Fold Change of Nuclear NF-κB Determines TNF-Induced Transcription in Single Cells

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Introduction
Motivation

• NF-κB deregulation is associated with disease.

• The nuclear NF-κB levels have considerable variability from cell to cell.

• What is the most important aspect of NF-κB changes? Which determines the TNF-induced transcription via NF-κB?
Methods

• Experiments
  – Cell line: HeLa
  – Immunofluorescence imaging and analysis
  – Live-cell imaging and analysis
  – smFISH microscopy and image analysis

• Model
  – I1-FFL (D2FC) model
  – direct promotion (D2F) model
Results

Method:

Fixed-cell RelA immunofluorescence imaging and analysis

Conclusion:

The timing and intensity of RelA translocation in response to TNF vary among cells.

Figure 1. TNF-Induced NF-κB Subcellular Localization Is Variable.
Results

Method:

Stably-expressing EGFP-RelA cell line
Living cell imaging and analysis.

CV (coefficient of variation)
= standard deviation / mean

Figure 2. TNF-Induced NF-κB Translocation Varies in Live Cells.
Results

Conclusion:

The ‘Descriptor’ is important to present the cell-to-cell variability in response to TNF.

The fold change of nuclear RelA is less variable than absolute RelA abundance.

Figure 2. TNF-Induced NF-κB Translocation Varies in Live Cells.
Results

Method:

Single-molecule fluorescent *in situ* hybridization (smFISH)

Conclusion:

The three targeted genes have distinct patterns of sensitivity to RelA abundance

RelA may not be an adequate descriptor of this transcription-inducing signal

**Figure 3.** Variability of TNF-Induced NF-κB-Dependent Transcription Is Transcript Specific.
Results

\[ y_i: \text{observed values;} \]
\[ f_i: \text{predictable values;} \]
\[ R^2: \text{Coefficient of determination.} \]

\[ SS_{\text{tot}} = \sum (y_i - \bar{y})^2 \quad SS_{\text{res}} = \sum (y_i - f_i)^2 \]
\[ R^2 \equiv 1 - \frac{SS_{\text{res}}}{SS_{\text{tot}}} \]

**Conclusion:**

NF-κB transcription regulation system is capable of fold-change detection.

**Figure 4.** Transcriptional Responses to TNF Are Determined by the Fold Change of Nuclear NF-κB
Results

Figure 5. An I1-FFL Model of NF-κB-Mediated Transcription Recapitulates Experimental Transcriptional Patterns

Direct transcription

\[ mRNA_i(t) = c1a_i \times \frac{(nNFkB(t))^{h_i}}{k_{NFkB_i} + 1} \]

I1-FFL-like transcription

\[ mRNA_i(t) = c1a_i \times \frac{(nNFkB(t))^{h_i}}{k_{NFkB_i} + (Competitor(t))^{h_i} + 1} \]
**Results**

**Figure 5.** An I1-FFL Model of NF-κB-Mediated Transcription Recapitulates Experimental Transcriptional Patterns

**Conclusion:**

High affinity of competitor for a promoter—inducible, depending on fold changes;  
Low affinity of competitor for a promoter—constitutive (like D2F)
Results

Method:

- siRNA knockdown
- qRT-PCR

Conclusion:

Knockdown of the competitor increased transcription of genes with high-affinity for competitor but less impact on the low affinity gene.
Results

**Figure 6.** Individual Genes Show Different Sensitivity to Knockdown of Candidate Competitors.

**Method:**
- siRNA knockdown
- qRT-PCR

**Conclusion:**
- The nuclear density of competitors, P50 and BCL3 changed correlativey with that of RelA in single cells;
Results

The establishment and prediction of I1-FFL-like model have to be hard-wired biochemical parameters, which are different case by case.

Noise of protein and epigenetic changes of the promoter of the competitor could alter the competitor:RelA ratio.

Figure 7. The Model Explains How Transcription Patterns Are Tuned by Changes to Competitor Affinity and Abundance
Summary

• The subcellular localization of NF-κB is important for its function as the transcriptional activator at the downstream of TNF pathway;

• Nuclear abundance of NF-κB is vary from cell to cell;

• However, the relationship among NF-κB, TNF, the transcription of the targeted genes can compose a I1-FFL-like motif, -with the competitors;

• The fold-change of NF-κB determines the TNF-induced transcription in single cell.
Thank you