Engineering Molecular Cell Biology
Lecture 25, Fall 2010

Literature Reading

Quantitative Analysis and Modeling of Gene Expression and Cell Signaling

Final Exam Presentation Format (I)

- Each presentation should include three sections
  - Background
  - Data presentation
  - Critical review
- Time allocation
  - Background section: no more than 15 minutes
  - Data presentation: ~ 45-60 minutes
  - Critical review section: no more than 10 minutes

Final Exam Presentation Format (II)

- Organization

  - For each group, generally one student → one section

  - Background section should be brief;
    Give details but be selective

  - Data presentation should include a slide summarizing main messages
    All figures in the main text must be covered

  - Critical review can accompany data presentation

  - Review section may include
    Whether the data and methods are sound
    Whether the logic development is sound
    Limitations, white space
    Writing style

Final Exam Presentation Format (III)

- Each presentation will be graded based on

  - Accuracy, clarity, logic, & completeness of presentation of all sections

  - Quality of slides (as the final report); Give proper citations

- For each group, the presentation PPT file will serve as the final report.

- Students not presenting should submit a one-page report that consists of two sections

  →Section I: critical comments on the paper
  →Section II: your questions

Literature Reading

1. Elowitz MB, Levine AJ, Siggia ED, Swain PS. Stochastic gene expression in a single cell. *Science, 297:1183-1186 (2002).*

2. Hoffmann, A., Levchenko, A., Scott, M.L. and Baltimore, D. The IκB-NF-κB signaling module: temporal control and selective gene activation. *Science, 298: 1241*-1245 (2002).

Stochastic Gene Expression in a Single Cell

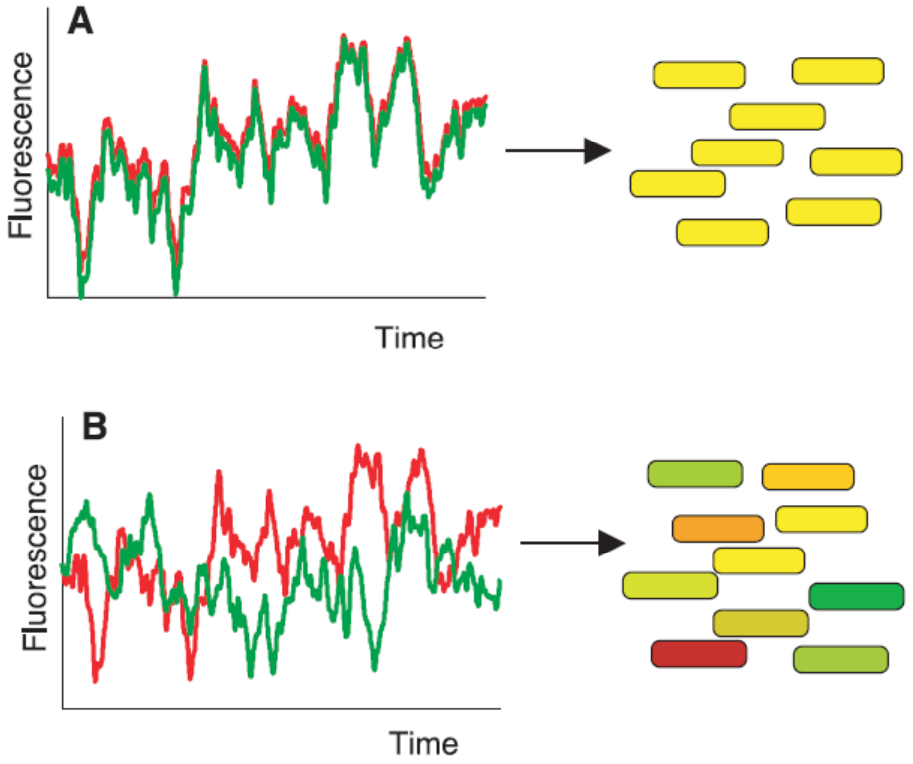
Michael B. Elowitz,[1,2]* Arnold J. Levine,[1] Eric D. Siggia,[2]
Peter S. Swain[2]

Main Messages

- Gene expression in a single bacteria cell exhibits both intrinsic and extrinsic noise.
- A method is developed to characterize intrinsic and extrinsic noise in gene expression.
- Intrinsic noise increases monotonically as the number of transcripts decreases.
- Extrinsic noise exhibits piecewise monotonic changes with a maximum as the number of transcript increases.

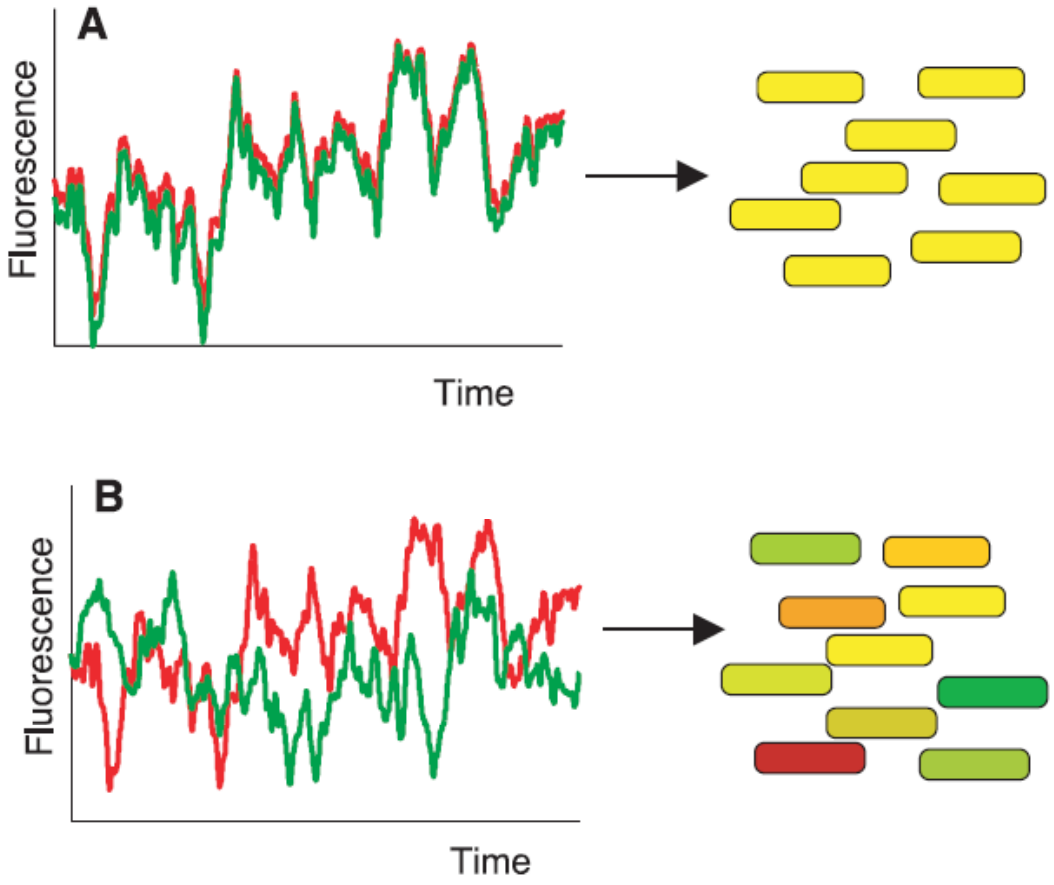
Stochastic Gene Expression: Fig. 1

- Differentiation and measurement of intrinsic and extrinsic noise.

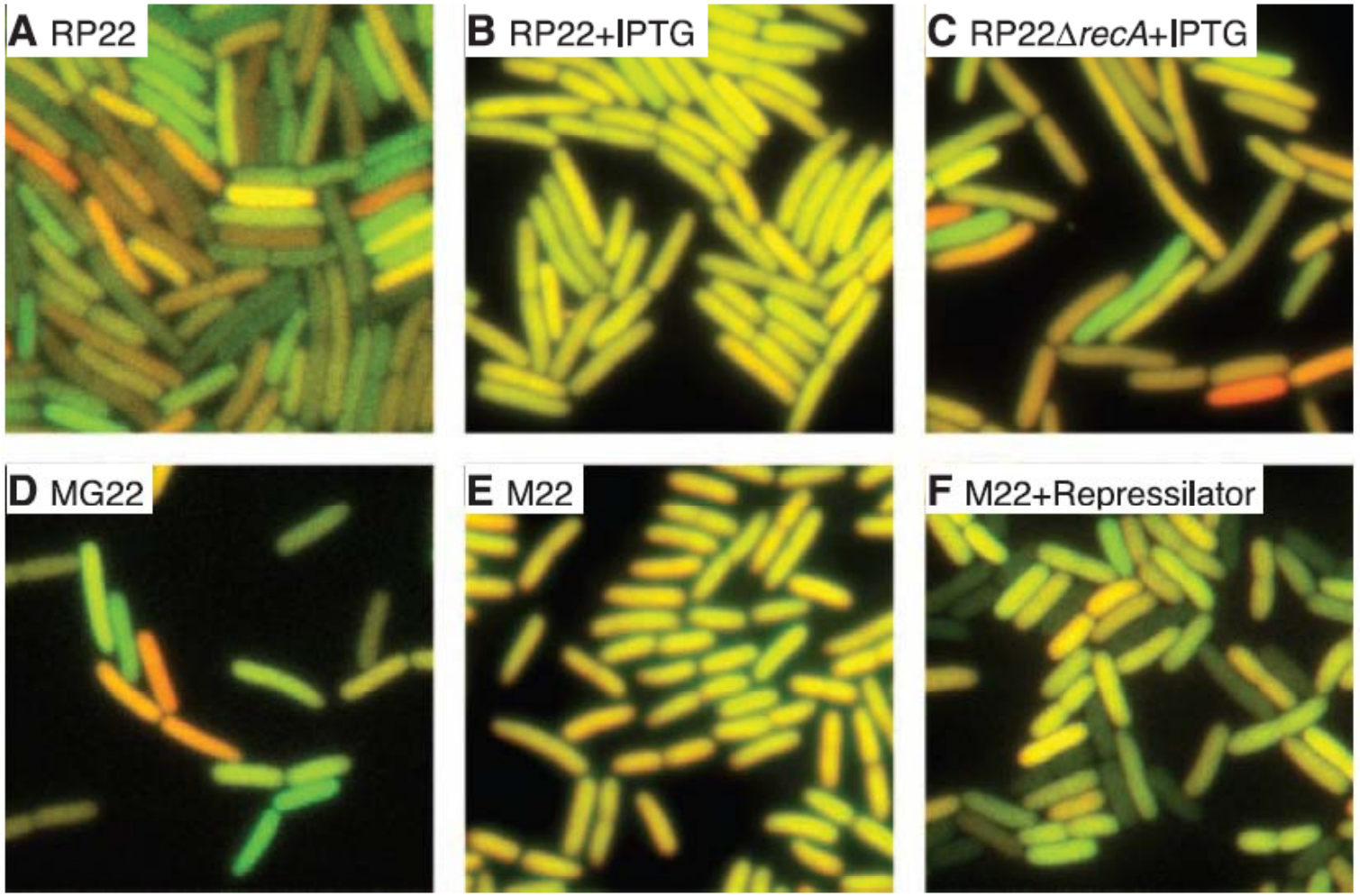


$$\eta_{int}^2 \equiv \frac{\langle (c-y)^2 \rangle}{2\langle c \rangle \langle y \rangle}; \qquad \eta_{ext}^2 \equiv \frac{\langle cy \rangle - \langle c \rangle \langle y \rangle}{\langle c \rangle \langle y \rangle} \qquad \eta_{tot}^2 \equiv \frac{\langle c^2 + y^2 \rangle - 2\langle c \rangle \langle y \rangle}{2\langle c \rangle \langle y \rangle}$$

Stochastic Gene Expression: Fig. 1



Stochastic Gene Expression: Fig. 2



Stochastic Gene Expression: Fig. 3

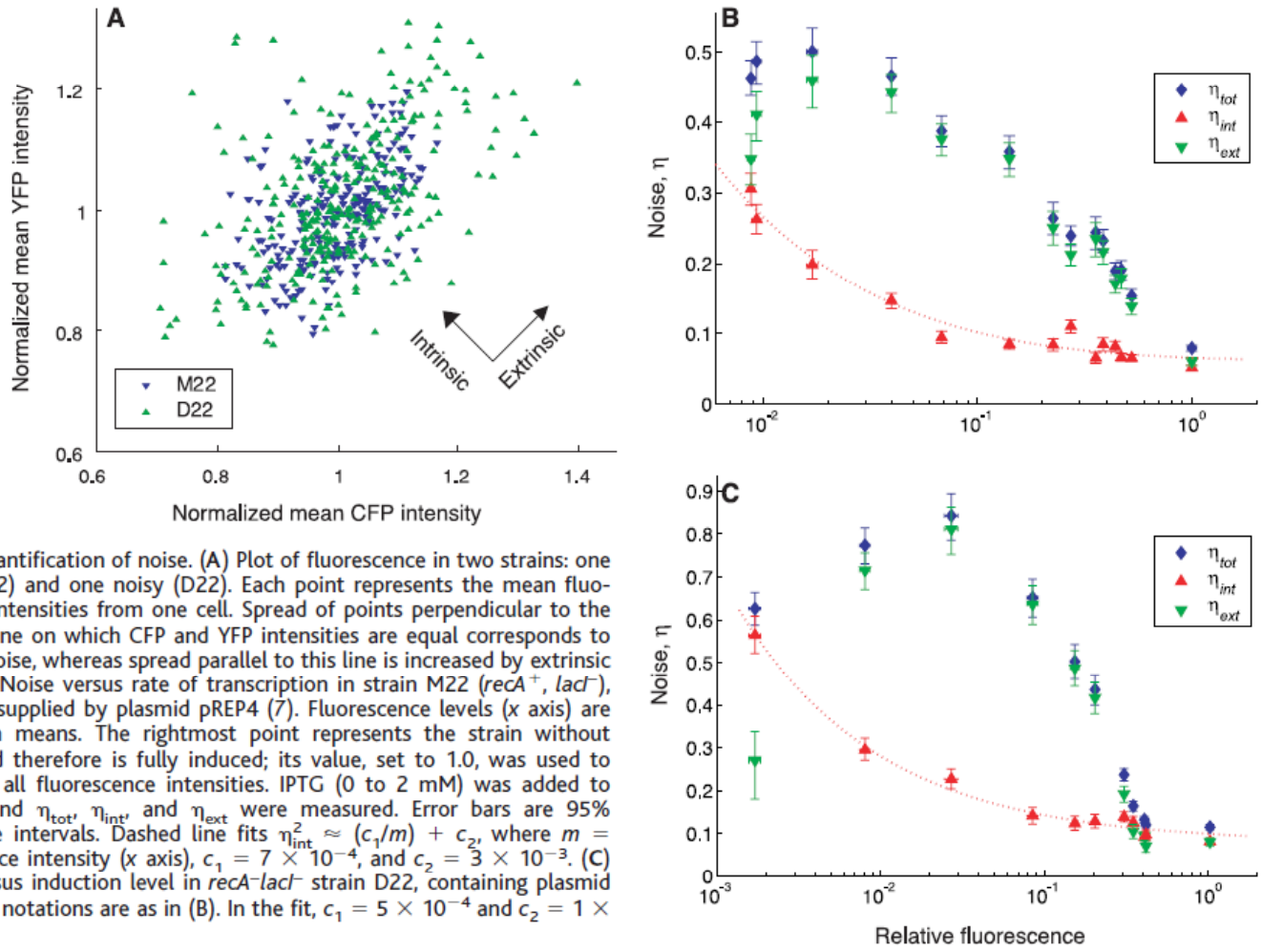


Fig. 3. Quantification of noise. (**A**) Plot of fluorescence in two strains: one quiet (M22) and one noisy (D22). Each point represents the mean fluorescence intensities from one cell. Spread of points perpendicular to the diagonal line on which CFP and YFP intensities are equal corresponds to intrinsic noise, whereas spread parallel to this line is increased by extrinsic noise. (**B**) Noise versus rate of transcription in strain M22 (*recA*$^+$, *lacI*$^-$), with LacI supplied by plasmid pREP4 (*7*). Fluorescence levels (*x* axis) are population means. The rightmost point represents the strain without pREP4 and therefore is fully induced; its value, set to 1.0, was used to normalize all fluorescence intensities. IPTG (0 to 2 mM) was added to cultures and η_{tot}, η_{int}, and η_{ext} were measured. Error bars are 95% confidence intervals. Dashed line fits $\eta^2_{int} \approx (c_1/m) + c_2$, where m = fluorescence intensity (*x* axis), $c_1 = 7 \times 10^{-4}$, and $c_2 = 3 \times 10^{-3}$. (**C**) Noise versus induction level in *recA*$^-$*lacI*$^-$ strain D22, containing plasmid pREP4. All notations are as in (B). In the fit, $c_1 = 5 \times 10^{-4}$ and $c_2 = 1 \times 10^{-2}$.

Stochastic Gene Expression: Table 1

Table 1. Measurements of noise in selected strains.

Modification*	Strain†	Intensity‡	Intrinsic noise, η_{int}§¶ ($\times 10^{-2}$)	Extrinsic noise, η_{ext}§ ($\times 10^{-2}$)	Total noise, η_{tot}§ ($\times 10^{-2}$)
Constitutive (*lacI*$^-$)	M22	1	5.5 (5.1–6)	5.4 (4.8–5.9)	7.7 (7.4–8.1)
	JM22	0.88	5.0 (4.6–5.4)	6.1 (5.5–6.7)	7.9 (7.4–8.4)
	MRR	1.21	5.1 (4.7–5.4)	5.6 (5.1–6.2)	7.6 (7.2–7.9)
Wild type (*lacI*$^+$)	MG22	0.057	19 (18–21)	32 (29–35)	37 (35–40)
	RP22	0.030	25 (22–27)	33 (30–35)	41 (39–43)
Wild type (*LacI*$^+$), +IPTG	RP22	1.00	6.3 (5.8–6.9)	9.8 (9.0–11)	11.7 (11–12.3)
lacI$^-$, Repressilator	M22	0.18	12 (11–13)	42 (37–45)	43 (39–47)
	MRR	0.16	11 (9.8–12)	57 (52–62)	58 (53–63)
Δ*recA*, *lacI*$^-$	D22	0.81	10.5 (9.6–11.4)	4.6 (2.8–5.8)	11.4 (10.8–12.1)
	M22ΔA	0.99	13 (12–15)	2.4 (0–5.3)	13.6 (12.8–14.5)
	JM22ΔA	0.92	14 (11–17)	2.5 (0–7.3)	15 (12–16.4)
Δ*recA*, *lacI*$^+$ +IPTG	RP22ΔA	1.22	17 (15–20)	12 (8.8–14)	21 (20–22)

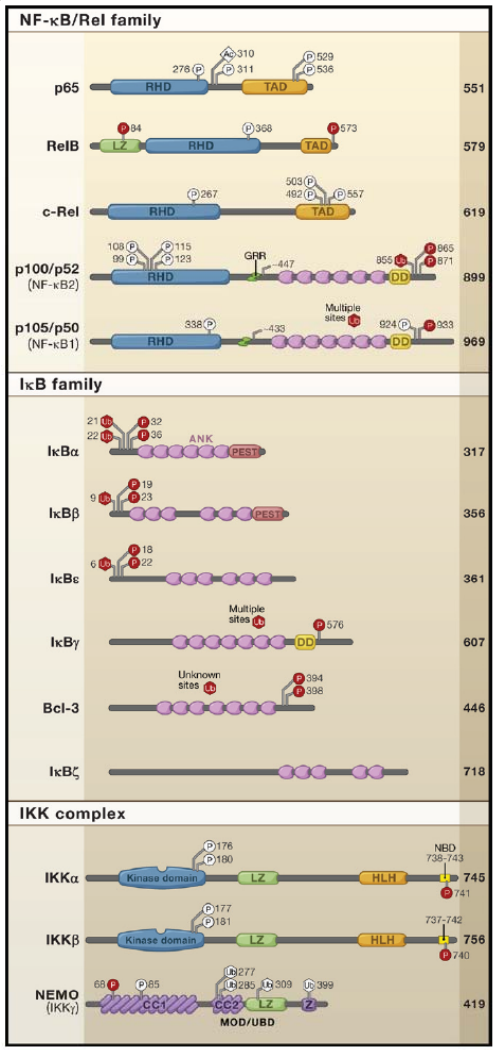
*Repressilator refers to Spect^R version of plasmid in (*16*); +IPTG indicates growth in the presence of 2 mM IPTG. †The following strain backgrounds were used: MC4100 (*22*) for M22, MRR, and M22ΔA; DY331 (*23*) for D22; JM2.300 (*E. coli* Genetic Stock Center) for JM22 and JM22ΔA; MG1655 for MG22; and RP437 (*24*) for RP22 and RP22ΔA. Each strain contains twin $P_L$lacO1 promoters (*9*), except MRR, which contains twin λP_R promoters (*25*). ‡Mean CFP value, relative to the intensity of strain M22. §95% confidence limits are in parentheses; see (*7*). ¶CFP and YFP are stable in *E. coli* (*26*); effective noise levels for unstable proteins would be greater (for example, a doubling of noise level for a protein half-life of ~0.3 cell cycle) (*8*).

Questions

- Is gene expression the sole source of cell-cell variation?

NF-kB Pathway (I)

- NF-kBs are transcription regulatory proteins.
- NF-kB are central to many stressful, inflammatory, and immune responses and to animal development.
- Misregulation of NF-kB leads to chronic inflammatory diseases and cancer.
- Most elements of NF-kB signaling pathway have been mapped.

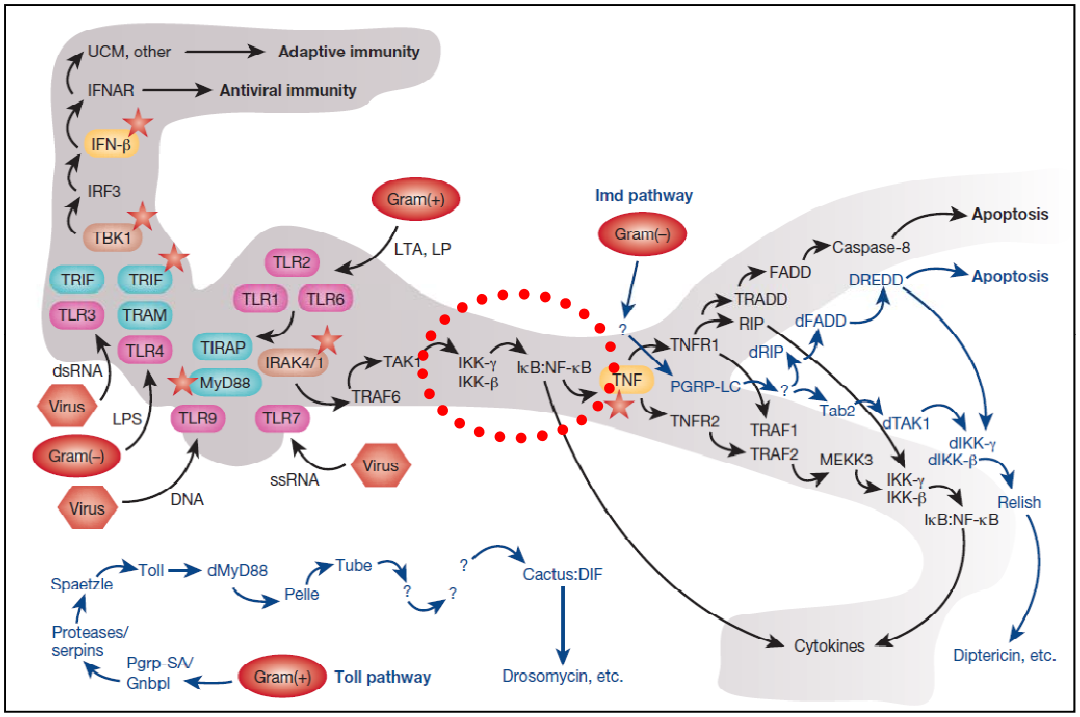


Hayden & Ghosh, *Cell*, 132:344, 2008.

NF-kB Pathway (II)

- NF-kB signaling pathway can be activated by many receptors
  - Toll-like receptors
  - TNF receptors
  - Cytokine receptors

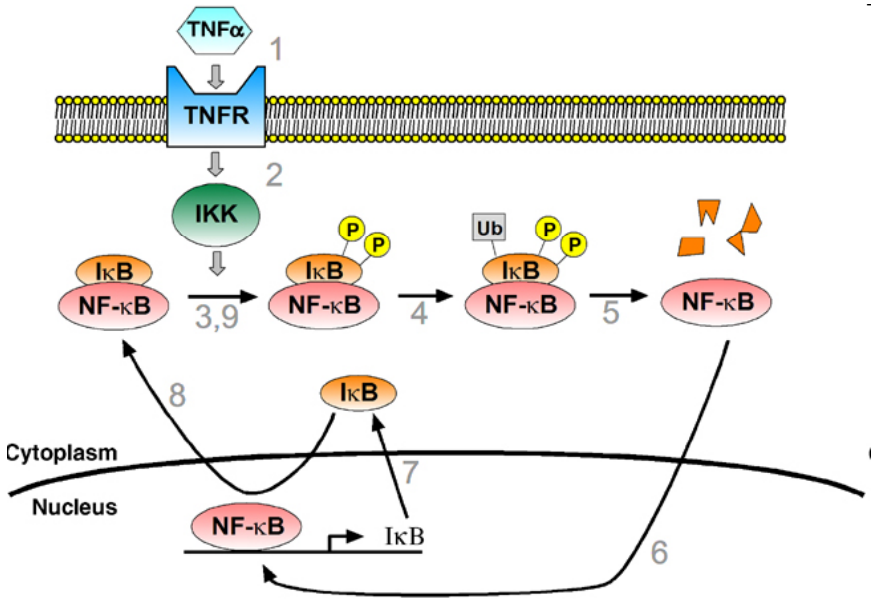
- Released NF-kB translocates into the nucleus and turns on the transcription of hundreds of genes related to stressful, inflammatory and immune responses.



Beutler, *Nature*, 430:257, 2004

NF-kB Signaling

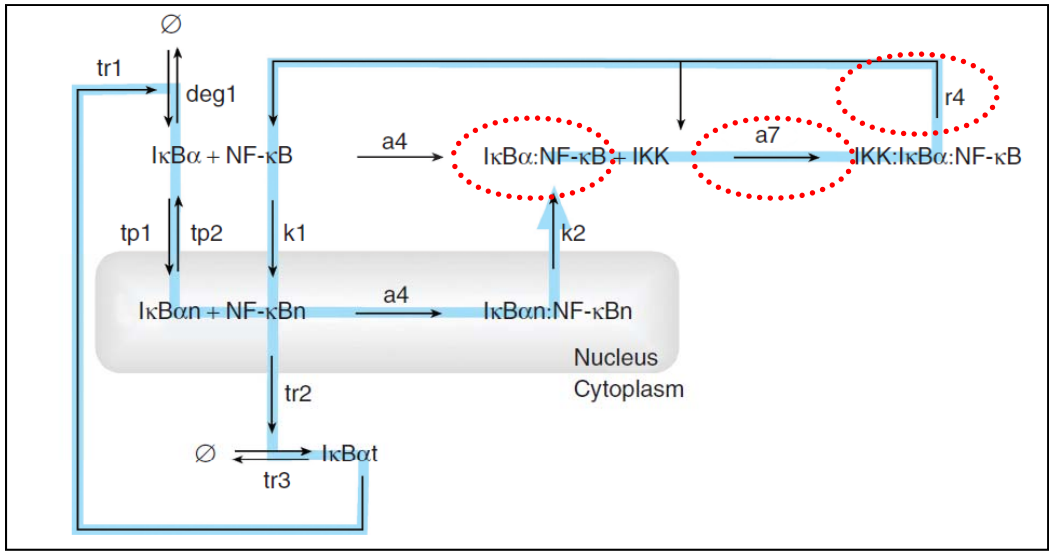
- Binding of IkB to NF-kB keeps NF-kB inactive.
- Phosphorylation of IkB by IKK triggers the degradation of IkB.
- Three isoforms of IkB
  - IkBα, IkBβ, IkBε
- Different isoforms of IkB play different functional roles.
- Triggered expression of IkB forms a negative feedback loop.



Cheong et al, *Mol. Sys. Biol.*, 4:192, 2008.

Modeling of NF-kB Signaling Using ODEs

- Some reactions are omitted.
- Phosphorylation, ubiquitination, and proteasomal degradation are lumped into one reaction.
- Input: a step increase in IKK
- Initiation strategy



$$\frac{d[\text{I}\kappa\text{B}\alpha : \text{NF-}\kappa\text{B}]}{dt} = a4[\text{I}\kappa\text{B}\alpha][\text{NF-}\kappa\text{B}] + k2[\text{I}\kappa\text{B}\alpha n : \text{NF-}\kappa\text{B}n] - a7[\text{I}\kappa\text{B}\alpha : \text{NF-}\kappa\text{B}][\text{IKK}]$$

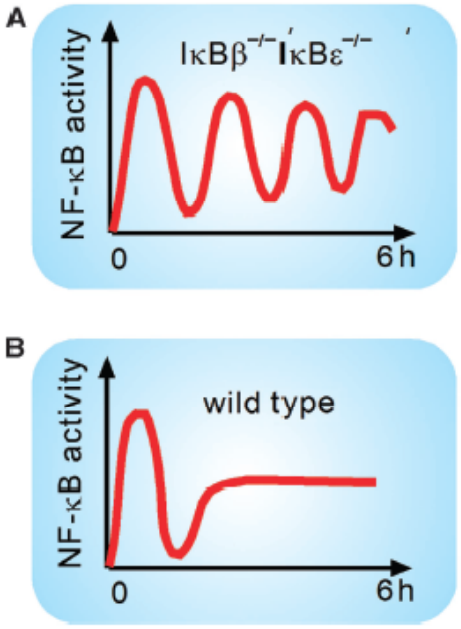
Cheong et al, *Mol. Sys. Biol.*, 4:192, 2008.

Main Results of Modeling Analysis (I)

- Initial model (Hoffmann et al; 2002) focuses on IkBα
- 34 parameters; 45 equations
- Main results
  - Different IkBs induce different reactions.
    - IkBα provides negative feedback and induces oscillation
    - IkBβ and IkBε dampens oscillation
  - Temporal responses
    - Short stimuli induce a short phase of NF-kB response
    - Long stimuli induces proportionally longer responses.
  - Differential activation of genes

Main Results of Modeling Analysis (II)

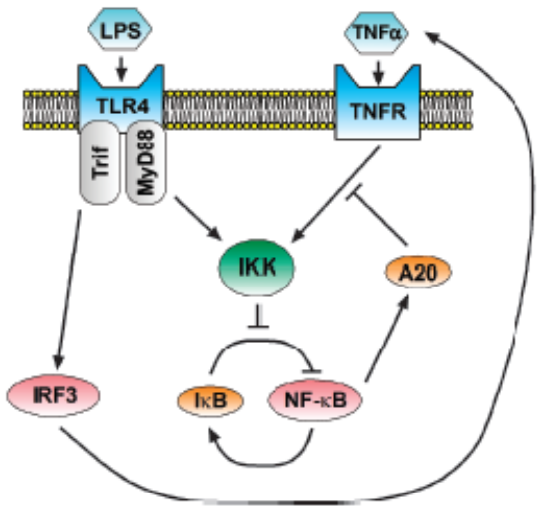
- Go to Biomodels
  http://www.ebi.ac.uk/biomodels-main/
- Model record
  http://www.ebi.ac.uk/biomodels-main/BIOMD0000000140
- SBML: system biology markup language



Cheong et al, *Mol. Sys. Biol.*, 4:192, 2008.

Extension of the Original Model (I)

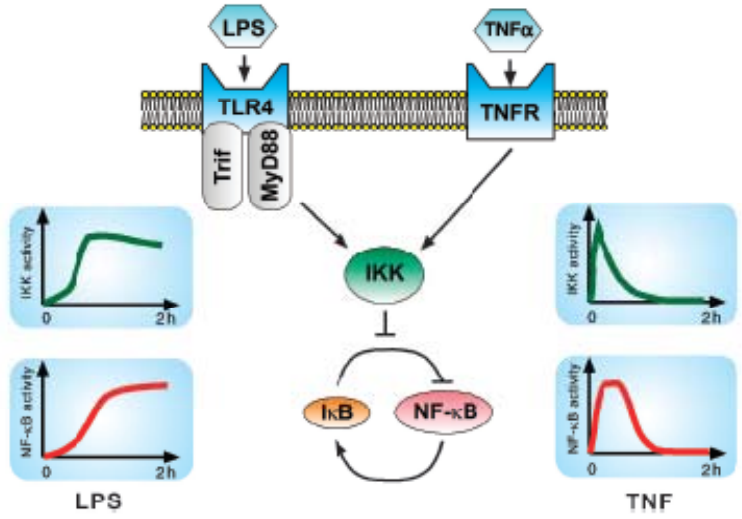
- Multiple intracellular feedback loops
  - IkBα and IkBε work in tandem to ensure fast response and oscillation suppression.
- Extracellular feedback loops
  - LPS (lipopolysaccharide) activates TLR4
  - Trif and MyD88 are activated asynchronously
  - MyD88: fast direct activation
  - Trif: slow indirect activation
- Many other possible feedback loops



Cheong et al, *Mol. Sys. Biol.*, 4:192, 2008.

Extension of the Original Model (II)

- IKK is chosen to be the input to the model.
- IKK activities are regulated.
- NF-kB dynamics is sensitive to timing and duration of IKK activities.
- Expression of targeted genes can be modulated by different temporal IKK signals.
- Crosstalk with many other pathways
  - LTβ
  - TGFα



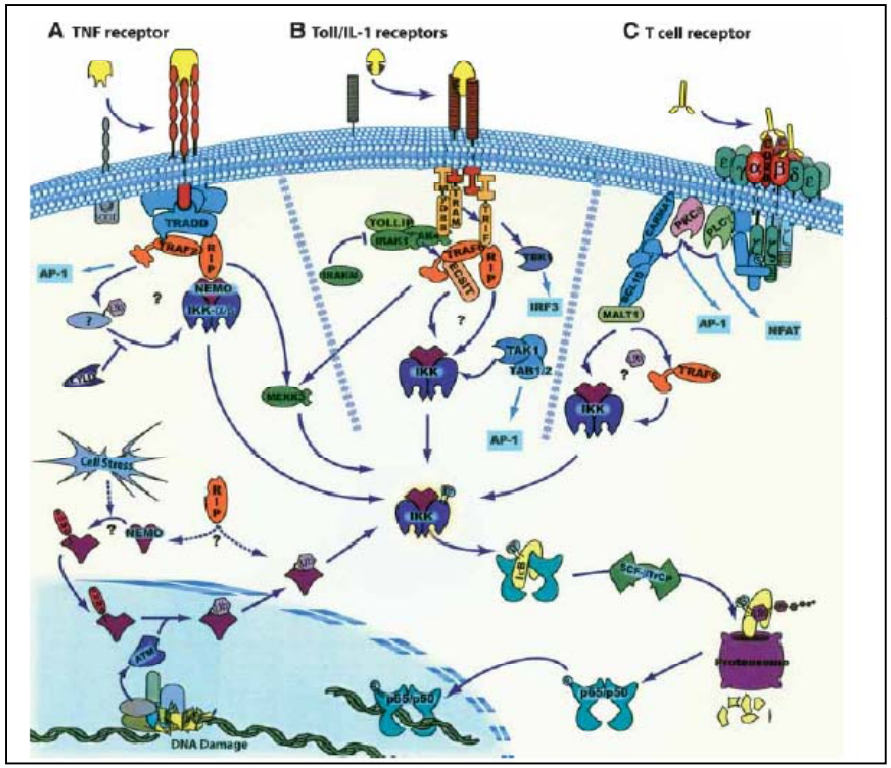
Cheong et al, *Mol. Sys. Biol.*, 4:192, 2008.

Outlook

- Encoding and decoding of spatial temporal communication
- Ration drug design through computer simulation
  - Outcome prediction
  - Efficiency analysis
- Integration with other signaling pathways
- Modeling method development
  - Parameter sensitivity analysis

Comments

- Definition of the module is critical
  - Complexity reduction
  - Functional independence
- Critical importance of integrating computational analysis and experiments
- Limitation of ODE-models
- How representative is the NF-kB pathway?



Hayden & Ghosh, *Genes & Dev.*, 18:2195, 2004

Questions?