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The N-terminal Truncated Isoform of SOCS3 Translated from an Alternative Initiation AUG Codon under Stress Conditions Is Stable Due to the Lack of a Major Ubiquitination Site, Lys-6*

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The suppressor of cytokine signaling-3 (SOCS3/CIS-33/SSI-3) is an important negative regulator of cytokine signaling. Here, we show that an N-terminal truncated isoform (ΔN-SOCS3) translated from the internal AUG codon 12 was profoundly induced by endoplasmic reticulum (ER) stress- or active double-stranded RNA-activated protein kinase PKR, as a result of induction of eukaryotic initiation factor 2α phosphorylation. ΔN-SOCS3 exhibited a stronger cytokine-inhibitory activity and a higher stability than WT-SOCS3 in Ba/F3 hematopoietic cells. A potential ubiquitination residue, Lys-6, at the N terminus is evolutionary conserved among SOCS3 species. The K6Q-SOCS3 mutant showed a much longer half-life than WT-SOCS3 in Ba/F3 cells. Furthermore, inhibition of the 26 S proteasome pathway increased both ubiquitination and protein levels of WT-SOCS3 but had no effect on K6Q-SOCS3. SOCS3 mutant lacking the carboxyl-terminal SOCS-box exhibited the same stability as K6Q-SOCS3. These observations suggest that the short form of SOCS3 is a naturally occurring stabilized inhibitory protein, whereas WT-SOCS3 is a short-lived protein modulated by Lys-6 ubiquitination and proteasome-dependent degradation. Our findings provide strong evidence for the first time that translational control plays an important role in stabilization and function of SOCS3.

Cytokines control a wide spectrum of biological responses, but the duration and intensity of their effects must be tightly regulated (1, 2). Cytokines induce oligomerization of specific cell-surface receptors and activate the Janus kinase/signal transducer and activator of transcription (JAK/STAT)[1] pathway (3, 4). The strength of cytokine signals is regulated, in part, by a family of endogenous JAK kinase inhibitor proteins referred to as suppressors of cytokine signaling (SOCS), cytokine-inducible SH2 proteins (CIS), or STAT-induced STAT inhibitors (SSI) (5–7). Both SOCS1 and SOCS3 have an N-terminal kinase inhibitory region and inhibit JAK kinase activity; SOCS1 directly binds to JAKs (8, 9), whereas SOCS3 inhibits JAKs through binding to cytokine receptor tyrosine residues (10–13). SOCS3 mRNA is induced by various cytokines, and SOCS3 protein negatively regulates immune and inflammatory responses *in vivo* (14, 15). However, cytokines exert their actions concomitantly in stressful situations including inflammation, ER stress, and viral infections (16, 17). Therefore, the response of SOCS proteins to cellular stress remains to be investigated.

When cells are under physiological and environmental stress, such as nutrient deprivation, disturbance of intracellular stores of Ca^{2+}, or virus infection, the accumulation of incorrectly folded proteins occurs, which induces a stress response, a process called the unfolded protein response (UPR). The UPR induces a rapid repression of protein synthesis to reduce unfolded protein levels or to interfere with viral replication (16, 17). This repression of protein synthesis occurs mainly through the phosphorylation of eIF2α on serine 51, which interferes with the formation of an active 40 S translation-initiation complex. Reduction of 40 S initiation complexes results in suppression of translation initiation of most mRNAs and reduces protein synthesis of many proteins (18, 19). However, not all of the proteins are translationally repressed by eIF2α phosphorylation. For example, in mammalian cells expression of transcription factor ATF4 is induced by eIF2α phosphorylation (20). Also, in yeast cells induction of eIF2α phosphorylation by the GCN2 kinase plays an important role in the translational induction of transcription factor GCN4 (21). The UPR also activates transcription factors ATF6 and XBP1, which induce the ER stress-response genes, chaperones, and folding catalysts as well as the proapoptotic gene, *CHOP/GADD153* (22–24).

In the present study, we demonstrate that an isoform of SOCS3 is generated by alternative translation initiation. This N-terminal truncated product of SOCS3, herein referred as ΔN-SOCS3, lacks the major ubiquitination site lysine 6 of the full-length protein, and therefore, it is resistant to 26 S protea-

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[1] The abbreviations used are: JAK, Janus kinase; STAT, signal transducer and activator of transcription; SOCS, suppressors of cytokine signaling; CIS, cytokine-inducible SH2 proteins; SSI, STAT-induced STAT inhibitors; ER, endoplasmic reticulum; UPR, unfolded protein response; eIF, eukaryotic initiation factor; PKR, protein kinase dsRNA-dependent; GFP, green fluorescence protein; EGFP, enhanced GFP; EGF, epidermal growth factor; IFN, interferon; IL, interleukin; EPO, erythropoietin; EPOR, erythropoietin receptor; HCV, hepatitis C virus; IRES, internal ribosome entry site.

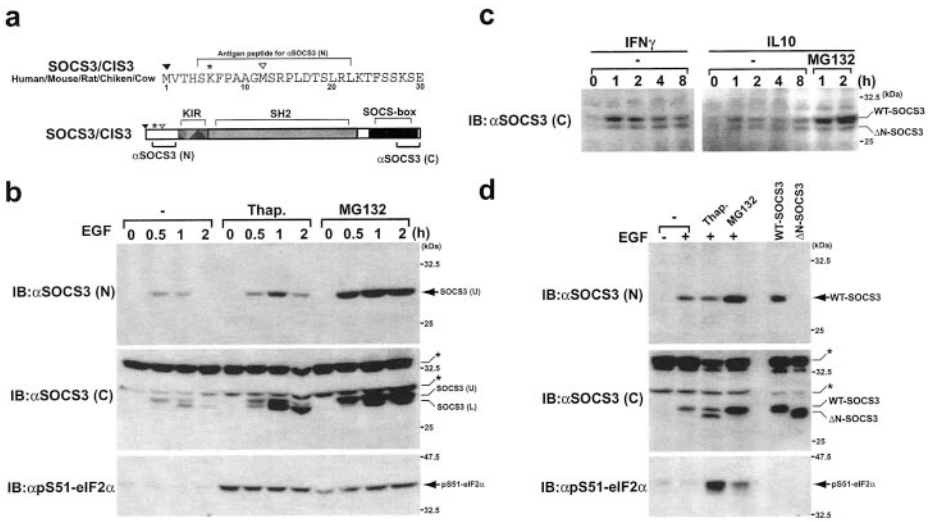

FIG. 1. **Detection of N-terminal truncated SOCS3 in Ba/F3 cells.** *a*, schematic model of the functional domains of SOCS3. The first Met and Met-12 are indicated as *solid* and *open triangles*, respectively. Conserved Lys-6 is shown with an *asterisk*. *b*, EGF-dependent BF-EGFR/EPOR cells were cultured without EGF for 8 h and then pretreated with Me_2SO (−), 5 μM thapsigargin (*Thap.*), or 50 μM MG132 for 15 min. Then cells were stimulated with 100 ng/ml EGF for the indicated times, and cell extracts were immunoblotted (*IB*) with indicated antibodies. The *asterisk* denotes nonspecific bands. Similar results were obtained in three independent experiments. *c*, RAW cells were pretreated with or without 50 μM MG132 for 15 min, then stimulated with 100 ng/ml IFNγ or IL-10 for the indicated times. The cell extracts were immunoblotted with anti-SOCS3(C) antibody. *d*, BF-EGFR/EPOR cells pretreated as in *b* were stimulated with 100 ng/ml EGF for 30 min and analyzed as in *b*. 293 cell lysate containing WT-SOCS3 or ΔN-SOCS3 (12–225 amino acids) were run on the same gel for comparison.

some-dependent degradation. We also provide evidence that ΔN-SOCS3 is induced by conditions that enhance eIF2α phosphorylation such as ER stress or activation of the protein kinase PKR. Furthermore, ΔN-SOCS3 appears to be more biologically active than the full-length protein in suppressing cytokine signaling. Our data provide strong evidence for a regulation of SOCS3 expression and function at the translational level.

EXPERIMENTAL PROCEDURES

Materials—SOCS3 mutants were generated by the standard PCR method and subcloned into pCDNA3 or pMX-IRES-EGFP as described (11, 12). The active PKR construct has been described (25). Human EGF, murine IFNγ, and IL-10 were purchased from PeproTech EC, Ltd. MG132 and thapsigargin were purchased from Sigma and dissolved in dimethyl sulfoxide (Me_2SO). Affinity-purified polyclonal rabbit anti-SOCS3(N) or anti-SOCS3(C) antibodies were generated against synthetic peptide SKFPAAGMSRPLDTSLRL or YEKVTQLPGPIREFLDQYDAPL, respectively (Immuno-Biological Laboratories Co., Ltd., Japan). Anti-GFP (FL) and anti-Myc (A-14 and 9E10) antibodies were purchased from Santa Cruz. Rabbit anti-phospho-S51-eIF2α antibody and mouse monoclonal anti-ubiquitin antibody were purchased from Cell Signaling and Zymed Laboratories Inc., respectively.

Immunochemical Analysis—Immunoprecipitation and immunoblotting analysis were performed as described (11, 12, 26). To detect WT-SOCS3 and ΔN-SOCS3, SDS-14% PAGE was used. For the detection of the ubiquitinated SOCS3, the proteins were transferred onto polyvinylidene difluoride membranes. The blots were incubated with Tris-buffered saline containing 0.5% glutaraldehyde for 30 min before blocking with 10% skim milk in Tris-buffered saline.

Cells and Mice—293T cells and RAW264.7 cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. The establishment of Ba/F3 cells expressing a chimeric receptor consisted of the extracellular domain of the EGF receptor, and the cytoplasmic domain of the EPO receptor (BF-EGFR/EPOR) was described previously (27). These cells were maintained in RPMI medium containing 10% fetal bovine serum and 100 ng/ml EGF. For thapsigargin or MG132 treatment, BF-EGFR/EPOR cells were cultured without EGF for 8 h and pretreated with 5 μM thapsigargin or 50 μM MG132 for 15 min. Then, cells were stimulated with 100 ng/ml EGF for the indicated times, and cell extracts were immunoblotted with anti-SOCS3(N) or anti-SOCS3(C) antibody. To assess the half-life of wild-type or mutant SOCS3 protein, infected cells were incubated with 100 μg/ml cycloheximide for various times. Cell extracts were then analyzed by immunoblotting with an anti-SOCS3(C) antibody specific to the C terminus of the protein. Transgenic mice expressing the hepatitis C virus (HCV) core protein were described previously (28).

Retrovirus Infection—The wild-type and mutant SOCS3 cDNAs subcloned in pMX-IRES-EGFP were transfected into a PLAT-E packaging cell line using the FuGENE 6 (Roche Molecular Biochemicals) to obtain the viruses (12). Ba/F3 cells expressing the EGFR-EPOR chimeras (2×10^5 cells) were infected with viruses on a RetroNectin (TaKaRa)-coated plate for 24 h in the presence of 100 ng/ml EGF. Cells were washed three times with phosphate-buffered saline, resuspended in RPMI-10% fetal calf serum containing 100 ng/ml EGF and incubated for the indicated times. Then, cells (1×10^4) were analyzed for EGFP fluorescence on a COULTER EPICS-XL flow cytometer.

RESULTS AND DISCUSSION

An N-terminal Truncated SOCS3 Form Is Induced by ER Stress—To examine the expression of SOCS3 protein by ER stress, we used BF-EGFR/EPOR cells in which treatment of EGF activates the EPO receptor cytoplasmic domain and the JAK2 pathway (12, 27). As shown in Fig. 1*b* (*upper panel*), upon EGF stimulation, the endogenous SOCS3 protein was detected as a single band by a rabbit polyclonal antibody against a peptide from the N terminus of SOCS3 (anti-SOCS3(N)) (Fig. 1*a*). SOCS3 was significantly down-regulated after a 2-h stimulation with EGF. Contrary to this, cells stimulated with EGF in the presence of the 26 S proteasome inhibitor MG132 showed a sustained increase in SOCS3 protein levels from 30 min to 2 h. Therefore, according to previous reports (29), our data verify that the proteasome plays a major role in the rapid degradation of SOCS3.

Thapsigargin is an inhibitor of the Ca^{2+}ATPase transporter known to induce the ER stress. We found that the thapsigargin rapidly induced the phosphorylation of eIF2α on serine 51 and slightly increased SOCS3 protein levels 1 h after EGF stimulation as detected by anti-N-terminal antibody (Fig. 1*b*). However, SOCS3 protein was detected as two bands by a rabbit anti-SOCS3(C) antibody, which recognizes the C terminus of SOCS3 (Fig. 1*b*, *middle panel*). The regions recognized by anti-SOCS3(N) and anti-SOCS3(C) antibodies are shown in Fig. 1*a*. The molecular weight and expression pattern of the two proteins suggested that the upper band recognized by the anti-SOCS3(C) antibody is identical to the protein detected by the anti-SOCS3(N) antibody. The levels of the lower band detected

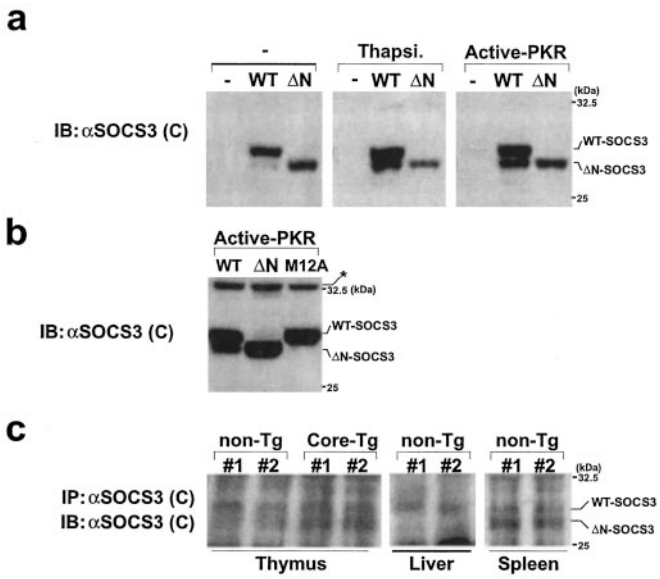

FIG. 2. **ΔN-SOCS3 is derived from an internal translational site, Met-12.** *a* and *b*, the WT-SOCS3, ΔN-SOCS3, or M12A-SOCS3 cDNA carrying no upstream untranslated region was transfected into 293 cells with or without active PKR plasmid. Cells were pretreated with 5 μM thapsigargin for 4 h (*a*, *center panel*). Cell extracts were then analyzed with an anti-SOCS3(C) antibody. The *asterisk* denotes a nonspecific band. Similar results were obtained in two independent experiments. *c*, tissue extracts (1 mg of protein/sample) from core-Tg or control littermates were immunoprecipitated (*IP*) and analyzed with an anti-SOCS3(C) antibody. Two separate mice were analyzed.

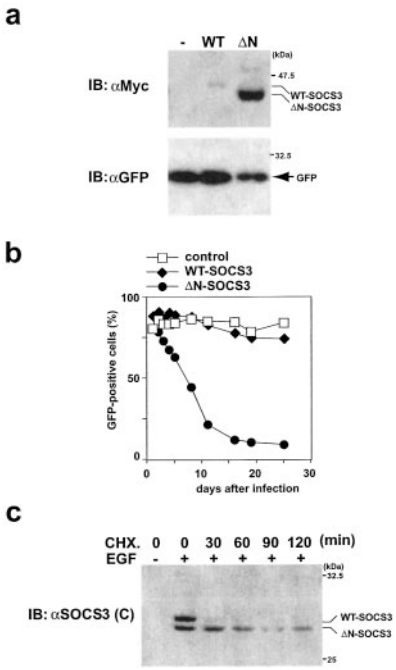

FIG. 3. **ΔN-SOCS3 is a long-lived protein and possesses a higher inhibitory effect than WT-SOCS3.** *a*, BF-EGFR/EPOR cells were infected with a control virus encoding EGFP alone (−) or with retrovirus encoding Myc-tagged WT-SOCS3 or ΔN-SOCS3. After 2 days, cells (1×10^5) were collected for Western blot analysis with anti-Myc or anti-GFP antibodies. The molecular weight of exogenous SOCS3 was higher than endogenous SOCS3 because of the 6×Myc tag. *b*, cells were maintained in culture in the presence of 100 ng/ml EGF, and the populations of EGFP-positive cells were counted by FACS analysis on indicated days after infection. *c*, BF-EGFR/EPOR cells were cultured without EGF for 8 h and stimulated with 100 ng/ml EGF for 1 h. The cells were then treated with 100 μg/ml cycloheximide (*CHX.*) for the indicated times, and cell extracts were analyzed with an anti-SOCS3(C) antibody.

by anti-SOCS3(C) were low after a 1-h EGF stimulation in the absence of the ER stress inducer. In contrast, this form of SOCS3 was profoundly elevated by the treatment with either thapsigargin or MG132 for 1 h after EGF stimulation (Fig. 1*b*). Similar results were obtained with a mouse monoclonal antibody that recognizes the C terminus of SOCS3 (data not shown). These data implied an involvement of ER stress-induced eIF2α phosphorylation in the induction of the N-terminal truncated SOCS3.

We also found that the two forms of SOCS3 were not only induced by IL-3 stimulation of Ba/F3 cells, but they were also induced in the mouse macrophage-like cell line RAW264.7 by either interferon (IFN) γ or IL-10 (Fig. 1*c*). The same two isoforms of SOCS3 were observed in v-*src* transformed 3T3 cells or granulocyte macrophage-colony-stimulating factor-stimulated UT-7 cells (data not shown). Thus, the N-terminal truncated SOCS3 form was generated not only by EGF/EPO receptor chimera but also by different cytokines.

The SOCS3 gene has no intron in the protein-coding region (30). Therefore, we speculated that N-terminal truncated SOCS3 was derived from an alternative translation initiation site starting from methionine at position 12 (Met-12) (Fig. 1*a*). To examine this possibility, the full-length (WT)-SOCS3 or an N-terminal 11-amino acid deletion mutant SOCS3 (ΔN-SOCS3) was expressed in 293 cells (Fig. 1*d*). We noticed that the upper and lower bands of SOCS3 in the Ba/F3 cell lysate migrated at the same positions corresponding to WT-SOCS3 and ΔN-SOCS3 expressed in 293 cells, respectively (*middle panel*). We also confirmed that the anti-SOCS3(C) antibody could recognize both WT- and ΔN-SOCS3 proteins, whereas the anti-SOCS3(N) antibody reacted only with WT-SOCS3 protein (Fig. 1*d*). These results indicated that an isoform of SOCS3 is expressed as a result of alternative translation initiation. Thus, phosphorylation of eIF2α may play a role in initiation of translation from the second AUG (Met-12), resulting in the generation of N-terminal truncated SOCS3.

Alternative Translation of SOCS3 mRNA Is Induced by Active PKR—To verify that N-terminal truncated SOCS3 was produced by alternative translation through eIF2α phosphorylation, we next examined the effect of the eIF2α kinase PKR (25) on SOCS3 expression. First, we examined whether a similar alternative translation initiation product is expressed in 293 cells transiently transfected with SOCS3 cDNA. As shown in Fig. 2*a*, *left* and *middle panels*, WT-SOCS3, as well as ΔN-SOCS3, was detected by an anti-SOCS3(C) antibody as a single band under normal conditions. On the other hand, WT-SOCS3 but not ΔN-SOCS3 was expressed as two bands in the presence of thapsigargin. Thus, alternative initiation in the presence of thapsigargin occurred even when SOCS3 cDNA was transiently expressed in 293 cells. When SOCS3 cDNA was cotransfected with human PKR cDNA, we found that expression of the low molecular weight form of SOCS3 was induced. The lower band detected in WT-SOCS3-transfected cells migrated at the same level as ΔN-SOCS3, indicating that N-terminal truncated SOCS3 is translated from full-length SOCS3 cDNA as a result of PKR activation and eIF2α phosphorylation. To further confirm that alternative translation from Met-12 occurred in the presence of active-PKR, we generated a SOCS3 mutant with substitution of Met-12 to Ala (M12A-SOCS3). Transient transfection in 293 cells showed that M12A-SOCS3 was detected as a single band even in the presence of active-PKR, whereas WT-SOCS3 was detected as two bands (Fig. 2*b*). Similar results were obtained by thapsigargin treatment of 293 cells transfected with M12A or WT-SOCS3 cDNA (data not shown).

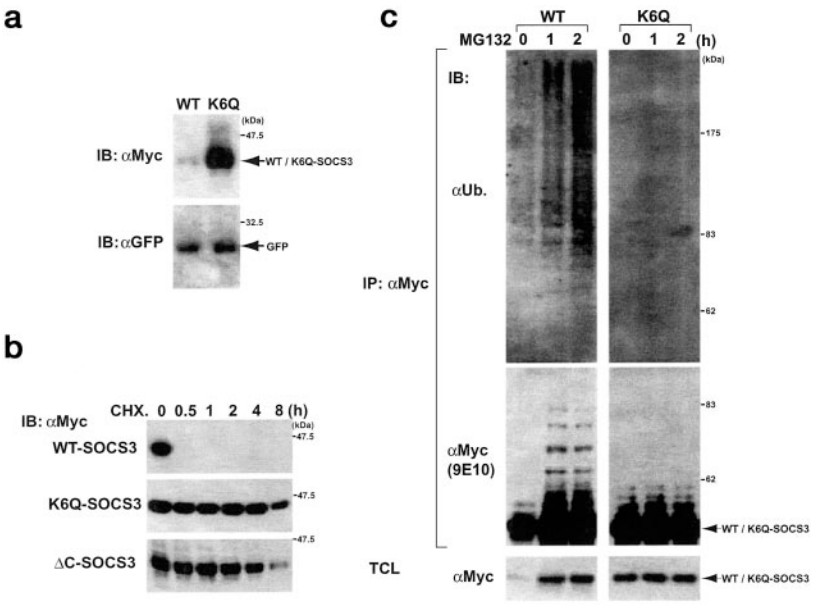

FIG. 4. **Mutation of Lys-6 diminished the ubiquitination of SOCS3 and conferred high stability.** *a*, BF-EGFR/EPOR cells were infected with a retrovirus encoding either Myc-tagged WT-SOCS3 or K6Q-SOCS3. After 2 days, 1×10^5 cells were collected for Western blot analysis with anti-Myc and anti-GFP antibodies. *b*, Ba/F3 cells (1×10^5) infected with the indicated viruses (infection efficiency was about 80%) were treated with 100 μg/ml cycloheximide (*CHX.*) for the indicated times in the presence of 100 ng/ml EGF, and cell extracts were then analyzed with anti-Myc antibody. To detect WT-SOCS3 protein, longer exposure of Western blot membrane was done. *c*, the infected cells (1×10^6) were treated with 50 μg/ml MG132 for the indicated times in the presence of 100 ng/ml EGF, and then cell extracts were immunoprecipitated with a rabbit anti-Myc antibody. They were then immunoblotted with mouse anti-Myc (9E10) and anti-ubiquitin (*Ub.*) antibodies.

ΔN-SOCS3 Is Produced in Vivo— To examine whether ΔN-SOCS3 is produced *in vivo*, we measured SOCS3 protein levels by immunoblotting in various tissues from wild-type (non-Tg) and transgenic (Tg) mice expressing the HCV core protein (28). We have recently demonstrated that HCV core protein associates with and activates STAT3, resulting in a higher production of SOCS3 protein (28). Furthermore, the core protein has been shown to activate PKR (31). As shown in Fig. 2*c*, in wild-type mice, WT-SOCS3 was predominately expressed in the liver and thymus, whereas ΔN-SOCS3 was highly expressed in the spleen. Because UPR has been shown to be activated during differentiation of B lymphocyte to the plasma cells (32), translation of ΔN-SOCS3 is likely to be induced in antigen-stimulated T- and B-lymphocytes in the spleen.

Core protein was expressed highly in the thymus of HCV core protein Tg mice (28). In the core Tg mouse thymus, the levels of ΔN-SOCS3 were much higher than those of WT-SOCS3 (Fig. 2*c*). These data suggested that HCV core protein not only induces SOCS3 expression through STAT3 activation but also stimulates alternative translation initiation of SOCS3 most probably through PKR. These results clearly indicated that expression of ΔN-SOCS3 takes place in physiological or pathological conditions *in vivo*.

The ΔN-SOCS3 Protein Is More Active and Stable Than WT-SOCS3 in Ba/F3 Cells—To assess possible functional differences between WT-SOCS3 and ΔN-SOCS3, SOCS3 genes were stably introduced into Ba/F3 cells with retrovirus vectors bearing EGFP under the control of an internal ribosome entry site (IRES). Because SOCS3 inhibits EPO receptor-JAK2 signaling, the cytokine-suppressing activity of SOCS3 can be assayed by measuring the reduction of EGFP-positive cells (12, 33). As shown in Fig. 3*a*, EGFP levels were similar after 2 days of infection, indicating a similar SOCS3-EGFP mRNA production in WT-SOCS3- and ΔN-SOCS3-infected cells. However, WT-SOCS3 protein levels were less than those of ΔN-SOCS3. Then we examined possible changes in the population of EGFP-positive cells (Fig. 3*b*). The population of EGFP-positive cells infected with control virus did not change, whereas EGF-positive cells expressing WT-SOCS3-infected cells decreased gradually. This indicated that the cytokine signal-suppressing activity of WT-SOCS3 is weak in Ba/F3 cells, probably due to its low expression. Contrary to this, the number of ΔN-SOCS3-infected cells decreased rapidly, indicating that the suppressing activity of ΔN-SOCS3 is higher than that of WT-SOCS3.

Because WT-SOCS3 protein levels were low in infected Ba/F3 cells, we compared the stability of WT-SOCS3 and ΔN-SOCS3 proteins. We measured half-life of the endogenous SOCS3 forms in Ba/F3 cells in the presence of the protein synthesis inhibitor cycloheximide. As shown in Fig. 3*c*, endogenous WT-SOCS3 is extremely unstable contrary to ΔN-SOCS3, which is more stable than WT-SOCS3. Similar results were obtained with transfected WT-SOCS3 or ΔN-SOCS3 in Ba/F3 cells (data not shown). As WT-SOCS3 half-life was significantly affected by MG132 (see Fig. 1*b*), these data suggested that ΔN-SOCS3 may not be subjected to proteasome-dependent degradation.

Lys-6 Is a Major Potential Ubiquitination Site of SOCS3—To understand the molecular mechanisms of high stability of ΔN-SOCS3, we examined carefully the amino acid sequence within the N-terminal region of WT-SOCS3. One lysine residue, a potential ubiquitination site, was found at position 6 (Lys-6) in SOCS3 (Fig. 1*a*). Thus, we created a mutant SOCS3 in which Lys-6 was substituted for glutamine (K6Q-SOCS3). Retroviruses carrying WT- or K6Q-SOCS3-IRES-EGFP were used to infect BF-EGFR/EPOR cells, and both protein expression and stability levels were compared and measured. The expression level of EGFP was similar after 2 days of infection; however, as in ΔN-SOCS3, the levels of K6Q-SOCS3 protein were much higher than those of WT-SOCS3 (Fig. 4*a*). Furthermore, K6Q-SOCS3 protein turnover was much slower than that of WT-SOCS3 (Fig. 4*b*). We also examined the half-life of C-terminal SOCS-box truncated SOCS3 (ΔC-SOCS3) using the same retrovirus infection system, based on previous findings that the SOCS-box interacts with Elongin BC complex to generate the E3 ubiquitin-ligase complex (29, 33). As expected, ΔC-SOCS3 was as stable as K6Q-SOCS3 (Fig. 4*b*). These results suggested that Lys-6 of SOCS3 is a key residue for regulating SOCS3 stability through the SOCS-box.

To further examine this hypothesis, we tested ubiquitination of WT-SOCS3 and K6Q-SOCS3 using Ba/F3 cells infected with SOCS3 viruses. As shown in Fig. 4*c*, the presence of MG132 increased the expression of WT-SOCS3 (*bottom panel*), which coincided with the appearance of high molecular weight bands containing WT-SOCS3 (*middle panel*). Ubiquitination of WT-SOCS3 protein was demonstrated by immunoblotting with anti-ubiquitin antibody (Fig. 4*c*, *upper panel*). In contrast, the expression level of K6Q-SOCS3 was not affected by MG132 (Fig. 4*c*, *bottom panel*), and the ubiquitination of K6Q-SOCS3

was not detected by anti-ubiquitin antibody. We cannot rule out the possibility that Lys-6 is not the only ubiquitination site because it may be involved in ubiquitination of other lysine residues in WT-SOCS3 protein. Nevertheless, our data definitely demonstrate that Lys-6 plays a major role in the ubiquitination of SOCS3.

It is also noteworthy that the protein stability of SOCS3 is varied in different cell lines. For example, we found that WT-SOCS3 is relatively stable in 293 cells and mouse epithelial cell lines, whereas it is highly unstable in hematopoietic cells including Ba/F3, Raw, and UT-7 cells (Fig. 2 and data not shown). Therefore, ubiquitination of Lys-6 and degradation of SOCS3 may be regulated by a cell type-specific factor(s) in addition to the SOCS box. Future studies on the mechanisms of SOCS3 ubiquitination as well as alternative translation initiation are likely to yield important new information about the regulatory pathways of cytokine signaling controlled by SOCS3.

In this study, we have demonstrated that ER stress induces the expression of ΔN-SOCS3 as a result of alternative translation initiation. It does so through the induction of eIF2α phosphorylation caused by the activation of the protein kinase PERK (17). This is also strongly supported by our data showing that activation of the eIF2α kinase PKR induces ΔN-SOCS3 expression. Inasmuch as PKR activity is induced in virus-infected cells, induction of ΔN-SOCS3 protein synthesis by PKR may provide evidence for a differential regulation of SOCS3 translation by viruses. Our study further substantiates previous findings showing that translational control plays a role in the regulation of SOCS proteins. That is, previous data demonstrated that translation of SOCS1 is suppressed by the presence of the 5′-untranslated region and is regulated in a cap-dependent manner by the activity of eIF4E-binding proteins (34). Taken together, these findings suggest that tight control of SOCS protein expression is required for efficient signaling in response to cytokines or environmental stress. Further studies with the use of ΔN-SOCS3 transgenic mice or M12A-SOCS3 knock-in mice are necessary to fully address the physiological relevance of translational control of SOCS3 *in vivo*.

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