Developing Improved Models of Signal Transduction Pathways via Systems Biology

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Overview

• Introduction
• Regulatory mechanism in signaling pathways stimulated by interleukin-6
• Sensitivity analysis & model refinement
• Introduction of quantitative measurement technique for transcription factor concentrations
• Conclusions
Introduction

- Systemic inflammatory mediators, e.g. IL-1 and IL-6, are involved in the regulation of the hepatic acute phase response (APR)

- An improved understanding of the molecular mechanisms involved in the APR can lead to improved treatment of complications arising from inflammatory disorders
Model Description

• Cell signaling induced by IL-6 in hepatocytes involves two pathways: JAK/STAT and MAPK

• The kinetic model is based on the IL-6 signaling pathway presented in Heinrich et al. (2003).
• The JAK/STAT part of model parameters have been adopted from Yamada et al. (2003) and MAPK pathway is based on the work presented by Schoeberl et al. (2002)

Model Description

- Model has 68 state variables and 118 parameters
- Presented model is based on mass action/Michaelis-Menten kinetics

Simulation Studies

- Simulations of the developed model are compared with experimental observations in literature.
- Dynamic response of signal transduction induced by IL-6.
- The presented model is used to analyze the effect of:
  - SOCS3 (suppressor of cytokine signaling 3)
  - SHP-2 (domain containing tyrosine phosphatase 2)
  - STAT3 nuclear phosphatase PP2
- Further, the interactions between the two pathways has been analyzed through simulation of the developed model.

Dynamic Response

• Comparison of nuclear STAT3 dynamics with experimental results

Dynamic Response

- SOCS3 acts as a feedback inhibitor and appears about 30 minutes after the IL-6 binding. SOCS3 reaches maximal concentration after about 2 hours.
Feedback Regulation

- Influence of SOCS3 on signal transduction through the JAK/STAT pathway is investigated in a SOCS3 knockout cell

Inhibitory Effect of SHP2

- SHP2 is not only one of the main components for initiating the MAPK pathway, it also acts as an inhibitor for the JAK/STAT pathway.
Inhibitory Effect of SHP2

• Comparison with experimental results

Nucleus-Cytosol Cycling of STAT3

• Nuclear export/import of STAT3 is important for signal transduction through JAK/STAT pathway
• Comparison of cell signaling in PP2 knockout, SOCS3 knockout and normal cell
Summary of Simulation Results

• Developed a model describing IL-6 signal transduction through the JAK/STAT and the MAPK pathways
• Model seems to describe semi-quantitative data with reasonable degree of accuracy
• SOCS3, SHP-2, and PP2 are important proteins affecting the signal transduction as shown by simulations with knockout cells
• Simulations seem to indicate significant interactions between the pathways
Parameter Sensitivity Analysis

- A more rigorous analysis than comparing simulations is required for refining the model
- Parameter sensitivity analysis is a tool which allows to quantitatively determine the affect that specific parameters have on the output
- Different approaches to parameter sensitivity analysis:
  - Local techniques (parameter output sensitivity matrix)
  - Global techniques (FAST, sampling-based approaches)
Parameter Sensitivity Analysis

• Summary of the results by the three Methods

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Parameter Sensitivity Analysis

• Summary of the Results by the Three Methods
  – Differential sensitivity analysis returns the same results as FAST for small perturbations in the parameters
  – Set of important parameters is similar for all three methods for small perturbations
  – Set of important parameters using FAST and the sampling-based method are very similar even for large perturbations
  – Results for small and for large perturbations can be quite different, e.g., significance of kf32 is strongly influenced by the perturbation size
    • Local sensitivity analysis may not be appropriate for a system with large uncertainties
  – The most important parameters can be directly linked to terms involved in the formation and disassociation of certain proteins
    • SOCS3 (Vm24, kf26, kf27),
    • PP2 (kf20, kf21),
    • SHP-2(kf48,kf32),

  as well as the initiation of signaling through the JAK/STAT pathway (kf7)
  – kf7 seems to play a major role in activity of the JAK/STAT vs. activity of the MAPK pathway

## Parameter Sensitivity Analysis

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Parameter Sensitivity Analysis

- The value of kf7 does have a profound impact not only on activity in the individual pathways but also on the rank of other parameters computed from sensitivity analysis.
- Many parameters associated with the MAPK pathway increase in importance for small values of kf7.
- Note that the output for all these investigations is STAT3 which is a “product” of the JAK/STAT pathway.
  - Indicator of strong interactions among the pathways.
  - Small changes in one part of the pathway can have a profound impact on the entire signaling activity.
- SHP-2, Grb2, and Sos play a major role for crosstalk between pathways as output is influenced by kf48, kf71, kb48, kf42.

Parameter Sensitivity Analysis

- It had been suggested (Orton et al., 2005) that there is a hidden feedback loop between ERKPP and Sos which was not modeled in the model proposed by Schoeberl et al. (2002)
- Since sensitivity analysis indicated that there is significant crosstalk between pathways and that (IL6-gp80-gp130-JAK*)2-SHP2*-Grb2-Sos is a key protein complex, modeling of this feedback loop can have influence of STAT3 concentration profile
- Implement loop where ERKPP phosphorylates Sos causing it to disassociate from the receptor complex

Modeling ERKPP-Sos Feedback Loop

- Updated model

- New model has 72 states and 124 parameters
Modeling ERKPP-Sos Feedback Loop

• Comparison of nuclear STAT3 for old and new model with results from the literature

Modeling ERKPP-Sos Feedback Loop

- Comparison of SOCS3 dynamics for old and new model with results from the literature

Analysis of Experimental Data and Comparison

- All experimental data shown so far came from the literature.

- Potential problem:
  - Western blots provide semi-quantitative data.
  - Western blots require that each sample is destroyed for taking one measurement.

  → Require a new measurement technique that returns quantitative information.

- Develop new measurement technique on TNF-α signaling pathway and then apply it to IL-6 signaling pathway.
TNF-α Signaling Pathway

- Cell stimulated with TNF-α show increased levels of the transcription factor NF-κB associated with this pathway\(^1\)
- Qualitative data exists in the form of Western blots for proteins involved in this pathway\(^1\)
- Several qualitative mathematical models have been presented\(^1,2,3\)
- Each of the existing models only describes part of the signal transduction pathway

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\(^{1}\) A. Hoffmann, A. Levchenko, M.L. Scott, and D. Baltimore (2002) Science


Fluorescence-based Reporter Systems

• Fluorescence-based reporter systems can be created where GFP is produced in addition to the proteins that are transcribed/translated by a particular transcription factor.

• GFP expression and fluorescence is observed only when a transcription factor binds to DNA.

• Compared to Western blots, GFP reporter systems allow continuous and non-invasive monitoring of transcription factors.

• Challenge: to obtain quantitative concentration profiles from experiments.

GFP-based TF reporter. (A) No fluorescence in the absence of TF binding, (B) Binding of TF leads to promoter activation and GFP fluorescence. Fluorescence images (C) without and (D) with NF-κB activation by TNF-α are shown.
Fluorescence-based Reporter Systems
Quantitative Measurement Technique for Transcription Factor Profiles

• Determining transcription factor profiles requires three tasks:
  – Generation of GFP reporter system and creation of experimental data
  – Determining fluorescence intensity profiles from fluorescence microscopy images taken at different points in time
  – Solution of an inverse problem to determine transcription factor concentration from the fluorescence intensity profile

• Focus here is on the 2\textsuperscript{nd} and 3\textsuperscript{rd} task
Image Analysis

- Image analysis extracts information from a time-series of images
- For each image, the following steps need to be performed:
  - Determine the area in the image representing cells where fluorescence can be seen
  - Compute the average fluorescence intensity over fluorescent area
Image Analysis based on K-means Clustering and PCA

- Principal component analysis can be used to distinguish regions of the image with similar features, e.g., brightness
- Form cluster for pixels with the same brightness
- Brightest clusters represent fluorescent cells
- Procedure: Compute
  \[ T = XP \]
  - Distance from PC1 is an indicator of variations in the cluster
  - \( T(:,1) \) represents the projection of points in the cluster onto PC1
Image Analysis based on K-means Clustering and PCA

- Perform principal component analysis on images to determine centroids of clusters.
- On the basis of the initial centroids of all clusters, perform K-means clustering to divide cluster.
- Cluster with largest percentage of variance of PCA 1 will be selected as cluster to be divided in the next step.
- Clusters with highest fluorescence intensity are considered to represent fluorescent cells, while other clusters are determined to be background.
Image Analysis based on K-means Clustering and PCA

- Procedure can provide information about different intensity levels (white region indicates points belonging to a particular cluster)
Computation of Fluorescence Intensity Profile

- Calculation of fluorescence intensity
  - Subtract background intensity to reduce measurement noise due to brightness variation
  - Subtract the intensity of (negative) control experiments to reduce other effects that can cause fluorescence

\[
\text{Intensity for the images with stimulation} = \text{Intensity of fluorescent cell regions} - \text{Intensity of background}
\]

\[
\text{Intensity for the control images without stimulation} = \text{Intensity of fluorescent cell regions} - \text{Intensity of background}
\]

Fluorescent intensity due to stimulation
Computation of Fluorescence Intensity Profile

- Example of calculation of fluorescence intensity profile (H35 cells with NF-κB-GFP)

Note:
- Red color represents the present image
- Blue color represents previous images
Image Analysis Applied to TNF-\(\alpha\) Signaling Pathway

- TNF-\(\alpha\) = 6 ng/ml
- TNF-\(\alpha\) = 10 ng/ml
- TNF-\(\alpha\) = 13 ng/ml
- TNF-\(\alpha\) = 19 ng/ml
Deriving Transcription Factor Concentrations from Images

• Derive a model that correlated transcription factor concentration with fluorescence intensity
• Model needs to include transcription, translation, and activation of GFP
• Implement a procedure of system inversion to compute transcription factor profile by using this model and the fluorescence data derived from image analysis

GFP model:

System inversion:
Model for GFP Dynamics

- Modify a model for GFP dynamics taken from the literature\(^1\) and adapt it to our experiments:
  - Amount of DNA remains constant
  - Effect of transcription factor concentrations on transcription rate is included
  - Estimate parameters C and \(\Delta\)

\[
\begin{align*}
\frac{dp}{dt} &= 0 \\
\frac{dm}{dt} &= S_m \frac{C_{NF-AB}}{C + C_{NF-AB}} p - D_m m \\
\frac{dn}{dt} &= S_n m - D_n n - S_f n \\
\frac{df}{dt} &= s_f n - D_n f \\
I &= \frac{f}{\Delta}
\end{align*}
\]

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<tr>
<th>Parameters</th>
<th>Variables</th>
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<td>(p): DNA concentration</td>
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<td>(D_m) (0.45 1/hr)</td>
<td>(m): GFP m-RNA, (\mu)M</td>
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<tr>
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<td>(n): non-fluorescent protein,</td>
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<td>(f): fluorescent protein</td>
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<tr>
<td>(C) (estimated)</td>
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\(^1\)Subramanian and Srienc (1996) J. Biotechnology
Model for GFP Dynamics

- Validate model by using transcription factor concentration profile taken from the literature\(^1\) with the new model describing GFP dynamics
- Fluorescence intensity profile matches experimental observation

\(^1\)A. Hoffmann, A. Levchenko, M.L. Scott, and D. Baltimore (2002) Science
Solving Inverse Problem to Obtain NF-κB Concentration

- Infer dynamics of NF-κB from fluorescence intensity data $I$

**Input:** $I$

**GFP model**

$$\frac{dm}{dt} = S_m \frac{C_{NF-κB}}{C + C_{NF-κB}} p - D_m m$$

$$\frac{dn}{dt} = S_n m - D_n n - S_j n$$

$$\frac{df}{dt} = s_j n - D_n f$$

$$I = f / \Delta$$

**Output:** $C_{NF-κB}$

NF-κB time-series profile
Solving Inverse Problem to Obtain NF-κB Concentration

• Define \( u(t) = \frac{C_{NF-\kappa B}}{C + C_{NF-\kappa B}} \) such that the relationship between \( u(t) \) and \( I(t) \) is given by a linear dynamic system.

\[
\begin{align*}
\frac{dm}{dt} &= S_m \frac{C_{NF-\kappa B}}{C + C_{NF-\kappa B}} \rho - D_m m \\
\frac{dn}{dt} &= S_n m - D_n n - S_f n \\
\frac{df}{dt} &= S_f n - D_n f \\
I &= f / \Delta
\end{align*}
\]
Solving Inverse Problem to Obtain NF-κB Concentration

- \(u(t)\) can be represented by a third order system to obtain the Laplace transform \(I(s)\) of \(I(t)\)

\[
U(s) = \frac{\omega_n^2}{s^2 + 2\varepsilon\omega_n s + \omega_n^2} \cdot \frac{T_\alpha}{s}
\]

\[
I(s) = \frac{1}{\Delta} \cdot \frac{S_f}{s + D_n} \cdot \frac{S_n}{s + D_n + S_f} \cdot \frac{S_m p}{s + D_m} \cdot \frac{\omega_n^2}{s^2 + 2\varepsilon\omega_n s + \omega_n^2} \cdot \frac{T_\alpha}{s}
\]

- Estimate parameters \(\varepsilon, \omega_n, T_\alpha\) by fitting \(I(t)\) to the fluorescence intensity data via nonlinear least squares optimization

\[
I(t) = A_1 + A_2 e^{-D_n t} + A_3 e^{-(D_n + S_f) t} + A_4 e^{-D_m t} + A_7 e^{-\varepsilon \omega_n t} \sin(\omega_n \sqrt{1 - \varepsilon^2} t + \varphi)
\]
Solving Inverse Problem to Obtain NF-κB Concentration

- $C_{\text{NF-κB}}$ is then given by

$$C_{\text{NF-κB}}(t) = \frac{CT_\alpha \sqrt{1-\varepsilon^2} - CT_\alpha e^{-\varepsilon \omega_n^t} \sin(\omega_n \sqrt{1-\varepsilon^2} t + \phi)}{(1-T_\alpha)\sqrt{1-\varepsilon^2} + T_\alpha e^{-\varepsilon \omega_n^t} \sin(\omega_n \sqrt{1-\varepsilon^2} t + \phi)}$$

where the parameters $\varepsilon, \omega_n, T_\alpha$ have the values that were estimated from fitting $I(t)$ to the fluorescence intensity data.
Solving Inverse Problem to Obtain NF-κB Concentration

- Estimated values for parameters $\varepsilon, \omega_n, T_\alpha$

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<th>$\omega_n$</th>
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<td>19 ng/ml</td>
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- Experiment data

- Estimated $f$ with the identified parameters
Solving Inverse Problem to Obtain NF-κB Concentration

- NF-κB profiles obtained from system inversion

Technique developed in this work

Western blot data (TNF-α = 10ng/ml)
Modeling of TNF-α Signal Transduction Pathway

- Model structure
  - Pathway from TNF-α to IKKn is taken from one source\(^1\) and pathway from IKKn to NF-κB is taken from a different paper\(^2\) to capture as much detail as possible


Parameter Estimation for TNF-α Signaling Pathway

- Sensitivity analysis is used to select parameter $C_3$, $k_{1p}$ and $k_r$ for estimation.
- Data set for 6 ng/ml, 13 ng/ml and 19 ng/ml of TNF-α are used for parameter estimation.
- $C_3$, $k_{1p}$ and $k_r$ are estimated to be 0.0132, 0.0666 and 2.40, respectively.
- Data set for 10 ng/ml of TNF-α is used as testing set.
Analysis of Experimental Data and Comparison

• Experimental results are in excellent agreement with results from the literature and model predictions

• Apply measurement technique to hepatocytes stimulated by IL-6 and with STAT3 GFP reporter system

• Hepatocytes stimulated over a 8 hours time interval with IL-6

• Record images of fluorescence every 30 minutes and quantify fluorescence
Analysis of Experimental Data and Comparison

- Results
Analysis of Experimental Data and Comparison

• Results
Conclusions

• A kinetic model for signal transduction of hepatocytes stimulated by IL-6 has been refined
• SOCS3, SHP-2, and PP2 are important proteins affecting the signal transduction as shown by simulations with knockout cells
• Sensitivity analysis was performed to quantitatively verify the conclusions drawn from the simulations
• Sensitivity analysis revealed a strong degree of cross-talk between the JAK/STAT and the MAPK pathway
• Based upon sensitivity analysis, a feedback loop where ERKPP phosphorylates Sos has been implemented
Conclusions

• Presented a method for quantitatively determining transcription factor concentration profiles from green fluorescent protein reporter systems
• Image analysis method has been investigated to obtain fluorescent intensity from images
• A model describing GFP dynamics is developed to describe the relationship between transcription factor and fluorescence intensity
• System inversion procedure has been developed to obtain quantitative data from fluorescence intensity profiles
• Updated model is in good agreement with data derived from GFP reporter systems
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Thank you for your attention!

Questions?