Signaling Networks and transcriptional control

- combinatorial control
- dynamic control
Virus Induction of Human IFNβ Gene Expression Requires the Assembly of an Enhanceosome

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The Mechanism of Transcriptional Synergy of an In Vitro Assembled Interferon-β Enhanceosome

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Modifying Gene Expression Programs by Altering Core Promoter Chromatin Architecture

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Figure 8. A Model for the IFNβ Gene Enhanceosome
The enhanceosome is a 60 bp segment of DNA bound to the transcriptional activators NF-κB (p50/p65), IRF-1, and ATF-2/c-Jun and HMG l(Y). HMG l(Y) is bound to the minor groove of DNA, while the transcriptional activators are bound to the major groove. The binding of HMG l(Y) to DNA and to NF-κB and ATF-2/c-Jun facilitates the conformational changes required to form the complex. Once assembled, the enhanceosome makes multiple contacts with the basal transcription complex. The relative sizes of the proteins and the length of the DNA covered are not drawn to scale.
Multiple TF sites and complex stimuli are required for β-IFN induction

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<td>1.4</td>
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</table>

Figure 1. Comparison of the Specificity and Strengths of Synthetic and Natural Virus-Inducible Enhancers

Comparison of the transcriptional activities of the intact IFNβ promoter (bottom) with the individual homopolymeric PRDs after transient transfection into L929 cells. Each of the elements responds to virus infection as well as to other extracellular signals. By contrast, the IFNβ promoter is induced only by virus. Numbers indicate fold induction.
**β-interferon enhancer: multiple TFs provide qualitatively different regulation than individual TFs**

Figure 1. Establishment of an In Vitro Transcription System for Synergistic Activation of the IFNβPromoter(A) Human IFNβgene promoter. The enhancer region contains four positive regulatory domains designated PRDI, II, III, and IV. Transcription factors that bind to each of the elements are shown: the ATF2/c-JUN heterodimer binds to PRDIV, an IRF family member protein binds to both PRDI and PRDIII, the p50/p65 heterodimer of NF-kB binds to PRDII, and HMG I(Y) binds to the three AT-rich sequences within the enhancer. All of the PRD-binding factors were purified by nickel affinity chromatography. The purified hexahistidine-tagged proteins were separated by SDS-PAGE and detected by staining with Coomassie blue.

(C) Titration of PRD-binding units. In vitro transcription was performed in the absence (lane 1) or presence (lanes 2–9) of each PRD-binding unit (ATF2/c-JUN, IRF1, HMG I[Y], and p50/p65) or all of the PRD-binding units (lanes 10 and 11) as indicated. The amounts of each recombinant PRD-binding factor were as follows: lanes 2, 4, 6, 8, and 10, 200 fmol and lanes 3, 5, 7, 9, and 11, 800 fmol.
Different TF binding sites are location coordinated

PRDIV to PRDII substitution. The transfected cells were induced with either Sendai virus, TNFα, IFNγ, or TNFα plus IFNγ. Fold induction is the ratio of CAT with and without the indicated inducers. Shown is the average of two independent experiments.

(B) The correct helical phasing of transcription factor–binding sites is required for virus induction of the IFNβ gene enhancer. Human HeLa cells were transfected with the wild-type IFNβ–CAT reporter construct or with the indicated insertion mutants. The transfected cells were either induced with Sendai virus or mock induced, and the CAT activity was measured from extracts of the transfected cells. Fold virus induction is the ratio of CAT with and without virus induction. Shown is the average of four independent experiments. Variability in virus induction among individual experiments and among individual constructs in the same experiment was less than 40% and 10%, respectively.
ATF2$_{195}$ binds specifically to PRDIV and interacts with HMG I(Y) and p50, and its affinity for PRDIV is enhanced by HMG I(Y). In contrast, ATF2$_{192}$ binds to PRDIV but does not interact with either HMG I(Y) or p50.
Histone like proteins facilitate cooperative binding of TFs
Nucleosome movement can be a rate limiting step.
Nucleosome position can impose combinatorial TF requirement
Molecular Determinants of Crosstalk between Nuclear Receptors and Toll-like Receptors

Sumito Ogawa,1 Jean Lozach,1 Chris Benner,1,2 Gabriel Pascual,1 Rajendra K. Tangirala,4,7 Stefan Westin,4 Alexander Hoffmann,5 Shankar Subramaniam,2 Michael David,3 Michael G. Rosenfeld,6 and Christopher K. Glass1,2,6
1Department of Cellular and Molecular Medicine
Interaction between NF-kB and IRF3 sensitizes genes to inhibitory effect of steroid
Dexamethasone receptor GR disrupts protein-protein interaction between NF-κB and IRF3
PC12 cell neuronal differentiation is associated with prolonged p21ras activity and consequent prolonged ERK activity.

**Qui MS, Green SH.** Department of Biology, University of Iowa, Iowa City 52242.

Expression of oncogenic ras in PC12 cells causes neuronal differentiation and sustained protein tyrosine phosphorylation and activity of extracellular signal-regulated kinases (ERKs), p42erk2 and p44erk1. Oncogenic N-ras-induced neuronal differentiation is inhibited by compounds that block ERK protein tyrosine phosphorylation or ERK activity, indicating that ERKs are not only activated by p21ras but serve as the primary downstream effectors of p21ras.

Treatment of PC12 cells with nerve growth factor or fibroblast growth factor results in neuronal differentiation and in a sustained elevation of p21ras activity, of ERK activity, and of ERK tyrosine phosphorylation.

**Epidermal growth factor**, which does not cause neuronal differentiation, stimulates only transient (< 1 hr) activation of p21ras and ERKs. These data indicate that transient activation of p21ras and, consequently, ERKs is not sufficient for induction of neuronal differentiation. Prolonged ERK activity is required: a consequence of sustained activation of p21ras by the growth factor receptor protein tyrosine kinase.
Figure 1. Signaling pathways for PACAP- and NGF-dependent PC12 cell differentiation. PACAP-dependent signaling is coded in blue, NGF-dependent signaling in red. Arrows are meant to convey major features of information flow through signaling pathways activated differentially by NGF and PACAP. Ras- and Rap1-dependent signaling are thought to account for immediate and sustained effects, respectively, of NGF mediated through ERK. Rap1-dependent B-Raf activation may also differ in intensity and duration in a stimulus-dependent fashion, perhaps accounting for PKA-dependent and PKA-independent aspects of signaling through ERK. Thus, although the TrkA and PACAP pathways activate several common cellular signaling components, their ultimate effects on gene transcription and cellular phenotype differ substantially.

Duration of ERK activity correlates with differentiation vs. proliferation.
Dynamic control of NF-κB Signaling

- Inflammatory inter-cellular signals
- Developmental inter-cellular signals
- Pathogen-derived substances
- Environmental stress
- Metabolic stress
- Genotoxic stress
- Signaling module
  - IKK
  - IκB
  - NF-κB
  - NF-κB
- Cytoplasm, nucleus
- E, F, G
- α, β
- NF-κB
- Inflammation
- Survival
- Proliferation
- Differentiation
- Apoptosis
- Cell cycle arrest

**Graphs:**
- IKK Activity
- WT LPSp
- WT TNFc
- Activity over 0 to 120 min
- TNF, LPS

**Legend:**
- WT
- LPSp
- irf3-/-
- LPSp
- WT
- TNFc
- 0 to 8 hr
Receptor-specific signaling pathways encode stimulus-specific dynamics of IKK activity.
TNF signaling is controlled by A20 negative feedback

Shannon Werner
Derren Barken
LPS activation of IKK/NF-κB involves TNF

IKK KA

CHX

tnf+/+

tnf−/

IKK KA

NF-κBn (nM)

EMSA

LPS activation of IKK/NF-κB involves TNF

IKK KA

control

NF-κB-deficient

IKK KA

EMSA

tnf+/+

tnf−/

EMSA

tnf+/+

tnf−/
TNF-mediated positive feedback in LPS signaling is essential for expression of specific inflammatory genes.
“computational modeling reveals hidden conversations within the cell”

Which parts/mechanisms within the IKK-NFκB signaling module impart the ability to distinguish different IKK profiles?
p53 oscillations
analog vs. digital  AM vs. FM
NF-kB activation by TNF shows one trough, but a two component model shows multiple troughs.

The diagram illustrates the model with the following kinetic equations:

- \( \frac{dx}{dt} = s - k_1.y - k_2.x \)
- \( \frac{dy}{dt} = k_3.x - k_4.y \)

The graphs show the activation profiles over time for different cell types:

- Human Jurkat T cells
- Human U937 monocytes
- Mouse fibroblasts
Three $\kappa B$ proteins control NF-$\kappa B$

- UV
- oxygen radicals
- $\kappa B_\epsilon$
- $\kappa B_\alpha$
- $\kappa B_\beta$
- TNF
- IL-1
- LPS
- Ag

Target genes:
- Inflammation
- Apoptosis/survival
- Growth/proliferation
Mathematical description of NF-κB regulation
- biochemical processes and parameters -

(1) synthesis

rate of constitutive mRNA synthesis

mRNA half-life

translation rate

rate of mRNA synthesis as a function of \((\text{NF}-\kappa\text{Bn})^2\)

constant level
Mathematical description of NF-κB regulation
- biochemical processes and parameters -

(2) NF-κB - IκB association

constants for
- association
- dissociation

IκB half-lifes
- when bound to NF-κB
- when free
Mathematical description of NF-κB regulation
- biochemical processes and parameters -

(3) NF-κB/IκB - IKK interactions

constants
- association
- dissociation
- catalysis

\[ k_M = \frac{k_d + k_{cat}}{k_a} \]

(“catalysis” is simplified to include phosphorylation, ubiquitination and degradation)
Mathematical description of NF-κB regulation
- biochemical processes and parameters -

(4) sub-cellular localization

transport rates
- nuclear import
- nuclear export
$I_k B \alpha_i'(t) = tr2a + tr2 \ NF–\kappa Bn(t)^2 - tr3 \ I_k B\alpha_i(t)$,

$\ I_k B\beta_i'(t) = tr2b - tr3 \ I_k B\beta_i(t)$,

$\ I_k B\epsilon_i'(t) = tr2e - tr3 \ I_k B\epsilon_i(t)$,

tr2a, b, e : constitutive synthesis
tr2: NF-kB responsive synthesis
tr3: mRNA degradation
Unbound cytoplasmic \( \kappa B\alpha \) proteins

\[
\begin{align*}
\frac{d[I\kappa B\alpha]}{dt} &= -a_1 \text{IKK}(t) [I\kappa B\alpha](t) + d_1 \text{IKK}[I\kappa B\alpha](t) \\
&\quad - a_4 [I\kappa B\alpha](t) \text{NF-}\kappa B(t) + d_4 [I\kappa B\alpha] \text{NF-}\kappa B(t) \\
&\quad + tr_1 [I\kappa B\alpha](t) - deg_1 [I\kappa B\alpha](t) - tp_1 [I\kappa B\alpha](t) + tp_2 [I\kappa B\alpha_n](t)
\end{align*}
\]

\( a \): association  \\
\( d \): dissociation  \\
\( tr \): translation  \\
\( deg \): degradation  \\
\( tp \): nuclear import/export
“Active” nuclear NF-$\kappa$B protein

$$NF-\kappa B_n'(t) = k1\;NF-\kappa B(t) - k01\;NF-\kappa B_n(t)$$

- $a4\;I\kappa B\alpha_n(t)\;NF-\kappa B_n(t) + d4\;I\kappa B\alpha_n\;NF-\kappa B_n(t)$
- $a5\;I\kappa B\beta_n(t)\;NF-\kappa B_n(t) + d5\;I\kappa B\beta_n\;NF-\kappa B_n(t)$
- $a6\;I\kappa B\varepsilon_n(t)\;NF-\kappa B_n(t) + d6\;I\kappa B\varepsilon_n\;NF-\kappa B_n(t)$

$r$ : IKK reaction rates
$k1$ : nuclear import
$k01$ : nuclear export
### Molecular Interactions

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### Synthesis/Degradation

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### Nuclear import/export

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- **measured values**
- **constrained values**
- **“parameter fitted” values**
Parameter estimation in genetically simplified systems

A

β−/−, ε−/−

α−/−, ε−/−

α−/−, β−/−

* *

min

0 2 10 30 60 90 2.5 3.5 4.5 5.5

hrs

B

nuclear κB binding activity (arbitrary units)

α−/−, ε−/−

α−/−, β−/−

C

NF-κBn (nM)

β−/−, ε−/−

α−/−, ε−/−

α−/−, β−/−
Oscillations in genetically modified cells

**knockouts**
(Hoffmann et al 2002)

\[ \text{iKB}^{\beta/-\epsilon/-} \]

**wild-type**

**transient transfections**
(Nelson et al 2004)

constitutive
Red-NF-\(\kappa\)B
+
inducible
GFP-\(\text{I}\kappa\Balpha\)

(Barken et al 2005)
In addition to $I\kappa B\alpha$, $I\kappa B\varepsilon$ expression is NF-$\kappa B$-responsive.
Delayed negative feedback by $I_{\kappa B}\epsilon$ dampens $I_{\kappa B}\alpha$-mediated oscillations in late NF-$\kappa B$ activity.
Computational Simulations vs. Experimental Results

Model vs. Experiment

- NFκBn / nM
- IκBα, μM
- IκBβ, μM
- IκBε, μM

(min 0-6) vs (hrs 0-6)

NFκB gelshift complex

Experimental Results