FEBS 29248 FEBS Letters 579 (2005) 1101–1108

In silico identification of the key components and steps in IFN-γ induced JAK-STAT signaling pathway

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Received 13 October 2004; revised 7 December 2004; accepted 5 January 2005

Available online 19 January 2005

Edited by Robert B. Russell

Abstract Systems biology efforts are increasingly adopting quantitative, mechanistic modeling to study cellular signal transduction pathways and other networks. However, it is uncertain whether the particular set of kinetic parameter values of the model closely approximates the corresponding biological system. We propose that the parameters be assigned statistical distributions that reflect the degree of uncertainty for a comprehensive simulation analysis. From this analysis, we globally identify the key components and steps in signal transduction networks at a systems level. We investigated a recent mathematical model of interferon gamma induced Janus kinase-signal transducers and activators of transcription (JAK-STAT) signaling pathway by applying multi-parametric sensitivity analysis that is based on simultaneous variation of the parameter values. We find that suppressor of cytokine signaling-1, nuclear phosphatases, cytoplasmic STAT1, and the corresponding reaction steps are sensitive perturbation points of this pathway.
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Keywords: Janus kinase-signal transducers and activators of transcription; Interferon gamma; Robustness analysis; Multi-parametric sensitivity analysis; Systems biology

1. Introduction

Considerable efforts have been made so far in the realm of systems biology for dynamical modeling and systems analysis of cellular signal transduction pathways and other networks. Quantitative mechanism-based models could allow researchers to predict the comprehensive behavior of the specified system over time and to track its dynamics for each set of fixed system parameters [1–8]. However, all of the parameters including rate constants and initial components concentrations in the mathematical models must be experimentally measured or inferred to specify the model. Even for those models with experimentally estimated parameters, it is still uncertain whether the particular set of parameters closely approximates the corresponding biological system because some of the kinetic parameters are usually taken or estimated from measurements reported by different laboratories using different in vitro models and conditions. Given the inherent uncertainties in the structure and parameter values of the models, parameters can be assigned statistical distributions that reflect the degree of uncertainty and then simulation analysis can be performed by sampling from the distributions. It is therefore of vital importance not only to study the dynamical properties governed by the particular kinetic parameters but also to further investigate the effects of their perturbations on the overall system. The purpose of this work is trying to answer the question: which signaling components and rate constants are more critical to the output behavior of the system? Investigation of such a question has been one of the major problems raised in systems biology [9].

In this study, we chose the interferon-γ (IFN-γ) induced Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway for analysis. IFN-γ, or type II IFN, was first identified in PHA-activated lymphocyte supernatants with distinctive antiviral activity [10] and is a pleiotropic cytokine widely involved in the regulation of both innate and adaptive immune responses. The IFN-γ induced JAK-STAT pathway is a stress-responsive mechanism that transduces signals from the cell surface to the nucleus. The binding of the cytokine to its cell-surface receptor results in receptor dimerization and the subsequent activation of JAK tyrosine kinases, which are constitutively associated with the receptors. The receptors are then phosphorylated by activated JAKs and serve as docking sites for the STAT1. STAT1 is phosphorylated by JAK, dimerizes, and subsequently leaves the receptor and translocates to the nucleus, where it activates gene transcription. The STAT1 dimers in the nucleus can be dephosphorylated to be STAT1 monomers and transported back to the cytosol by nuclear export [11,12]. Dysregulation of JAK-STAT

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Abbreviations: IFN-γ, interferon gamma; JAK, Janus kinase; STAT, signal transducers and activators of transcription; IFNR, interferon-γ receptor; RJ, IFNR–JAK complex; STAT1, signal transducer and activator of transcription 1; SHP-2, SH2 domain-containing tyrosine phosphatase 2; SOCS1, suppressor of cytokine signaling-1; PPN, nuclear phosphatase; PPX, unidentified phosphatase in the cytoplasm; MAPK, mitogen-activated protein kinase; MPSA, multi-parametric sensitivity analysis

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doi:10.1016/j.febslet.2005.01.009

signaling is associated with various immune disorders and cancers. The signaling strength, kinetics, and specificity of the JAK-STAT pathway are modulated at many levels by distinct regulatory proteins including the suppressor of cytokine signaling (SOCS) proteins, SH2 domain-containing tyrosine phosphatase 2 (SHP-2), and various cytoplasmic and nuclear protein tyrosine phosphatases (PTPs) [13–16]. In this study, we use STAT to represent STAT1 in particular, in the absence of kinetic data distinguishing rate constants for the different STAT isoforms. The basic steps and regulatory scheme of JAK-STAT pathway are shown in Fig. 1.

Here, we propose a global approach for systematic analysis of the JAK-STAT signaling pathway against variations in kinetic parameters and initial concentrations of signaling proteins. The multi-parametric sensitivity analysis (MPSA) method used in this study is based on a Monte-Carlo method over a broad range of simultaneous variation of parameters in uniform distribution followed by a statistical assessment. With this method, we globally identify the key components and steps that are critical to the dynamical behaviors of this signaling pathway.

2. Materials and methods

2.1. The mathematical model

We employ the mathematical model developed by Satoshi Yamada et al. in 2003 [17] for the IFN-γ induced JAK-STAT signaling pathway in liver cells. Since it does not include synthesis of new transcription factors, the direct transcriptional activation by this signaling pathway is to be referred to as the primary IFN-γ response [16]. Fig. 1 and Supplementary Figure 1 show the dynamic scheme of this pathway and all the biochemical reactions included in the model. The model is constructed by ordinary differential equations composed of 32 state variables and 51 parameters. Detailed chemical reactions as well as their parameters are described in Supplementary Table 1.

Experimental studies have shown that phosphorylated STAT1 dimers in the nucleus (STAT1n*–STAT1n*) mediates and is necessary, although not sufficient, for the induction of IFN-γ-inducible genes [11,18,19]. Therefore, STAT1n*–STAT1n* can be regarded as an indirect indicator for target gene activation and we considered STAT1n*–STAT1n* as the output of the signal transduction system in our analysis. The simulated time course of STAT1n*–STAT1n* using the reference set of parameters shows that it is detected within 15 min and reaches its maximum between 30 min and one hour, and then it decreases by SOCS1 action (see Fig. 2). Longer simulations showed that STAT1n*–STAT1n* arrives at a steady state after 8 h.

2.2. Multi-parametric sensitivity analysis

The MPSA method was proposed by Hornberger and Chang [20,21] and further developed by Choi et al. [22] in the field of hydrology. MPSA is a tool that can be used to define the relative importance of the factors related to the model [23]. The idea of MPSA is to inject uncertainty of the parameters into the model by randomly selecting parameter values from probability distributions rather than using fixed values. This is achieved using a Monte-Carlo method in which the model is run repeatedly using sets of parameters drawn randomly from the distributions. Because the natural distributions of parameter values for real biological systems are unknown, we used uniform probability distribution [24]. The range of the parameter distributions are usually determined from the available literature or guided by the experiences of the researchers. For the MPSA with respect to the rate constants, due to the large number (51) of parameters to vary simultaneously, it was necessary to sample a representative set from all possible combinations of parameter values. Latin hypercube sampling method was used to generate random sets of parameter values for simulations in this case (see below). A criterion is coded into the algorithm to classify the output of each model simulation as either acceptable or unacceptable. The final step of MPSA is statistical evaluation of the occurrences of the acceptable and unacceptable cases, summarized for each parameter. The larger the difference between the cumulative frequencies of the two cases, the more significant is the given parameter. The detailed procedure of MPSA is described in the following:

Step 1. Select the parameters to be tested.

Step 2. Set the range of each selected parameter large enough to cover all feasible variations.

Step 3. For each parameter, generate a series of independent, random numbers from a uniform distribution within the defined range and obtain parameter combinations (see below for sampling methods).

Step 4. Simulate the model for each chosen set of parameter values and calculate the corresponding objective function. The objective function is defined as the sum of squared errors between the observed and perturbed system output values. That is

$$f_{\text{obj}}(k) = \sum_{i}^{n} (x_{\text{obs}}(i) - x_{\text{cal}}(i,k))^2 \tag{1}$$

where f_{obj} is the objective function that describes how much the system output deviates from the observed data by varying the parameters, $x_{\text{obs}}(i)$ denotes an observed system output value at the ith sampling time (this is to be substituted by the simulation result from the reference parameter values), $x_{\text{cal}}(i,k)$ denotes the perturbed system output value at the ith sampling time for the parameter variation set k, and n is the number of sampling time points. We set 50 sampling time points in our analysis.

Step 5. Determine whether the chosen set of parameter values is 'acceptable' or 'unacceptable' by comparing the objective function value to a given threshold. If the objective function value is greater than the threshold, the set of parameter values is classified as 'unacceptable'. If the value is less than the threshold, it is classified as 'acceptable'. A previous work [22] indicated that MPSA results are not affected by the choice of a subjective threshold and here we used the average of the objective function over all parameter variations as the threshold value.

Step 6. Statistically evaluate the parameter sensitivity. To this end, we quantitatively compare the distributions of the individual parameter values associated with the acceptable and the unacceptable cases. For each selected parameter, the cumulative frequency is computed for both acceptable and unacceptable cases. We evaluate the sensitivity by a direct measure of the separation of the two cumulative frequency distributions. We use the following Kolmogorov–Smirnov (K–S) statistic:

$$\text{K–S} = \sup_{x} |S_{\text{a}}(x) - S_{\text{u}}(x)| \tag{2}$$

where S_{a} and S_{u} are the cumulative frequency functions corresponding to acceptable cases and unacceptable cases, respectively, and x is the given parameter. The statistic K–S is determined as the maximum vertical distance between the cumulative frequency distribution curves for n acceptable and m unacceptable cases. A larger value of K–S indicates that the system is sensitive to variation in the given parameter.

In Step 3 for the 51 rate constants, selecting just two values for each parameter would generate 2^{51} simulations to run, which is not practical. Instead, we used the Latin hypercube sampling method to sample 2000 random parameter vectors while evenly covering individual parameter ranges (some background information about Latin hypercube sampling method is available in the Supplementary material). This way we could computationally manage the large number (51) of rate constants being varied simultaneously, while ensuring maximal sampling through each parameter dimension [25]. Briefly, for the jth parameter, we divide the range of the parameter into N (= 2000) subintervals of equal size. Then randomly sample N values (p_{ij}, $i = 1, \ldots, N$), one from each subinterval, for the jth parameter. To combine these values of individual parameters to generate sets of parameter values, we randomly permute the N values for each parameter to get the parameter vectors, i.e., we individually permute the elements of each column of the matrix p_{ij} and use the N rows as the parameter

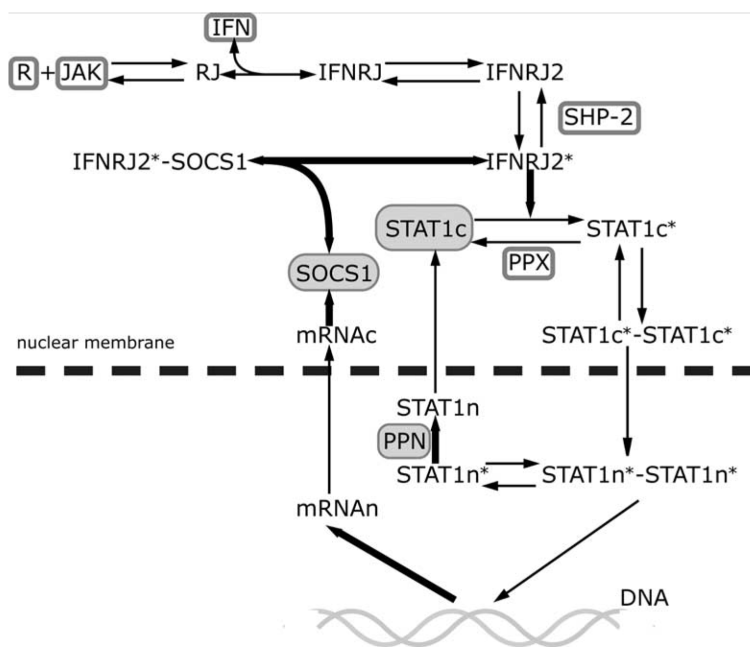

Fig. 1. IFN-γ-induced JAK-STAT1 signal transduction pathway. Different forms of the STAT1 protein are represented as: STAT1c, STAT1c∗, STAT1c∗- STAT1c∗, corresponding to the STAT1 monomers, phosphorylated STAT1 monomers, and phosphorylated dimers, respectively, in the cytoplasm. In the symbols for the nuclear counterparts, "n" replaces "c". This diagram is developed from Yamada, S. et al. The relatively important proteins that were identified in our sensitivity analysis are marked with gray shading boxes. Other signaling proteins with non-zero initial concentrations are marked with open boxes, whereas proteins with zero initial concentration in the unstimulated cell are not. Reaction steps that we identified to be critical for the perturbation of the system are in thick arrows.

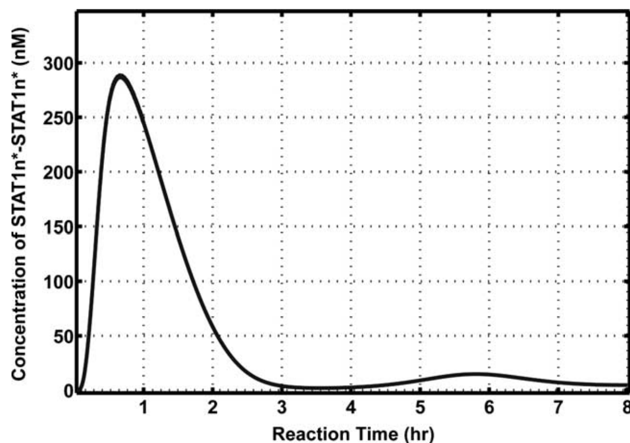

Fig. 2. Simulated time course profile of STAT1n∗–STAT1n∗ activated by continuous exposure to IFN-γ using reference kinetic parameter values. The concentration of IFN-γ was set to be 1 nM.

vectors for our simulations. This sampling method was implemented by the MATLAB command 'lhsdesign' to produce 2000 parameter vectors. Covering the individual parameter ranges evenly is desirable for good summary statistics in the final step of MPSA.

3. Results

3.1. Key signaling components of the pathway

Since a simulation requires not only the kinetic parameter values but also the initial concentrations of various signaling components that could affect the overall system behavior, we first applied MPSA to pinpoint those critical components possessing dominant effects. So we examined the influences of variations in the initial concentrations of the components in the system. The primary molecular species, namely, the receptor, IFN-γ, JAK, STAT1c, PPX (unidentified phosphatase), SHP-2, PPN (TC45), and SOCS1 were chosen for the MPSA analysis. The reference values for the initial concentrations of these signaling proteins and the variation ranges for simulation are shown in Table 1. The initial concentrations of the other proteins were set to zero. The cumulative frequency distributions for the acceptable and unacceptable cases of the initial concentrations of proteins are shown in Fig. 3. The relative sensitivity is reflected by the difference of the two distributions. For each signaling protein, K–S statistic was used to evaluate the statistical difference between the two distributions. The larger the value of K–S is, the more important the protein is for the output of this signaling pathway. The results of MPSA are summarized in Table 1. The MPSA results indicate that SOCS1, nuclear phosphatase TC45, and STAT1 proteins in the cytoplasm are relatively important components in the IFN-γ induced JAK-STAT signaling pathway. On the other hand, the concentrations of JAK, the receptor, SHP-2, and the cytoplasmic phosphatase PPX are less important for the output of this system.

3.2. Critical kinetic parameters of the pathway

We further investigated the system to identify the key steps of the JAK-STAT signaling pathway, i.e., the most sensitive targets for perturbation. The kinetic rate constant parameters for our MPSA analysis and their variation ranges for simulation are shown in Table 2. Because the significant parameters were not known a priori, we decided to vary all 51 parameters. We ran the 2000 simulations randomly chosen by the Latin

Table 1
Results of MPSA with respect to variations in the initial concentrations of signaling proteins

Signaling protein	K–S	Reference initial concentration (nM)	Range of variation (nM)
SOCS1	0.71	0	0–100
STAT1c	0.64	1000	200–5000
PPN (nuclear phosphatase TC45)	0.41	60	12–300
JAK proteins	0.19	12	2.4–60
Receptor	0.18	12	2.4–60
Interferon	0.03	1	0.2–5
SHP-2 (cytoplasmic phosphatase)	0.02	100	20–500
PPX (cytoplasmic phosphatase)	0.01	50	10–250

Higher K–S values indicate the system behavior is more sensitively affected by changes in the corresponding protein level.

hypercube sampling method for MPSA on all 51 rate constants (see Section 2).

Consistent with the previous K–S statistic results, the parameters related to the identified key signaling components had relatively significant effects on the time course of STAT1n∗–STAT1n∗, whereas variations of the parameters concerning SHP-2, PPX, receptor, JAK, and IFN-γ had relatively minor effects on the output of this system (see Table 2). The cumulative frequency distributions for the acceptable and unacceptable cases of all the rate constants are shown in the supplementary material in the website. Specifically, reactions involved in the phosphorylation of STAT1c, negative regulation by SOCS1, the synthesis and degradation of SOCS1 at the mRNA and at the protein level, and regulation by PPN had the highest K–S values. These reactions are all molecular events that affect the level of signaling most directly. Changes in the reaction kinetics of these steps had a more pronounced effect on the system behavior of this pathway. Our results also underscore the importance of SOCS1 with numerous high K–S reactions involving SOCS1.

3.3. Comparison to local sensitivity analysis

We examined the extent to which MPSA reveals distinct system features that are not readily obtained by the conventional local sensitivity analysis. Regarding parameter changes involving initial concentrations of signaling molecules, we performed simulations with variations either in single parameters or in multiple parameters, which are used in local sensitivity analysis and MPSA, respectively. For single parameter changes (with the other parameter values fixed), the time course of STAT1n∗–STAT1n∗ concentration is shown in Fig. 4A–H for each signaling protein whose concentration was varied. The corresponding plot for some multiple simultaneous parameter changes is in Fig. 4I. Despite the fact that (I) shows only a few typical simulations from the MPSA, we see that the temporal activity of nuclear STAT1 dimers depends dramatically on whether the variation was in single or in multiple simultaneous parameters. It is apparent that single parameter changes provide very limited view of the wide range of possible system behavior. It may be useful to assess the effect of variations of a single parameter for certain purposes. For example, in Fig. 4, PPN stands out as the signaling

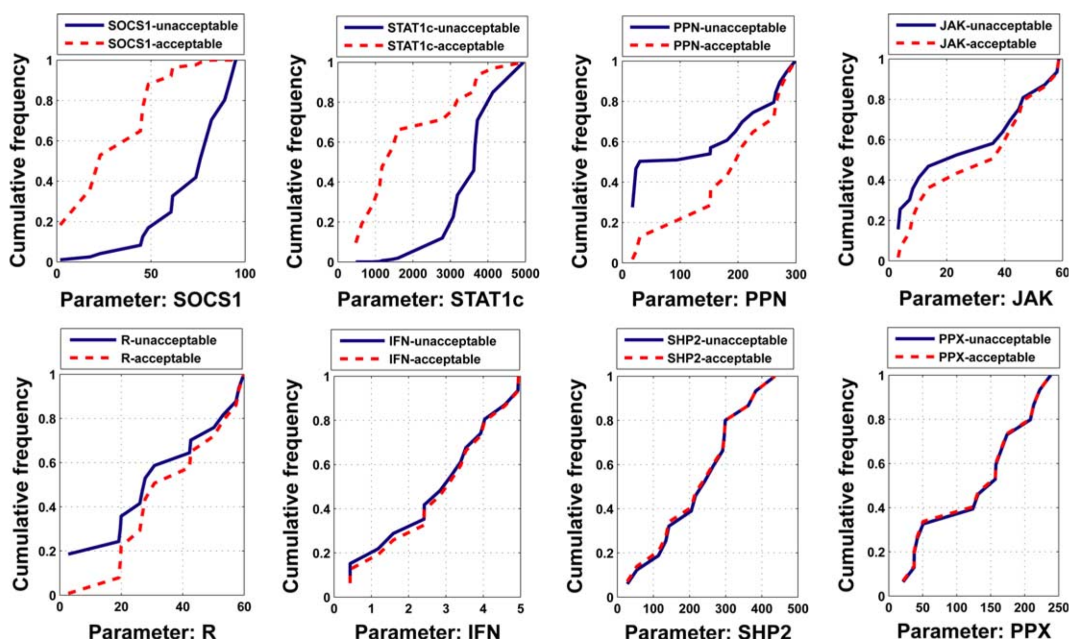

Fig. 3. Cumulative frequency distributions of the MPSA with respect to the initial concentrations of the proteins. Solid curve denotes the unacceptable cases and the dashed curve indicates the acceptable cases. The maximum vertical difference between the two curves is the K–S statistic for the parameter.

Table 2
Results of MPSA with respect to variations in the kinetic parameter values

Rate constant	K–S	Relative step	Reference value	Range of variation	Unit
k_{f5}	0.28	STAT1c phosphorylation	8	0.8–80	10^6 M^{-1} s^{-1}
k_{f21}	0.27	SOCS1 negative regulation	20	2–200	10^6 M^{-1} s^{-1}
k_{f18}	0.27	SOCS1 mRNA synthesis	0.01	0.001–0.1	nM/s
k_{b30}	0.24	SOCS1 negative regulation	0.8	0.08–8	s^{-1}
k_{f6}	0.23	STAT1c phosphorylation	0.4	0.04–4	s^{-1}
k_{f31}	0.21	SOCS1 negative regulation	1	0.1–10	10^6 M^{-1} s^{-1}
k_{f22}	0.2	SOCS1 mRNA degradation	0.0005	0.00005–0.005	s^{-1}
k_{f30}	0.2	SOCS1 negative regulation	8	0.8–80	10^6 M^{-1} s^{-1}
k_{f28}	0.2	PPN regulation	0.05	0.005–0.5	s^{-1}
k_{f20}	0.2	SOCS1 protein synthesis	0.01	0.001–0.1	s^{-1}
k_{f23}	0.18	SOCS1 protein degradation	0.0005	0.00005–0.005	s^{-1}
k_{b31}	0.17	SOCS1 negative regulation	0.2	0.02–2	s^{-1}
k_{b18}	0.16	SOCS1 mRNA synthesis	400	40–4000	nM
k_{f15}	0.15	PPN regulation	1	0.1–10	10^6 M^{-1} s^{-1}
k_{b21}	0.14	SOCS1 negative regulation	0.1	0.01–1	s^{-1}
k_{f33}	0.14	SOCS1 negative regulation	0.0005	0.00005–0.005	s^{-1}
k_{f2}	0.12	Interferon stimulation	20	2–200	10^6 M^{-1} s^{-1}
k_{b5}	0.11	STAT1c phosphorylation	0.8	0.08–8	s^{-1}
k_{f16}	0.1	PPN regulation	0.005	0.0005–0.05	s^{-1}
k_{b8}	0.099	STAT1c dimerization	0.1	0.01–1	s^{-1}
k_{f27}	0.099	PPN regulation	1	0.1–10	10^6 M^{-1} s^{-1}
k_{b2}	0.094	Interferon stimulation	0.02	0.002–0.2	s^{-1}
k_{b27}	0.094	PPN regulation	0.2	0.02–2	s^{-1}
k_{b3}	0.088	IFN-R-JAK dimerization	0.2	0.02–2	s^{-1}
k_{b26}	0.086	Nuclear STAT1 dimerization	0.5	0.05–5	s^{-1}
k_{b15}	0.084	PPN regulation	0.2	0.02–2	s^{-1}
k_{f14}	0.084	STAT1 nuclear import	0.005	0.0005–0.05	s^{-1}
k_{b24}	0.083	PPX regulation	0.2	0.02–2	s^{-1}
k_{f3}	0.082	IFN-R-JAK dimerization	40	4–400	10^6 M^{-1} s^{-1}
k_{f8}	0.08	STAT1c dimerization	20	2–200	10^6 M^{-1} s^{-1}
k_{f32}	0.079	SOCS1 negative regulation	0.003	0.0003–0.03	s^{-1}
k_{f9}	0.076	SHP2 regulation	1	0.1–10	10^6 M^{-1} s^{-1}
k_{f12}	0.075	PPX regulation	0.003	0.0003–0.03	s^{-1}
k_{f17}	0.071	STAT1 nuclear export	0.05	0.005–0.5	s^{-1}
k_{b29}	0.068	STAT1∗–STAT1∗ formation	0.2	0.02–2	s^{-1}
k_{b9}	0.067	SHP2 regulation	0.2	0.02–2	s^{-1}
k_{f1}	0.067	R-JAK formation	100	10–1000	10^6 M^{-1} s^{-1}
k_{b11}	0.065	PPX regulation	0.2	0.02–2	s^{-1}
k_{f25}	0.065	PPX regulation	0.003	0.0003–0.03	s^{-1}
k_{f13}	0.06	STAT1∗–STAT1∗ formation	0.0002	0.00002–0.002	10^6 M^{-1} s^{-1}
k_{f26}	0.058	Nuclear STAT1 dimerization	5	0.5–50	10^6 M^{-1} s^{-1}
k_{b7}	0.057	STAT1c phosphorylation	0.5	0.05–5	s^{-1}
k_{b13}	0.052	STAT1∗–STAT1∗ formation	0.2	0.02–2	s^{-1}
k_{f11}	0.052	PPX regulation	1	0.1–10	10^6 M^{-1} s^{-1}
k_{f10}	0.051	SHP2 regulation	0.003	0.0003–0.03	s^{-1}
k_{f24}	0.049	PPX regulation	1	0.1–10	10^6 M^{-1} s^{-1}
k_{f4}	0.046	IFN-R-JAK dimerization	0.005	0.0005–0.05	s^{-1}
k_{f19}	0.046	SOCS1 mRNA nuclear export	0.001	0.0001–0.01	s^{-1}
k_{b1}	0.043	R-JAK formation	0.05	0.005–0.5	s^{-1}
k_{f7}	0.036	STAT1c phosphorylation	5	0.5–50	10^6 M^{-1} s^{-1}
k_{f29}	0.036	STAT1∗–STAT1∗ formation	0.0002	0.00002–0.002	10^6 M^{-1} s^{-1}

protein whose concentration variation can impact both the response time (time to reach the peak) and amplitude of STAT1 activity. However, local sensitivity analysis cannot give us comprehensive, combined effects that are only possible from simultaneous variations of the parameter values.

MPSA not only makes use of variations in multiple parameters but also provides a statistical summary of the large number of simulations in the form of the K–S values, which helps to rank the parameters by importance. Since local sensitivity analysis can also produce quantitative assessment of the impact of single parameter changes, we compared the relative importance of signaling proteins obtained by both measures. The quantity that is often used in local sensitivity analysis is the control coefficient [28]. For our JAK-STAT system, the control coefficients are defined as follows:

$$C_{p_i}^{\text{STAT1n}^*\text{–STAT1n}^*} = \frac{p_i}{\text{STAT1n}^*\text{–STAT1n}^*} \times \frac{\partial(\text{STAT1n}^*\text{–STAT1n}^*)}{\partial p_i} \quad (3)$$

The coefficients for the local analysis near the reference values of initial concentrations of signaling components are listed in Table 3. From this, we can see the relative importance is not accurately assessed by these coefficients. For example, the control coefficient for PPN had a smaller absolute value than that for SHP-2, whereas our MPSA, based on a much larger number of simulations with simultaneously varying parameter values, showed that PPN is in fact more important than SHP-2. Control coefficients from a local sensitivity analysis are inherently limited because they are obtained from small one-parameter changes. When the rate constants were considered as varying parameter

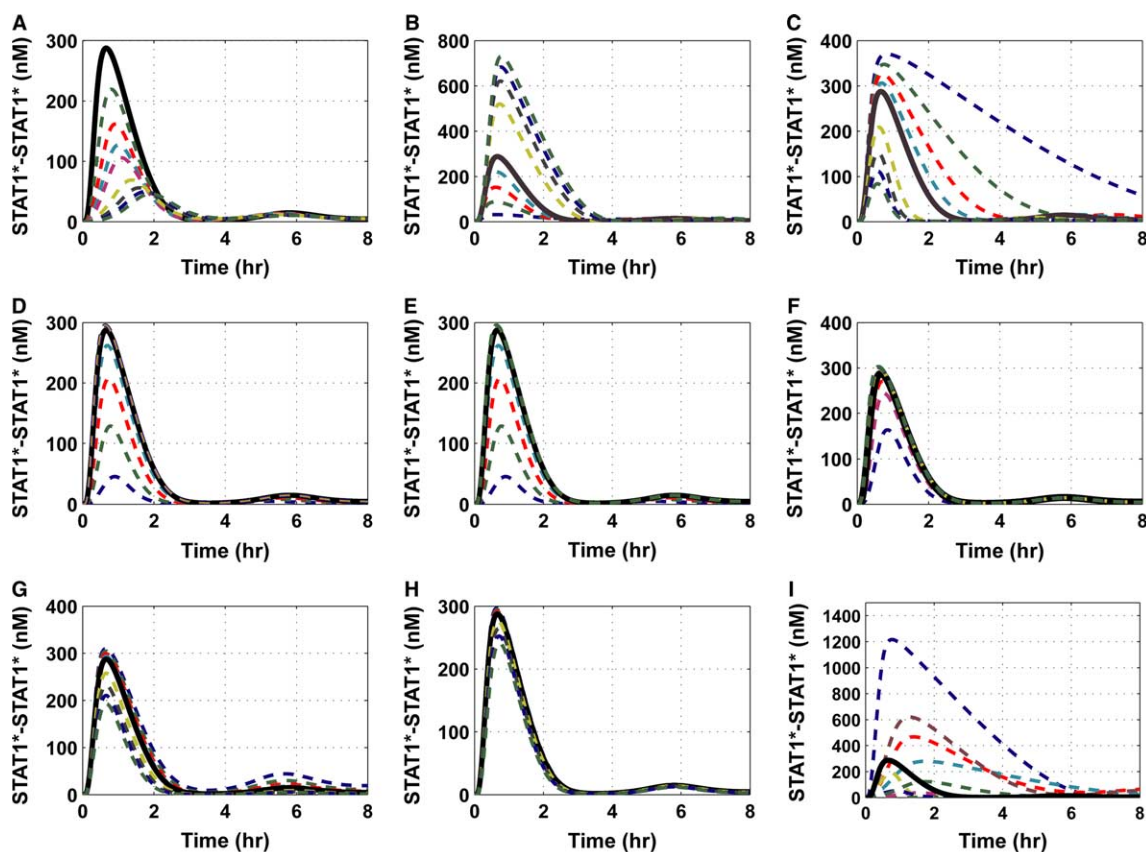

Fig. 4. Dependency of STAT1 activity on single concentration changes in the signaling components and on simultaneous concentration variations in multiple components. The time course of nuclear STAT1n∗–STAT1n∗ for various initial concentrations of (A) SOCS1 (0–100 nM), (B) STAT1c (1/5–5 times), (C) PPN (1/5–5 times), (D) JAK (1/5–5 times), (E) Receptor (1/5–5 times), (F) Interferon (1/5–5 times), (G) SHP-2 (1/5–5 times), (H) PPX (1/5–5 times), and (I) typical simultaneous variations of signaling components used in MPSA. The bold line shows the time course from the simulation using the reference parameter values. The concentration range in the y axis is different for each plot.

Table 3
Control coefficients for the concentration of STAT1n∗–STAT1n∗ relative to changes in the concentrations of pathway components

Signaling protein	Control coefficient
STAT1c	1.1565
JAK proteins	0.3333
Receptor	0.3333
SHP-2 (cytoplasmic phosphatase)	−0.2689
Interferon	0.1013
PPN (nuclear phosphatase TC45)	−0.0629
PPX (cytoplasmic phosphatase)	−0.0629

The control coefficients were obtained by simulations of response to a change of initial concentrations by 1%.
Using relative changes of initial concentrations less than 1% did not lead to a significant improvement of the precision of the control coefficient value.
The coefficients were evaluated at the reference concentrations shown in Table 1, column 3.

values, however, the difference between the local sensitivity analysis and MPSA was not as striking (data not shown).

4. Discussion

It is useful to employ a computational model to systematically identify the specific perturbations that have significant effects on the system behavior, especially when conducting numerous experiments on the living system is not practical. Sensitivity analysis has been used as an in silico investigation method to identify critical parameters in signal transduction [26,27]. For example, Lee et al. [28] investigated the variations of parameters for the Wnt pathway using control coefficients. This and many other approaches have been based on local sensitivity analysis, which deals with only small perturbations of the reference model and/or allows only one parameter to vary for each simulation. Importantly, traditional local sensitivity analysis pertains to a particular point in the parameter space. However, there is probably not a single 'true' point that occurs in nature. It is likely that cells use a 'repertoire' of points or regions in the parameter space depending on their genetic and cellular types. In addition, rate constants and concentrations of diverse molecules may vary extensively in an interactive manner among different cellular environments. For these reasons it would be more appropriate to explore, in a probabilistic context, possibilities of non-linear effects from simultaneous parameter variations of arbitrary magnitudes by means of a global sensitivity analysis [29]. The MPSA method proposed in this study is based on this idea to investigate the influence of the uncertainty of parameters on the behaviors of the signaling pathway. Fig. 5 shows schematically the comparison between the local and global sensitivity analysis approach. Suppose a model has two parameters k_1 and k_2. In the absence

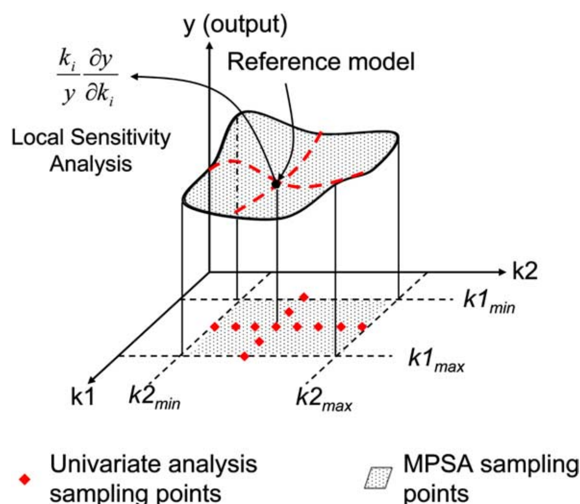

Fig. 5. Schematic illustration of the difference between local sensitivity analysis and MPSA. For simplicity, only two axes (k_1 and k_2) represent the high dimensionality of the parameter space. Within the interval bounded by maximum and minimum parameter values, representative values are sampled for each parameter to produce input parameter vectors for simulations. In a local sensitivity analysis, sampling points (large dots) are chosen only along the individual parameter directions. A global sensitivity analysis such as MPSA is based on model simulations using multi-variate points (small dots) with comprehensive coverage of the parameter domain.

of a priori knowledge of the probability distribution of k_1 and k_2, we simply use a uniform distribution to implement global sensitivity analysis ('maximum entropy'). Then resulting range of uncertainty in the output are computed and the distribution of the possible behaviors is statistically analyzed. On the other hand, control coefficients from local sensitivity analysis about k_1 and k_2 represent the (scale-free) slopes of the surface in the two coordinate directions at the point corresponding to the reference model. From the very limited sampling of the parameter space it is evident that a local sensitivity analysis may miss the full range of system behaviors that are possible by simultaneous variations of multiple parameters. Indeed, we confirmed this difference in our comparison of the two analyses on the JAK-STAT pathway. Recently, Bentele et al. [30] illustrated an extended use of local sensitivity analysis to identify critical systems parameters in the CD95-induced apoptosis pathway. They employed local sensitivity analysis for different locations in the parameter space and statistically analyzed the distributions of the 'sensitivity matrix', eventually revealing some intrinsic characteristics of this system.

Through the global sensitivity analysis of the IFN-γ induced JAK-STAT signaling pathway, we have identified that SOCS1, cytoplasmic STAT1, and nuclear phosphatase TC45 are critical components for the perturbation of the system output. The result that the nuclear phosphatase and SOCS1 are more critical than SHP-2 and the cytoplasmic phosphatase underscores the importance of downstream (hence, more direct upon STAT1) negative regulators compared to upstream regulators. Recently it was experimentally observed that increased expression of SOCS1 and STAT1c occurred in IFN-γ signaling sensitization without being accompanied by changes in expression of the receptors or JAK proteins [31] and that SOCS1 deficiency in mice is perinatally lethal [32,33]. The SOCS proteins are generally expressed at low levels in unstimulated cells and become rapidly induced by cytokines, thereby blocking continued signaling and forming a classic negative-feedback loop [12,34]. Such experimental results imply that JAK-STAT signaling may use variation of key components to achieve different behaviors such as IFN-γ signaling sensitization, for example. Our analysis result is also consistent with a previous work, in that the nuclear phosphatase, PPN, is more important than other regulatory proteins such as SHP-2 and cytoplasmic phosphatase PPX in this signal transduction system [17]. Moreover, Swameye et al. [27] developed a simpler model of JAK-STAT signaling pathway by data-based modeling and found that STAT1 nucleocytoplasmic shuttling is most sensitive for the perturbation of this system. The importance of nucleocytoplasmic shuttling process of STAT1 matches well with our finding that the nuclear phosphatase TC45 (which promotes the nuclear export) is an important signaling component.

Since oncogenic tyrosine kinases or autocrine loops result in constitutively activated STATs contributing to malignant transformation and tumor progression, identification of key components and steps in the corresponding signal transduction system provides useful knowledge for drug discovery and cancer therapy [27]. This strategy allows, for example, identification of multiple targets that would enable the use of two or more drugs in smaller dosage [35]. In summary, our work implicates STAT1c, SOCS1, nuclear phosphatase TC45, and their corresponding reactions as effective targets for intervention of the IFN-γ induced JAK-STAT signaling pathway. The MPSA approach proposed in this study is applicable to the analysis of metabolic pathways or other signaling pathways such as mitogen-activated protein kinase (MAPK), bacterial chemotaxis, TNFα-mediated NF-κB, and Wnt signaling pathways.

Acknowledgments: The authors acknowledge helpful discussions with Dr. Satoshi Yamada and Dr. George Hornberger. They also thank Institute of High Performance Computing in Tsinghua University for providing us with the use of the super computer. Financial support for this work was provided by the National Key Foundational Research Grant in China (973-2003CB715903; 863-2002AA234041, 2002AA271031; NSFC-90303017). K.-H. Cho acknowledges the support received by a grant from the Korea Ministry of Science and Technology (Korean Systems Biology Research Grant, M103090000-0603B5000-00211) and also by a Grant No. (R05-2004-000-10549-0) from the Korea Ministry of Science and Technology.

Appendix A. Supplementary data

Supplementary materials are available at http://www.bio-info.tsinghua.edu.cn/~zzk/JAK-STAT. Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.febslet.2005.01.009.

References

[1] Angeli, D., Ferrell Jr., J.E. and Sontag, E.D. (2004) Detection of multistability, bifurcations, and hysteresis in a large class of biological positive-feedback systems. Proc. Natl. Acad. Sci. USA 101, 1822–1827.

[2] Asthagiri, A.R. and Lauffenburger, D.A. (2001) A computational study of feedback effects on signal dynamics in a mitogen-

activated protein kinase (MAPK) pathway model. Biotechnol. Prog. 17, 227–239.
[3] Bhalla, U.S. and Iyengar, R. (1999) Emergent properties of networks of biological signaling pathways. Science 283, 381–387.
[4] Bhalla, U.S. and Iyengar, R. (2001) Robustness of the bistable behavior of a biological signaling feedback loop. Chaos 11, 221–226.
[5] Cho, K.H., Shin, S.Y., Lee, H.W. and Wolkenhauer, O. (2003) Investigations into the analysis and modeling of the TNF alpha-mediated NF-kappa B-signaling pathway. Genome Res. 13, 2413–2422.
[6] Hoffmann, A., Levchenko, A., Scott, M.L. and Baltimore, D. (2002) The IkappaB-NF-kappaB signaling module: temporal control and selective gene activation. Science 298, 1241–1245.
[7] Huang, C.Y. and Ferrell Jr., J.E. (1996) Ultrasensitivity in the mitogen-activated protein kinase cascade. Proc. Natl. Acad. Sci. USA 93, 10078–10083.
[8] Kholodenko, B.N., Demin, O.V., Moehren, G. and Hoek, J.B. (1999) Quantification of short term signaling by the epidermal growth factor receptor. J. Biol. Chem. 274, 30169–30181.
[9] Kitano, H. (2002) Systems biology: a brief overview. Science 295, 1662–1664.
[10] Wheelock, E.F. (1965) Interferon-like virus inhibitor induced in human leukocytes by phytohemagglutinin. Science 149, 310–311.
[11] Darnell Jr., J.E. (1997) STATs and gene regulation. Science 277, 1630–1635.
[12] Levy, D.E. and Darnell Jr., J.E. (2002) Stats: transcriptional control and biological impact. Nat. Rev. Mol. Cell Biol. 3, 651–662.
[13] Shuai, K. and Liu, B. (2003) Regulation of JAK-STAT signalling in the immune system. Nat. Rev. Immunol. 3, 900–911.
[14] Shuai, K. (1999) The STAT family of proteins in cytokine signaling. Prog. Biophys. Mol. Biol. 71, 405–422.
[15] O'Shea, J.J., Gadina, M. and Schreiber, R.D. (2002) Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. Cell 109 (Suppl.), S121–S131.
[16] Boehm, U., Klamp, T., Groot, M. and Howard, J.C. (1997) Cellular responses to interferon-gamma. Annu. Rev. Immunol. 15, 749–795.
[17] Yamada, S., Shiono, S., Joo, A. and Yoshimura, A. (2003) Control mechanism of JAK/STAT signal transduction pathway. FEBS Lett. 534, 190–196.
[18] Chatterjee-Kishore, M., Wright, K.L., Ting, J.P. and Stark, G.R. (2000) How Stat1 mediates constitutive gene expression: a complex of unphosphorylated Stat1 and IRF1 supports transcription of the LMP2 gene. EMBO J. 19, 4111–4122.
[19] Mahboubi, K. and Pober, J.S. (2002) Activation of signal transducer and activator of transcription 1 (STAT1) is not sufficient for the induction of STAT1-dependent genes in endothelial cells. Comparison of interferon-gamma and oncostatin M. J. Biol. Chem. 277, 8012–8021.
[20] Hornberger, G. and Spear, R. (1981) An approach to the preliminary analysis of environmental systems. J. Environ. Manage. 12, 7–18.
[21] Chang, F.-J. and Delleur, J.W. (1992) Systematic parameter estimation of watershed acidification model. Hydrol. Processes 6, 29–44.
[22] Choi, J., Hulseapple, S.M., Conklin, M.H. and Harvery, J.W. (1998) Modeling CO_2 degassing and pH in a stream–aquifer system. J. Hydrol. 209, 297–310.
[23] Cho, K.-H., Shin, S.-Y. and Kolch, W.a.W.O. (2003) Experimental design in systems biology based on parameter sensitivity analysis using a Monte Carlo method: a case study for the TNF alpha-mediated NF-kappaB signal transduction pathway. Simulation: Trans. Soc. Model. Simulation Int. 79, 726–739.
[24] Alves, R. and Savageau, M.A. (2000) Systemic properties of ensembles of metabolic networks: application of graphical and statistical methods to simple unbranched pathways. Bioinformatics 16, 534–547.
[25] McKay, M.D., Beckman, R.J. and Conover, W.J. (1979) A comparison of three methods for selecting values of input variables in the analysis of output from a computer code. Technometrics 21, 239–245.
[26] Schoeberl, B., Eichler-Jonsson, C., Gilles, E.D. and Muller, G. (2002) Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors. Nat. Biotechnol. 20, 370–375.
[27] Swameye, I., Muller, T.G., Timmer, J., Sandra, O. and Klingmuller, U. (2003) Identification of nucleocytoplasmic cycling as a remote sensor in cellular signaling by databased modeling. Proc. Natl. Acad. Sci. USA 100, 1028–1033.
[28] Ethan, L., Adrian, S., Roland, K., Reinhart, H. and Marc, W.K. (2003) The roles of APC and axin derived from experimental and theoretical analysis of wnt pathway. PLOS Biol. 1, 116–132.
[29] Tilde, J.W., Costanza, V. and McRae, G.J.A.S.J.H. (1981) Sensitivity analysis of chemically reacting systems in: Modelling of Chemical Reaction Systems (Ebert, K.H., Deuflhard, P. and Jager, W., Eds.), pp. 69–91, Springer, Berlin, Heidelberg, New York.
[30] Bentele, M., Lavrik, I., Ulrich, M., Stosser, S., Heermann, D.W., Kalthoff, H., Krammer, P.H. and Eils, R. (2004) Mathematical modeling reveals threshold mechanism in CD95-induced apoptosis. J. Cell Biol. 166, 839–851.
[31] Hu, X., Herrero, C., Li, W.P., Antoniv, T.T., Falck-Pedersen, E., Koch, A.E., Woods, J.M., Haines, G.K. and Ivashkiv, L.B. (2002) Sensitization of IFN-gamma Jak-STAT signaling during macrophage activation. Nat. Immunol. 3, 859–866.
[32] Marine, J.C., Topham, D.J., McKay, C., Wang, D., Parganas, E., Stravopodis, D., Yoshimura, A. and Ihle, J.N. (1999) SOCS1 deficiency causes a lymphocyte-dependent perinatal lethality. Cell 98, 609–616.
[33] Alexander, W.S., Starr, R., Fenner, J.E., Scott, C.L., Handman, E., Sprigg, N.S., Corbin, J.E., Cornish, A.L., Darwiche, R., Owczarek, C.M., Kay, T.W., Nicola, N.A., Hertzog, P.J., Metcalf, D. and Hilton, D.J. (1999) SOCS1 is a critical inhibitor of interferon gamma signaling and prevents the potentially fatal neonatal actions of this cytokine. Cell 98, 597–608.
[34] Greenhalgh, C.J. and Hilton, D.J. (2001) Negative regulation of cytokine signaling. J. Leukoc. Biol. 70, 348–356.
[35] Endy, D. and Brent, R. (2001) Modelling cellular behaviour. Nature 409, 391–395.