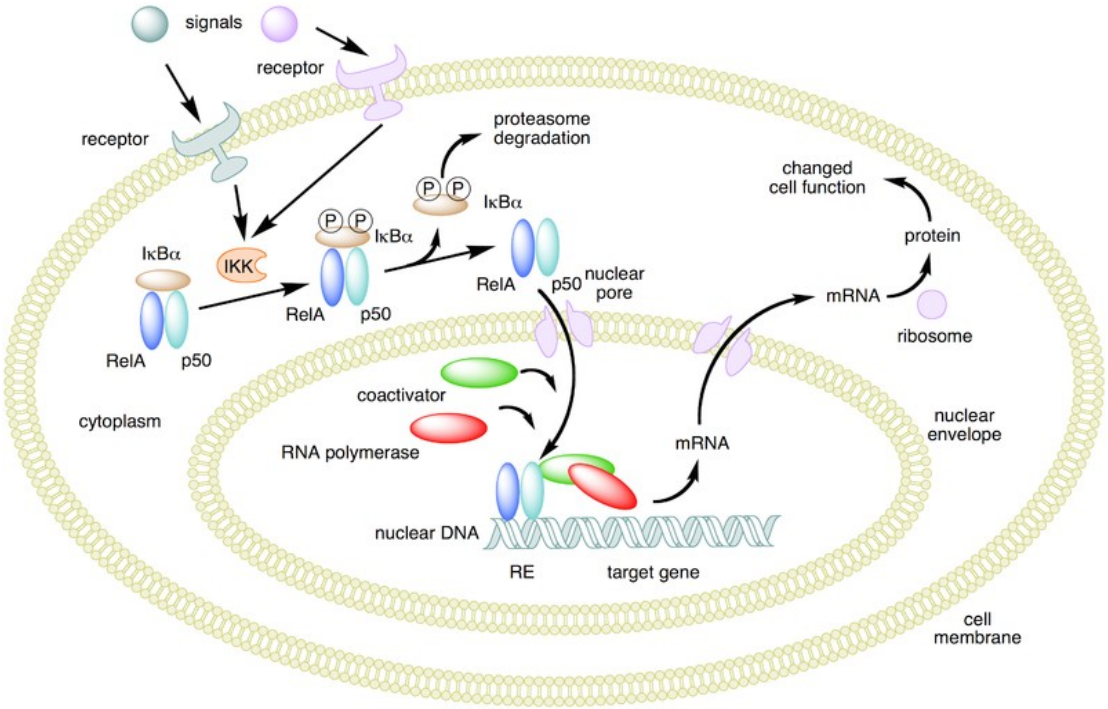
	IkB	IkB--NF-kB	IKKa	NF-kBn	IkBn	IkBt	S	A20	A20t	IKKi	IKKne
t_0	0	2	0	0	1	0	1	0	0	0	2
					↓						
t_1	0	2	2	0	1	0	1	0	0	0	1

$$f : A \subseteq \{0,1,2\}^{11} \rightarrow \{0,1,2\}^{11}$$

As $\#\{0,1,2\}^{11} = 3^{11} < \infty$, we can think $f : A \subseteq \mathbb{F}_3^{11} \to \mathbb{F}_3^{11}$, with f_i a polynomial in 11 variables for $i = 1, \ldots, 11$.

Moreover, each node depends on at most 4 other nodes. So we can think $f_i : A_i \subseteq \mathbb{F}_3^{k_i} \to \mathbb{F}_3$ for some $1 \leq k_i \leq 4$.

For instance, $I\kappa B$ only depends on itself, $IKKa$, $I\kappa Bn$ and $I\kappa Bt$.
Hence, $f_{I\kappa B} : \mathbb{F}_3^4 \to \mathbb{F}_3$.



Which are the nodes with only two levels? A20 and S.

$$Im(f_{A20}) \subseteq \{0,1\}, \quad Im(f_S) \subseteq \{0,1\}$$

How do we find the polynomials that depend on A20 or S?

Toy example:

$x \leftrightsquigarrow \{0,1\}, \quad y \leftrightsquigarrow \{0,1,2\}$

In $\mathbb{F}_3$:

$$a^3 = a \quad \forall a$$

$$0^2 = 0,\ 1^2 = 1 \Rightarrow x^2 = x$$

$$g(x,y) = \alpha_1 + \alpha_2 x + \alpha_3 y + \alpha_4 xy + \alpha_5 y^2 + \alpha_6 xy^2$$

6 unknowns, $\quad 6 = \#(\{0,1\} \times \{0,1,2\})$

How we constructed the functions:

IκB	IκBn	NF-κBn		$f_{IκBn}$(IκB; IκBn; NF-κBn)
0	0	0	→	0
1	0	0	→	1
2	0	0	→	2
0	1	0	→	1
1	1	0	→	1
2	1	0	→	2
0	2	0	→	1
1	2	0	→	2
2	2	0	→	2
0	0	1	→	0
1	0	1	→	1
2	0	1	→	2
0	1	1	→	0
1	1	1	→	1
2	1	1	→	1
0	2	1	→	1
1	2	1	→	1
2	2	1	→	2
0	0	2	→	0
1	0	2	→	1
2	0	2	→	2
0	1	2	→	0
1	1	2	→	1
2	1	2	→	2
0	2	2	→	0
1	2	2	→	1
2	2	2	→	2

```
////////////////////////////
////////////////////////////    IkBn:
////////////////////////////
//
matrix b16[27][1];
b16[1,1]=0; //the image of (IkB=0,IkBn=0,NF-kBn=0)
b16[2,1]=1; //the image of (1,0,0)
b16[3,1]=2; //the image of (2,0,0)
[...]
//q=a1+a2x+a3y+a4z+a5x^2+a6y^2+a7z^2+a8xy+a9xz+a10yz+[...]
matrix A16[27][27];
int i=1;
for(int l=3; l<=5; l=l+1)
{
for(int k=3; k<=5; k=k+1)
{
for(int j=3; j<=5; j=j+1)
{
   A16[i,1]=1; A16[i,2]=j; A16[i,3]=k; A16[i,4]=l; A16[i,5]=j^2; A16[i,6]=k^2;
   A16[i,7]=l^2; A16[i,8]=j*k; [...]
   i=i+1;
}
}
}
matrix B16=inverse(A16);
matrix C16=B16*b16;
poly q16=C16[1,1]+C16[2,1]*x+C16[3,1]*y+[...]+C16[27,1]*x^2*y^2*z^2;
poly f16=subst(q16,x,x1,y,x16,z,x15);
//
//
```

For example:

$$f_{I\kappa Bt} : \mathbb{F}_3^2 \to \mathbb{F}_3$$

$$f_{I\kappa Bt} = (1 + x_{I\kappa Bt})^2 x_{NF-\kappa Bn} + x_{I\kappa Bt}(x_{I\kappa Bt} + 2)(1 + x_{NF-\kappa Bn}^2)$$

- We consider synchronous updates.

- And make many biological assumptions.

Our results:

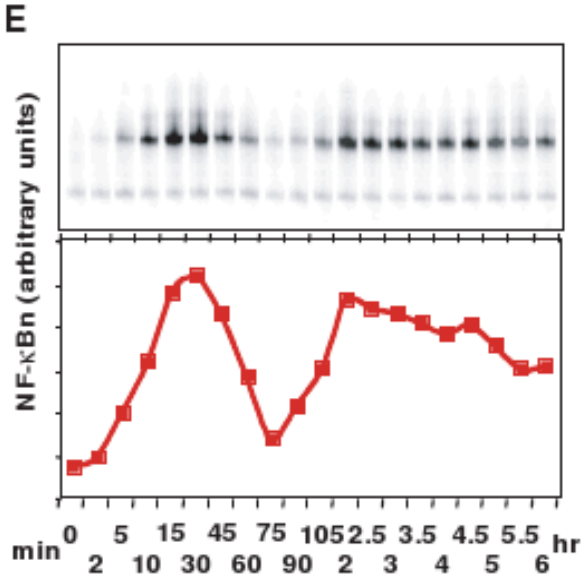
Transitions in the wild type case with persistent stimulus:

IkB	IkB--NF-kB	IKKa	NF-kBn	IkBn	IkBt	S	A20	A20t	IKKi	IKKn
0	2	0	0	1	0	1	0	0	0	2
0	2	2	0	1	0	1	0	0	0	1
0	0	2	2	1	0	1	0	0	2	1
0	1	2	2	0	2	1	0	2	2	1
0	0	2	2	0	2	1	1	2	2	1
0	0	1	2	0	2	1	1	2	2	1
2	0	1	2	0	2	1	1	2	2	1
2	0	1	2	2	2	1	1	2	2	1
2	2	1	0	2	2	1	1	2	2	1
2	1	1	1	2	1	1	1	1	2	1
1	1	1	1	2	1	1	1	1	2	1
1	1	1	1	1	1	1	1	1	2	1

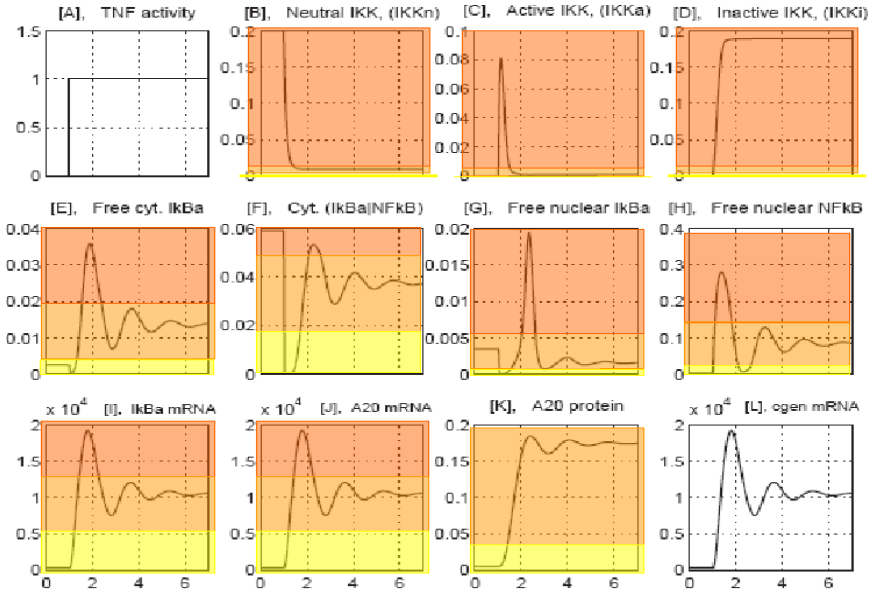
The IκB—NF-κB Signaling Module: Temporal Control and Selective Gene Activation

A. Hoffmann, A. Levchenko, M. L. Scott, D. Baltimore

SCIENCE VOL 298 8 NOVEMBER 2002, 1243



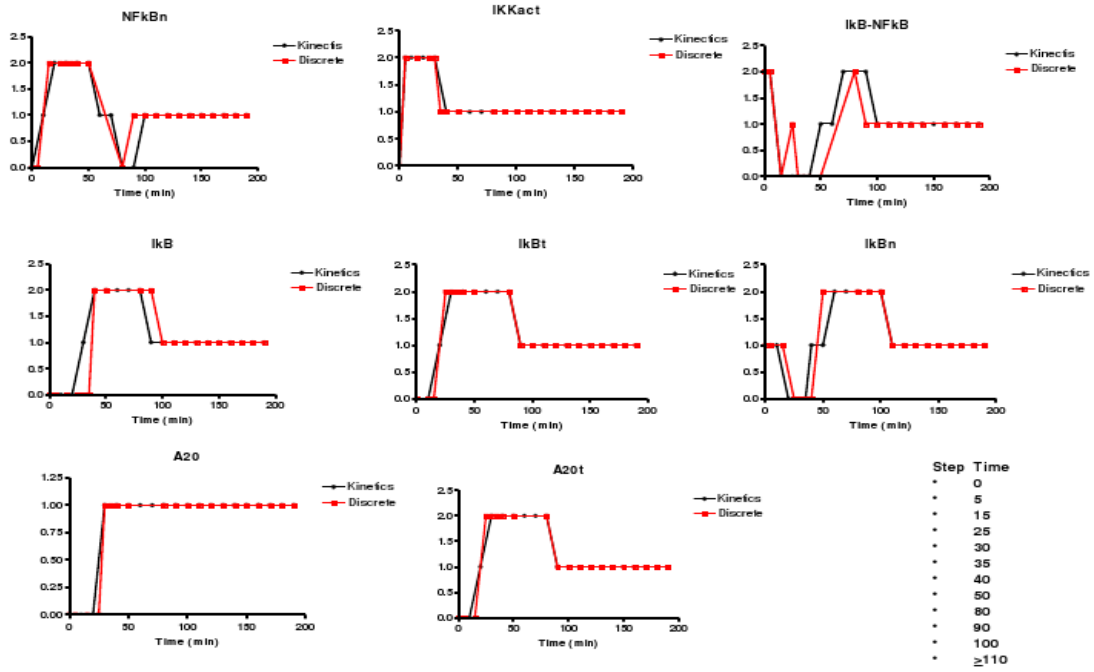
(**E**) Biochemical analysis of NF-κB and IκB isoforms in wild-type fibroblasts. NF-κBn (top) assayed by EMSA at the indicated times after persistent stimulation with TNF-α. The specific NF-κB–specific mobility shift was quantitated by phosphoimager and normalized and graphed at the indicated nonlinear time scale. Western blots of corresponding cytoplasmic fractions are probed with anti-bodies specific to IκBα and -β (bottom) and IκBε (above). (**F**) Verifica-

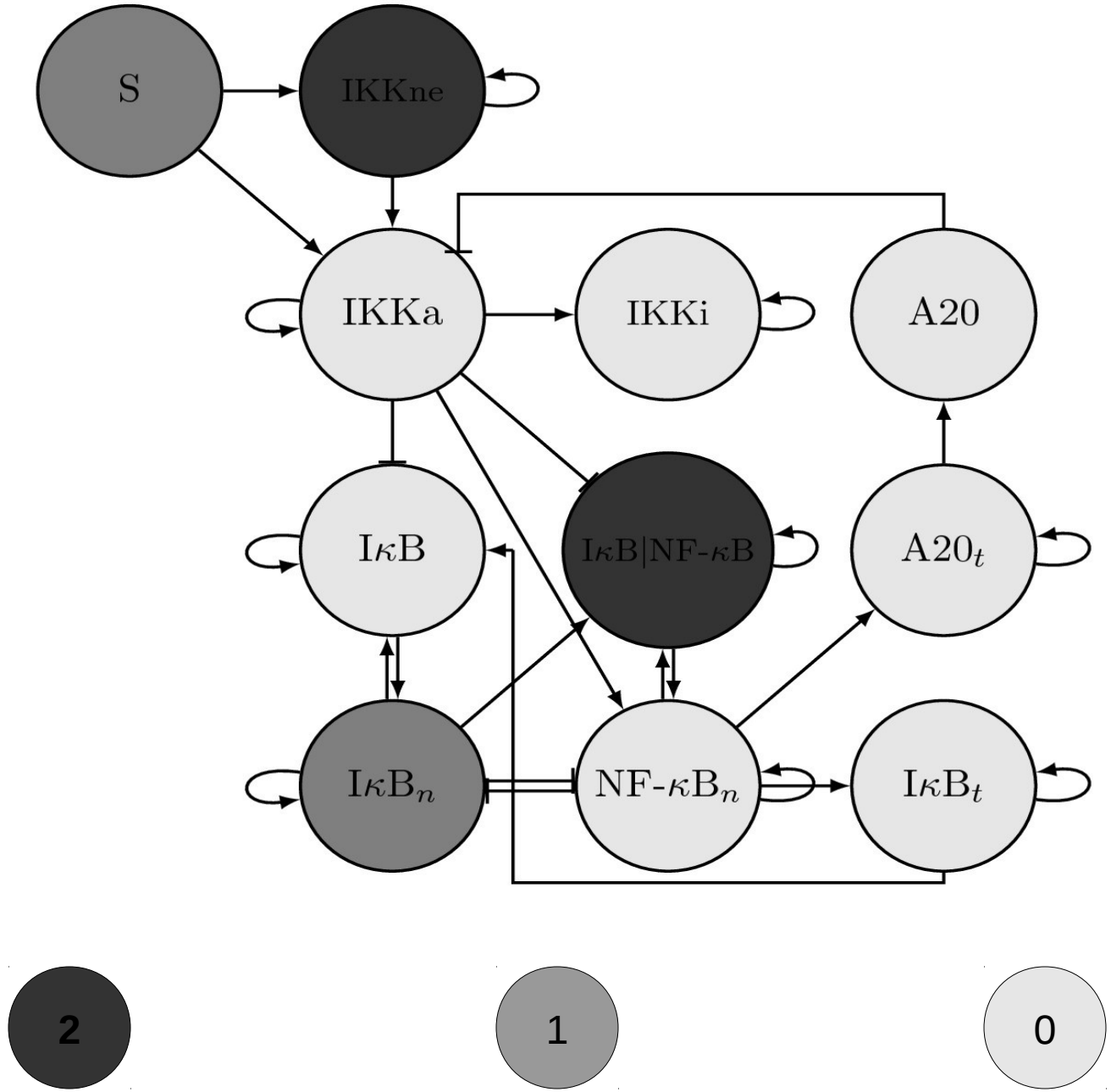


Mathematical model of NF-κB regulatory module

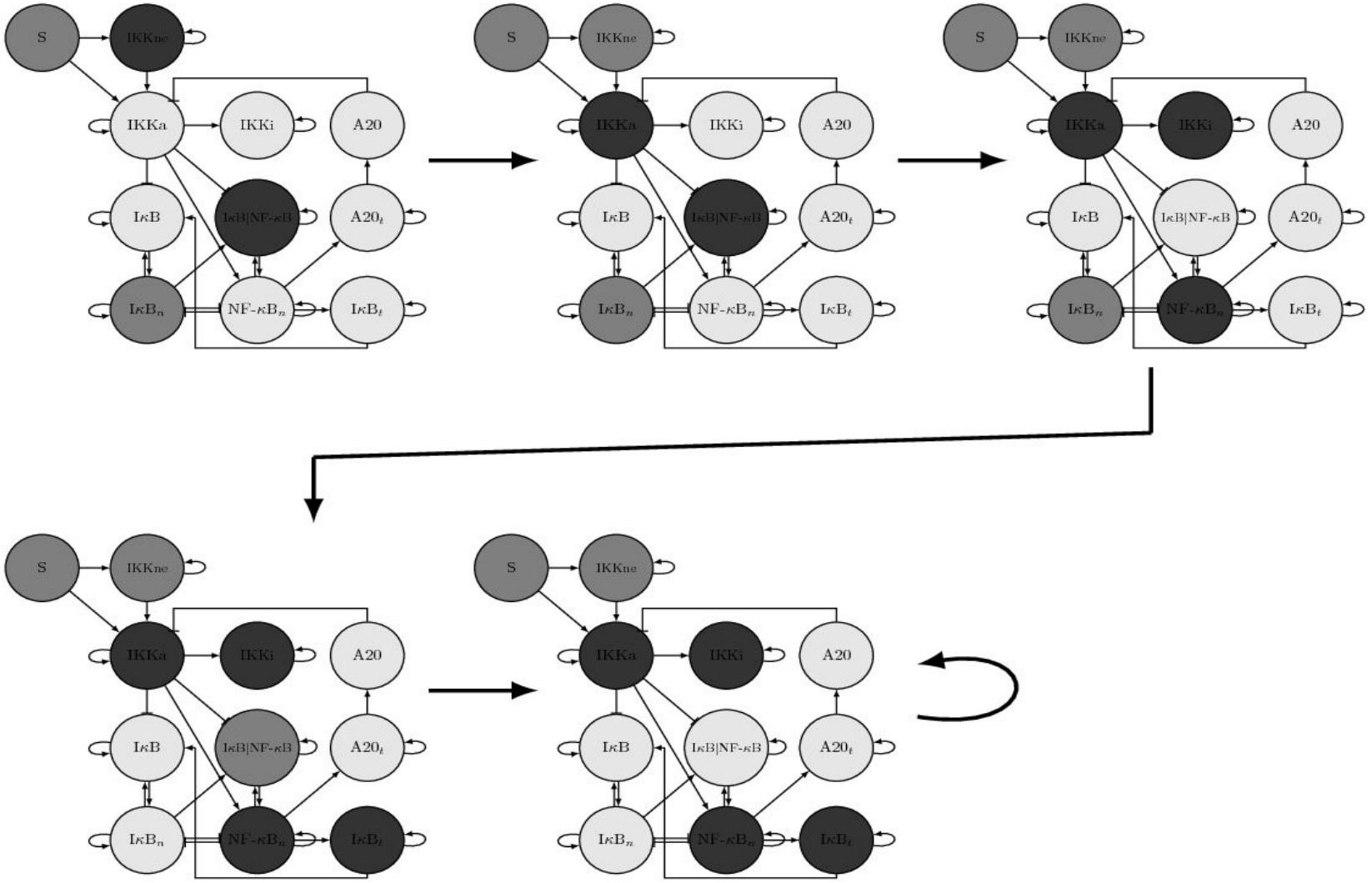
T. Lipniacki, P. Paszek, A. R. Brasier, B. Luxon, M. Kimmel.

Journal of Theoretical Biology 228 (2004) 195–215




S
IKKne
IKKa
IKKi
A20
IκB
IκB|NF-κB
A20$_t$
IκB$_n$
NF-κB$_n$
IκB$_t$
2
1
0

Evolution of the A20 knock-out system:



Failure to Regulate TNF-Induced NF-κB and Cell Death Responses in A20-Deficient Mice

E. G. Lee, D. L. Boone, S. Chai, S. L. Libby, M. Chien, J. P. Lodolce, A. Ma

SCIENCE VOL 289 29 SEPTEMBER 2000, p.2353

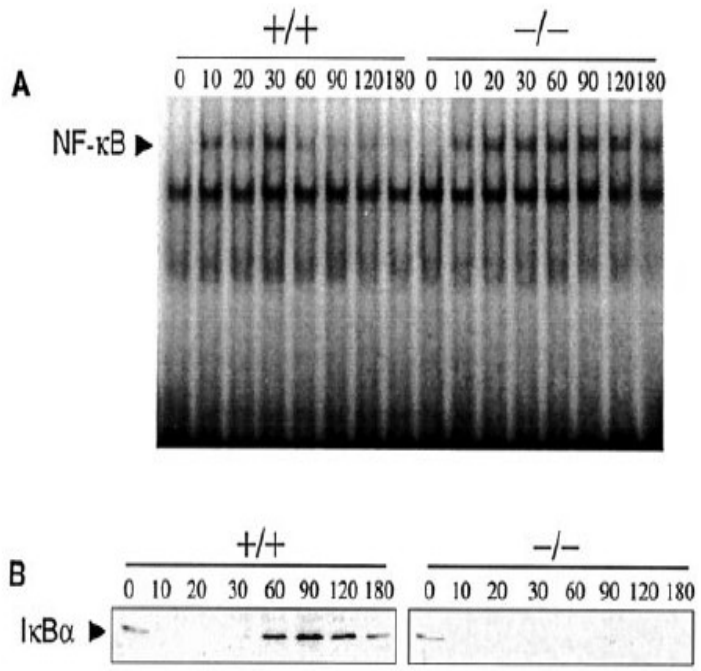
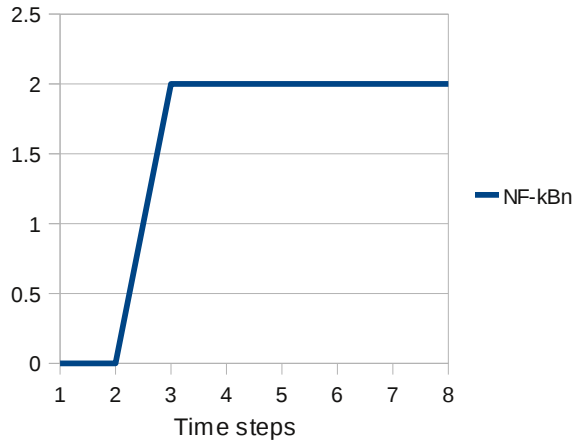
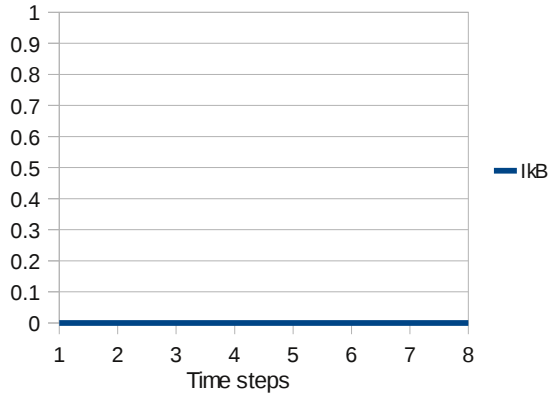


Fig. 3. Prolonged NF-κB responses to TNF in $A20^{-/-}$ MEFs. Electrophoretic mobility-shift assay (EMSA), Western, and Northern blot analyses of $A20^{+/+}$ and $A20^{-/-}$ MEFs treated repeatedly with TNF and harvested at the indicated time points. (**A**) EMSA analyses of NF-κB activity, using an NF-κB consensus oligonucleotide (SCB). (**B**) Western blot analysis of IκBα expression. (**C**) Northern blot analyses of IκBα and glyceraldehyde phosphate de-

A20 -/-, our result



A20 -/-, our result



Mathematical model of NF-κB regulatory module

T. Lipniacki, P. Paszek, A. R. Brasier, B. Luxon, M. Kimmel.

Journal of Theoretical Biology 228 (2004) 195–215

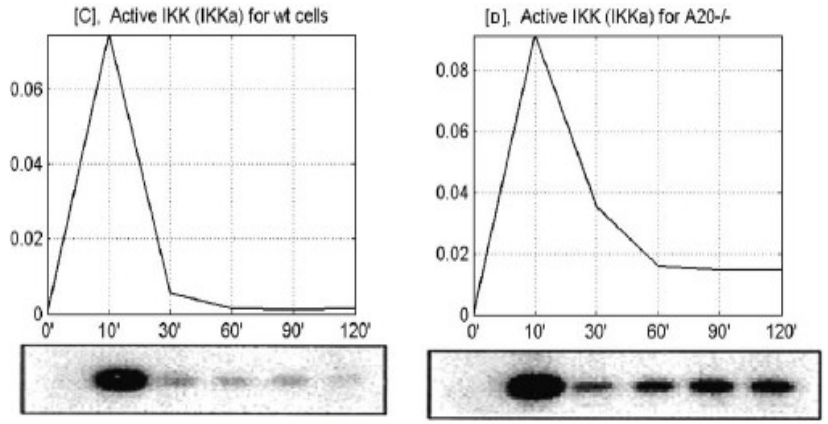
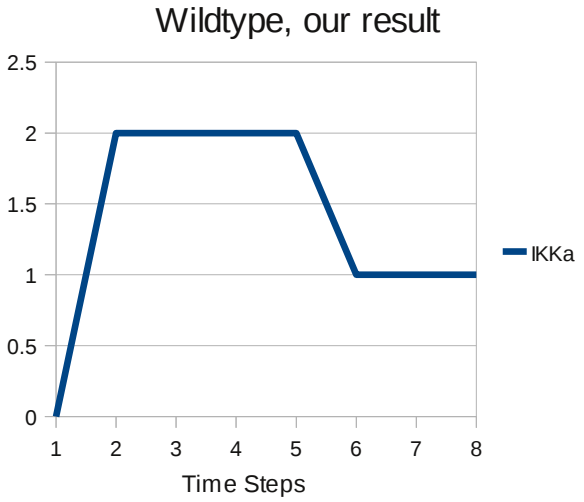
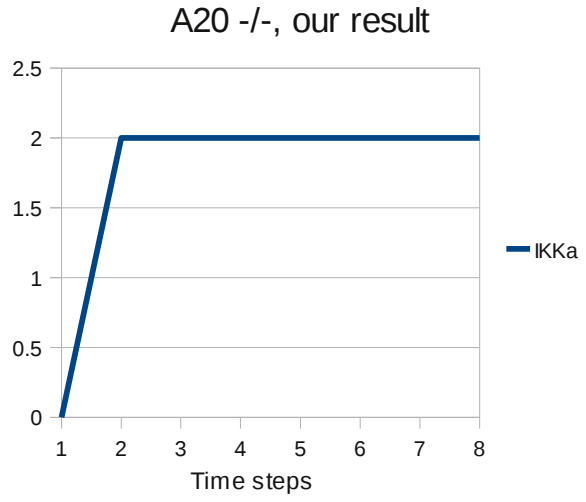
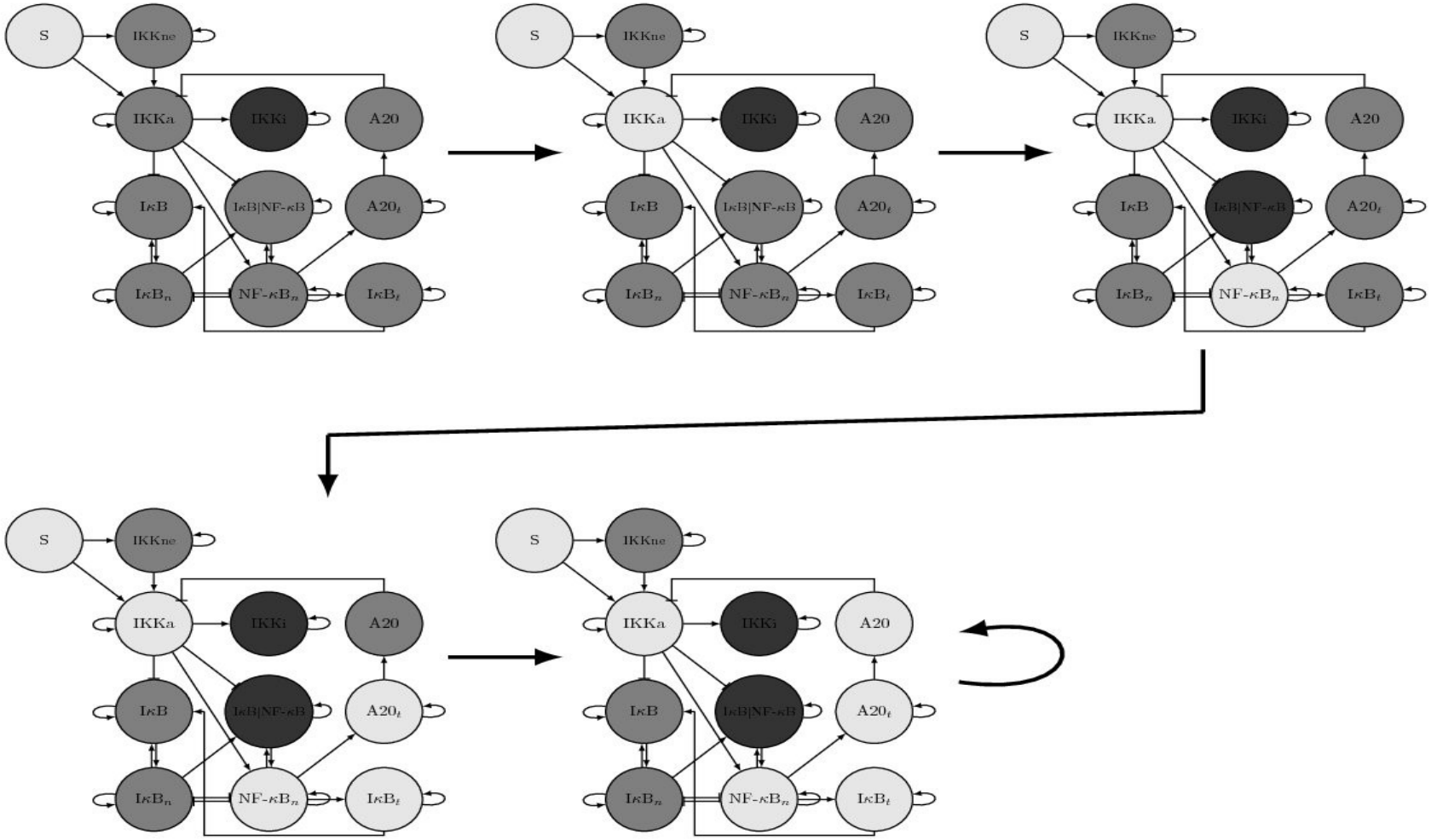


Fig. 6. Model predictions versus measurements by Lee et al. (2000) on wild-type cells and A20 deficient cells with persistent TNF stimulation. The time coordinate is rescaled, and the values of corresponding functions are calculated based on the fitted parameters, at the same time points as in the experiment. The concentrations are given in μM. (A) A20 mRNA for wild-type cells. (B) Model-predicted total $IKK = IKKn + IKKa + IKKi$ versus experiment, wild type in upper panel, and A20−/− in lower panel. According to the model total IKK remains constant. (C and D) Model-predicted IKKa versus IKK kinase activity, respectively for wild-type and A20−/− cells.





Evolution of the system when the stimulus is removed at steady state:



The evolution of the system when IKKa is inhibited at the steady state:

IkB	IkB--NF-kB	IKKa	NF-kBn	IkBn	IkBt	S	A20	A20t	IKKi	IKKne
1	1	0	1	1	1	1	1	1	2	1
1	2	0	0	1	1	1	1	1	2	1
1	2	0	0	1	0	1	1	0	2	1
1	2	0	0	1	0	1	0	0	2	1

The evolution of the system when the stimulus is removed at a certain time step:

The first time step, with S=0.

IkB	IkB--NF-kB	IKKa	NF-kBn	IkBn	IkBt	S	A20	A20t	IKKi	IKKne
0	2	0	0	1	0	0	0	0	0	2

The second time step, with S=0.

IkB	IkB--NF-kB	IKKa	NF-kBn	IkBn	IkBt	S	A20	A20t	IKKi	IKKne
0	2	2	0	1	0	0	0	0	0	1
0	0	2	2	1	0	0	0	0	2	1
0	1	2	2	0	2	0	0	2	2	1
0	0	2	2	0	2	0	1	2	2	1
0	0	1	2	0	2	0	1	2	2	1
2	0	0	2	0	2	0	1	2	2	1
2	0	0	2	2	2	0	1	2	2	1
2	2	0	0	2	2	0	1	2	2	1
2	2	0	0	2	1	0	1	1	2	1
2	2	0	0	2	0	0	1	0	2	1
2	2	0	0	2	0	0	0	0	2	1

The evolution of the system when the stimulus is removed at the steady state, in the A20 knockout case:

IkB	IkB--NF-kB	IKKa	NF-kBn	IkBn	IkBt	S	A20	A20t	IKKi	IKKne
0	0	2	2	0	2	0	0	0	2	1

Model advantages

• This model does not depend on affinity and catalytic constants, which are usually difficult to determine and require a deep understanding of the system (which, except for the NF-κB regulatory module and some other special cases, is rare).

Model advantages

- This model can be built with the information biologists normally handle: IκB binds to NF-κB and prevents it from entering the nucleus, IKKa induces IκB degradation, etc.

Model advantages

- And despite the fact that it simply requires this kind of data, it can render even more information, such as predictions in mutant cases.

What else we should do...

- Study more cases (other mutants, over-activations, etc.)
- Study the asynchronous case.
- Improve the code.
- ...


Thanks!
Finland
Iceland
Sverige
Sweden
Norge
Norway
Россия
Russia
Canada
United Kingdom
Polska
Poland
Deutschland
Germany
Україна
Ukraine
Kazakhstan
Mongolia
France
Italia
Italy
España
Spain
Türkiye
Turkey
United States
North Atlantic Ocean
Iraq
Afghanistan
Pakistan
China
대한민국
S Korea
日本
Japan
Algeria
Libya
Saudi Arabia
India
México
Mauritania
Mali
Niger
Chad
Thailand
Venezuela
Colombia
Indonesia
Papua
Perú
Bolivia
Namibia
Botswana
Madagascar
Indian Ocean
Australia
South Pacific Ocean
Chile
South Atlantic Ocean
South Africa
Argentina
A
B
©2010 Google - Datos de mapa ©2010 AND, Geocentre Consulting, MapLink, Tele Atlas, MapData Sciences Pty Ltd, PSMA - Términos de uso