"Dynamics and function of the NF-kappaB signalling system: How mathematical modelling can assist experimental biology"

COMBINE 2011

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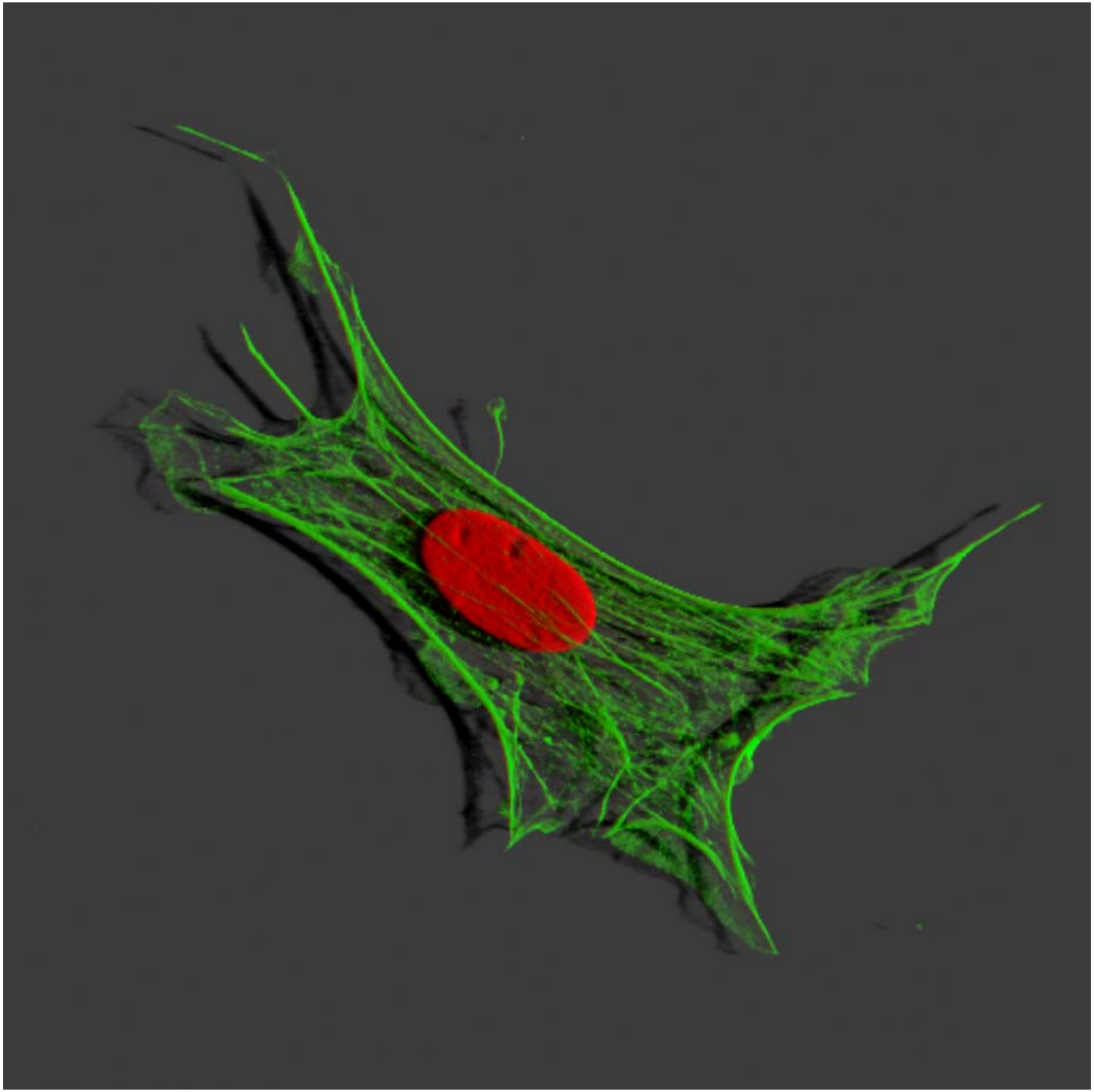
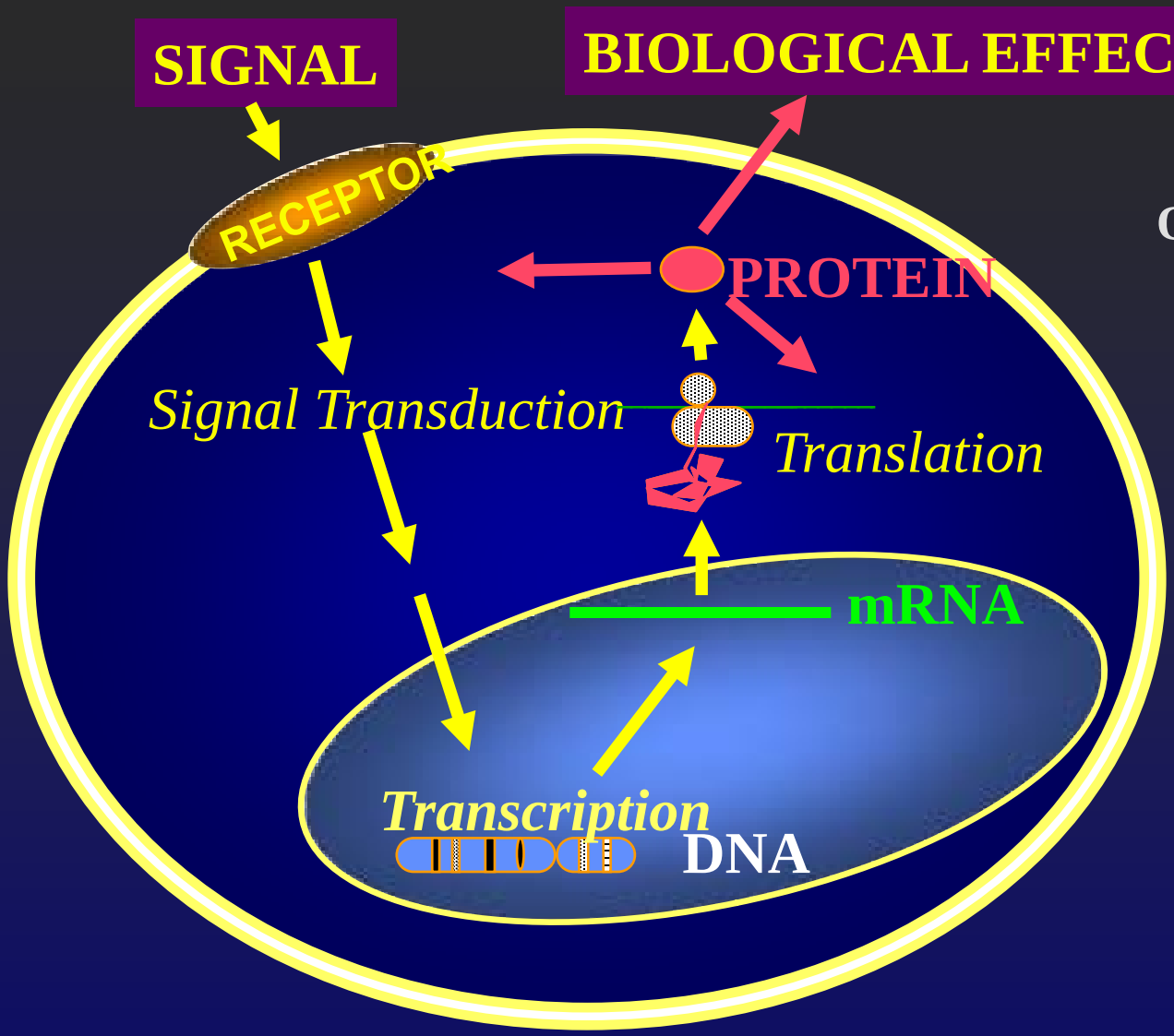
Nicolas Le Novere

- “Throughout the last decade, your work on NF-kB has been a shining example of interplay between modelling and experiment. We would be delighted if you were willing to spend some time with us and provide a lecture on the topic of your choice (as far as it involves modelling, for the audience, and signalling for my own enjoyment”



SIEMENS



Quantitative measurement

New Understanding

Mathematical modellimg

See: Spiller et al., *Nature* 465: 736-745

Is molecular biology yet a science?

The great successes of the past few years suggest that living processes consist of well-ordered events executed under strict control, but a few numbers would give a different and more fuzzy impression.

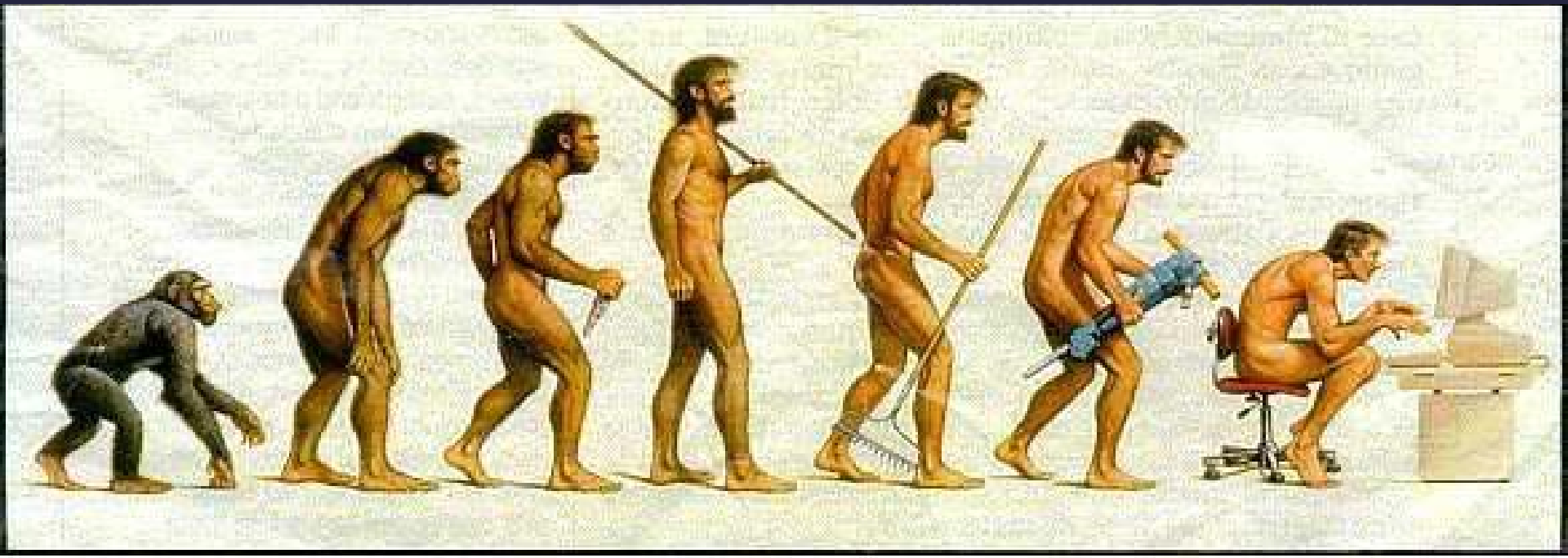
There is now an army of people called molecular biologists whose published papers are innocent of references to whole plants and animals and which may have little to say about their physiology either. For these people, experimental data may consist largely of what are called, in the trade, 'gels' — perhaps simply strips of filter paper allowing the comparison of the molecular weights of large molecules with those of similar materials by the relative rate at which they move under the influence of an electric field. Such techniques are deliciously simple, at least in skilled hands. It is marvellous that so much continues to be learned from them.

J. Maddox, Nature 1992

Systems Biology

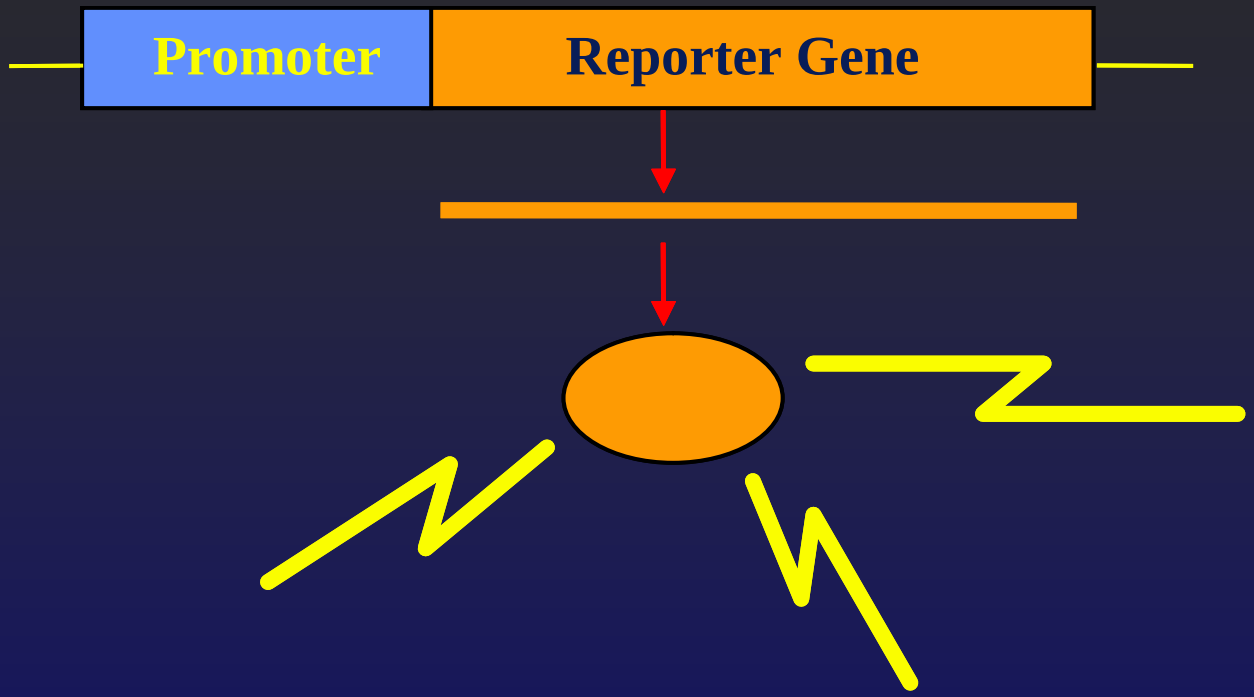
- **BBSRC**
- ***"… seeks to understand the workings of biological systems as a whole, placing a greater emphasis on the interactions between components, and the consequences of such interactions, than on the components themselves (which, in a post-genomic era, are largely known)."***
  Web site (2008)

Evolution of the microscopist!



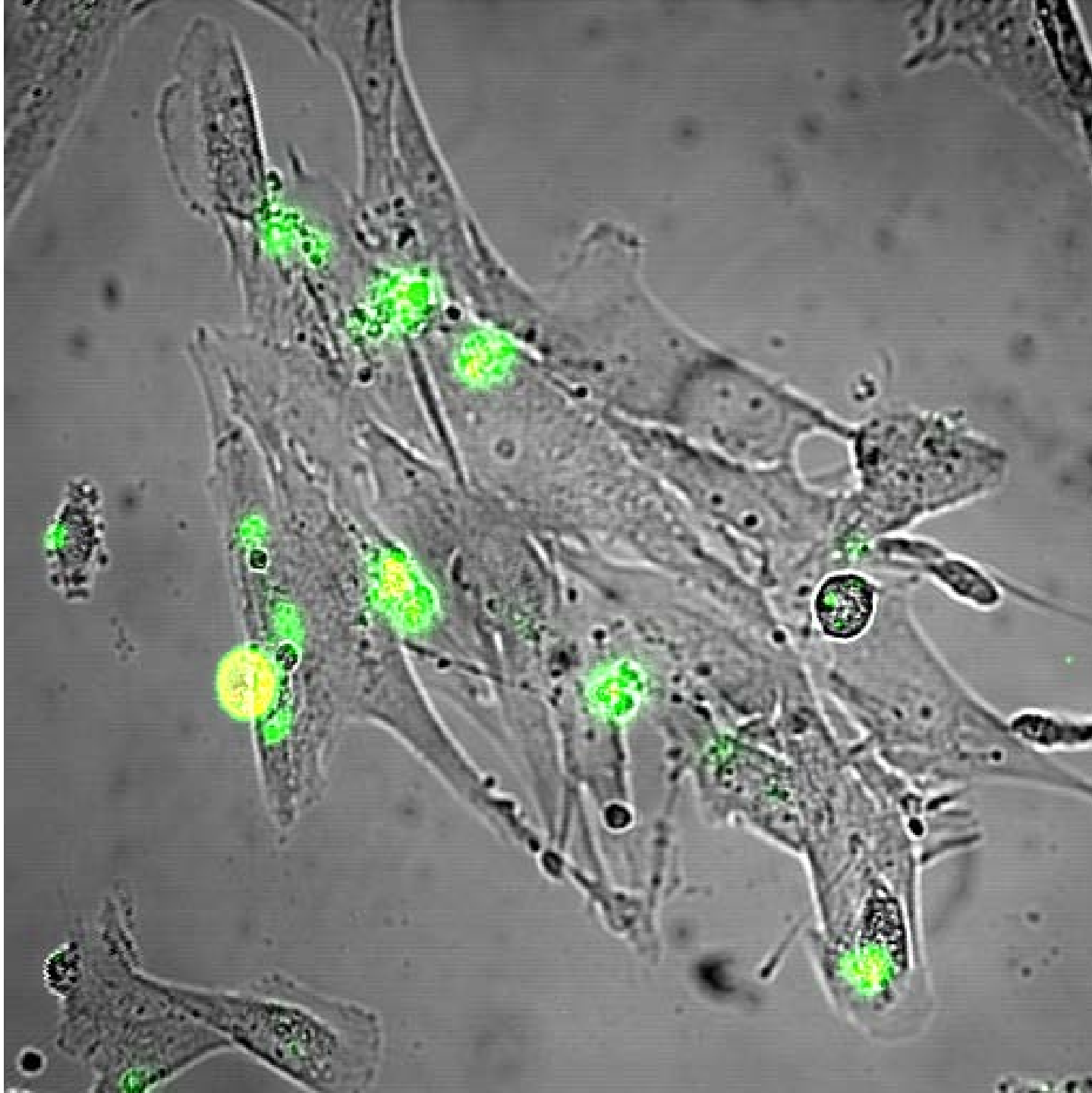
Somewhere, something went terribly wrong

Imaging of single cell transcription dynamics



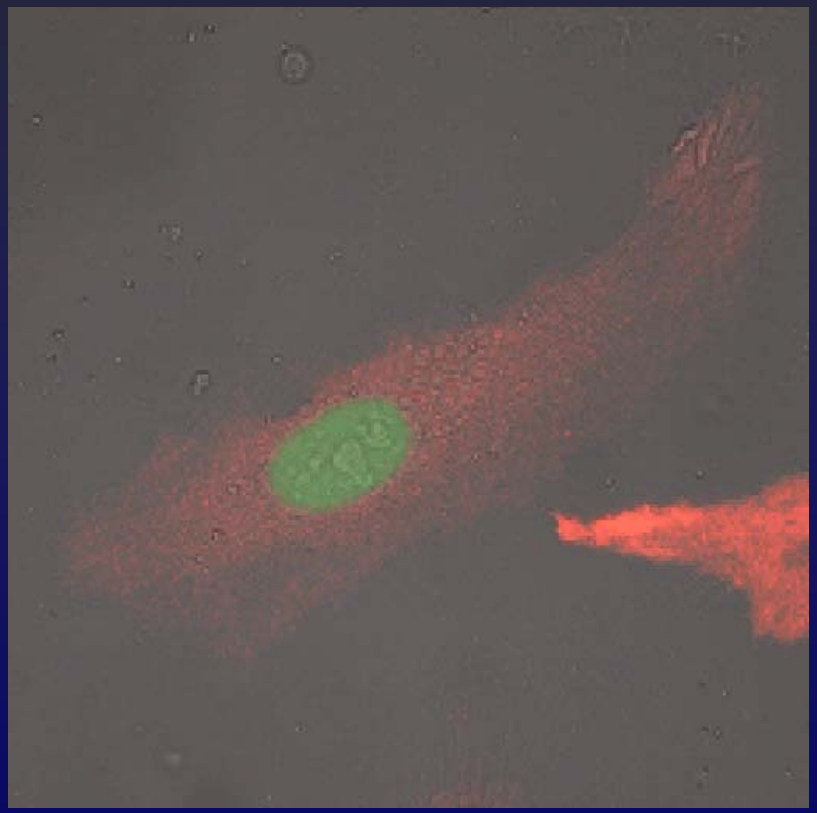
Real-time Cell death Measurement

UV irradiated cells

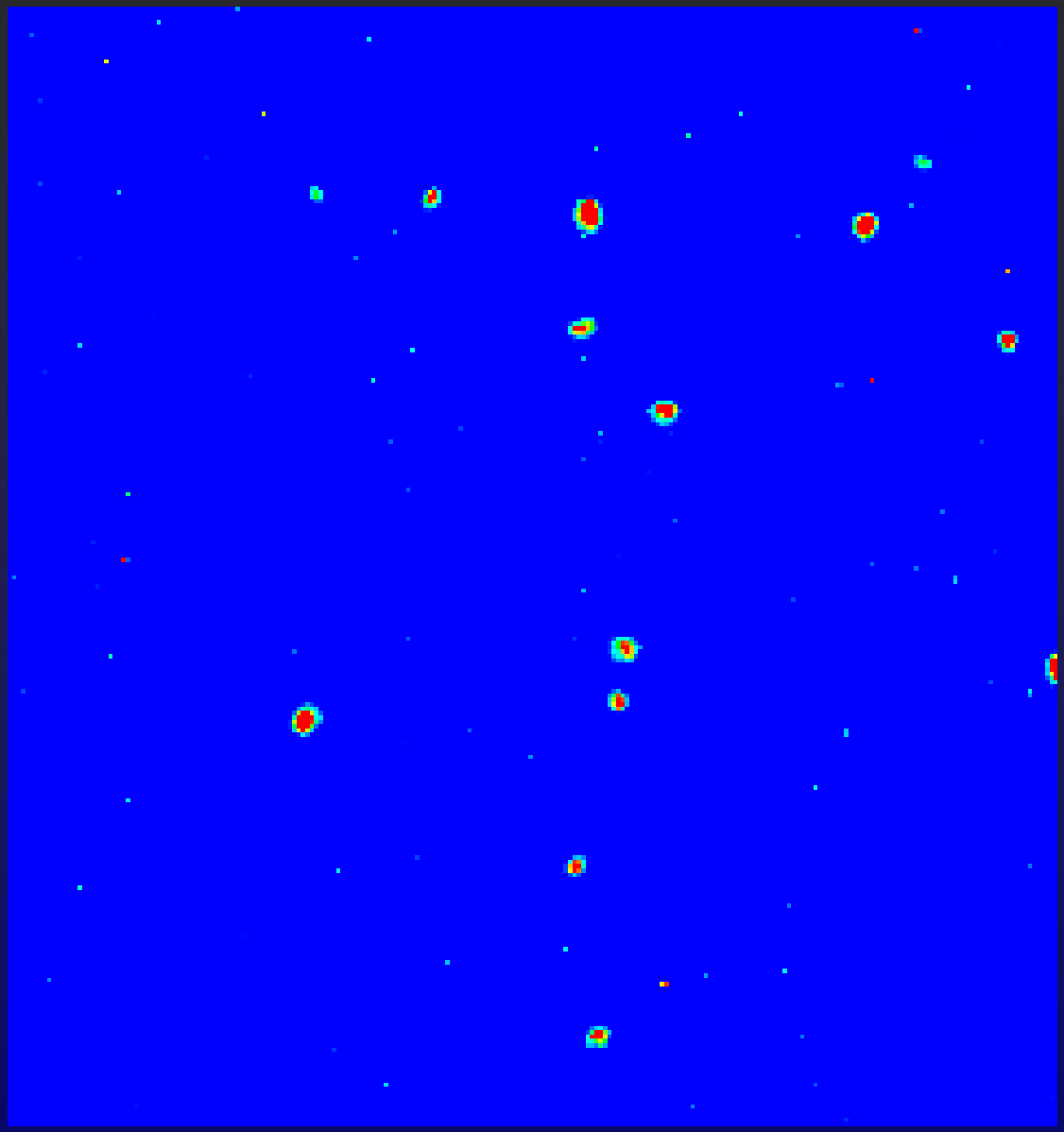


24-61 h after treatment.

Cells are not always predictable!



Imaging of single cell transcription dynamics

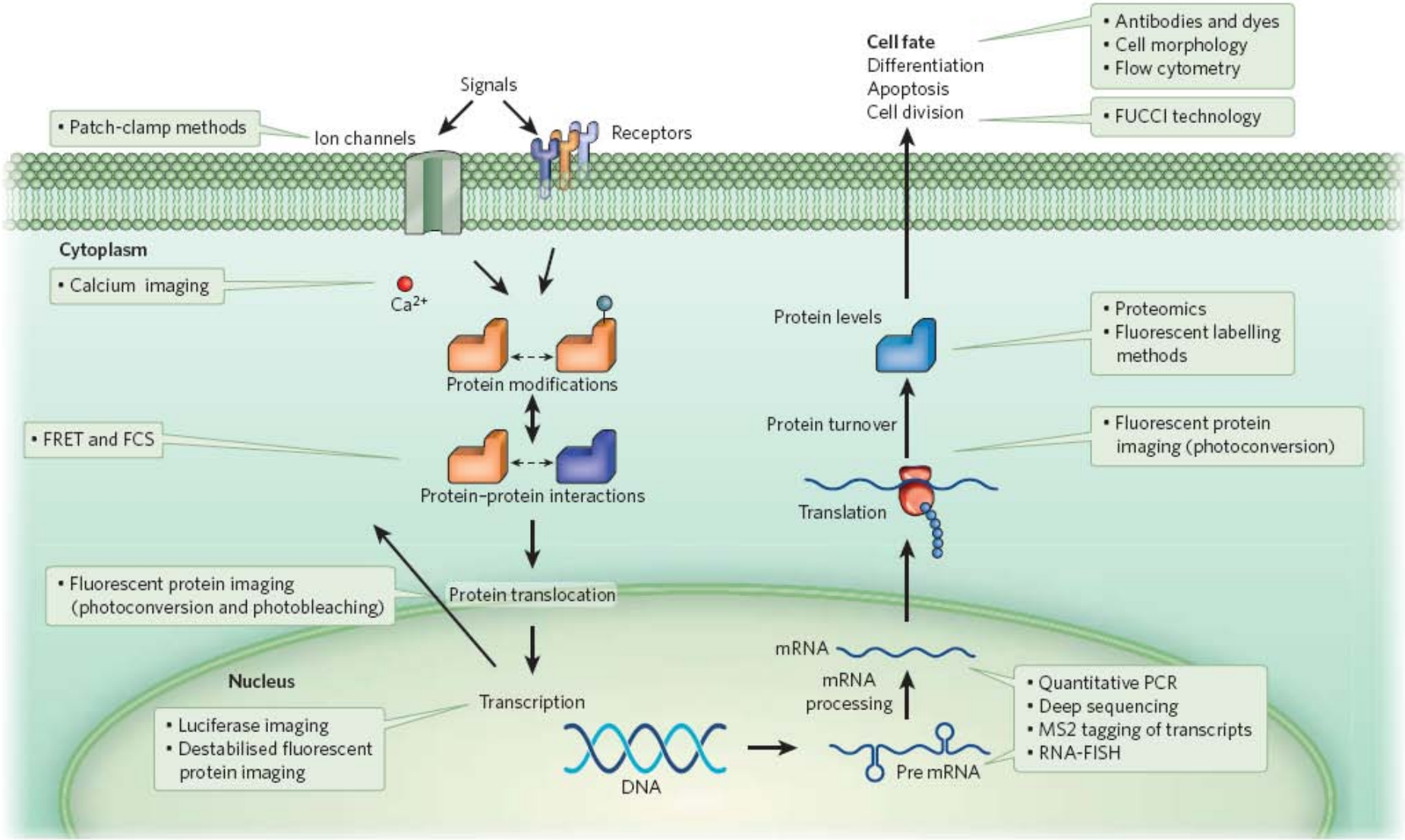


Primary rat pituitary cells
Prolactin - luciferase

Luciferase imaging
Gene expression constant at the population level

Harper *et al.*, (2010) *PLOS Biol*, 9(4):e1000607

Requirements for quantitative intracellular measurements

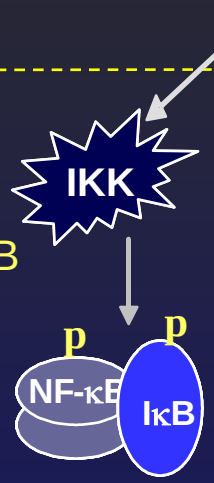


See: Spiller et al., *Nature* 465: 736-745

Questions

- How is extracellular information encoded within the cell?
- Why do we see heterogeneity in cell signalling processes?

NF-κB:



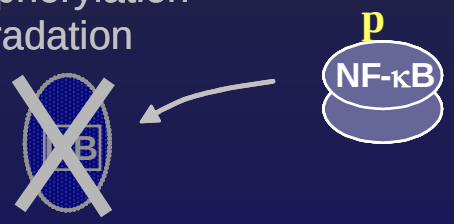
IKK complex phosphorylates IκB

NF-κB (RelA/p65) discovered ~25 y ago
associated with antibody gene expression

IκB protein(s) hold RelA/p65 in the cytoplasm

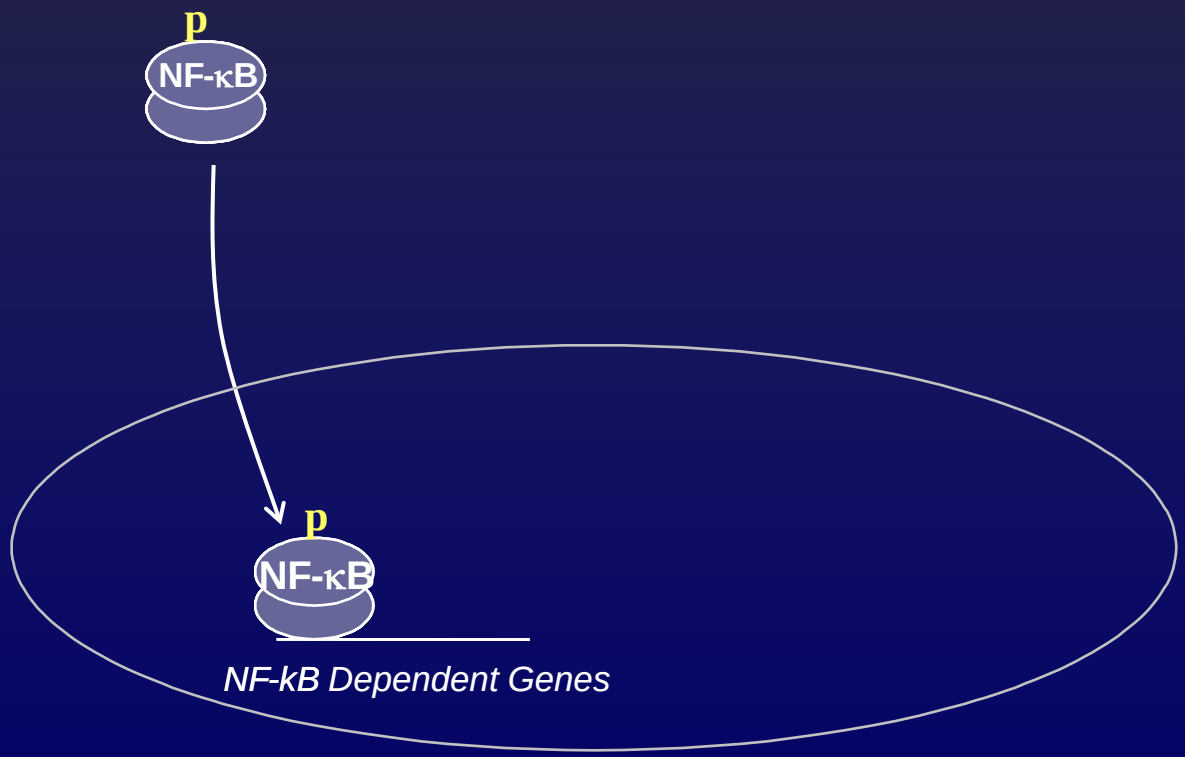
NF-κB:

IκB Phosphorylation & Degradation

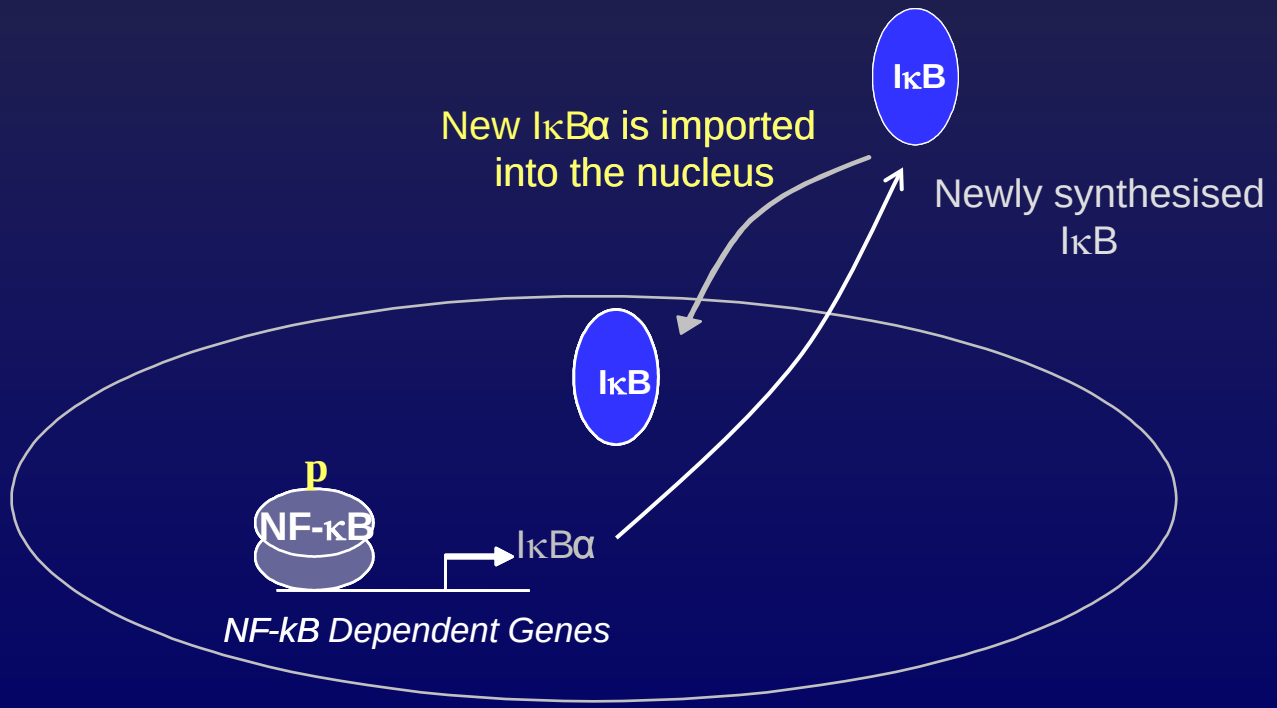


NF-kB Dependent Genes

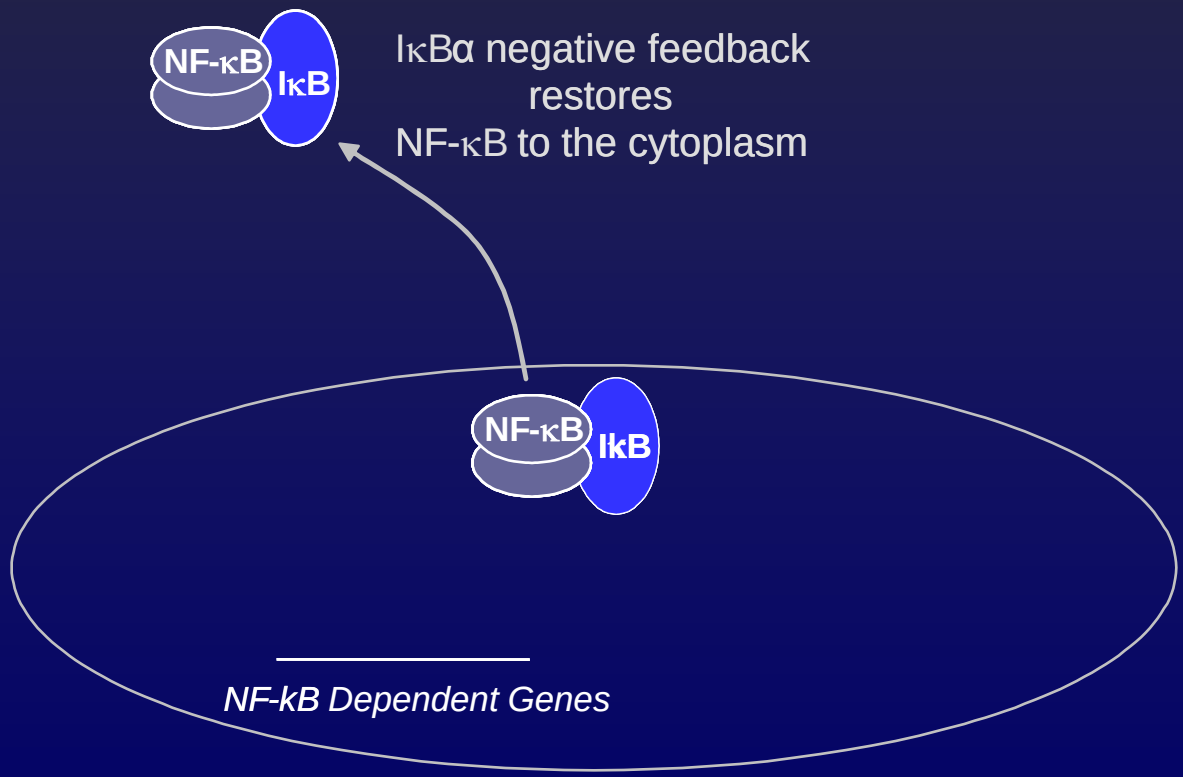
NF-κB:



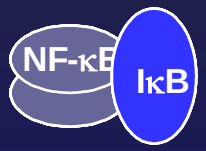
NF-κB:


IκB
New IκBα is imported into the nucleus
Newly synthesised IκB
IκB
p
NF-κB
IκBα
NF-kB Dependent Genes

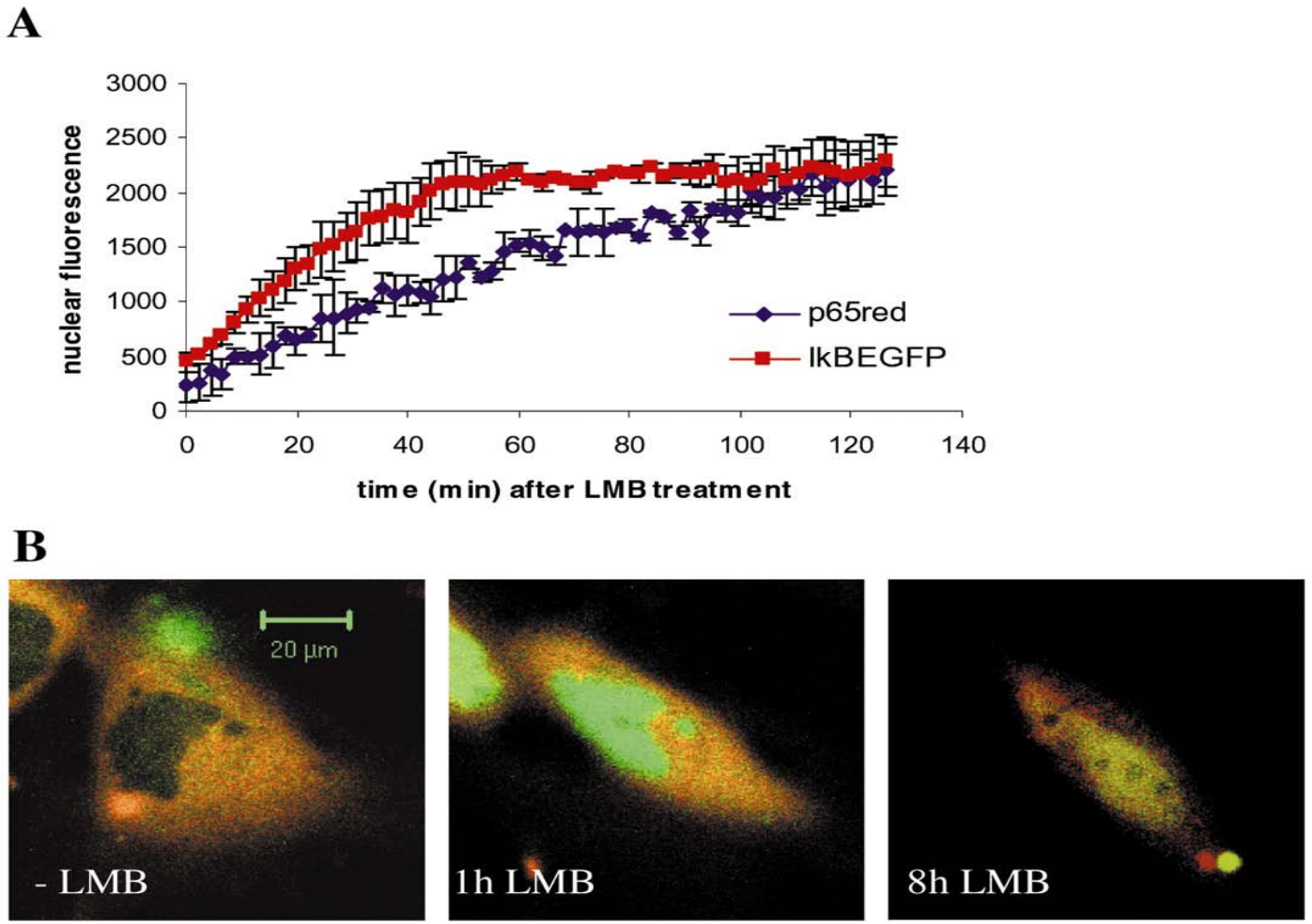
NF-κB:


NF-κB
IκB
IκBα negative feedback
restores
NF-κB to the cytoplasm
NF-κB
IκB
NF-kB Dependent Genes

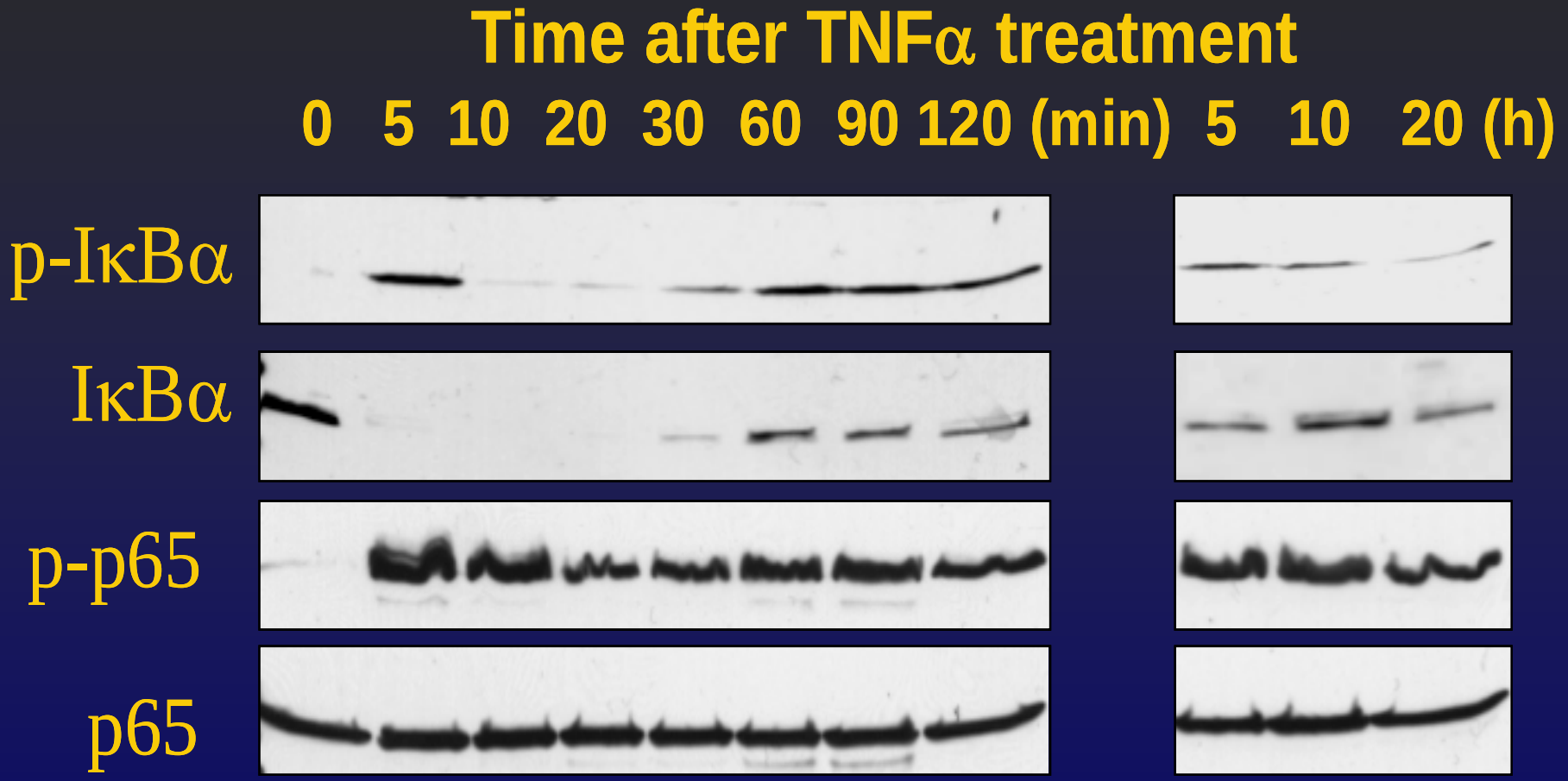
NF-κB:



Quantification of differential accumulation of p65-dsRed and IκBα-EGFP in the nucleus following treatment of dual-transfected cells with leptomycin B.

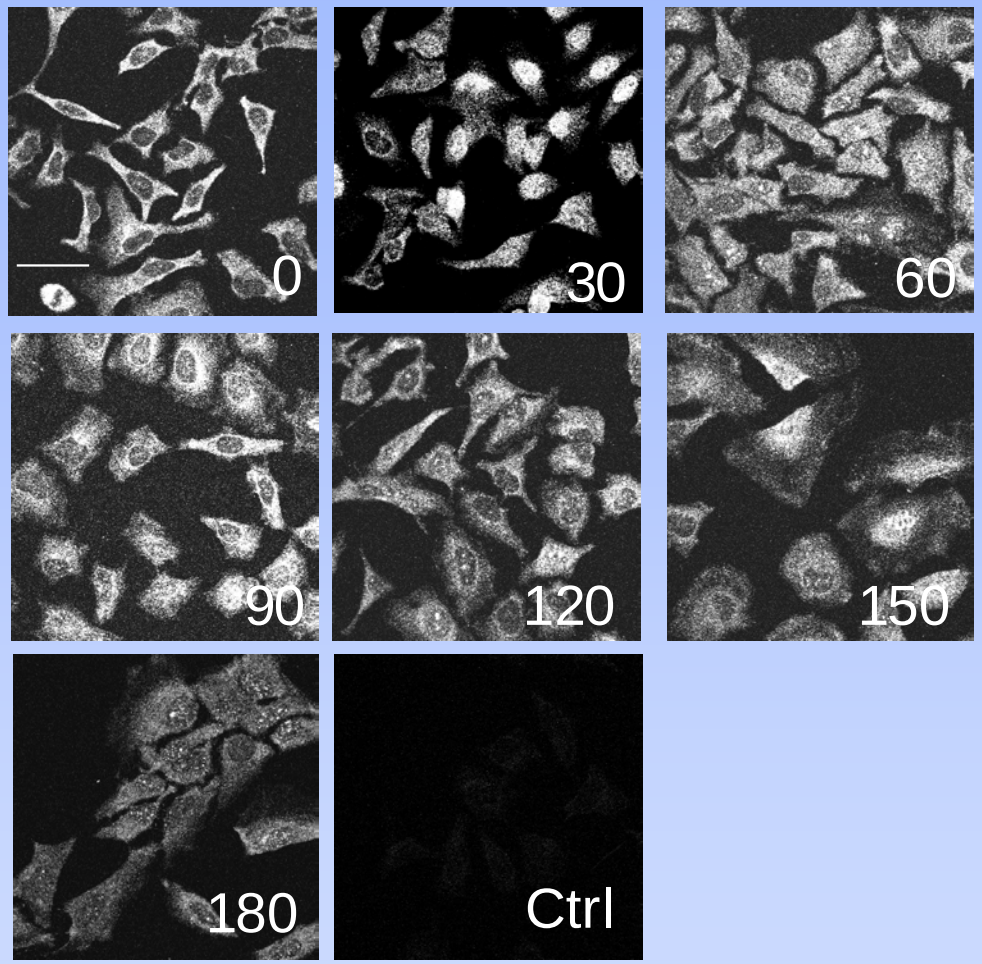


Nelson G et al. J Cell Sci 2002;115:1137-1148

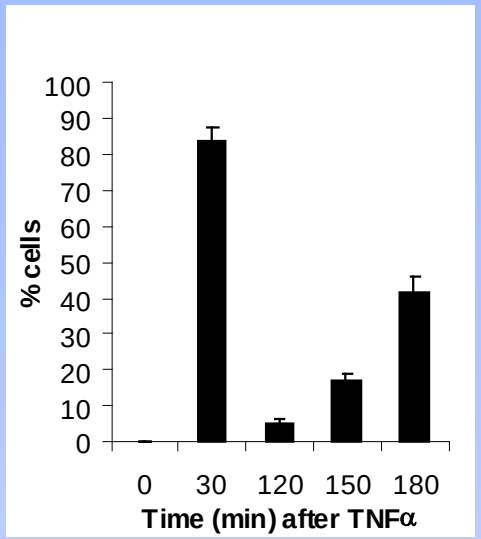

Time after TNFα treatment
0 5 10 20 30 60 90 120 (min) 5 10 20 (h)
p-IκBα
IκBα
p-p65
p65

Observing p65 movement in single cells

A



B

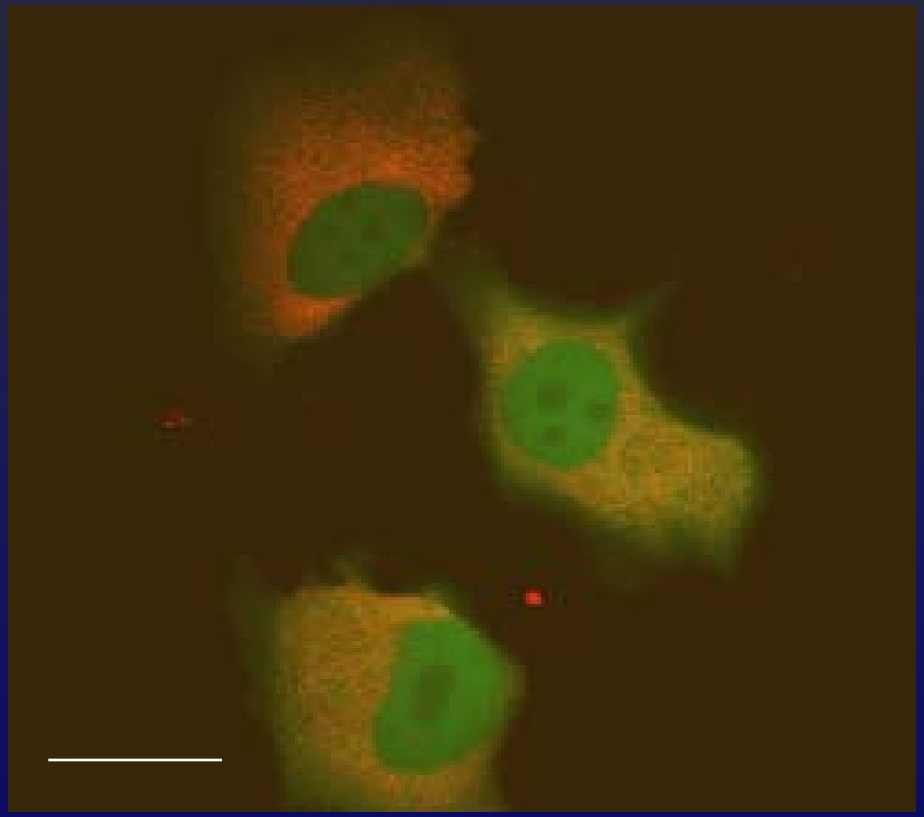


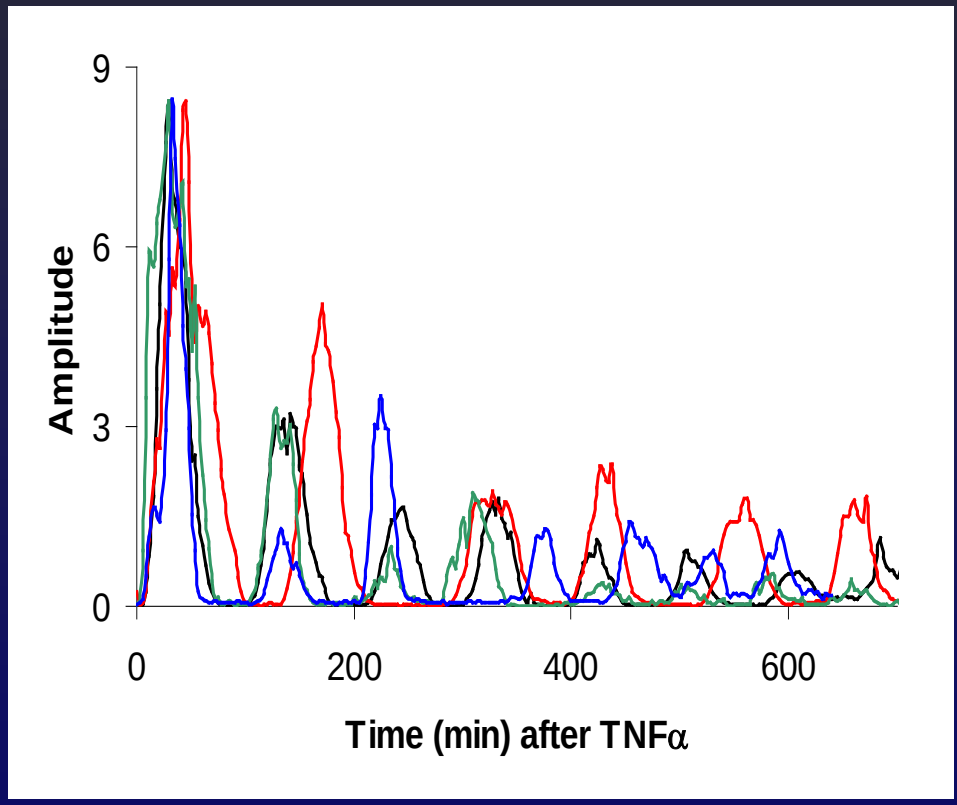
C

Time	N<C	N>C	N=C
0	100	0	0
30	16.2	44.7	39.1
60	54.5	16.9	28.6
90	89.6	4.5	5.9
120	94.6	1.2	4.1
150	82.7	5.6	11.7
180	58.5	9.1	32.4

Live cell imaging shows that NF-κB oscillates asynchronously between the cytoplasm and nucleus in single cells

SK-N-AS cells expressing p65-dsRedxp and EGFP

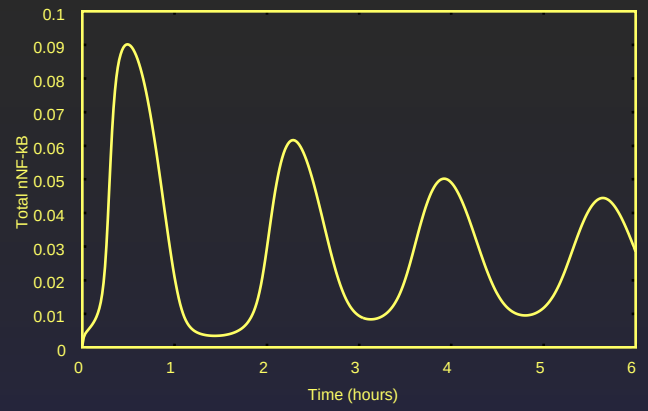




D. Nelson et al., (2004) Science 306: 705-708

NF-κB:

Computational model



Signal, e.g. TNF-α

IKK

IκBα Phosphorylation & Degradation

NF-κB

IκB

IκBα negative feedback restores NF-κB to the cytoplasm

New IκBα is imported into the nucleus

Newly synthesised IκBs

Nuclear Translocation

IκBα

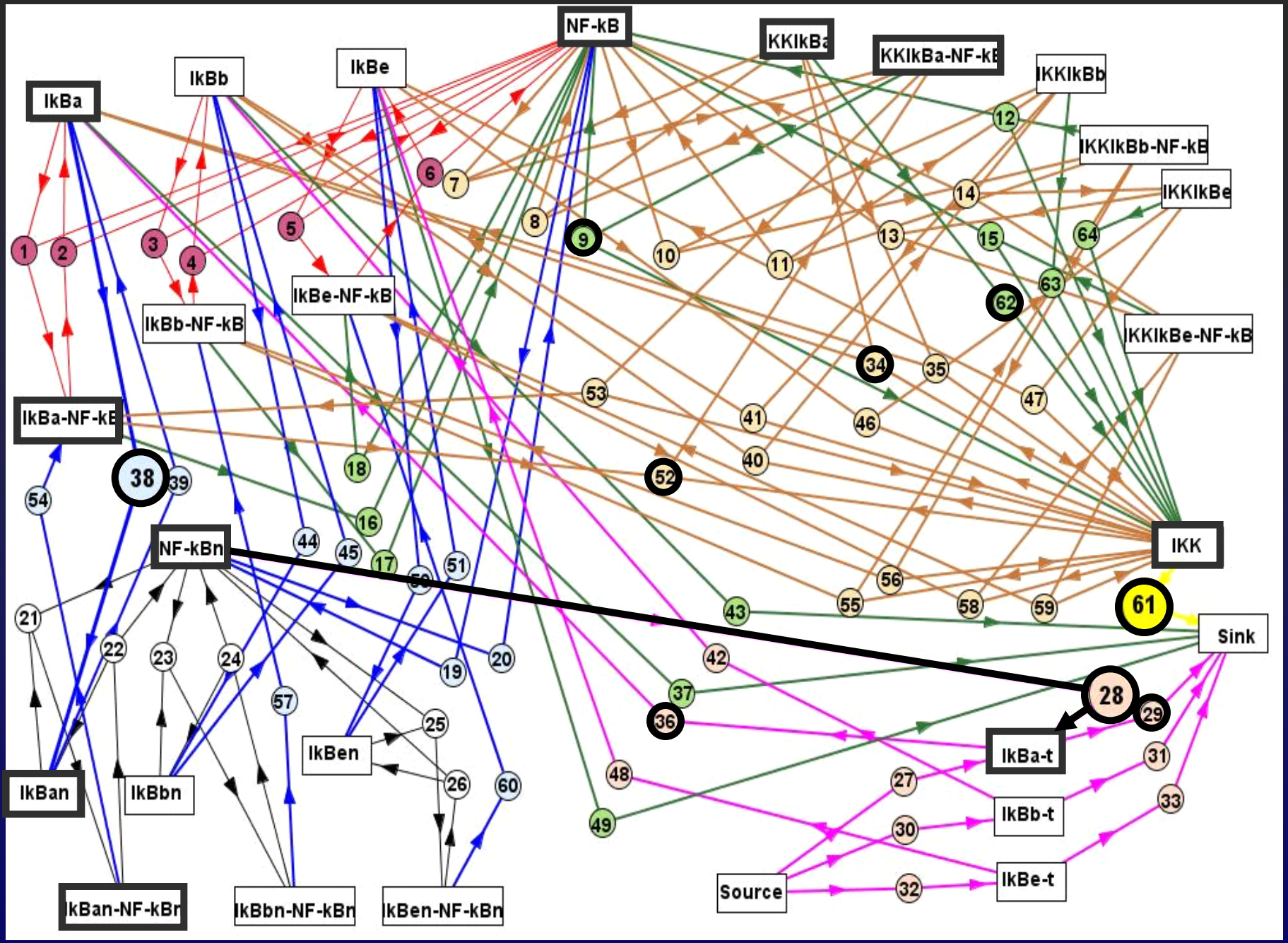
IκBβ

IκBε

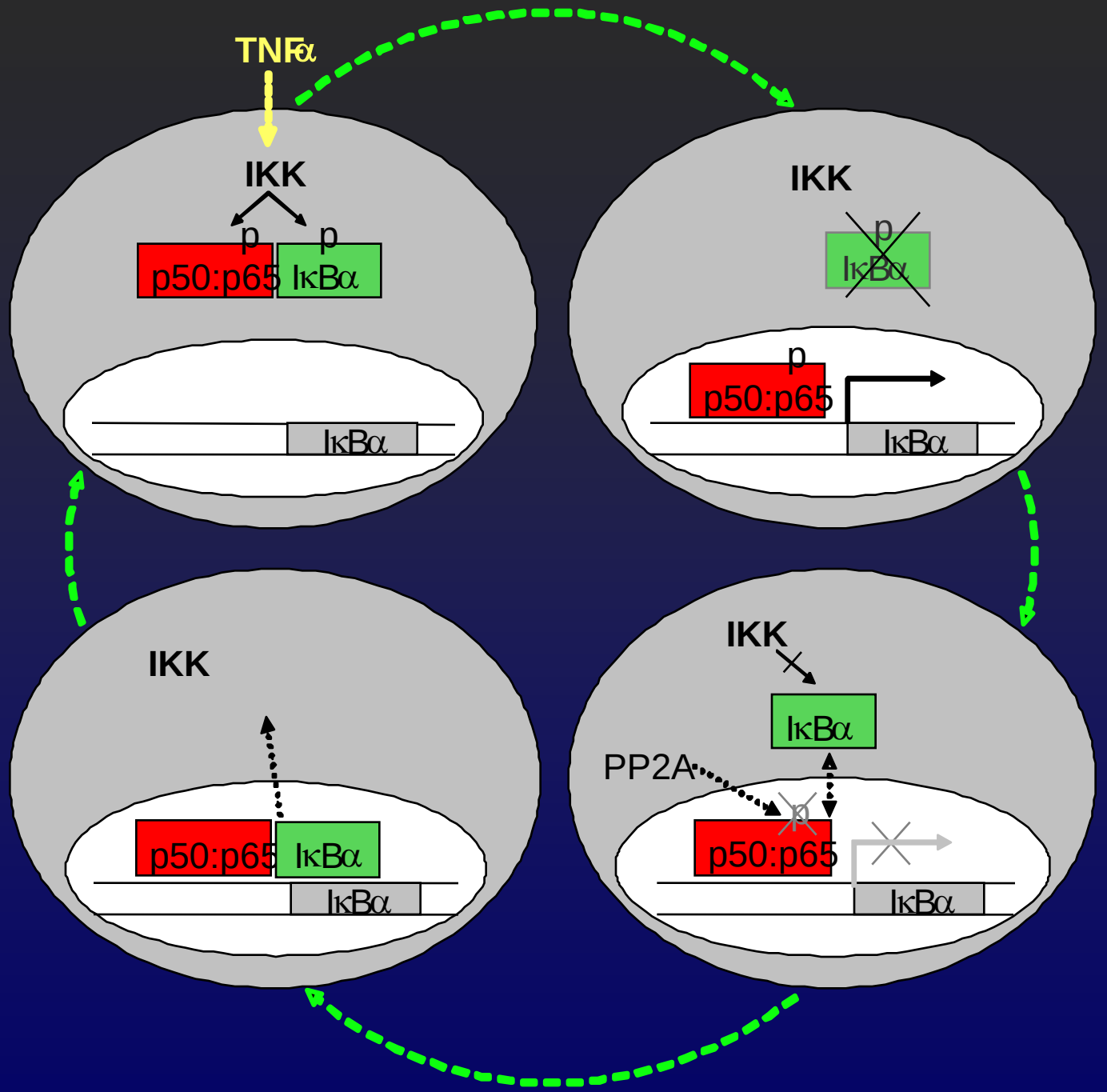
Constitutive Synthesis

NF-kB Dependent Genes

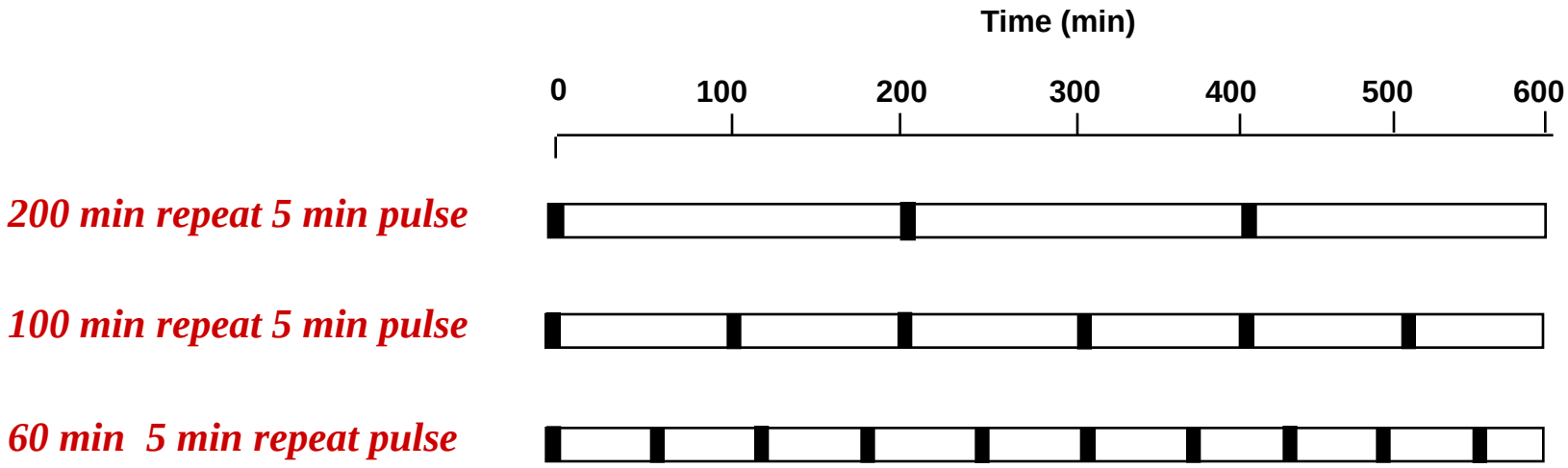
Hoffmann *et al*., (2002)
Science **298:** 1241



A. Ihekwaba and D. Kell


TNFα
IKK
p
p
p50:p65
IκBα
IκBα
IKK
p
IκBα
p
p50:p65
IκBα
IKK
p50:p65
IκBα
IκBα
IKK
IκBα
PP2A
p
p50:p65
IκBα

TNFα repeat pulse protocol



Cell Assays:

Imaging of RelA/p65 dynamics by fluorescence imaging of single cells

Semi-quantitative Western blot analysis of IκBα, p-IκBα and p-RelA/p65 levels

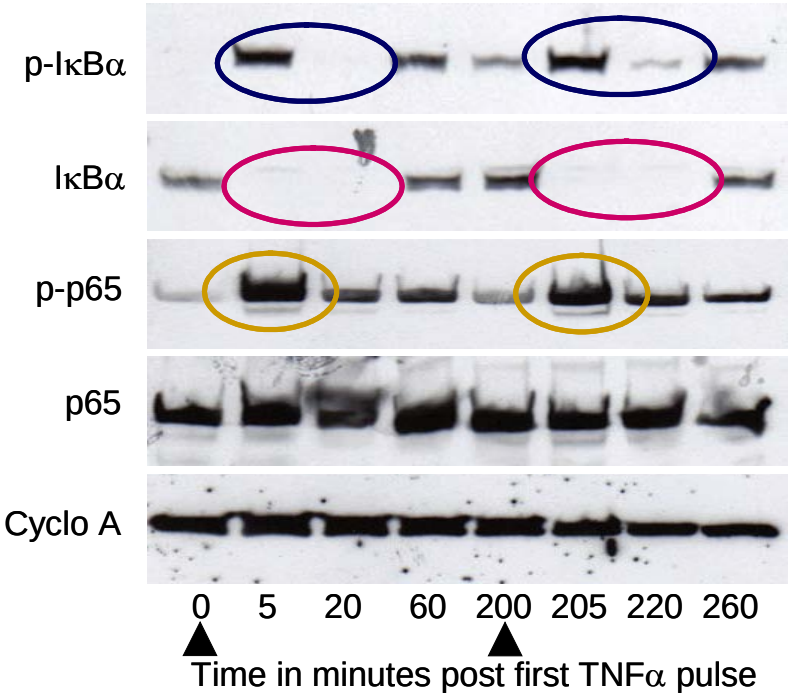
An experimental model for dynamic cell to cell cytokine signalling

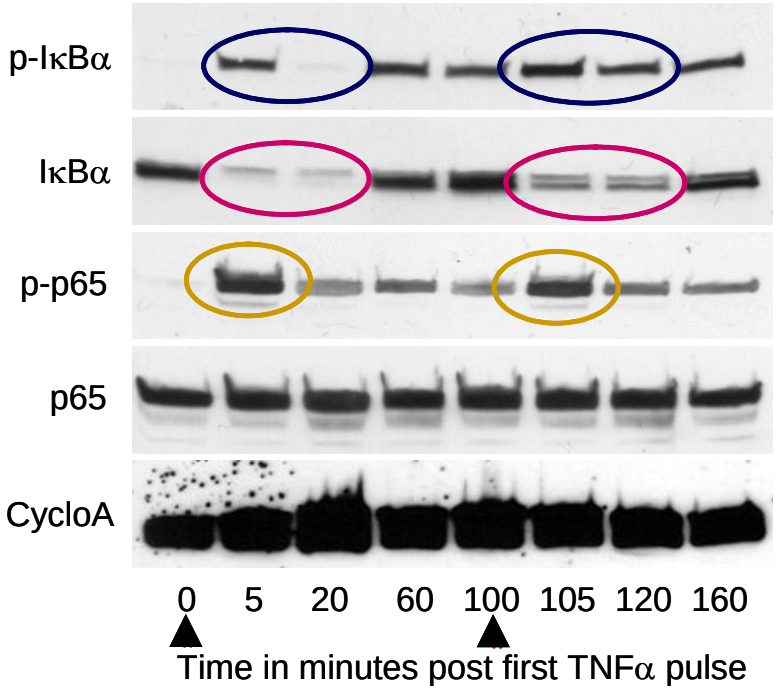
Ashall *et al.*, *Science*, (2009) 324: 242

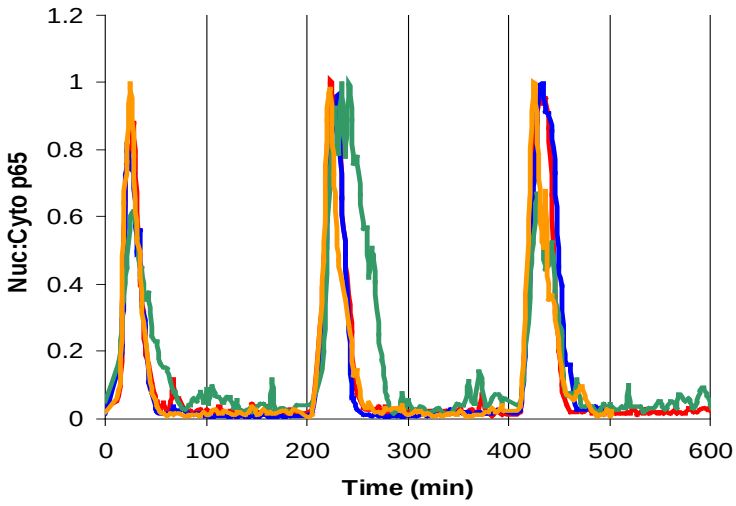
D. Nelson and L. Ashall

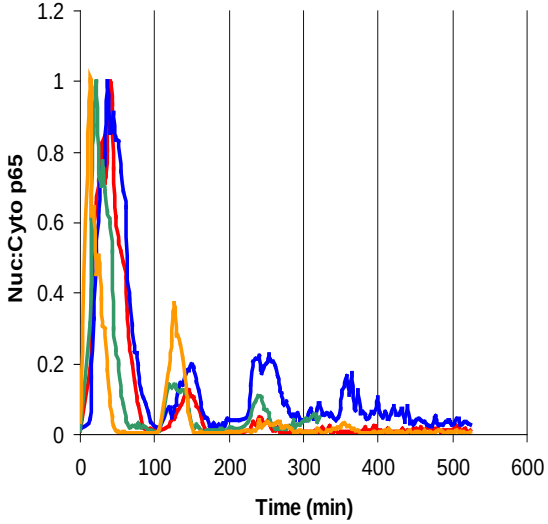
Can repetitive pulsing be used to synchronise populations of cells?

SK-N-AS Cells



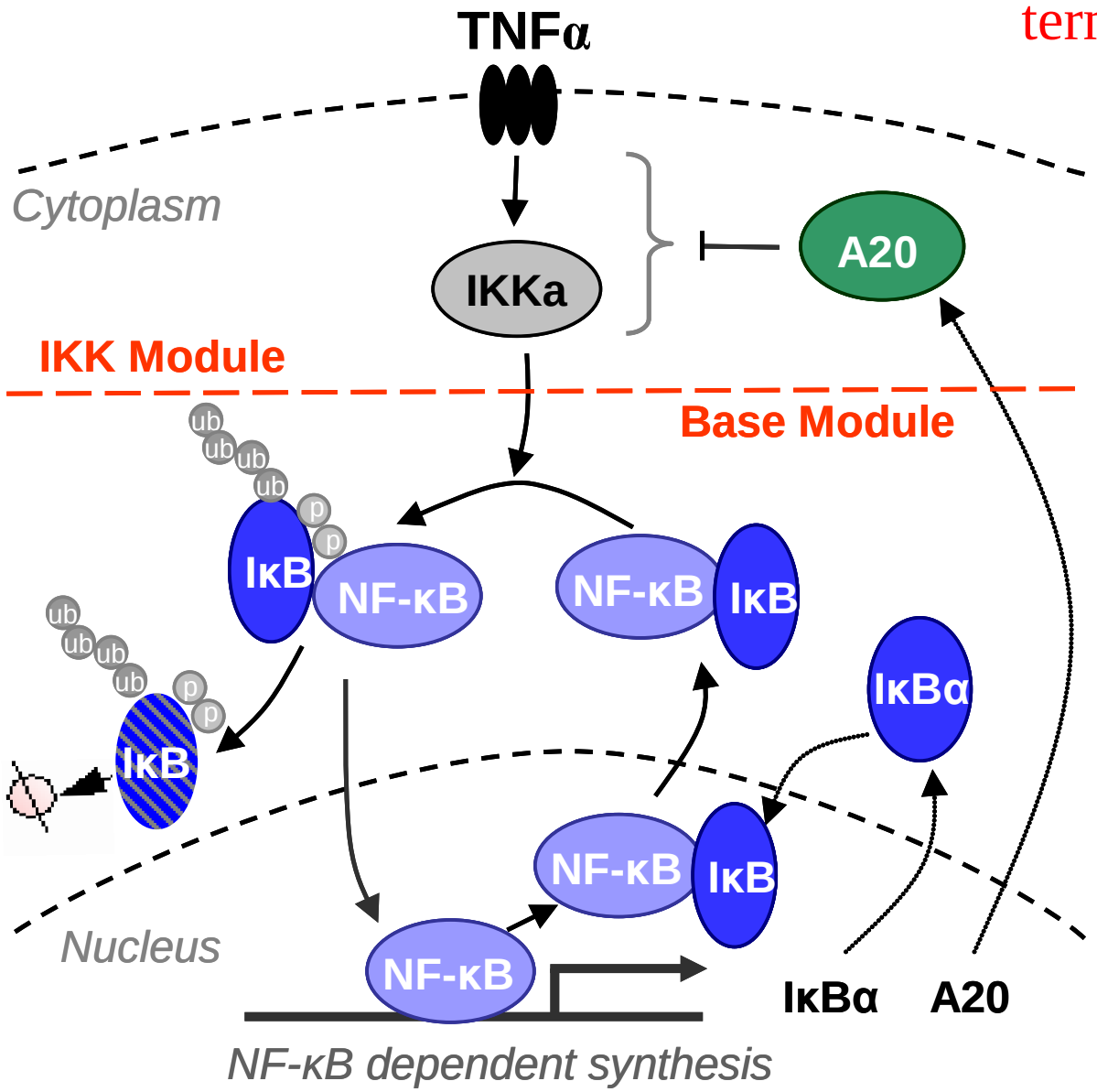






Ashall *et al.*, *Science*, (2009) 324: 242

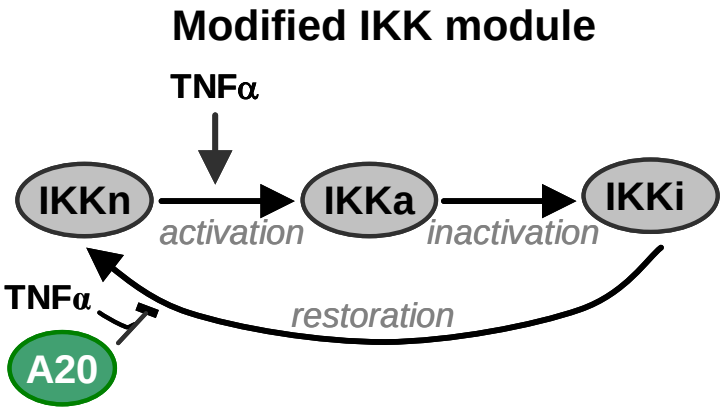
A20-/- mice fail to terminate inflammation



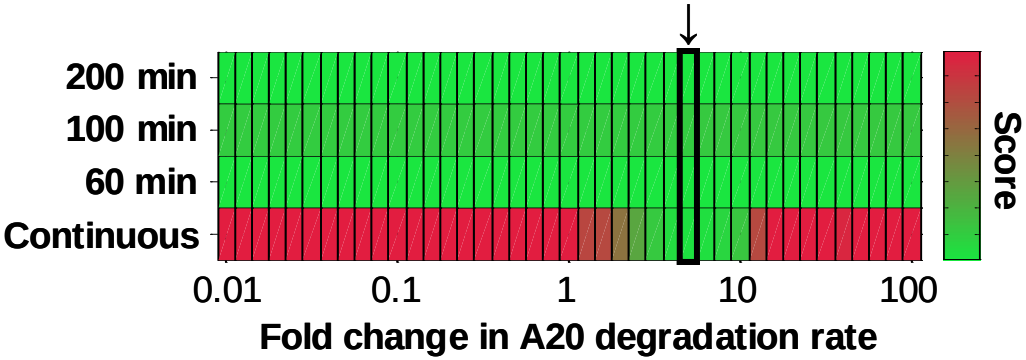
Ashall *et al.*, *Science*, (2009) 324: 242

C. Horton

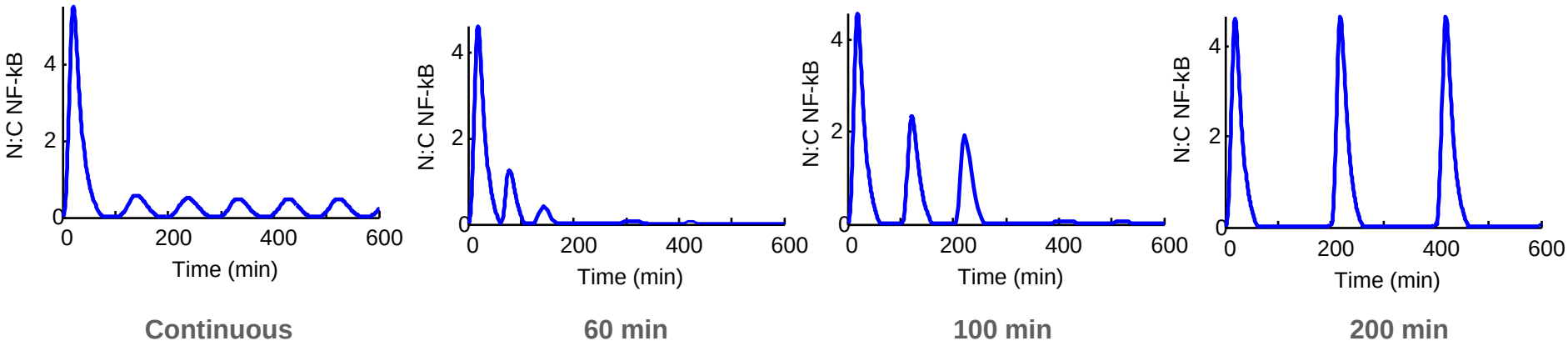
Modifications with minimal assumptions to the model yielded a successful variant



Green = good fit defined by formal criteria from experimental data



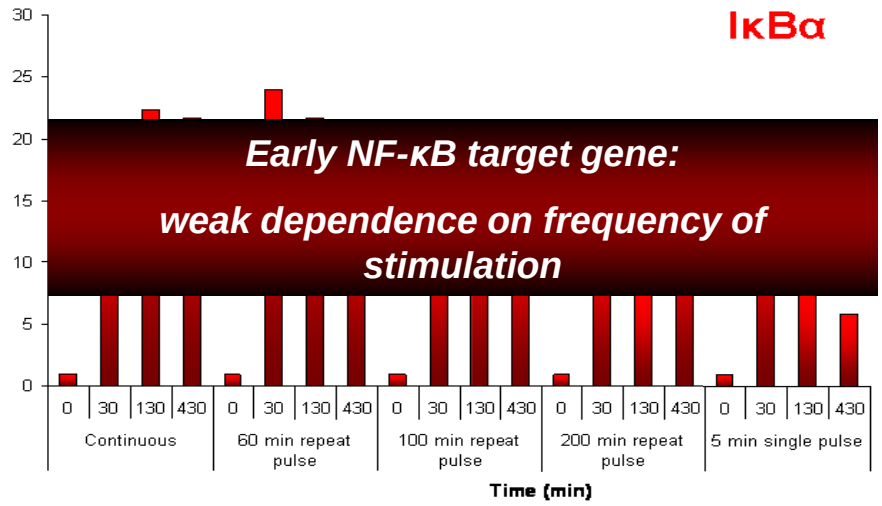
A single parameter set could then simulate all experimental outputs with a reasonable fit

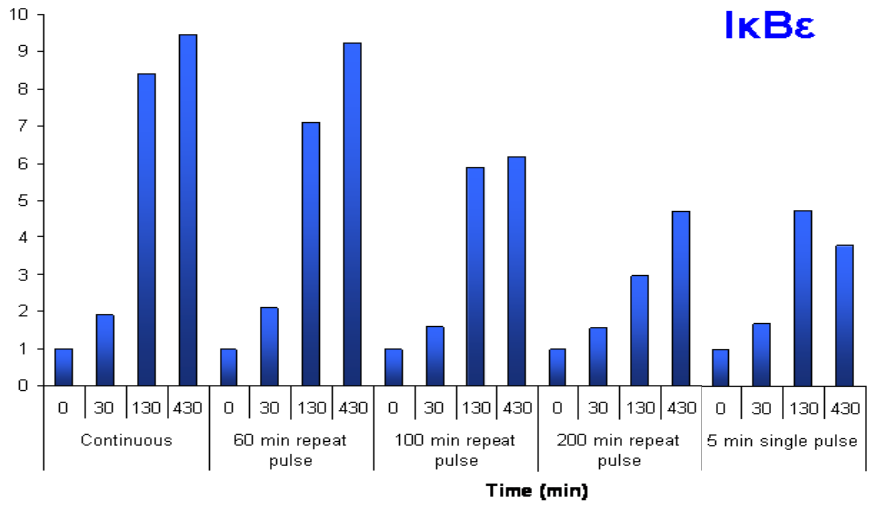


Ashall *et al.*, *Science*, (2009) 324: 242

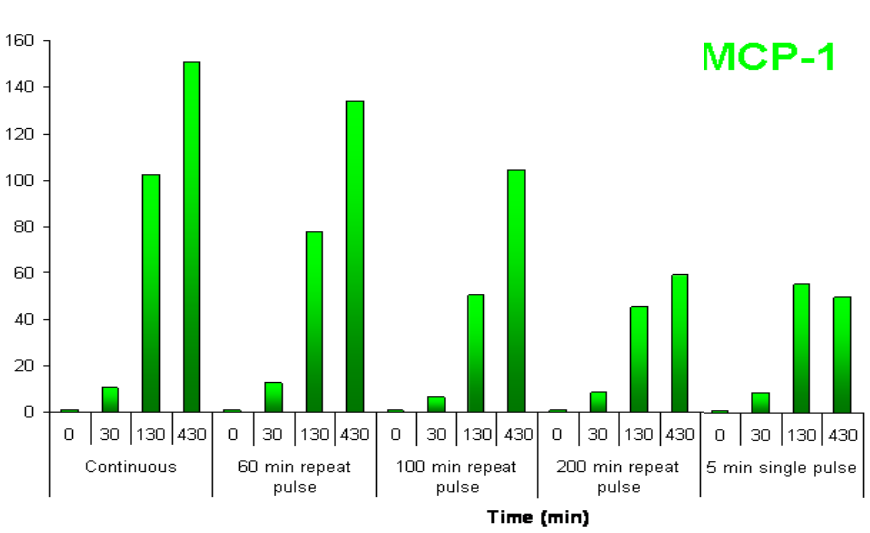
C. Horton

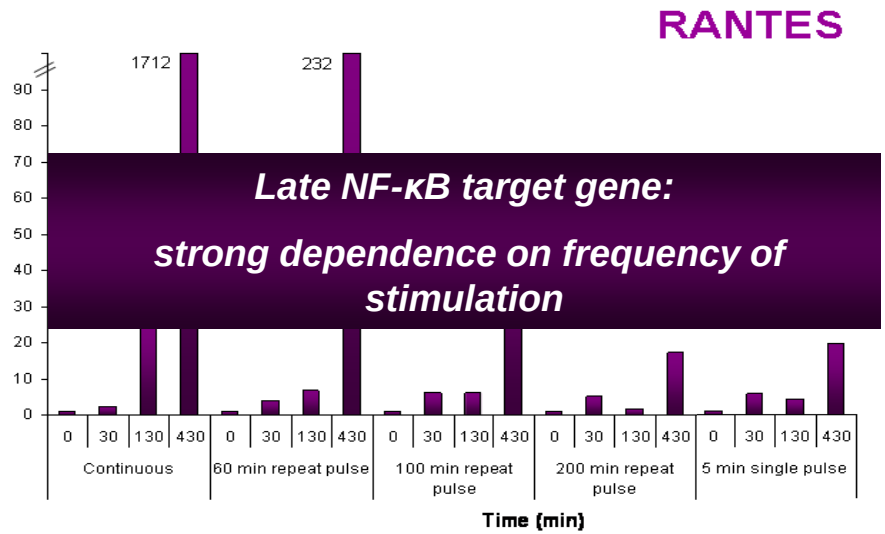
Differential gene expression





high frequency ⟶ **low frequency**





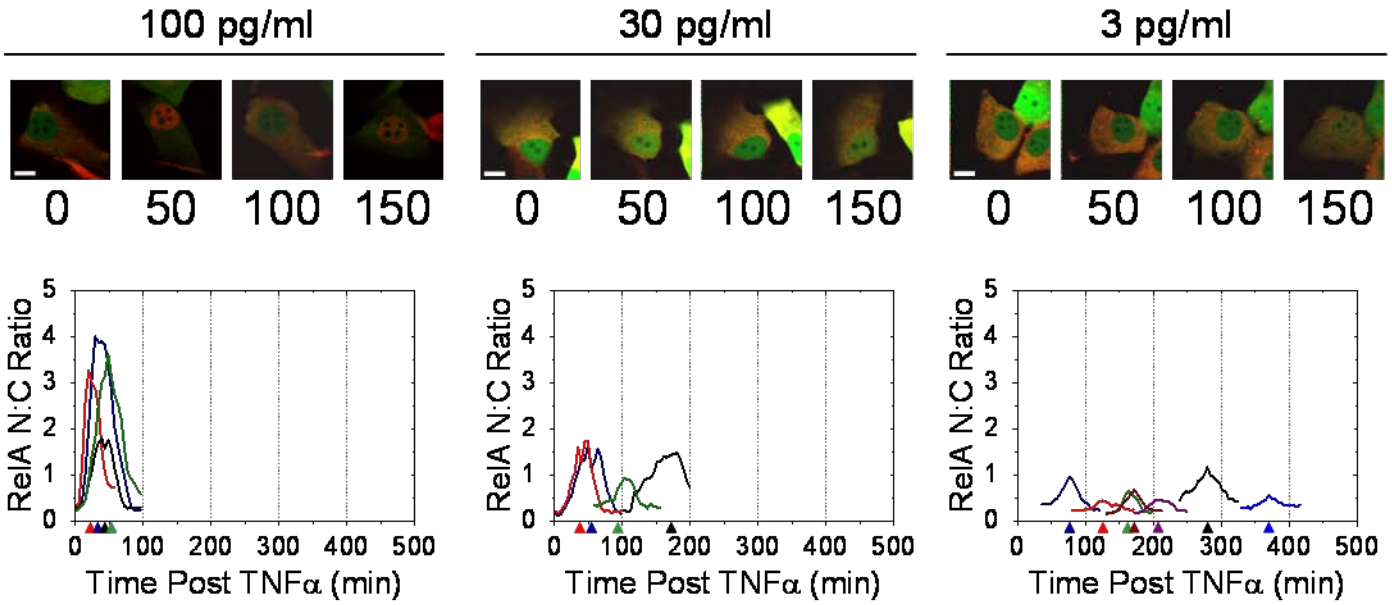
- Stimulation frequency determines differential gene expression.

Ashall et al., Science, (2009) 324: 242

NF-κB dynamics

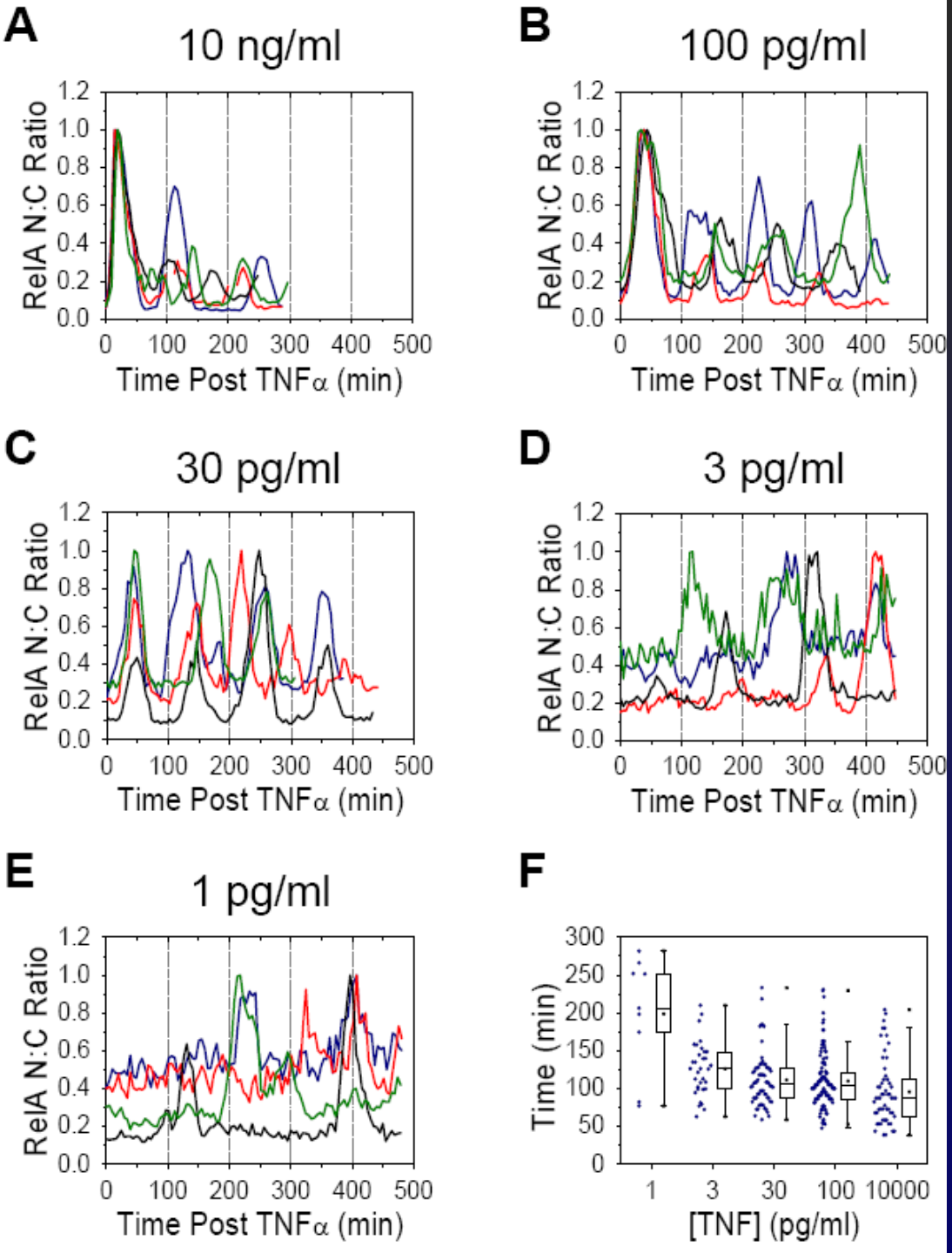
- What is the effect of stimulation dose on the responsiveness of the system?

Low dose data

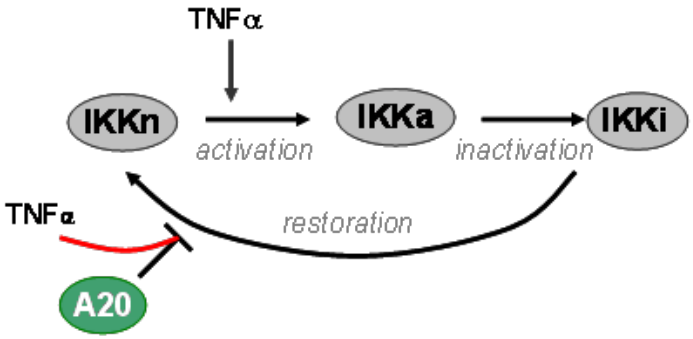


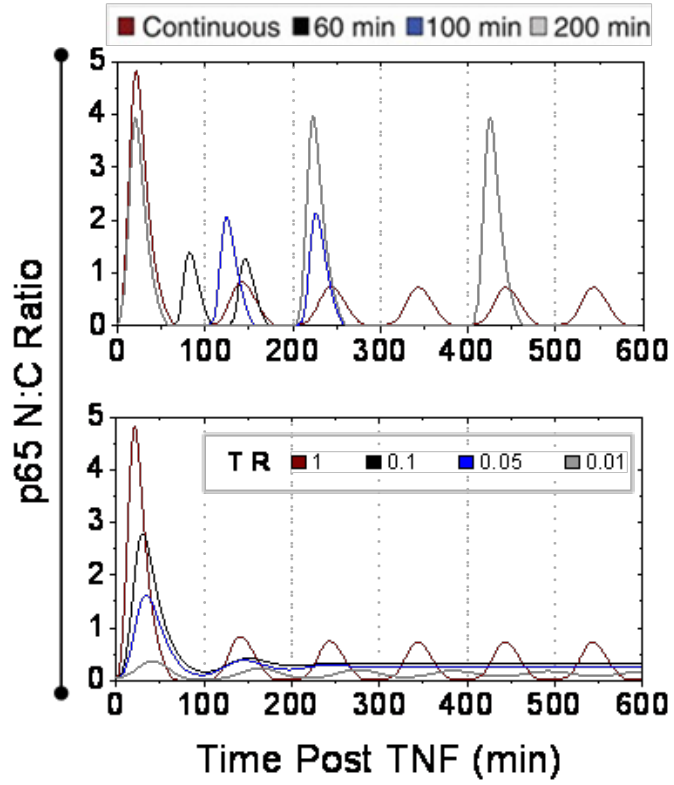
- Decreasing TNFa dose
  - Reduction in amplitude
  - Increase in heterogeneity (timing)

D. Turner *et al.*, *J. Cell Sci.* 123: 2834-2843
See also: Tay et al., *Nature* 466: 267


A
10 ng/ml
RelA N:C Ratio
Time Post TNFα (min)
B
100 pg/ml
RelA N:C Ratio
Time Post TNFα (min)
C
30 pg/ml
RelA N:C Ratio
Time Post TNFα (min)
D
3 pg/ml
RelA N:C Ratio
Time Post TNFα (min)
E
1 pg/ml
RelA N:C Ratio
Time Post TNFα (min)
F
Time (min)
[TNF] (pg/ml)

Original model structure

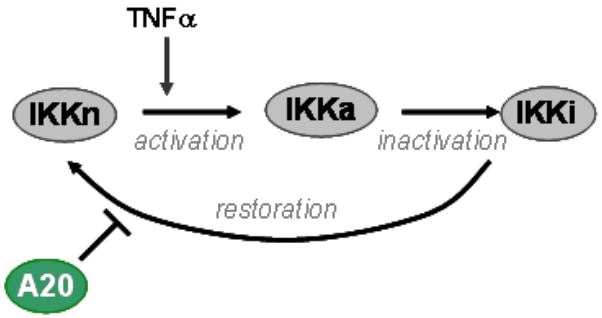


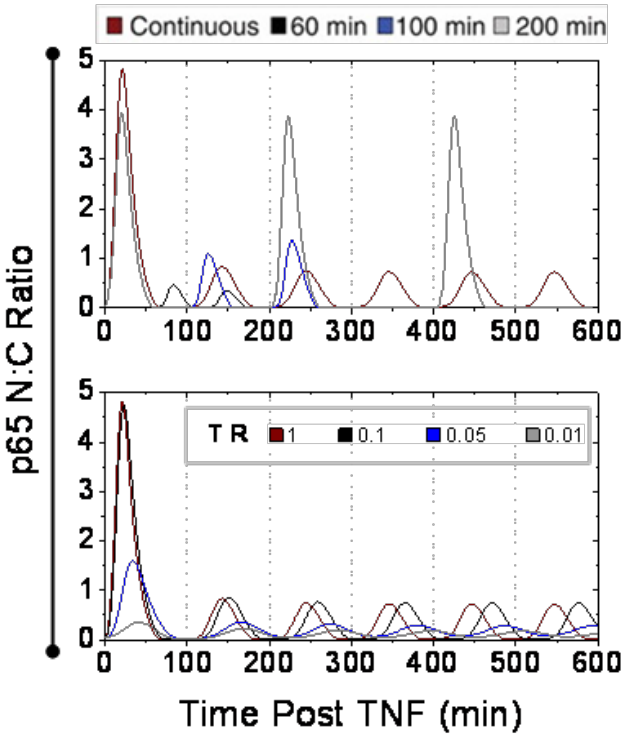


- TNFα required for A20 inhibition
  - Can fit repeat pulse data
  - Reducing the dose (parameter *TR*) fails to maintain oscillations

D. Turner *et al.*, *J. Cell Sci.* 123: 2834-2843

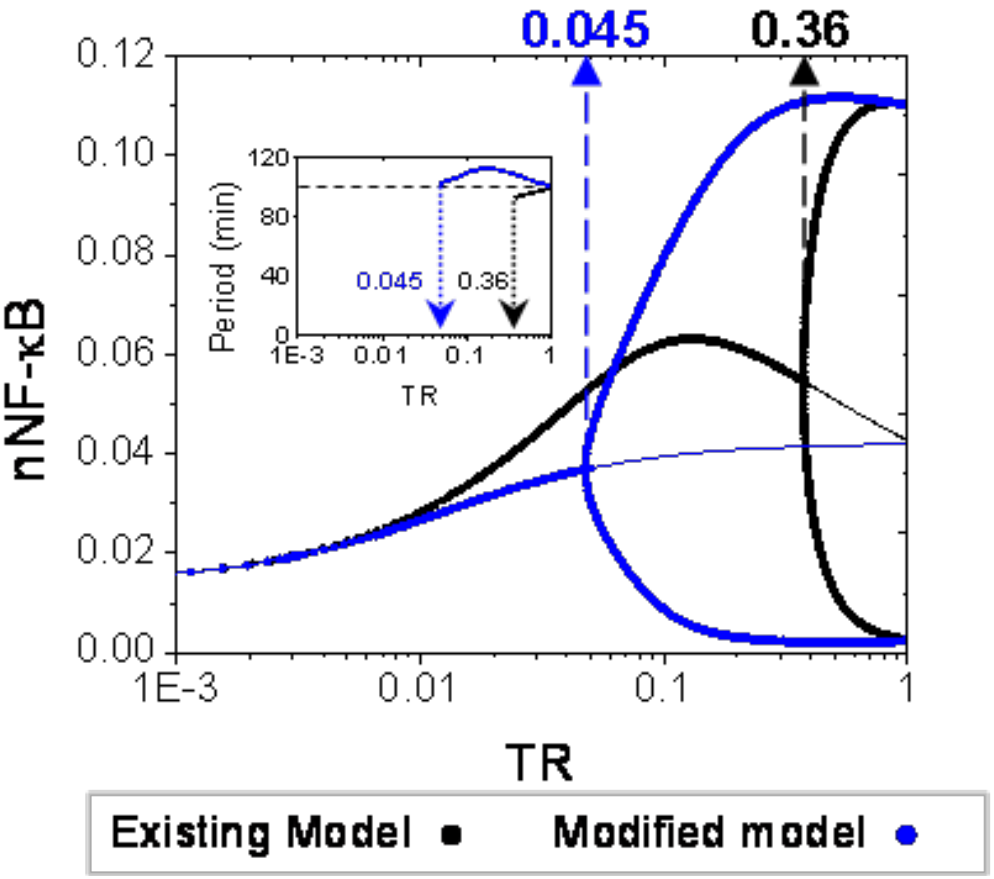
Modification of model structure





- Remove dependence of A20 inhibition on TNFα stimulation
  - Can still fit repeat pulse data
  - Reducing the dose (parameter *TR*) allows maintenance of oscillations to much lower doses

D. Turner *et al.*, *J. Cell Sci.* 123: 2834-2843



- Removal of dependence of A20 inhibition on TNFα stimulation
  - Shift of Bifurcation point to lower *TR*
  - Stable oscillations can be maintained for lower doses

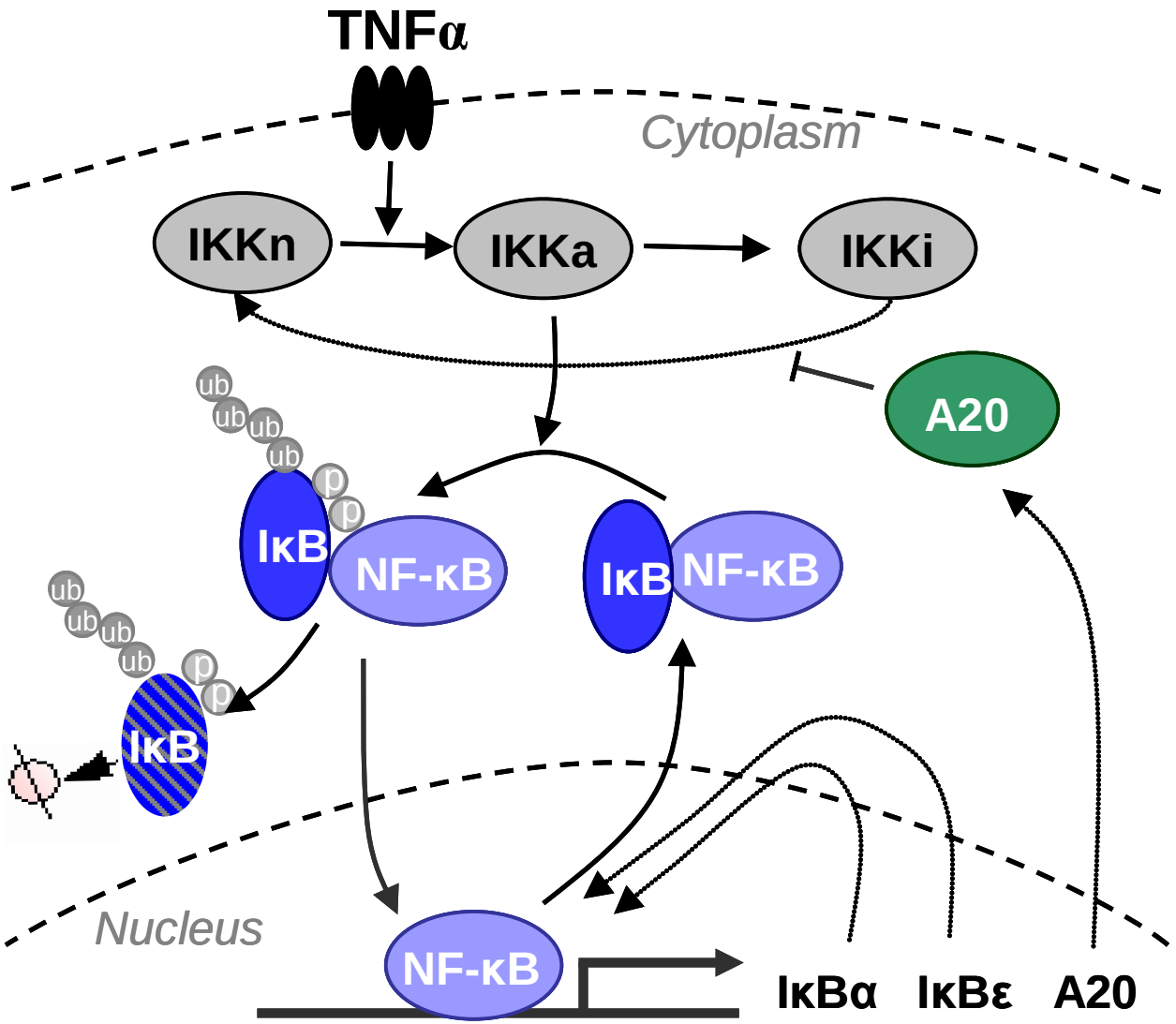
D. Turner *et al.*, *J. Cell Sci.* 123: 2834-2843

NF-κB dynamics

- What is the source of cell to cell heterogeneity?

Cells like to be different!

NF-κB Pathway: IκBε negative feedback



"All models are wrong… some are useful"

– Prof. George Box

Ashall *et al.*, *Science*, (2009) 324: 242

P. Paszek

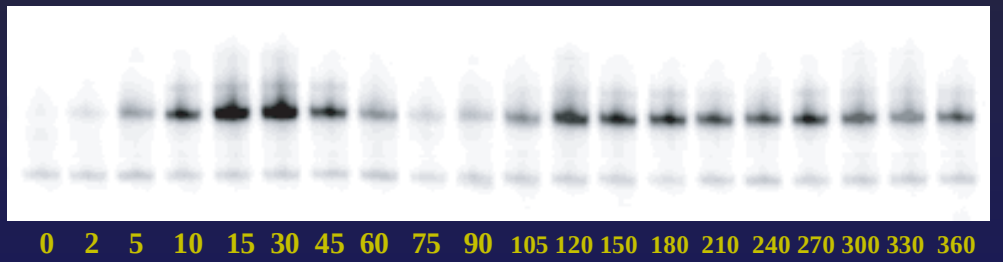
Knock out IκBε–/– MEFS show oscillations at the population level

- EMSA assays,
- Constant TNFα stimulation

Hoffmann *et al*., (2002)
Science **298:** 1241

IκBβ-/-. ε-/-

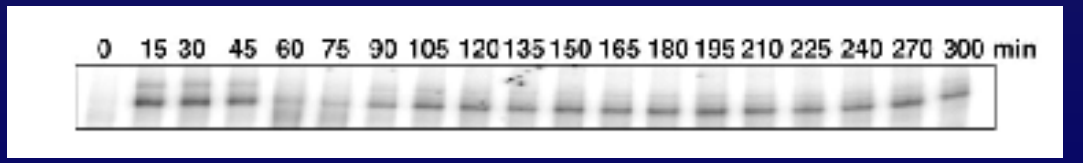
WT



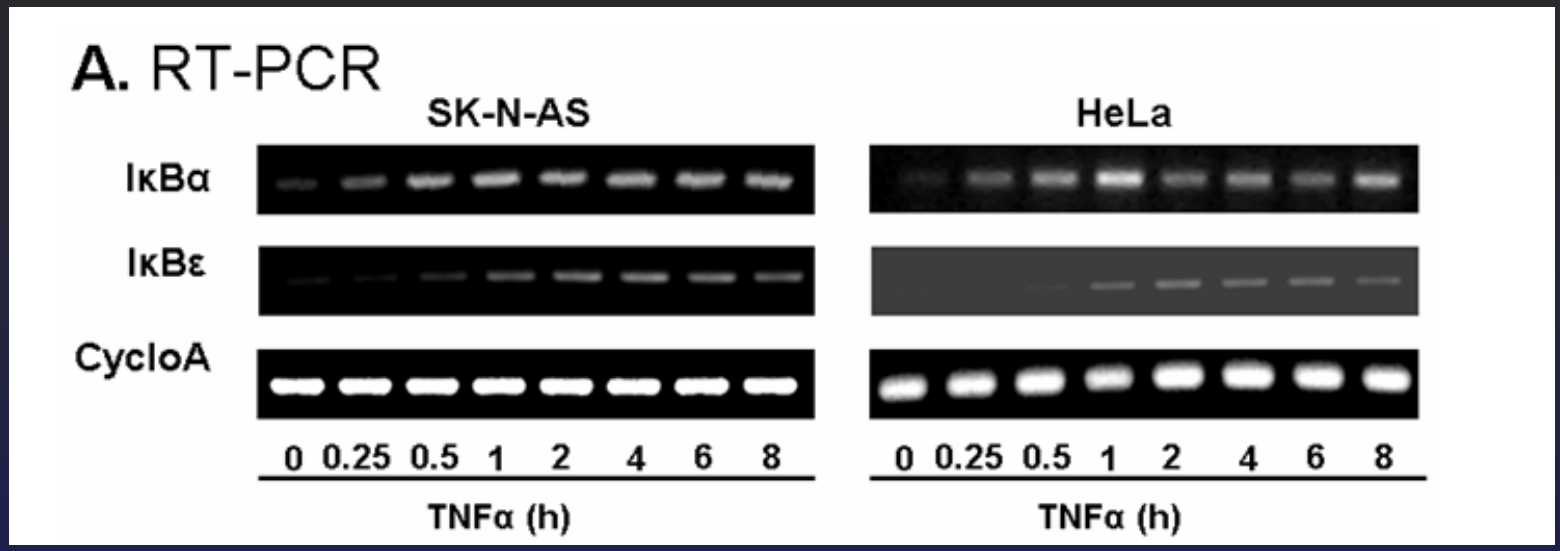
IκBε-/-

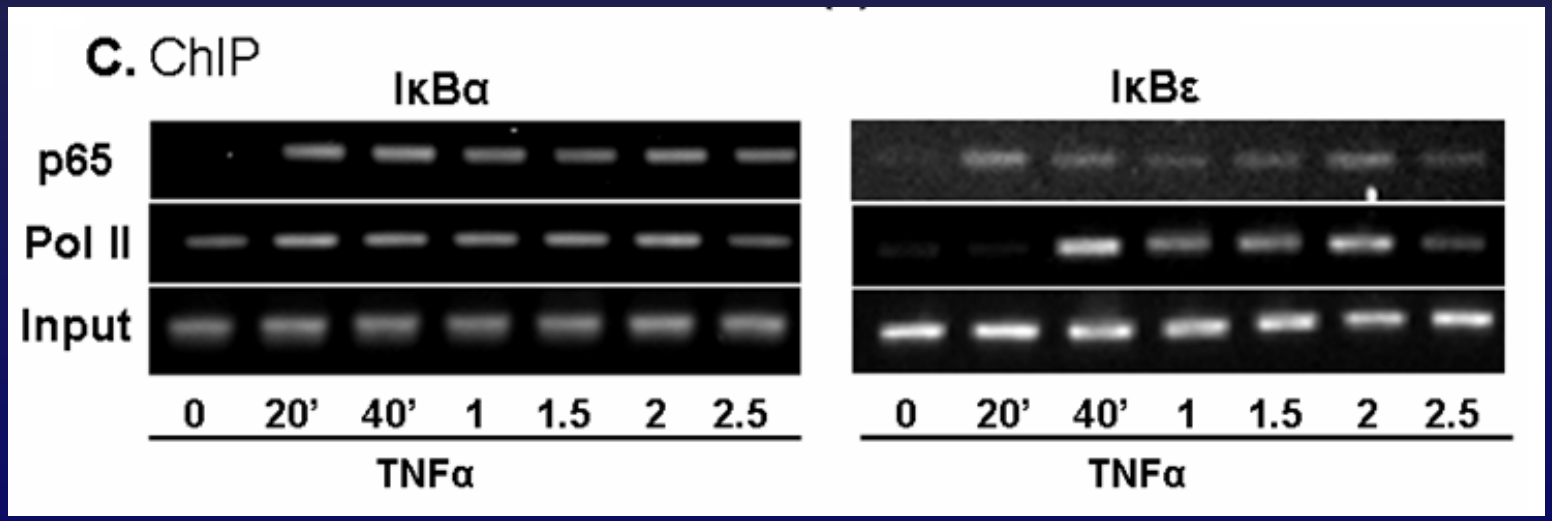
Kearns *et al*., (2006)
J. Cell Biol. **173**: 659

WT



Mechanism of delayed IκBε transcription

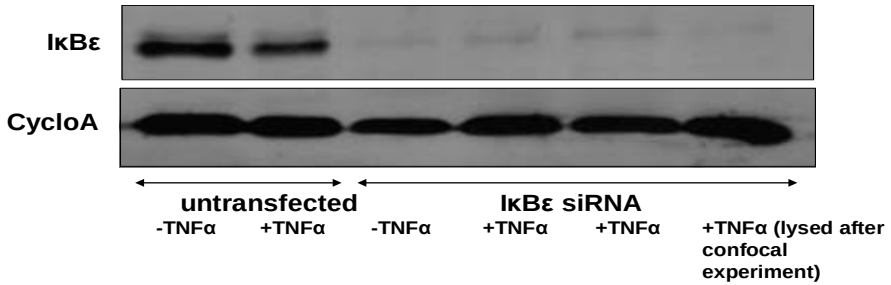


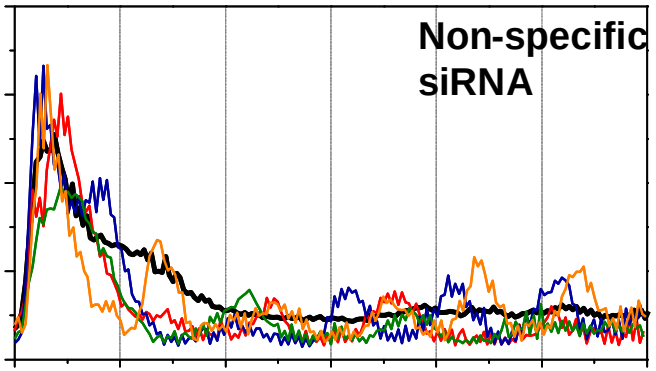


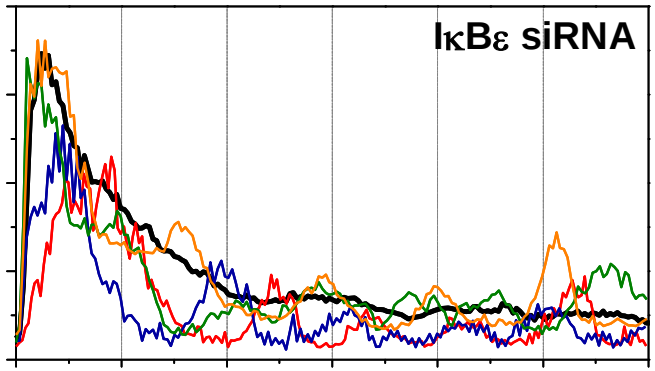
Ashall *et al.*, *Science*, (2009) 324: 242

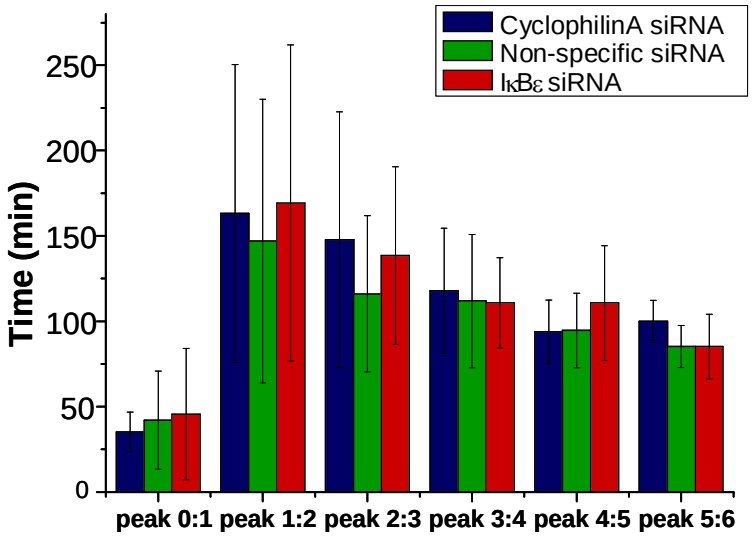
L. Ashall

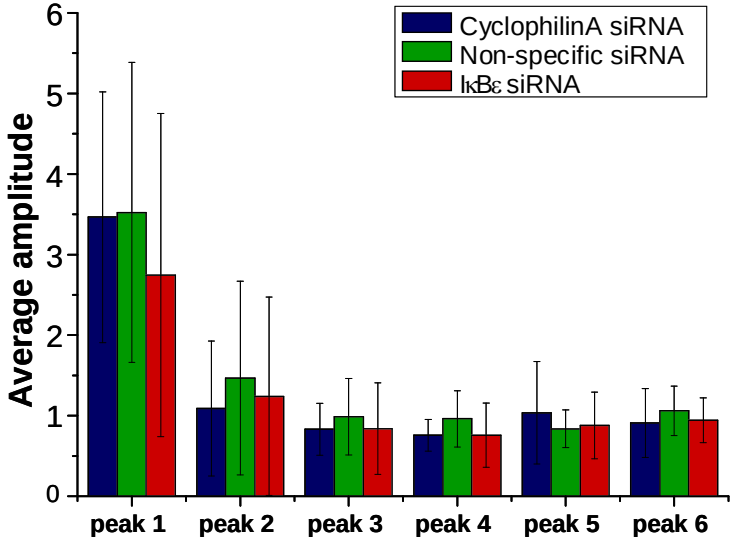
Knockdown of IκBε expression does not affect oscillation amplitude or period in response to TNFα



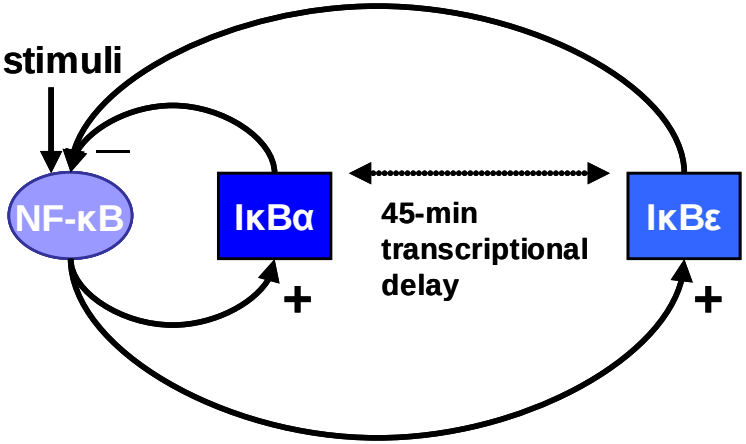


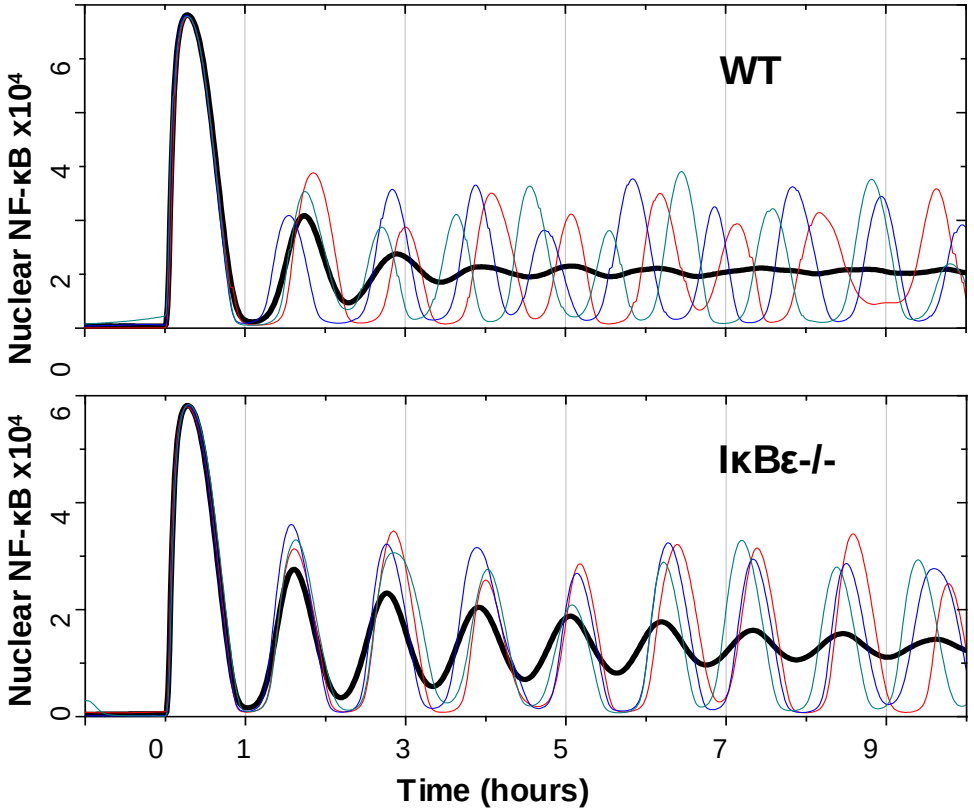






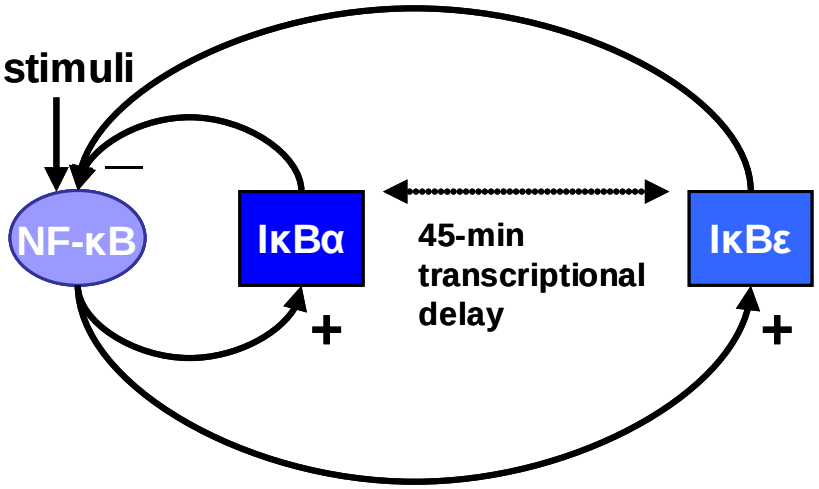
The IκBε feedback loop generates noise creating phase differences in different cells

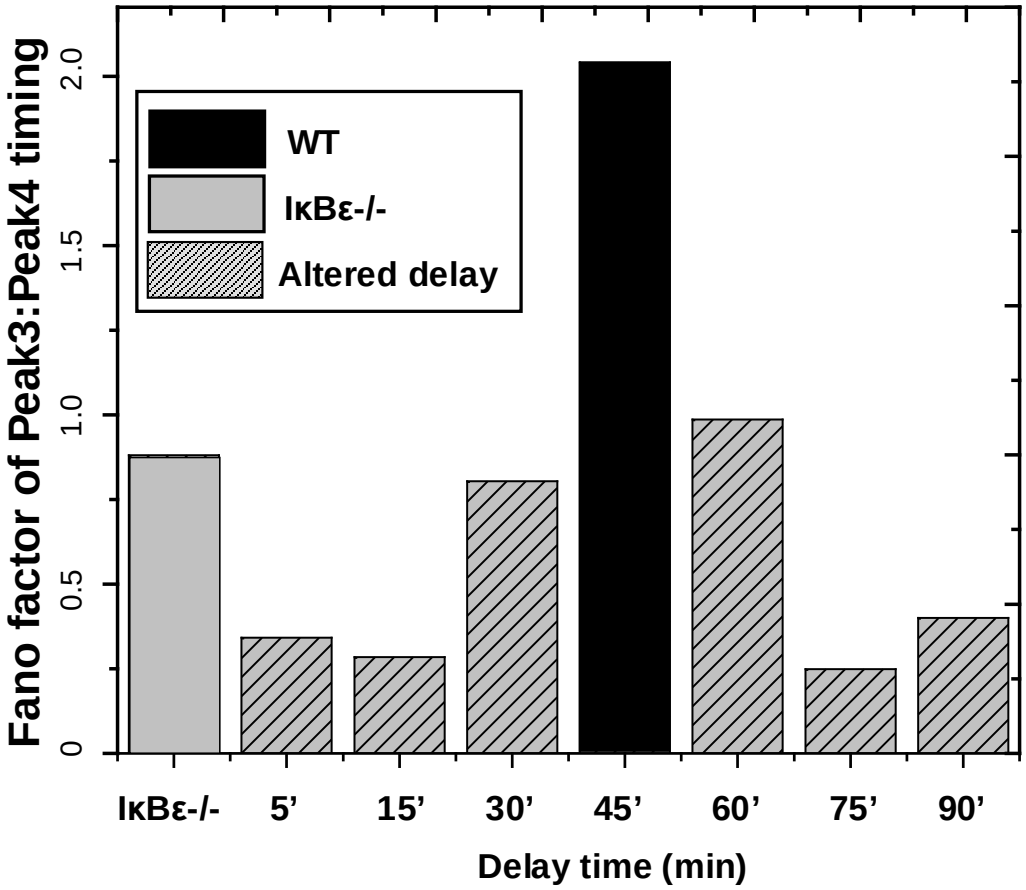




Ashall *et al.*, *Science*, (2009) 324: 242

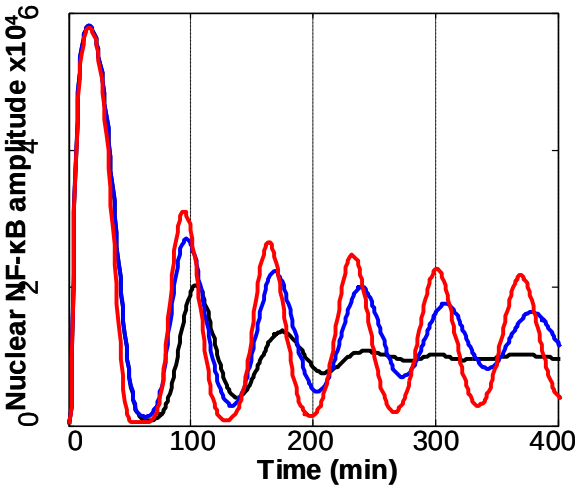
A counter-intuitive prediction: The IκBε feedback loop has optimised delay timing for the generation of maximal cell to cell heterogeneity

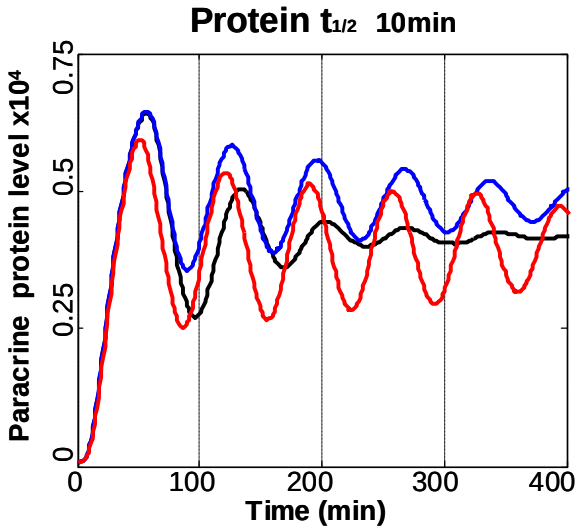


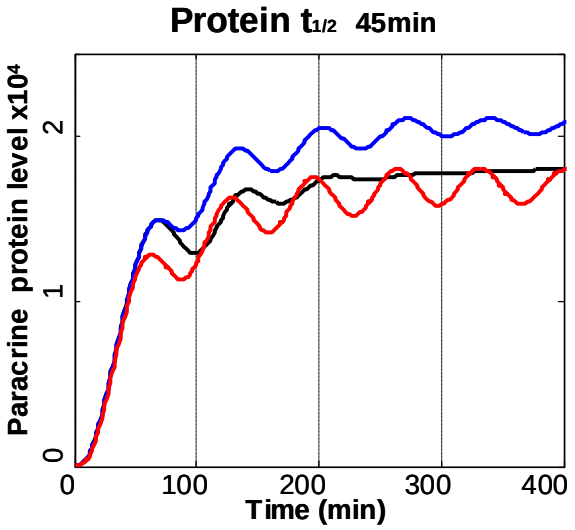


P. Paszek, *et al.*, *PNAS*, (2010) 107:11644

Simulated effect of the IκBε feedback loop on population-level fluctuations in TNFα expression.







P. Paszek, *et al.*, *PNAS*, (2010) 107:11644

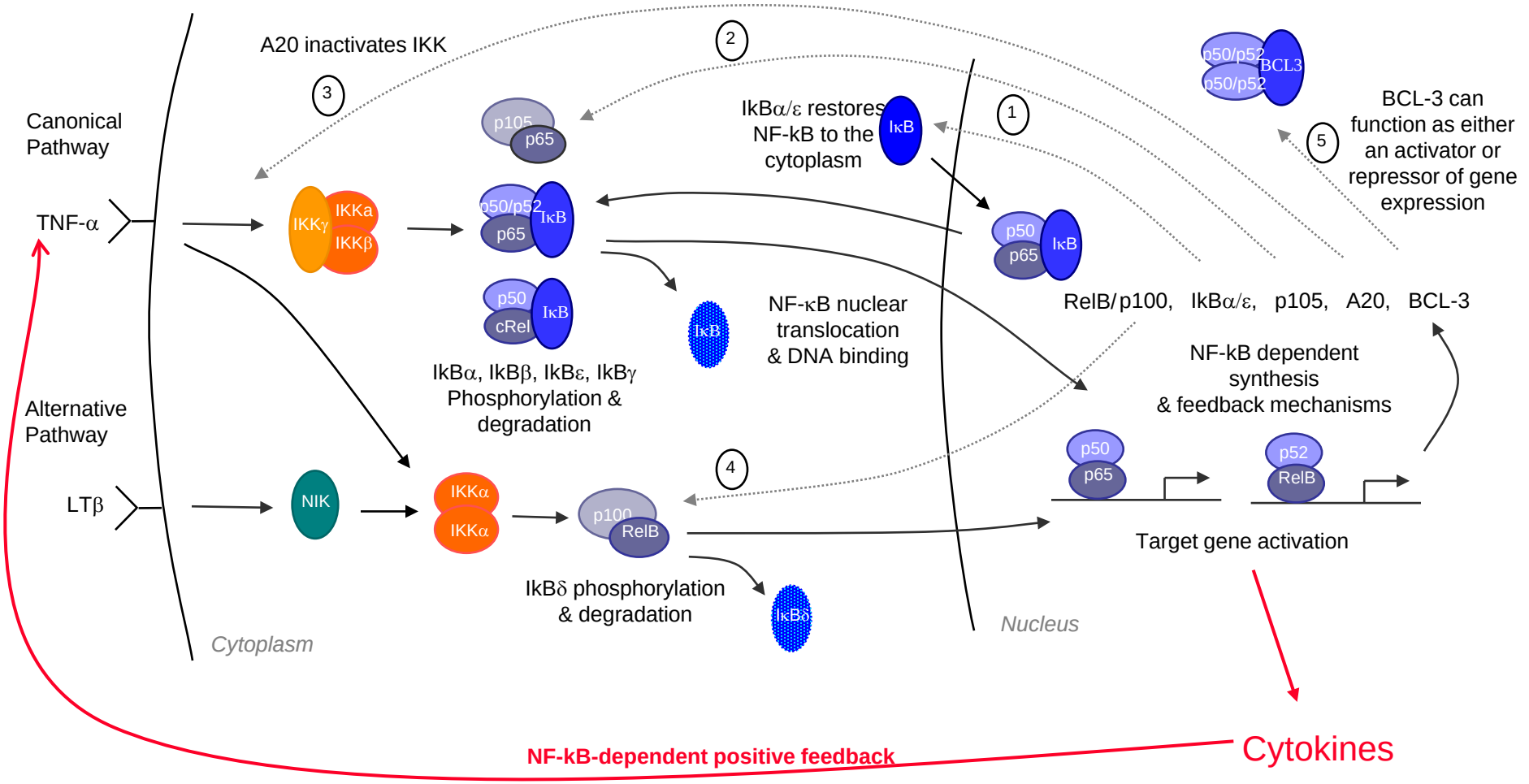
Engineered heterogeneity - hypotheses

- Cells use oscillations to accurately control gene expression
- TNFα is controlled by NF-κB and may be secreted in pulses with each oscillation.
- If cells are synchronous, then the single cell oscillations could lead to fluctuations in TNFα concentration.
- Tissue level fluctuations in TNFα could be dangerous due to positive feedback

NF-κB as a complex timing system:

NF-κB Family Members & Additional Feedback Loops

Activating Stimuli | Dimer Pairs & Exchange | Feedback Regulation | Combined Functional Response



Complexity

- Canonical vs alternative pathway
- NF-κB modifications
- Dimerisation and protein interactions
- Signal dependent IκB processing

Oscillatory Systems

- Calcium -s/mins (many pubs - Dolmetsch *et al.*, *Nature*, 1997)
- MSN2 ~6 min (Jacquet et al.)
- ERK ~15 min (Shankaran *et al.*, *Mol. Sys. Biol.* 2009)
- Transcription cycles ~ 10s of min (e.g. Metivier, *et al.*, *Cell* 2003)
- NF-κB ~100 min (Nelson *et al.*, *Science* 2004)
- N-FAT / Crz1 mins/random (Cai *et al.*, *Nature* 2008)
- Segmentation (Notch/Wnt/FGF ~100 min (Palmeirim, *et al.*, *Cell* 1997)
- STATs ~2h (Yoshiura *et al.*, *PNAS*, 2007)
- p53 ~5-6 h (Lahav, *et al.*, *Nature Genetics* 2004)
- Cell cycle/E2F ~18 h **E2F/NF-κB cross-talk**
- Circadian Rhythms 1 day
- Seasonal Rhythms months/year

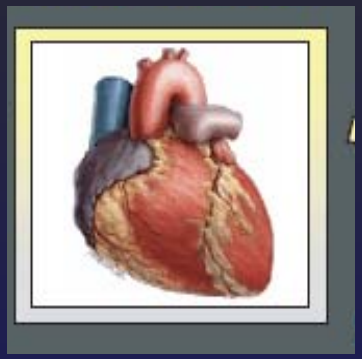
Coupled oscillators

- Driven oscillators – e.g. TNFα pulsing
- Gated oscillators = One oscillation permits the other only during a particular phase - (NF-κB-E2F1)
- Loosely coupled oscillators- Mathematics predicts non-linear and unexpected effects on timing.

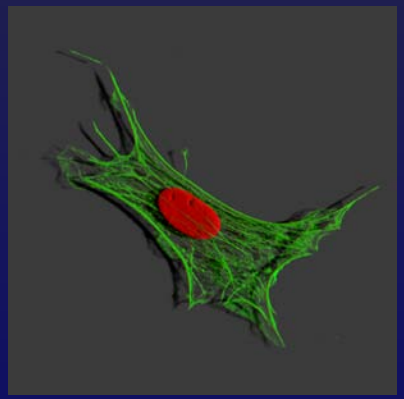
Working Across Biological scales

–Length scales and time scales

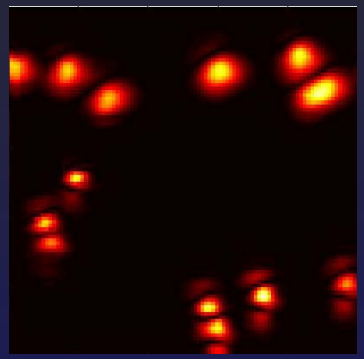
1 m



0.10 m



100 μm



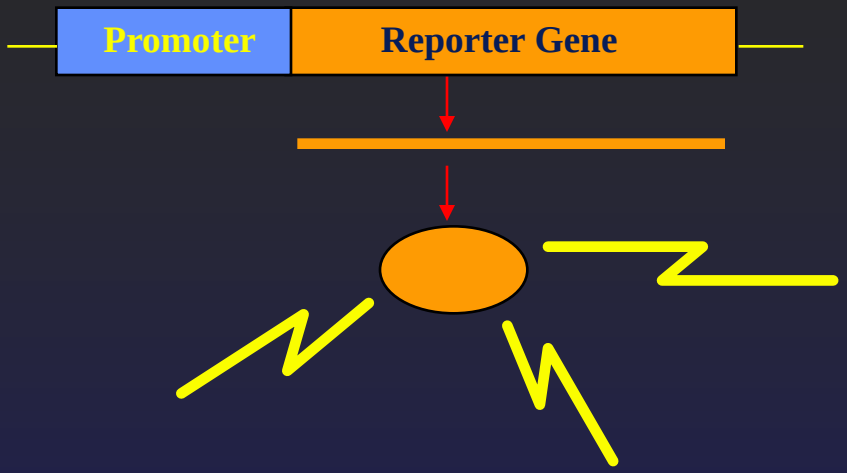
5 nm

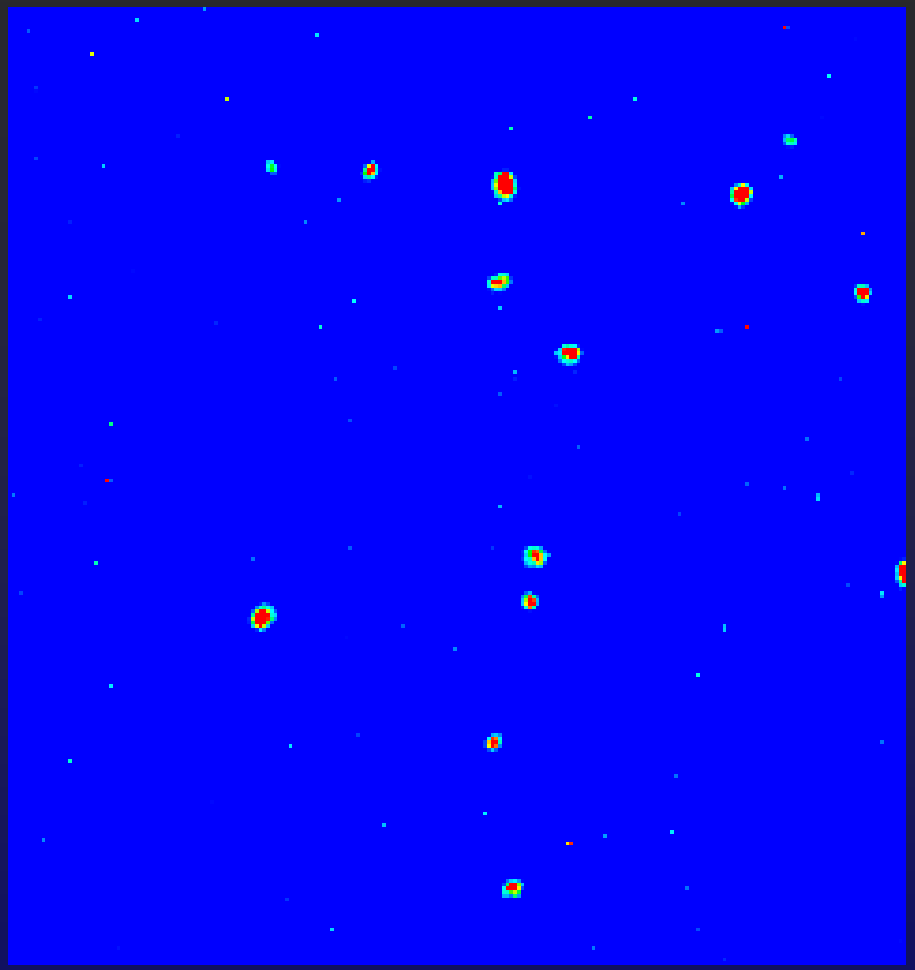
Organism – organ – cell - molecule

Gene Expression

- Gene inactive – wrapped in chromatin
- Transcription factor binds to specific DNA sequence close to the gene.
- Accessory factors form transcription complex
- RNA polymerase binds and begins to move along the DNA making RNA

Imaging of single cell transcription dynamics





White *et al.*, (1990 *Technique*, **2: 194-201**

Craig *et al.*, (1991) *Biochem. J.* **276: 637-641**

White, *et al.*, (1995) *J. Cell Sci.* 108: 441

Rutter, et al., (1995) *Curr. Biol.*, **5: 890-899**

Takasuka *et al., (1998) Endocrinol.* **139: 1361-1368**

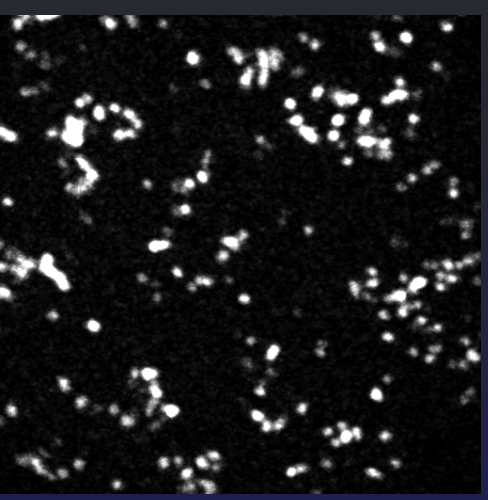
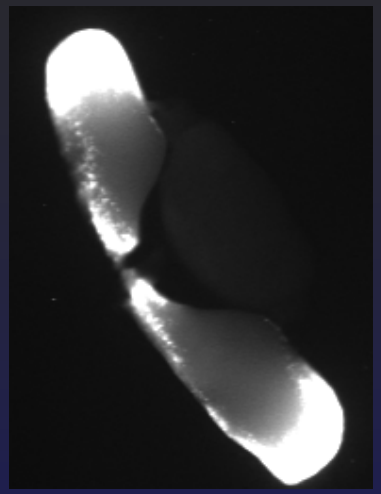
Faria *et al., (2000) PNAS* **97: 3862-3867**

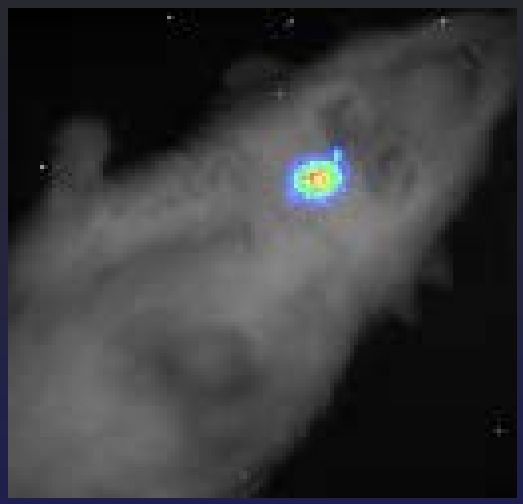
Faria *et al., (2001) Nat. Biotechnol.* **19: 40-44.**

McFerran *et al.*, (2001) *Endocrinol*, **142: 3255-60**

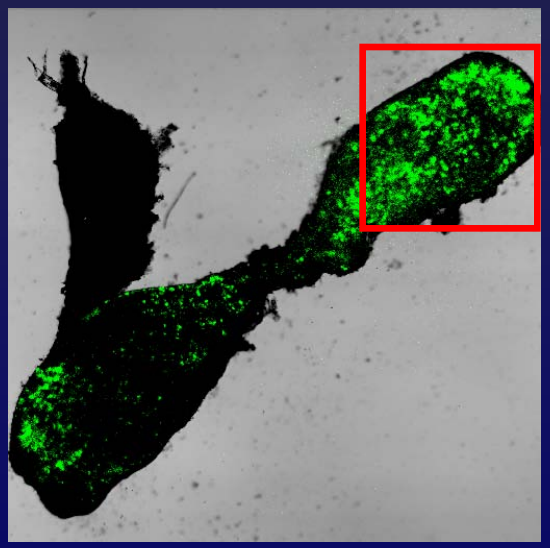
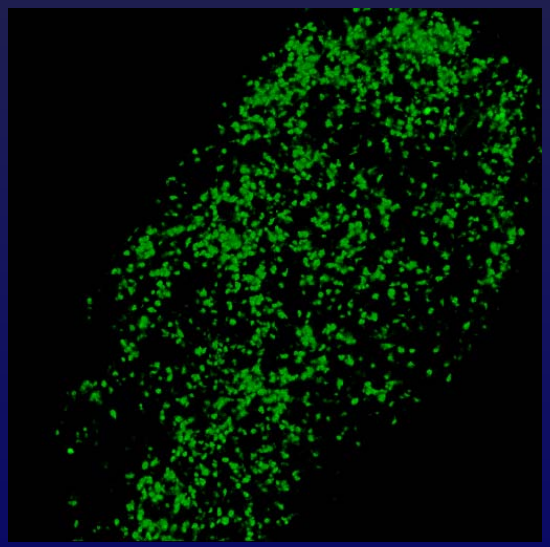
Cell, tissue and in vivo imaging in PRL transgenic rats

hPRL-Luciferase

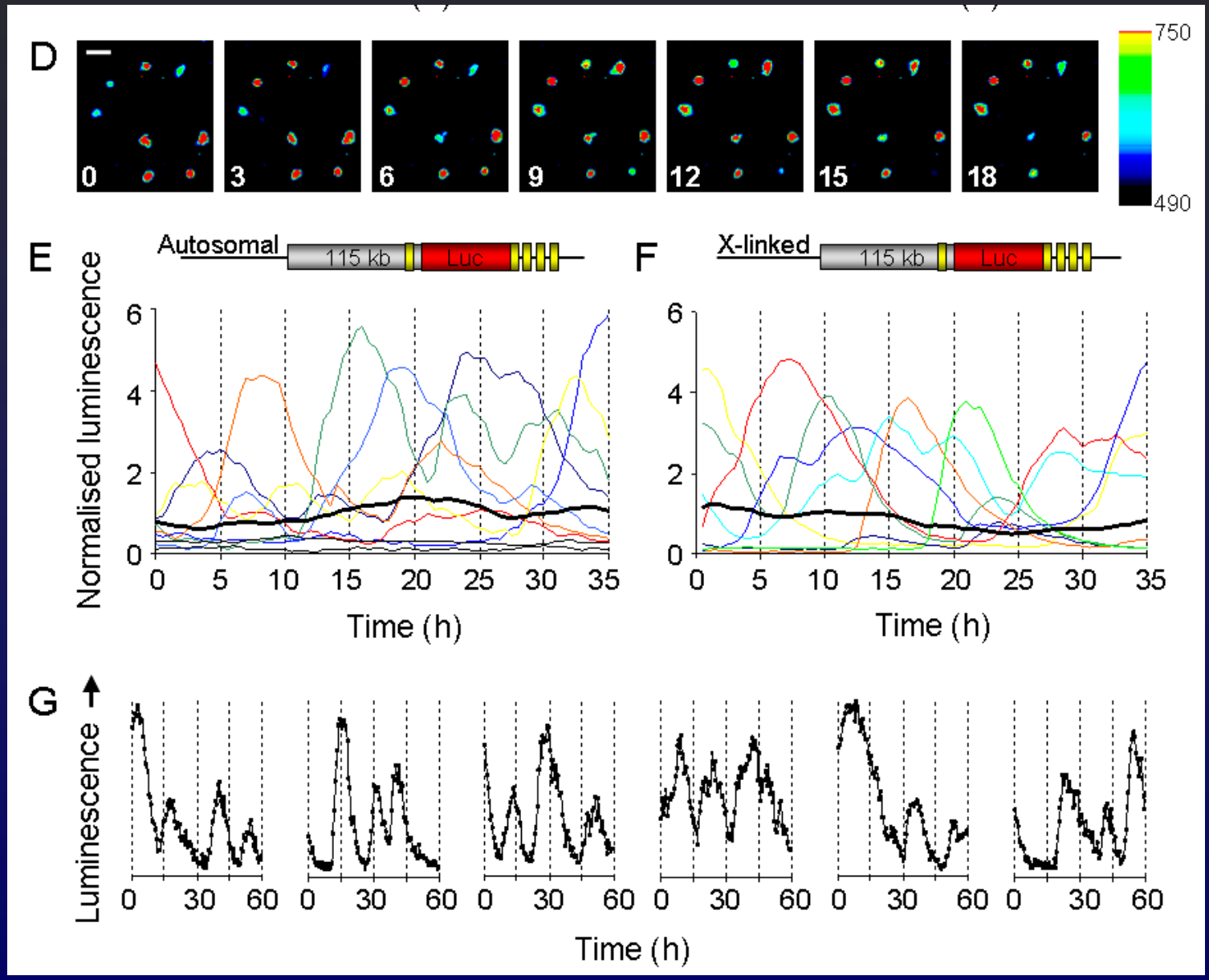


hPRL-dGFP

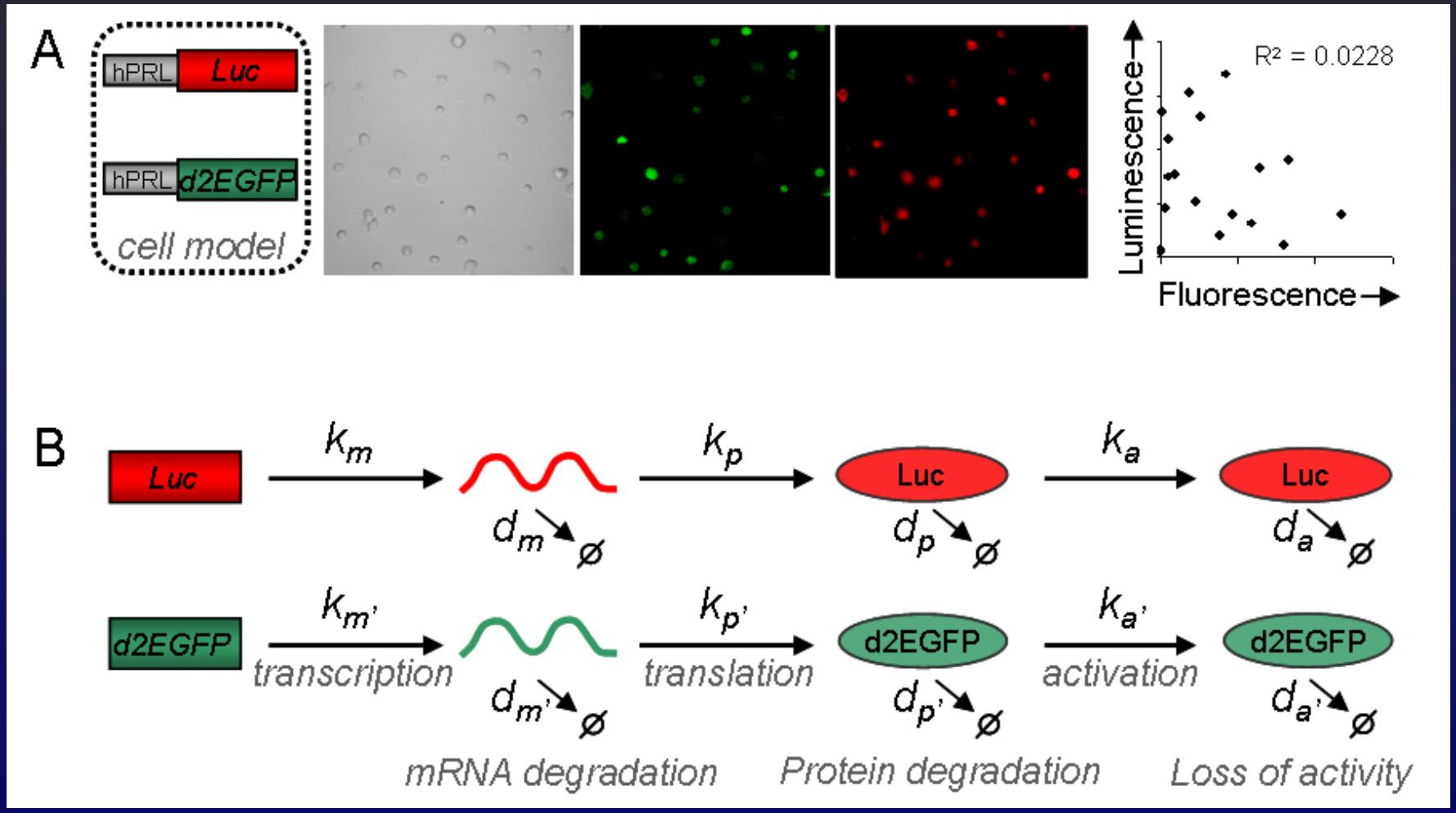
Semprini *et al.* (2009) *Mol. Endocrinol.* **23**, 529
Harper *et al.*, (2010) *J. Cell Sci.* **123**: 424

Apparent cycles in luciferase expression from the PRL promoter in primary cells (and cell lines)



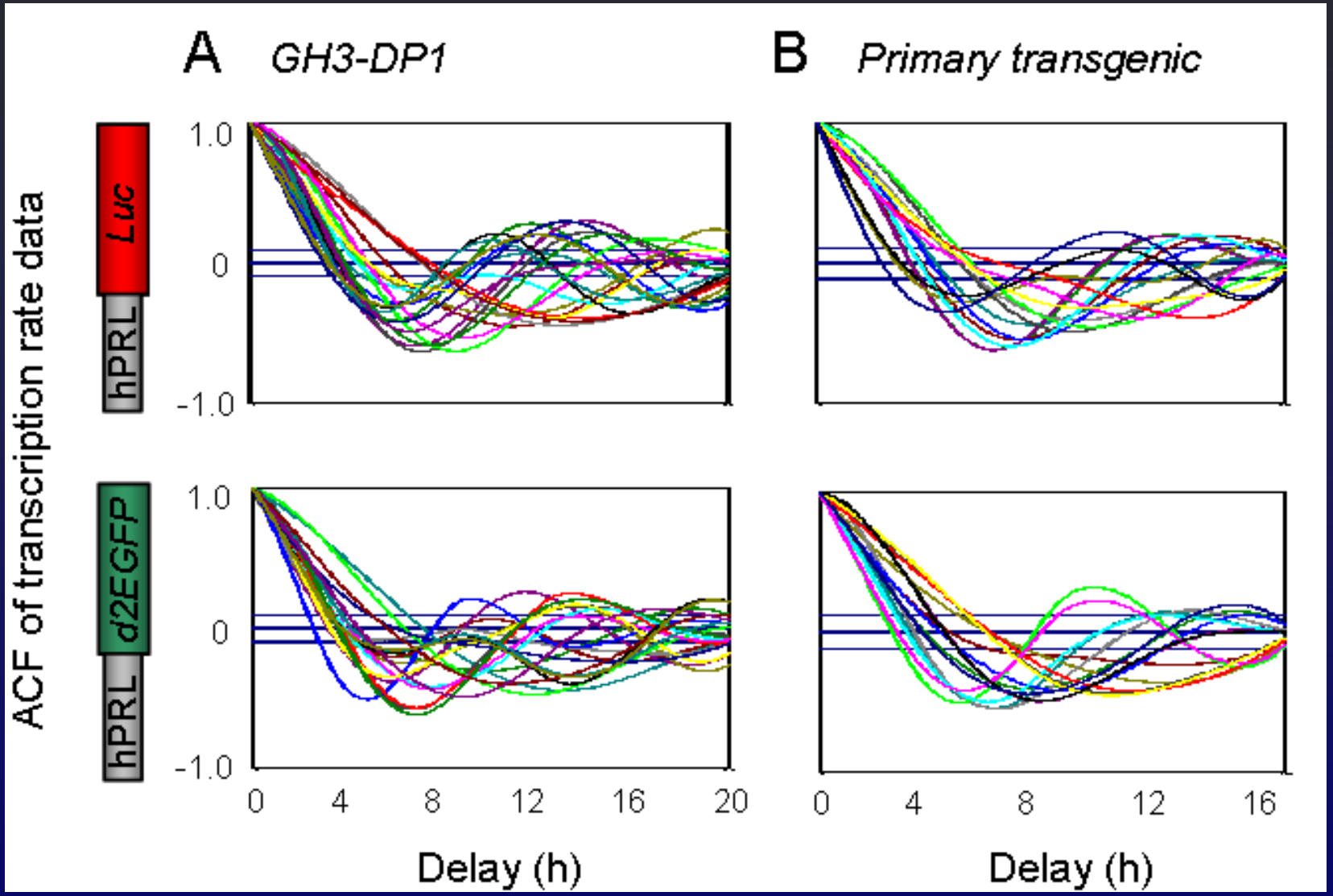
C. Harper

A model for estimating transcription rate from reporter imaging data



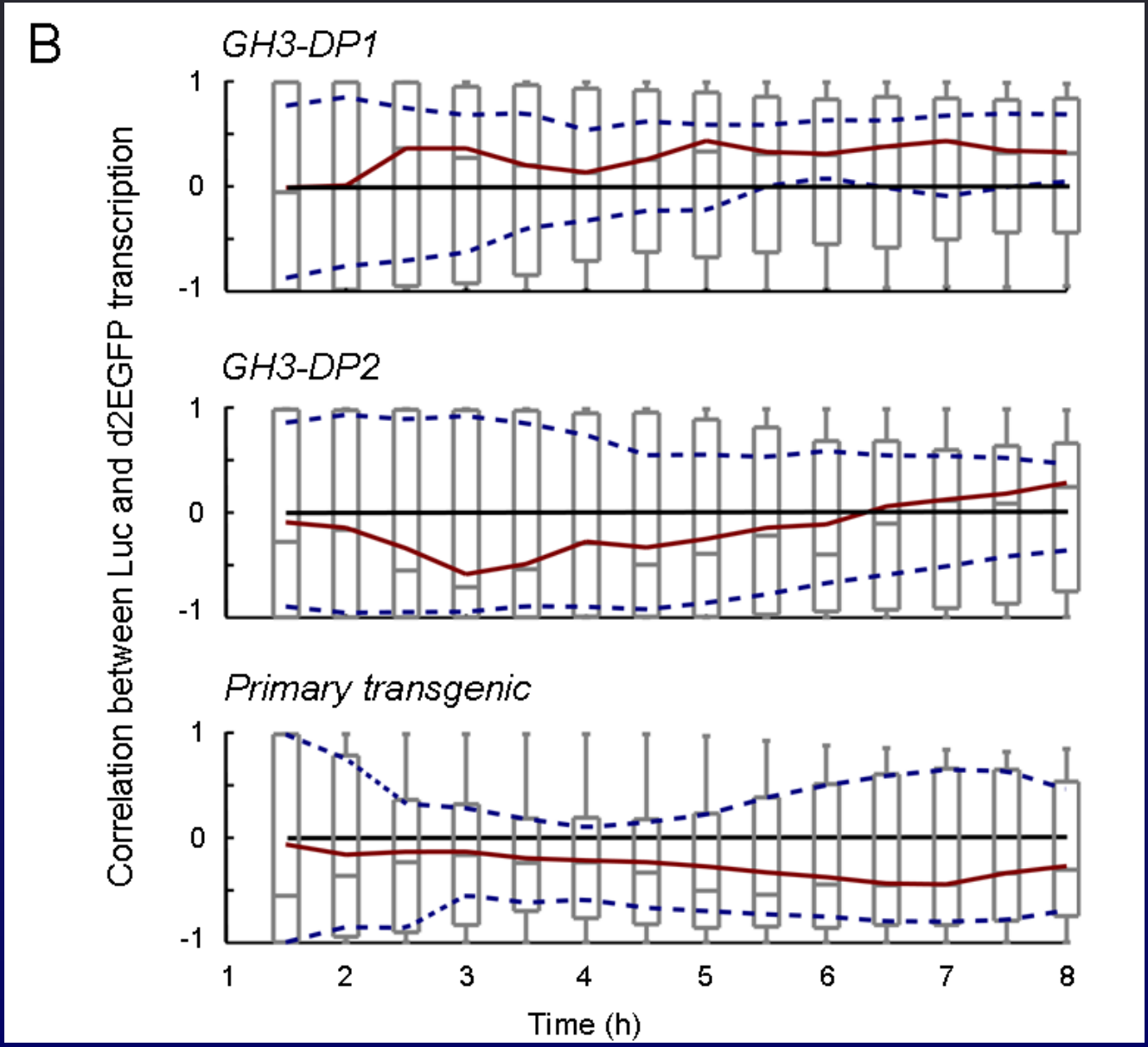
Finkenstadt et al., (2008) *Bioinformatics* 24: 2901

Regular cycles in luciferase and dEGFP expression in cell lines and primary cells

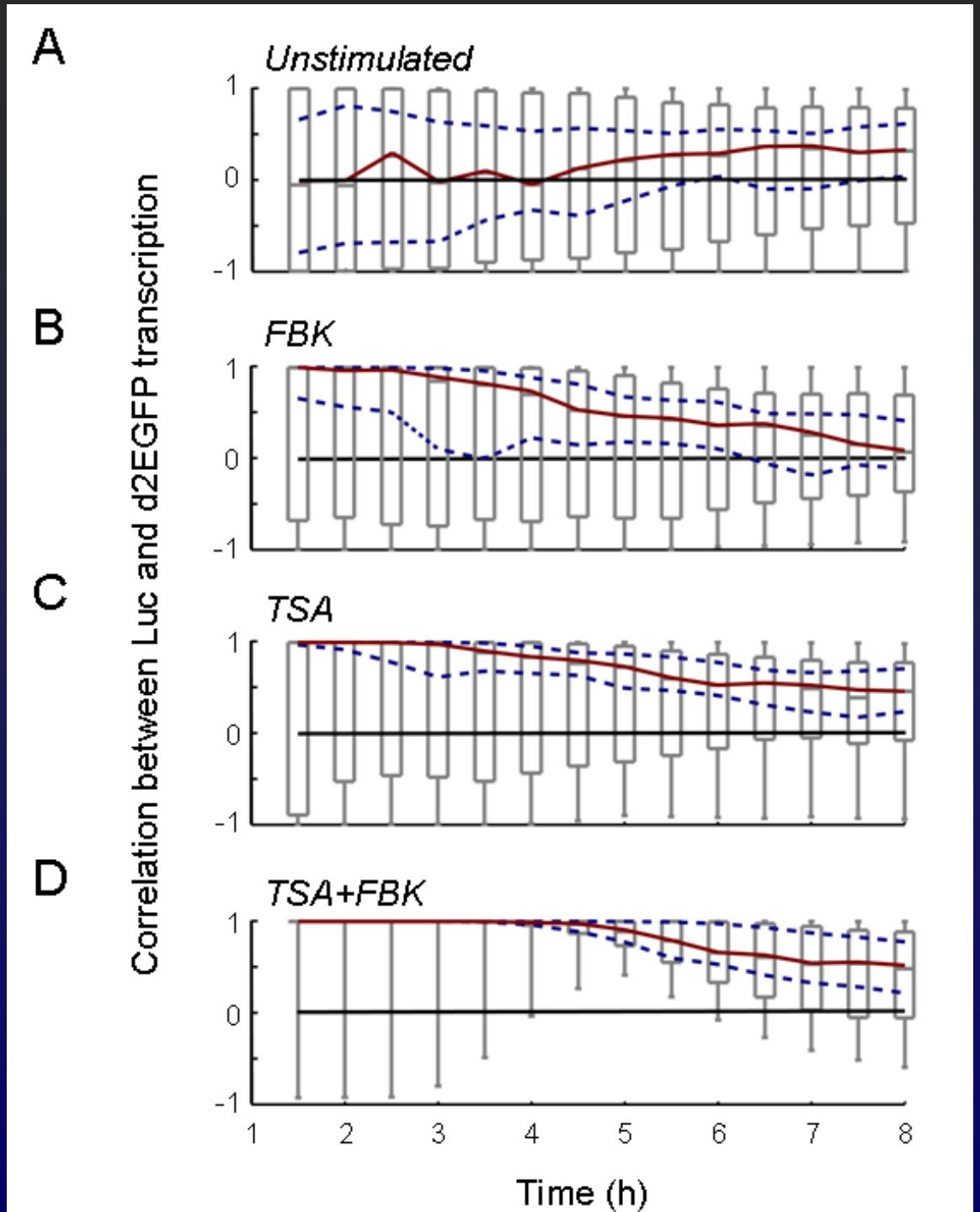


C. Harper et al., (2010) PLOS Biol, 9(4):e1000607

No correlation between dynamics of transgene expression in single pituitary cells



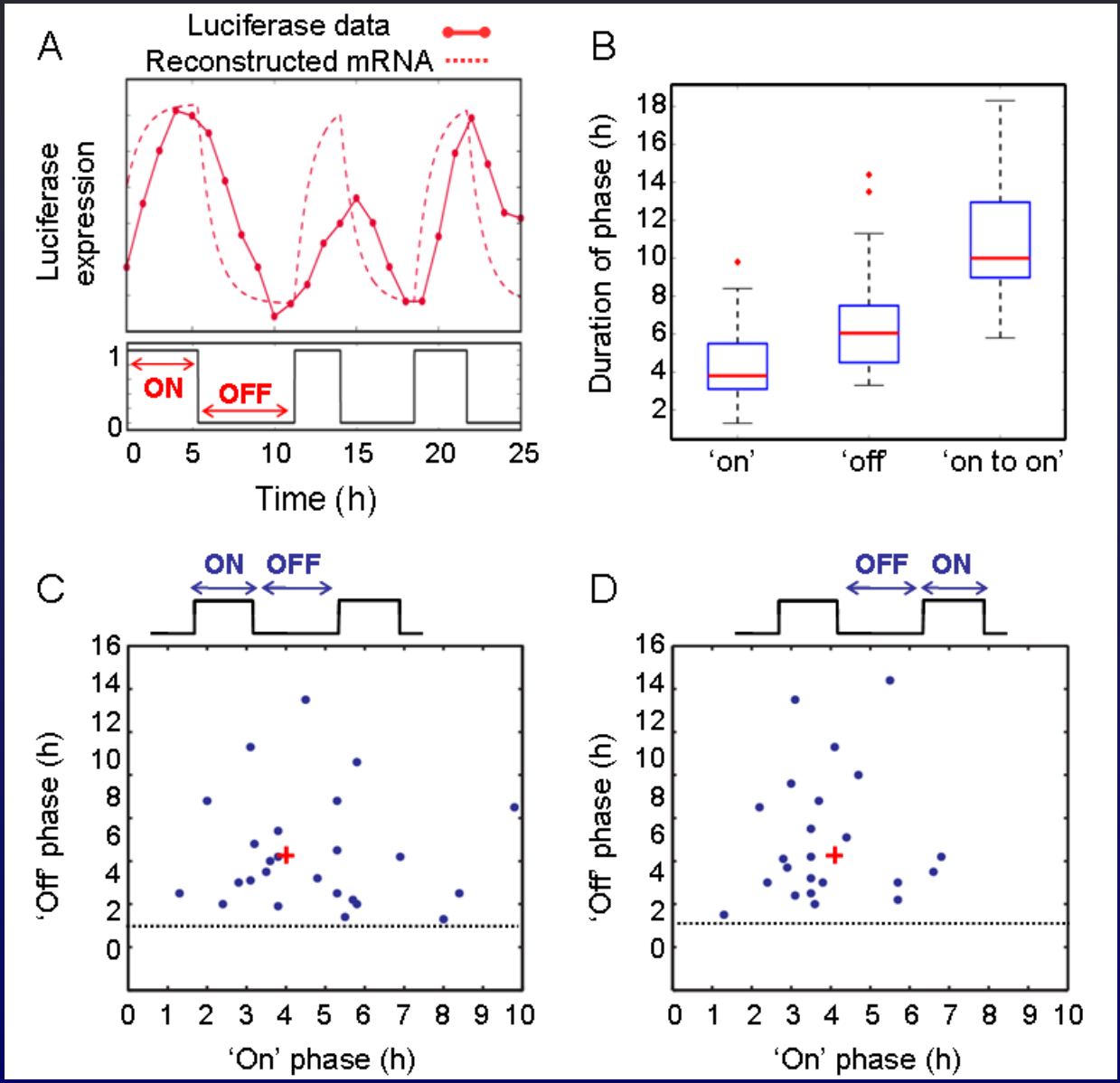
C. Harper
D. Woodcock
B. Finkenstadt



Role of chromatin?

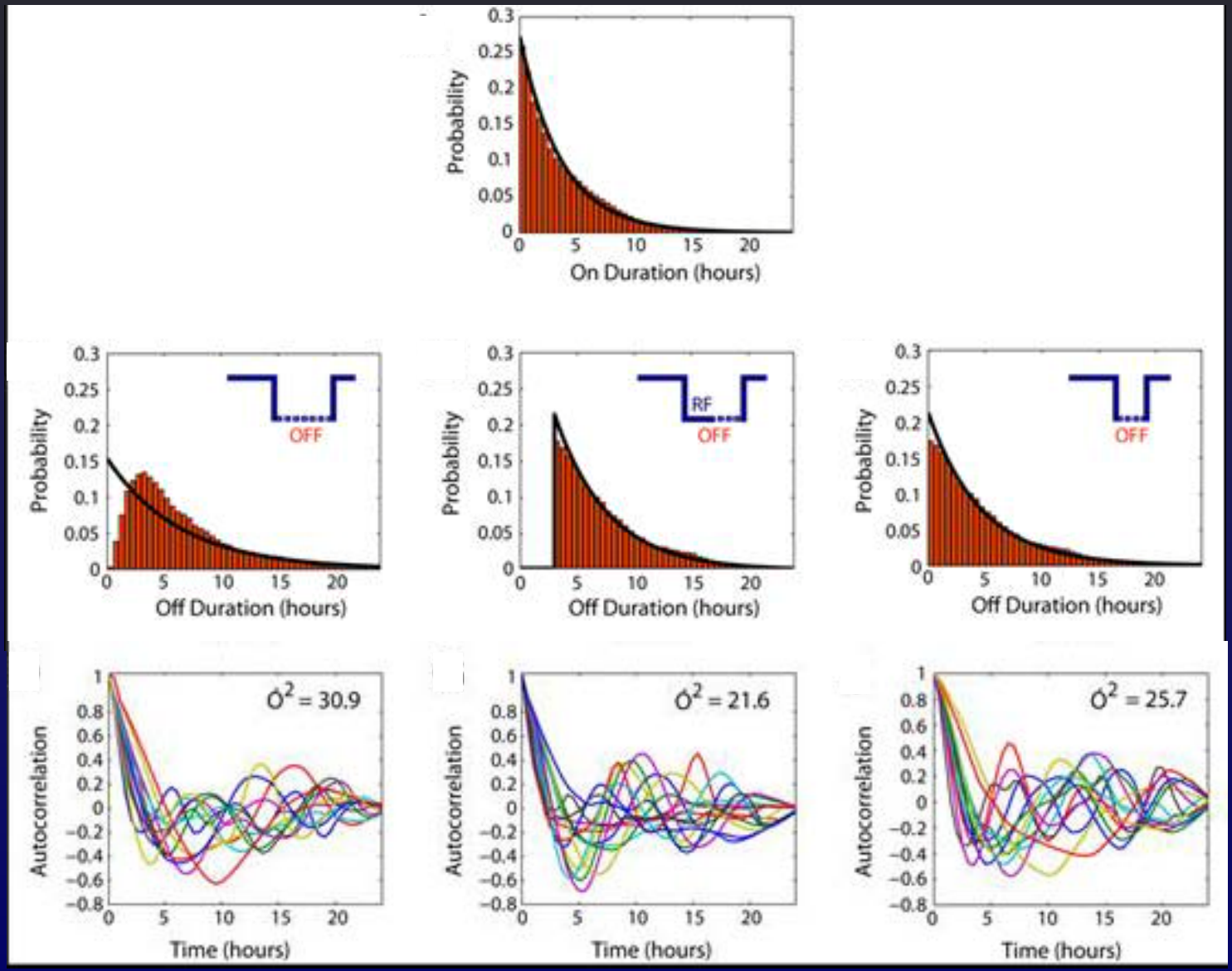
C. Harper
D. Woodcock
B. Finkenstadt

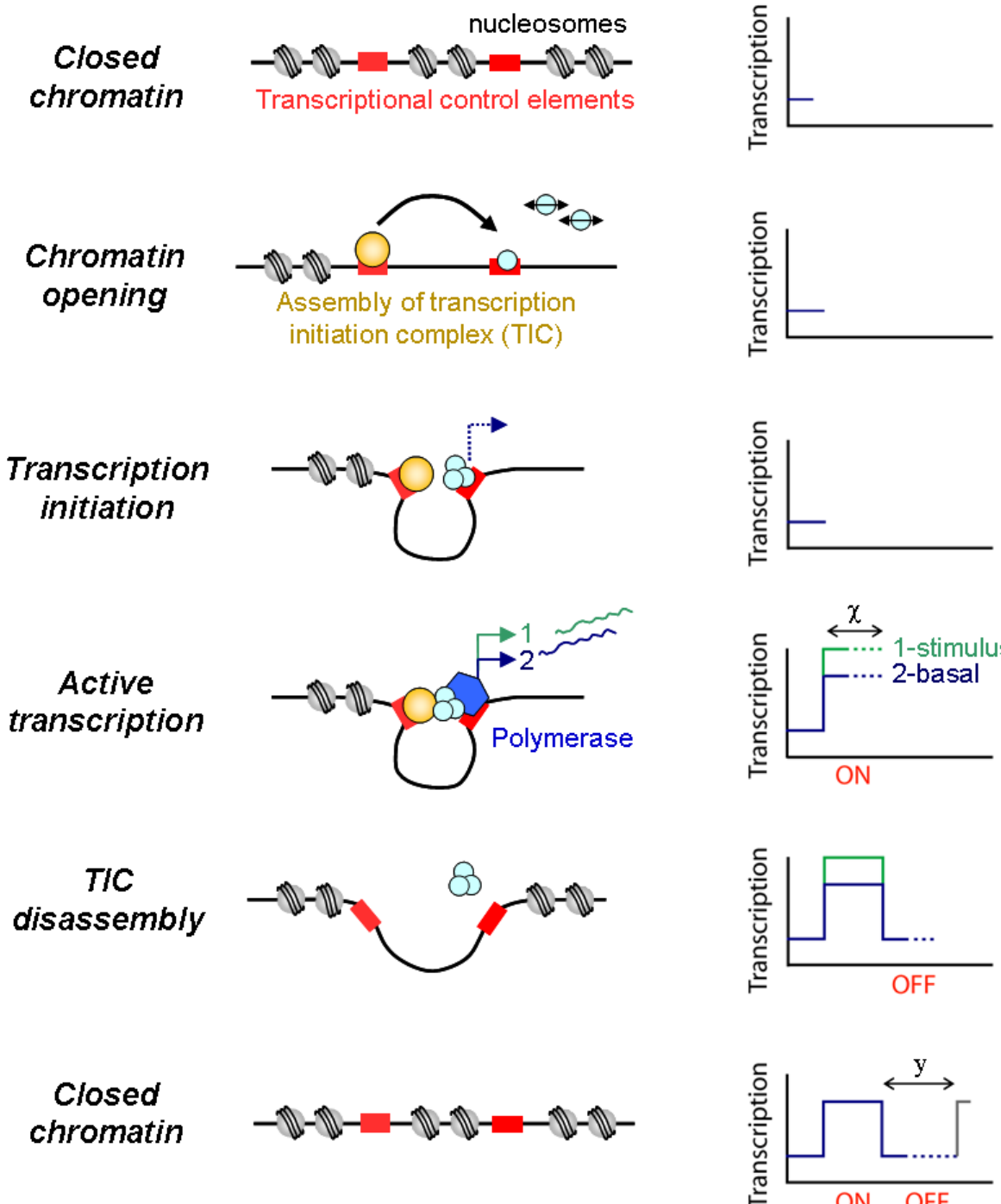
Switch model indicates a refractory period in the transcription cycle



C. Harper
D. Woodcock
B. Finkenstadt

A regular cycle arises from a minimum refractory off-phase




Closed chromatin
nucleosomes
Transcriptional control elements
Transcription
Chromatin opening
Assembly of transcription initiation complex (TIC)
Transcription
Transcription initiation
Transcription
Active transcription
1
2
Polymerase
χ
1-stimulus
2-basal
Transcription
ON
TIC disassembly
Transcription
OFF
Closed chromatin
y
Transcription
ON
OFF

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Chris Sanderson

Dean Jackson
Claire Eyers

Julian Davis
John Mullins

Dave Spiller

(Caroline Horton)
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John Ankers
Dan Woodcock
Sascha Ott
Boris Noyvert
Daniel Jameson

Doug Kell
Dave Broomhead
Mark Muldoon
Adoaha Ihekwaba
Barbel Finkenstadt
Rachel Bearon
Norman Paton

David Rand

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Wellcome Trust

Astra-Zeneca – *J. Unitt*