FEBS Letters 534 (2003) 190–196 FEBS 26886

Control mechanism of JAK/STAT signal transduction pathway

Satoshi Yamada[a,*], Satoru Shiono[a], Akiko Joo[b], Akihiko Yoshimura[b]

[a] *Advanced Technology R&D Center, Mitsubishi Electric Corporation, 8-1-1, Tsukaguchi-Honmachi, Amagasaki, Hyogo 661-8661, Japan*
[b] *Medical Institute of Bioregulation, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*

Received 28 October 2002; revised 10 December 2002; accepted 10 December 2002

First published online 19 December 2002

Edited by Judit Ovádi

Abstract Suppressor of cytokine signaling-1 (SOCS1) was identified as the negative regulator of Janus kinase (JAK) and signal transducer and activator of transcription (STAT) signal transduction pathway. However, the kinetics and control mechanism of the pathway have not yet been fully understood. We have developed the computer simulation of the JAK/STAT pathway. Without nuclear phosphatase, SOCS1's binding to JAK did not cause the decrease in nuclear phosphorylated STAT1. However, without SH2 domain-containing tyrosine phosphatase 2 (SHP-2) or cytoplasmic phosphatase, it did. So nuclear phosphatase is considered to be the most important in this system. By changing parameters of the model, dynamical characteristics and control mechanism were investigated.

Key words: Interferon-γ; Janus kinase; Signal transducer and activator of transcription; Suppressor of cytokine signaling-1; Nuclear tyrosine phosphatase

1. Introduction

Recently, many proteins and protein interactions in signal transduction pathways have been identified. Since the phosphorylation of serine/threonine or tyrosine residues is a key reaction in the signal transduction pathway, protein phosphatases must have an essential role similarly to protein kinases. However, protein phosphatases have been paid less attention, compared to protein kinases, especially from the dynamical point of view.

In the Janus kinase (JAK) and and signal transducer and activator of transcription (STAT) signal transduction pathway, which transduces the class I/II cytokine receptor signals and has been considerably studied [1,2], SH2 domain-containing tyrosine phosphatase 2 (SHP-2) was identified as a phosphatase for the dephosphorylation of receptors and JAK, and its role in the pathway has been studied [3,4]. The phosphorylated STAT homo- or hetero-dimers were translocated to the nucleus and worked as the transcription factors [5]. But the behavior of the STAT after the translocation and the control mechanism of its nuclear concentration have not been discussed. The JAK-binding protein [JAB/suppressor of cytokine signaling-1 (SOCS1)] inhibits JAK signaling in cells. We demonstrated that JAB specifically binds to the tyrosine residue in the activation loop of JAKs whose phosphorylation is required for the activation of kinase activity. The SOCS1 SH2 domain and N-terminal 12 amino acid region (kinase inhibitory region) is required for the binding to JAKs and the inhibition of its activity. Gene disruption studies demonstrated that JAB/SOCS1 negatively regulates interferon-γ (IFN-γ) signaling [6–10]. However, the control mechanism of this system has not been clarified. Furthermore, although a nuclear phosphatase was reported to be necessary for the JAK/STAT pathway [11] and identified recently [12], its role in the pathway has not been fully understood.

In order to investigate the control mechanism and the factors influencing the kinetics of JAK/STAT pathway, we have developed a computer simulation of the JAK/STAT signal transduction pathway. The IFN-γ pathway in liver cells was selected as a typical example. Our analysis indicates that nuclear phosphatase is the most important phosphatase in this system. And our analysis also shows the stability of the time course of active transcription factors against the addition of STAT1, receptor, and JAK proteins to the system. The reason why induced protein is used for the feedback control is also investigated.

2. Model description

The kinetic scheme presented in Fig. 1 forms the basis for the computational analysis of the JAK/STAT signaling network. In step 1, JAK binds to the intracellular domain of the IFN-γ receptor (IFNR) and forms the IFNR–JAK complex (designated as RJ in the kinetic scheme). Although JAK1 and JAK2 bind to IFNR, both JAK's are treated as JAK in this model for simplicity. IFN-γ binds to the extracellular domain of the RJ complex and forms the IFN-γ–IFNR–JAK complex (designated as IFNRJ) (step 2). IFN binding drives the association of two receptor monomers into a receptor dimer (IFNRJ2) (step 3). The dimerization of the RJ complex leads to the phosphorylation of several tyrosine residues by JAK (step 4) [1,2], yielding a form as IFNRJ2*. The STAT1 binds to IFNRJ2* and is phosphorylated by JAK (step 5) [1,2,13]. The phosphorylated STAT1 forms a homo-dimer (step 6) [14]. The phosphorylated dimers of STAT1 are translocated to the nucleus (step 7) and work as the transcription factors (step 8) [5]. The SOCS1 is induced by JAK/STAT pathway (step 9). The SOCS1 binds to the activated receptor–JAK and inhibits its kinase activity (step 10) [6–10].

The SHP-2 is known to be a phosphatase for the RJ complex[3].

*Corresponding author. Fax: (81)-6-6497 7289.
E-mail addresses: yamada.satoshi@wrc.melco.co.jp (S. Yamada), shiono.satoru@wrc.melco.co.jp (S. Shiono), ajoo@bioreg.kyushu-u.ac.jp (A. Joo), yakihiko@bioreg.kyushu-u.ac.jp (A. Yoshimura).

Abbreviations: IFN-γ, interferon-gamma;; IFNR, interferon-γ receptor; JAK, Janus kinase; 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; STAT1*D_n, phosphorylated STAT1 dimers in the nucleus; PPN, nuclear phosphatase; PPX, unidentified phosphatase in the cytoplasm; JAB, JAK-binding protein

0014-5793/02/$22.00
doi:10.1016/S0014-5793(02)03842-5

However, phosphatases for phosphorylated STATs in the cytoplasm and the nucleus have been paid less attention to, although nuclear phosphatase was reported to have an important role in JAK/STAT pathway [11] and identified recently [12]. These phosphatases are assumed as PPX and PPN for phosphatases in the cytoplasm and the nucleus, respectively. Although nuclear STAT1 protein tyrosine phosphatase was identified as TC45 [12], it is called as PPN in this model. The binding of STAT1, SHP-2, and SOCS1 to IFNRJ2* are not competitive. All binding forms are considered in this model, and SHP-2 works even if the other proteins bind to IFNRJ2*.

In this kinetic analysis, Michaelis–Menten equation is not used, because in the signal transduction pathway the condition that the substrate concentration is much larger than the enzyme is not usually satisfied. All reactions are represented by mass-action kinetics. A cell is divided into two compartments, the cytoplasm and the nucleus. The phosphorylated STAT1 dimers were reported to be translocated to the nucleus and dephosphorylated STAT1 monomer was transported from the nucleus to the cytoplasm [15]. Other transports through the nuclear membrane except mRNA's transport to the cytoplasm are ignored. The translocation rate of STAT protein and mRNA is approximated to be proportional to their concentrations. The transcription rate depends on the concentration of the active transcription factors and has maximal rate because of the limited number of RNA polymerase complexes. The transcription rate v_t is approximated by the following equation [16]:

$$v_t = \frac{V_{max}[Tr]}{K_{tr} + [Tr]}$$

where $[Tr]$, V_{max}, and K_{tr} denote the concentration of the active transcription factor, the maximal transcription rate, and the constant, respectively. Because the translation rate is not a rate-limit step, it is approximated to be proportional to the mRNA concentration in the cytoplasm.

Since SOCS1 was reported to be degraded by the proteasome [17] and mRNA is not stable in the cytoplasm, the degradation reaction of SOCS1 and mRNA are included in this kinetic scheme, and are approximated to be proportional to their concentrations.

The dissociation and kinetic constants and the protein concentration were set based on the experimental results. The parameter values were set in step order. The dissociation constant for IFN-γ and IFNR was set to values similar to the dissociation constants of other cytokines (0.1–1 nM). Then, the binding and kinetic constants for receptor–JAK phosphorylation were set to fit the JAK phosphorylation to the experimental results [18]. And then, the binding constants and kinetic constants for STAT phosphorylation and STAT translocation were set to fit those to the STAT phosphorylation and distribution change of green fluorescent protein–STAT [5]. The dissociation constants of SOCS1 and IFNRJ2* were set based on the experimental results [8,19]. Detailed chemical reactions and their parameters are described in Appendix.

These reactions are described in the differential equations and solved mathematically by using Runge–Kutta–Gill method. The simulation program was written in C by us, using commonly used Runge–Kutta–Gill subroutine.

3. Results and discussion

The cytokine binding to its receptor induces receptor-dimerization and autophosphorylation of receptor and receptor-bound JAK. The phosphorylated JAK was detected at 15 min after the exposure to IFN and reached a maximum at about 30 min. STATs are phosphorylated by JAK, and make homo- or hetero-dimers. The phosphorylated STATs were detected at 30 min. and reached a maximum between 1 and 2 h, and then decreased by the inhibition of induced SOCS1. Fig. 2 demonstrates that the simulated time course shows the above characteristics. In SOCS1 knock-out cells, the phosphorylated STAT1 were kept in a high concentration. The simulated time course also shows the accumulation of the phosphorylated STAT1 in the nucleus (Fig. 2F), and was consistent with the reported experimental data [18]. The phosphorylation rate of JAKs and STATs is dependent on the cell types and cytokines. Cells stimulated by IFN showed the above time courses [20]. Cells stimulated by other cytokines (e.g. interleukin-2) showed faster time courses [20]. The model having more receptors and JAKs as well as that with faster phosphorylation rate by JAK showed faster time courses (data not shown).

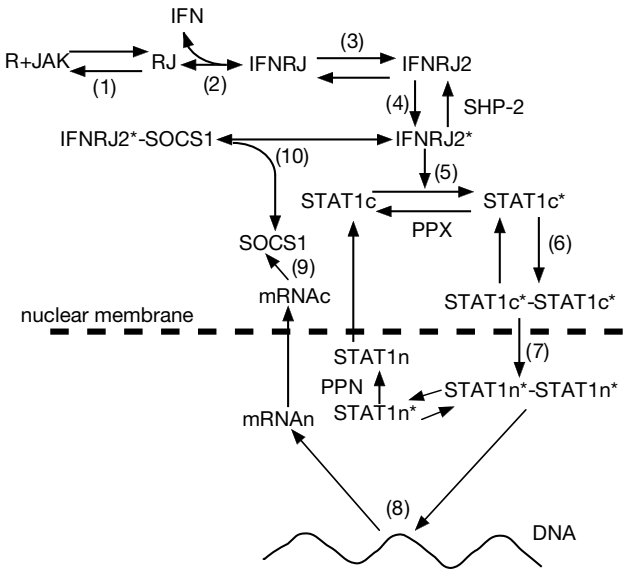

Fig. 1. Schematic representation of the computer simulation of JAK/STAT signal transduction pathway. Numbers in parentheses denote the number of reaction steps.

This system involves three phosphatase, SHP-2 and two phosphatases for STAT1. The dependency of the time course of phosphorylated STAT1 dimers in the nucleus ($STAT1*D_n$) on the phosphatase concentration was investigated in order to evaluate their role in this system. Fig. 3A–C shows the time course of $STAT1*D_n$ for various PPN (A), PPX (B), and SHP-2 (C) concentrations. The changes in PPN concentration caused the most considerable changes in the time course among the three phosphatases. Fig. 3D shows the dependency of peak concentration and Fig. 3E shows the steady-state concentration of $STAT1*D_n$. In the low concentration of PPN, $STAT1*D_n$ did not decrease after the peak, and almost all STAT1 were kept as active transcription factors in the nucleus, which was similar to the time course in the SOCS1 knock-out cells. However, in the low concentration of PPX and SHP-2 the time courses of $STAT1*D_n$ were similar to that under the normal condition. In the high concentration of these three phosphatases, the signal transduction of JAK/STAT pathway was inhibited. These results indicate that PPN is the most important phosphatase in the inhibitory action of SOCS1.

The concentration of $STAT1*D_n$ is determined by the balance of STAT1*D's influx to the nucleus and its efflux. Without SOCS1 production (Fig. 2L,N), the influx exceeds the efflux until 1.5 h, and the STAT1 is accumulated in the nucleus, and then the influx and the efflux are balanced. With SOCS1 inhibition (Fig. 2K,M), the phosphorylation of STAT1 is inhibited by the binding of SOCS1 to JAK and the influx decreases. Since the efflux exceeds the influx from 1 to 4 h, $STAT1*D_n$ decreases after 1 h. In order to decrease $STAT1*D_n$, not only the decrease in the influx but also the

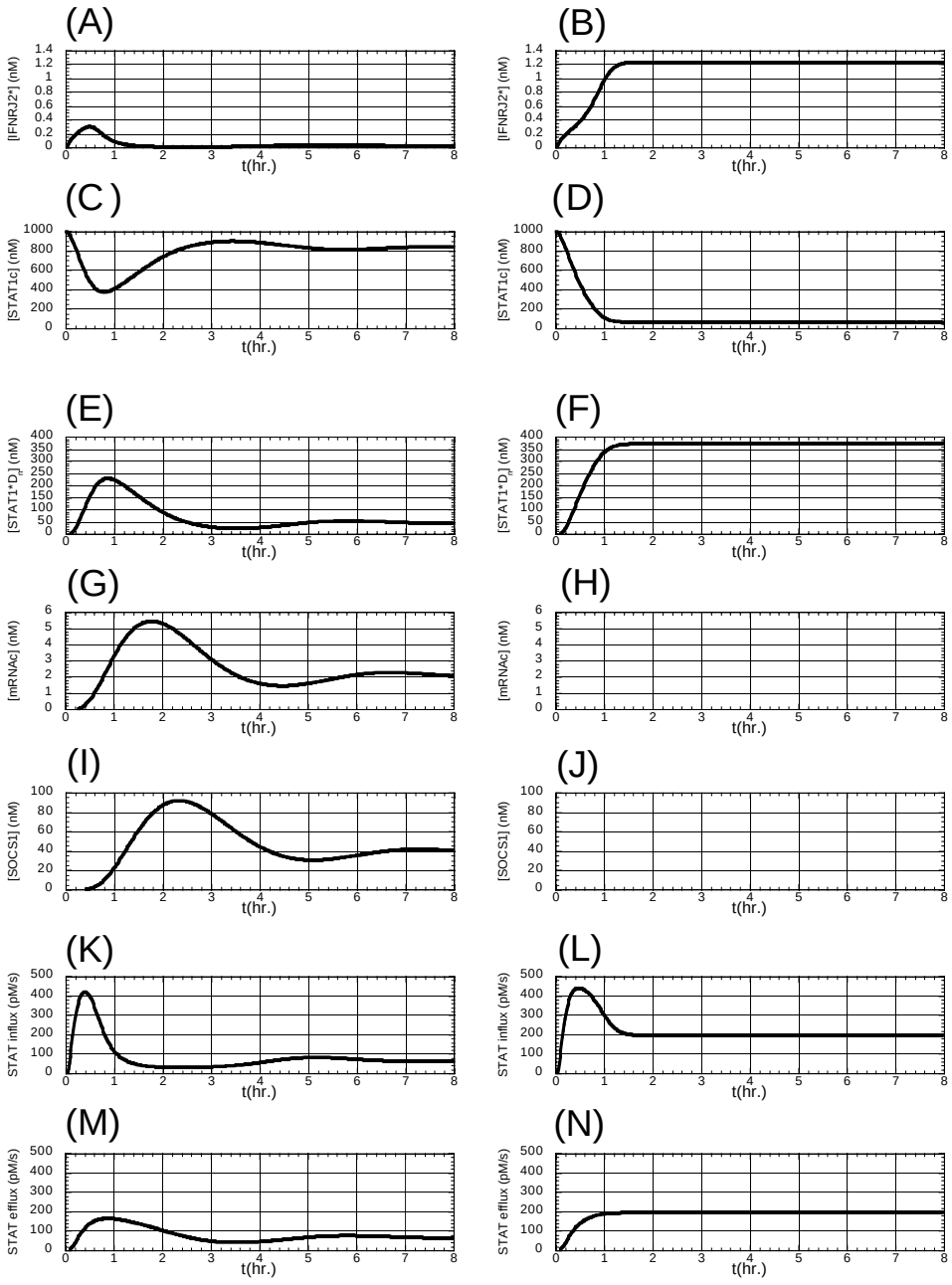

Fig. 2. Simulated time course of JAK/STAT activation in liver cells over 8 h continuous exposure to IFN-γ in normal cells (A,C,E,G,I,K,M) and SOCS1 knock-out cells (B,D,F,H,J,L,N). The time course of activated IFNRJ (A,B), dephosphorylated STAT1 in the cytoplasm (C,D), STAT1*D_n (E,F), mRNA in the cytoplasm (G,H), SOCS1 in the cytoplasm (I,J), STAT1 influx to the nucleus (K,L), and STAT1 efflux from the nucleus (M,N).

efflux is needed. The efflux reactions consist of the dephosphorylation in the nucleus and the translocation from the nucleus. Because the dephosphorylation in the nucleus is catalyzed by PPN, PPN is necessary for the efflux and more important than the other phosphatases. Without PPN, the binding of SOCS1 to JAK did not cause the decrease in STAT1*D_n (Fig. 3A).

The stability of responses (the peak and steady state concentration of active transcription factors (STAT1*D_n)) was investigated. Responses with various initial concentrations or parameter values were compared. If the phosphorylation reactions were decelerated, which corresponds to the decreases in initial STAT1 (Fig. 4A,B), receptor, and JAK (Fig. 4C), or the increases in dissociation constant between them (data not shown), the responses decreased as the phosphorylation rate decreased. On the contrary, even if the phosphorylation reactions were accelerated (Fig. 4A, bold lines), the responses were similar to that under the normal condition. Because the peak concentration of STAT1*D_n under the normal condition is close to the maximum, the peak values are changed a little by the addition of STAT1. The steady state concentration is determined by the balance of the influx and efflux. The influx

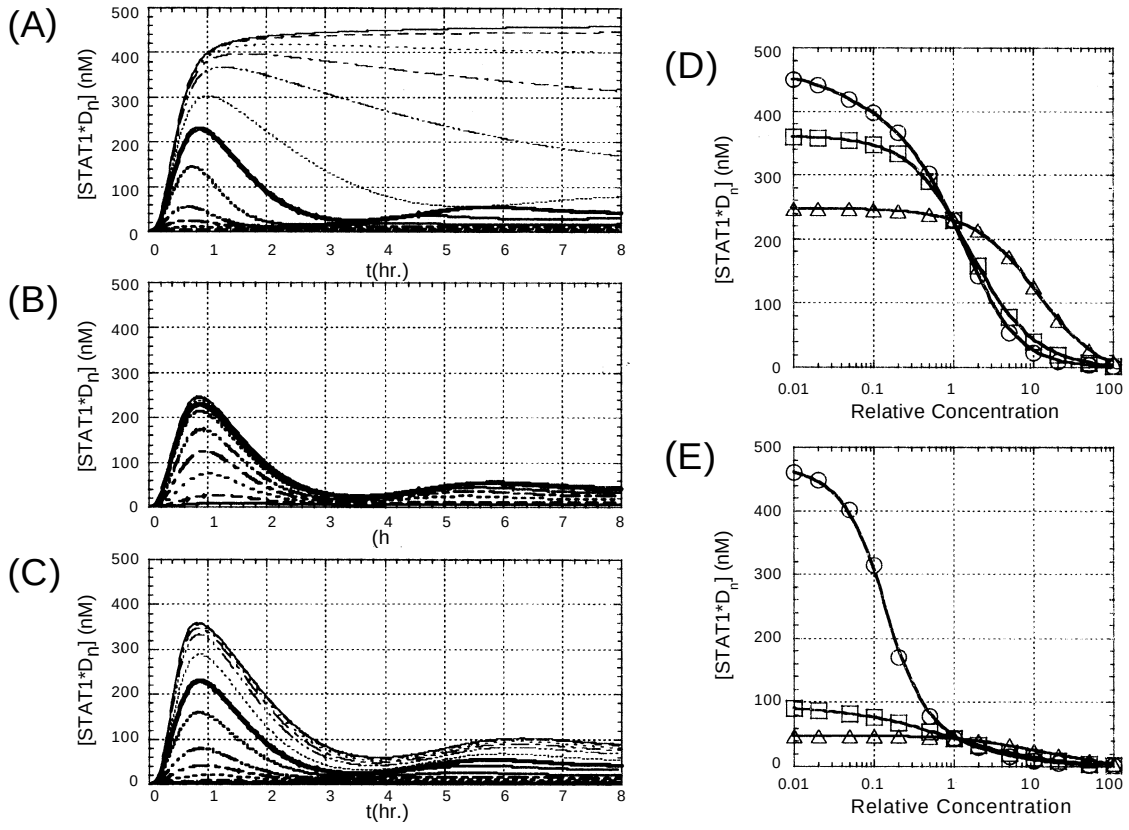

Fig. 3. Dependency on the phosphatase concentration. The time course of STAT1*D_n for various concentrations of (A) PPN (1/100–100 times), the bold line shows the time course of the normal condition, (B) PPX, and (C) SHP-2. D: The dependency of the peak concentration of STAT1*D_n on the phosphatase concentration. E: The dependency of the steady state concentration (8 h) of STAT1*D_n on the phosphatase concentration. ○, PPN; □, SHP-2; △, PPX.

in the steady state is determined by the concentration of free (not bound with SOCS1) JAK–receptor complexes. So the increase in the single factor or the affinity between them does not induce the efficient increase in the complex concentration. Fig. 4B,C shows that the steady state concentration of STAT1*D_n does not change by the addition of STAT, JAK, and receptor. If the two of those factors simultaneously increase (Fig. 4D), the initial concentrations of both receptor and JAK increase), the steady state concentration of STAT1*D_n increases. The responses of JAK/STAT signal transduction pathway are stable against the addition of STAT1, JAK, and receptor.

Why JAK/STAT pathway is controlled by an induced protein? In the signal transduction pathway, the transduced signal must be controlled to have adequate size and duration. If phosphatase activity is stronger than kinase activity, a transient signal is formed [21]. In JAK/STAT pathway, a kinase activity is stronger than a phosphatase activity, since phosphorylated STAT1 was accumulated in the nucleus in SOCS1 knock-out cells. The feedback control by activated protein in the pathway is another candidate of the control scheme. The Ras–MAP kinase cascade was reported to be controlled by such a scheme [22], the activation of Ras was inhibited by the activated ERK. In order to investigate the validity of feedback control of activated protein, the kinetics of a tentative pathway shown in Fig. 5A was simulated. In this tentative model, phosphorylated STAT1 dimers are assumed to bind to JAK kinases and inhibit them. This scheme showed no transient signals (Fig. 5B). Since inhibitory binding occurs without time-lag, the concentration of active transcription factors shows a simple time course of the saturation and no transient peak. Furthermore, since the concentration of STAT1 dimers in the cytoplasm is low because of the translocation to the nucleus, the inhibitory action by STAT1 dimers will not be effective. On the contrary, an induced protein (SOCS1) showed transient kinetics under various conditions (Fig. 5C shows the time course with several dissociation constants), since the inhibitory factors appear with a time-lag. In the MAPK cascade active ERK appears with time-lag, therefore feedback control by the activated existing factors is useful. The Smad pathway is another pathway of one-step activation of transcription factors, it is also controlled by the induced inhibitory Smad (I-Smad) [23].

4. Conclusions

Through a quantitative analysis of a computer simulation of JAK/STAT signal transduction pathway in liver cells, we have presented the SOCS1 inhibitory mechanism and the importance of a nuclear phosphatase. Simulation of SOCS1 −/− was consistent with the reported experimental data [18]. In the JAK/STAT pathway, SHP-1 or SHP-2 has been studied as a negative regulator. However, from the simulation result, a nuclear phosphatase (PPN in our model) is the most important phosphatase in this system. Although the signal transduction was inhibited by the high concentration of SHP-2,

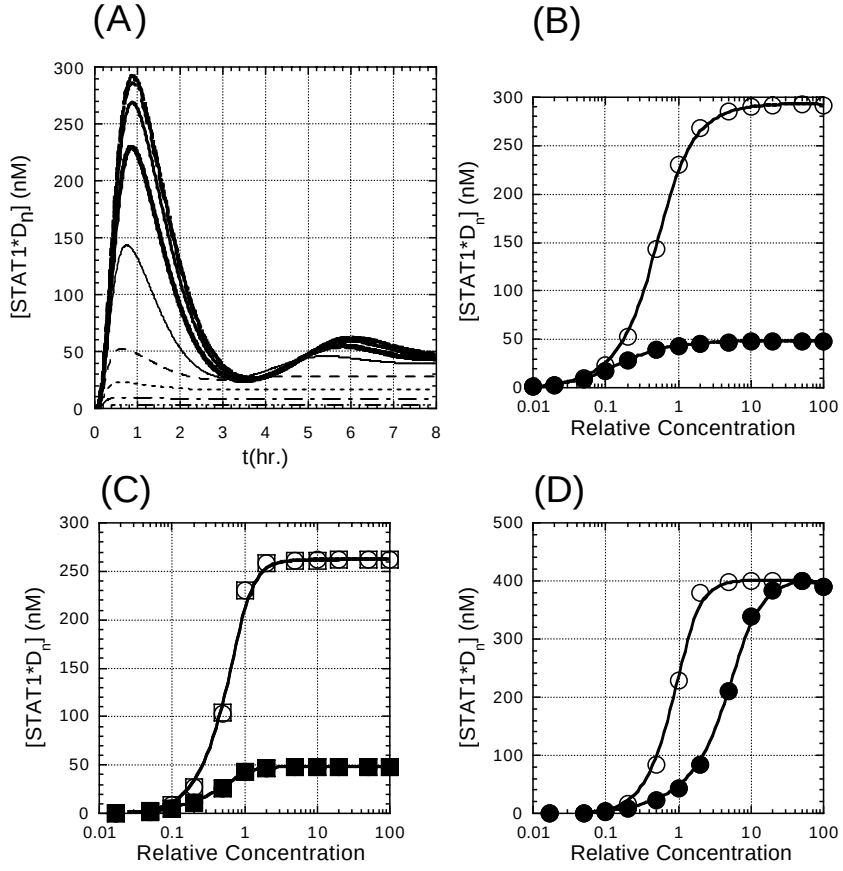

Fig. 4. Dependency on the STAT1, receptor, and JAK concentration. A: The time course of STAT1*D_n for various STAT1 concentrations (1/100–100 times), bold line shows the time course of the normal condition. B: The dependency of the peak concentration (○) and steady state (8 h, ●) of STAT1*D_n on the STAT1 concentration. C: The dependency on the receptor (peak, ○; 8 h, ●)and JAK concentration (peak, □; 8 h, ■). D: The dependency on receptor–JAK concentration (both receptor and JAK concentrations are changed; peak, ○; 8 h, ●).

that without SHP-2 was similar to that under the normal condition. However, the signal transduction without PPN was similar to that in SOCS1 knock-out cells.

The further addition of receptor, JAK, or STAT1 did not cause the increase in the active transcription factors, especially in the steady state. The feedback control of the system can keep the signal size and duration against the addition of actuator proteins.

The JAK/STAT and Smad pathways share a similar scheme with one-step activation of transcription factors. Since the inhibition by the activated protein themselves is not effective, these systems are controlled by induced inhibitory proteins, SOCS1 or I-Smad. The duration of the signal is one to several hours because a protein synthesis takes about 1 h.

The merit of the model study is to be able to investigate the role of any factors and any parameters in the system by the simulation with various values. We investigated the control mechanism by changing the model.

Appendix. Chemical reactions and their parameter values

First and second order rate constants are expressed in units of second^{-1} and 10^6 molar^{-1} second^{-1}, respectively. The dissociation constants for binding reactions are also written in parentheses in units of nM. Initial concentrations of proteins are expressed in units of nM.

[R]+[JAK]↔[RJ] $k_1 = 100$, $k_{-1} = 0.05$ ($k_{d1} = 0.5$)
[IFN]+[RJ]↔[IFNRJ] $k_2 = 20$, $k_{-2} = 0.02$ ($k_{d2} = 1$)
2[IFNRJ]↔[IFNRJ2] $k_3 = 40$, $k_{-3} = 0.2$ ($k_{d3} = 5$)
[IFNRJ2]→[IFNRJ2*] $k_4 = 0.005$
[IFNRJ2*]+[STAT1c]↔[IFNRJ2*-STAT1c] $k_5 = 8$, $k_{-5} = 0.8$ ($k_{d5} = 100$)
[IFNRJ2*-STAT1c]→[IFNRJ2*]+[STAT1c*] $k_6 = 0.4$
[IFNRJ2*]+[STAT1c*]↔[IFNRJ2*-STAT1c*] $k_7 = 5$, $k_{-7} = 0.5$ ($k_{d7} = 100$)
2[STAT1c*]↔[STAT1c*-STAT1c*] $k_8 = 20$, $k_{-8} = 0.1$ ($k_{d8} = 5$)
[IFNRJ2*]+[SHP-2]↔[IFNRJ2*-SHP-2] $k_9 = 1$, $k_{-9} = 0.2$ ($k_{d9} = 200$)
[IFNRJ2*-SHP-2]→[IFNRJ2]+[SHP-2] $k_{10} = 0.003$
[PPX]+[STAT1c*]↔[PPX-STAT1c*] $k_{11} = 1$, $k_{-11} = 0.2$ ($k_{d11} = 200$)
[PPX-STAT1c*]→[PPX]+[STAT1c] $k_{12} = 0.003$
[PPX]+[STAT1c*-STAT1c*]↔[PPX-STAT1c*-STAT1c*] k_{11}, k_{-11}, (k_{d11})
[PPX-STAT1c*-STAT1c*]→[PPX]+[STAT1c-STAT1c*] k_{12}
[STAT1c]+[STAT1c*]↔[STAT1c-STAT1c*] $k_{13} = 0.0002$, $k_{-13} = 0.2$ ($k_{d13} = 1000000$)
[STAT1c*-STAT1c*]→[STAT1n*-STAT1n*] $k_{14} = 0.005$
2[STAT1n*]↔[STAT1n*-STAT1n*] k_7, k_{-7}, (k_{d7})
[PPN]+[STAT1n*]↔[PPN-STAT1n*] $k_{15} = 1$, $k_{-15} = 0.2$ ($k_{d15} = 200$)
[PPN-STAT1n*]→[PPN]+[STAT1n] $k_{16} = 0.005$

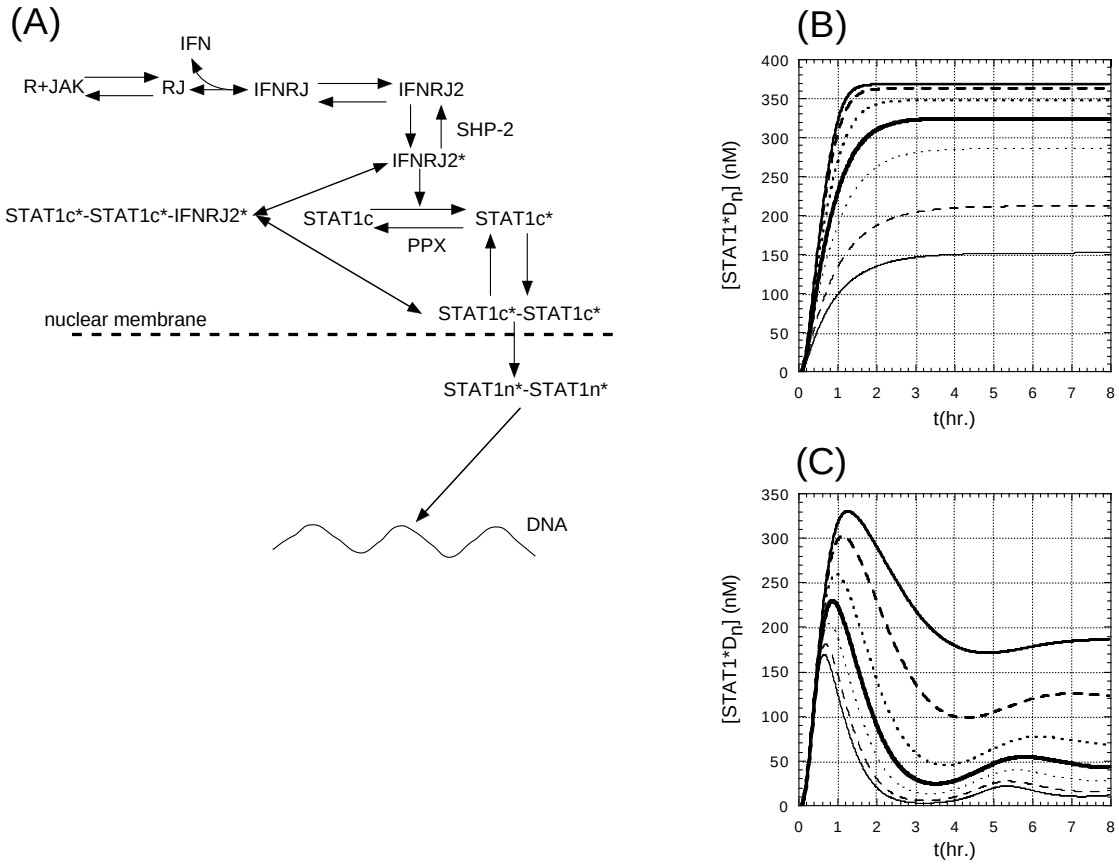

Fig. 5. Comparison of feedback mechanism. A: Tentative scheme in which phosphorylated STAT1 dimers bind to JAK's and inhibit them. B: The time course of STAT1*D_n in the tentative scheme shown in panel A with various dissociation constants (1–100 nM) of phosphorylated STAT1 dimers and JAK. C: The time course of STAT1*D_n in the normal model shown in Fig. 1 with various dissociation constants (0.5–50 nM) of SOCS1 and JAK.

[PPN]+[STAT1n*-STAT1n*] ↔ [PPN-STAT1n*-STAT1n*] k_{15}, k_{-15}, (k_{d15})
[PPN-STAT1n*-STAT1n*] → [PPN]+[STAT1n-STAT1n*] k_{16}
[STAT1n]+[STAT1n*] ↔ [STAT1n-STAT1n*] k_{13}, k_{-13}, (k_{d13})
[STAT1n] → [STAT1c] $k_{17} = 0.05$
d[mRNAn]/d$t = k_{18a}$[STAT1n*-STAT1n*]/(k_{18b}+[STAT1n*-STAT1n*]) $k_{18a} = 0.01$ nM/s, $k_{18b} = 400$ nM
[mRNAn] → [mRNAc] $k_{19} = 0.001$
d[SOCS1]/d$t = k_{20}$[mRNAc] $k_{20} = 0.01$
[SOCS1]+[IFNRJ2*] ↔ [SOCS1-IFNRJ2*] $k_{21} = 20$, $k_{-21} = 0.1$ ($k_{d21} = 5$)
d[mRNAc]/d$t = -k_{22}$[mRNAc] $k_{22} = 0.0005$
d[SOCS1]/d$t = -k_{23}$[SOCS1] $k_{23} = 0.0005$
[STAT1c]+[SOCS1-IFNRJ2*] ↔ [SOCS1-IFNRJ2*-STAT1c] k_5, k_{-5}, (k_{d5})
[SHP-2]+[SOCS1-IFNRJ2*-STAT1c] ↔ [SOCS1-IFNRJ2*-STAT1c-SHP-2] k_9, k_{-9}, (k_{d9})
[SOCS1-IFNRJ2*-STAT1c-SHP-2] → [SOCS1]+[IFNRJ2]+[STAT1c]+[SHP-2] k_{10}
[SOCS1-IFNRJ2*-STAT1c-SHP-2] → [IFNRJ2*-STAT1c-SHP-2] k_{23}
$[R]_0 = 12$, $[JAK]_0 = 12$, $[STAT1c]_0 = 1000$, $[SHP-2]_0 = 100$, $[PPX]_0 = 50$, $[PPN]_0 = 60$

References

[1] Shuai, K. (2000) Oncogene 19, 2638–2644.
[2] Imada, K. and Leonard, W.J. (2000) Mol. Immunol. 37, 1–11.
[3] You, M., Yu, D.H. and Feng, G.S. (1999) Mol. Cell. Biol. 19, 2416–2424.
[4] Bousquet, C., Susini, C. and Melmed, S. (1999) J. Clin. Invest. 104, 1277–1285.
[5] Köster, M. and Hauser, H. (1999) Eur. J. Biochem. 260, 137–144.
[6] Endo, T.A., Masuhara, M., Yokouchi, M., Suzuki, R., Sakamoto, H., Mitsui, K., Matsumoto, A., Tanimura, S., Ohtsubo, M., Misawa, H., Miyazaki, T., Leonor, N., Taniguchi, T., Fujita, T., Kanakura, Y., Komiya, S. and Yoshimura, A. (1997) Nature 387, 921–924.
[7] 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) Cell 98, 597–608.
[8] Yasukawa, H., Misawa, H., Sakamoto, H., Masuhara, M., Sasaki, A., Wakioka, T., Ohtsuka, S., Imaizumi, T., Matsuda, T., Ihle, J.N. and Yoshimura, A. (1999) EMBO J. 18, 1309–1320.
[9] Yasukawa, H., Sasaki, A. and Yoshimura, A. (2000) Annu. Rev. Immunol. 18, 143–164.
[10] Cooney, R.N. (2002) Shock 17, 83–90.
[11] Shuai, K. (1999) Prog. Biophys. Mol. Biol. 71, 405–422.
[12] ten Hoeve, J., de Jesus Ibarra-Sanchez, M., Fu, Y., Zhu, W., Tremblay, M., David, M. and Shuai, K. (2002) Mol. Cell. Biol. 22, 5662–5668.
[13] Stahl, N., Farruggella, T.J., Boulton, T.G., Zhong, Z., Darnell, J.E. and Yancopoulos, G.D. (1995) Science 267, 1349–1353.
[14] Shuai, K., Horvath, C.M., Huang, L.H., Qureshi, S.A., Cowburn, D. and Darnell, J.E. (1994) Cell 76, 821–828.
[15] McBride, K.M., McDonald, C. and Reich, N.C. (2000) EMBO J. 19, 6196–6206.
[16] Kyoda, K.M., Muraki, M. and Kitano, H. (2000) in: Pacific Symposium on Biocomputing 2000 (Altman, R.B., Dunker,

A.K., Hunter, L., Lauderdale, K. and Klein, T.E., Eds.), pp. 317–328, World Scientific, Singapore.
[17] Kamizono, S., Hanada, T., Yasukawa, H., Minoguchi, S., Kato, R., Minoguchi, M., Hattori, K., Hatakeyama, S., Yada, M., Morita, S., Kitamura, T., Kato, H., Nakayama, I.K. and Yoshimura, A. (2001) J. Biol. Chem. 276, 12530–12538.
[18] Brysha, M., Zhang, J.G., Bertolino, P., Corgin, J.E., Alexander, W.S., Nicola, N.A., Hilton, D.J. and Starr, R. (2001) J. Biol. Chem. 276, 22086–22089.
[19] Nicholson, S.E., Souza, D.D., Fabri, L.J., Corbin, J., Willson, T.A., Zhang, J.G., Silva, A., Asimakis, M., Farley, A., Nash, A.D., Metcalf, D., Hilton, D.J., Nicola, N.A. and Baca, M. (2000) Proc. Natl. Acad. Sci. USA 97, 6493–6498.
[20] Hanada, T., Yoshida, T., Kinjyo, I., Minoguchi, S., Yasukawa, H., Kato, S., Mimata, H., Nomura, Y., Seki, Y., Kubo, M. and Yoshimura, A. (2001) J. Biol. Chem. 276, 40746–40754.
[21] Heinrich, R., Neel, B.G. and Rapoport, T.A. (2002) Mol. Cell 9, 957–970.
[22] Brightman, F.A. and Fell, D.A. (2000) FEBS Lett. 482, 169–174.
[23] Itoh, F., Asao, H., Sugamura, K., Heldin, C.-H., Dijke, tenP. and Itoh, S. (2001) EMBO J. 20, 4132–4342.