

COMMENTARY

SOCS: physiological suppressors of cytokine signaling

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SUMMARY

Cytokines regulate cellular behavior by interacting with receptors on the plasma membrane of target cells and activating intracellular signal transduction cascades such as the JAK-STAT pathway. Suppressors of cytokine signaling (SOCS) proteins negatively regulate cytokine signaling. The SOCS family consists of eight proteins: SOCS1-SOCS7 and CIS, each of which contains a central Src-homology 2 (SH2) domain and a C-terminal SOCS box. The expression of CIS, SOCS1, SOCS2 and SOCS3 is induced in response to stimulation by a wide variety of cytokines, and overexpression of these proteins in cell lines results in inhibition of cytokine signaling. Thus, SOCS

proteins appear to form part of a classical negative feedback loop. The analysis of mice lacking SOCS1 has revealed that it is critical in the negative regulation of IFN γ signaling and in the differentiation of T cells. Additionally, the analysis of mouse embryos lacking SOCS3 suggests that SOCS3 negatively regulates fetal liver erythropoiesis, probably through its ability to modulate erythropoietin (Epo) signaling. Thus, the use of gene targeting has confirmed that SOCS proteins regulate cytokine signaling in a physiological setting.

Key words: SOCS, Cytokine, Signal transduction, JAK, STAT

INTRODUCTION

Cytokines constitute a large family of secreted proteins that regulate fundamental biological processes including immunity, wound healing and hematopoiesis. Cytokines convey information about the biological status of the animal to target cells by interacting with receptors on the cell surface. Cellular responses to cytokine stimulation depend on the type of cytokine and the nature of the target cell and include proliferation, differentiation, effector function and survival (Nicola, 1994).

As a result of intensive study, a clear picture of the way in which cytokines activate target cells has emerged. Although cytokine receptors lack intrinsic kinase activity, they are constitutively associated with members of the Janus kinase (JAK) family of protein tyrosine kinases, which includes JAK1, JAK2, JAK3 and TYK2. The interaction between a cytokine and its receptor induces receptor dimerization or oligomerization, which results in the juxtaposition of JAKs; these then cross-phosphorylate, causing enzymatic activation. A key target of JAK activity is the cytoplasmic domain of the cytokine receptor, which becomes tyrosine-phosphorylated at multiple residues, creating docking sites for signaling proteins containing SH2 or phosphotyrosine binding (PTB) domains. The association between signaling proteins and cytokine receptors serves to initiate multiple signaling pathways, such as those regulated by RAS, phosphatidylinositol 3-kinase (PI3K) and the signal transducers and activators of

transcription (STATs). Together, these pathways culminate in the regulation of gene expression in the nucleus, resulting in an appropriate cellular response to the cytokine.

The STAT family of transcription factors plays a critical role in regulating physiological responses to cytokine stimulation. Members of the STAT family bind tyrosine-phosphorylated cytokine receptors through their SH2 domains. Once bound to the receptor, STATs are phosphorylated by JAKs, which causes them to dissociate from the receptor and form homo- or heterodimers. STAT dimers then translocate to the nucleus, where they interact with specific DNA elements in the promoters of target genes and thus regulate transcription (reviewed by Darnell, 1997).

It is well established that cytokine-induced signaling pathways are negatively regulated with respect to both duration and intensity. However, only recently has our understanding of this process advanced. It is now known that at least three different classes of negative regulator contribute to cytokine inhibition: the SH2-containing protein tyrosine phosphatase 1 (SHP1), the protein inhibitors of activated STATs (PIAS) and the suppressors of cytokine signaling (SOCS) proteins (reviewed by Hilton, 1999). Here, we discuss the SOCS family of proteins and how they negatively regulate cytokine signaling.

CLONING OF SOCS FAMILY MEMBERS

Socs1 was cloned simultaneously by three groups, who used

three different approaches. Starr et al. (1997) cloned *Socs1*, using a functional screen for inhibitors of cytokine signaling. In this screen, the myeloid leukemia cell line M1 was infected with a retroviral library and M1 clones that could no longer differentiate in response to IL-6 were isolated. The cDNA in one IL-6-unresponsive M1 clone encoded an SH2-domain-containing protein that Starr et al. (1997) named suppressor of cytokine signaling 1 (SOCS1). Endo et al. (1997) identified SOCS1 as a protein that interacts with the kinase domain of JAK2 in a yeast two-hybrid screen and named it JAK-binding protein (JAB). *Socs1* was also cloned by Naka et al. (1997), during a screen for novel STAT family members. In this study, a monoclonal antibody that recognized the STAT3 SH2 domain was used to screen an expression library and was found to recognize the gene product of *Socs1*. Here, the authors named the protein STAT-induced STAT-inhibitor (SSI). In each of these studies, SOCS1 was implicated in the negative regulation of cytokine signaling.

Database searches using the predicted amino acid sequence of SOCS1 revealed that at least 20 proteins in human and mouse share sequence similarity within a 40-residue C-terminal motif referred to as the SOCS box (Starr et al., 1997; Hilton et al., 1998). One of these genes had been previously identified and named cytokine-inducible SH2-domain-containing protein (CIS; Yoshimura et al., 1995). Interestingly, although all of the newly identified proteins contain a C-terminal SOCS box, they contain different domains in their central regions. Proteins containing a central SH2-domain were termed SOCS (SOCS1-SOCS7 and CIS), proteins containing WD-40 repeats were termed WSB (WSB1-WSB2), proteins containing ankyrin repeats were termed ASB (ASB1-ASB3) and proteins containing SPRY domains were termed SSB (SSB1-SSB3). In addition, a subfamily containing a central GTPase domain was identified. Although SOCS orthologs probably exist in other species, at present only a *Drosophila* ortholog of SOCS5 has been identified (Nicholson et al., 1999).

Members of the SOCS family contain N-terminal regions of variable length. For example, CIS, SOCS1, SOCS2 and SOCS3 have relatively short (50-80 residue) N-terminal regions, whereas SOCS4, SOCS5, SOCS6 and SOCS7 have longer N-terminal regions of up to 380 residues (Hilton et al., 1998). The N-terminal regions of SOCS family members contain no recognizable motifs, the exception being SOCS7 which contains a putative nuclear localization signal (Matsuoka et al., 1997). Currently, three naming systems are in place for the SOCS genes/proteins (Fig. 1).

THE EXPRESSION OF SOCS mRNAs CAN BE INDUCED BY CYTOKINES

Transcripts encoding SOCS1, SOCS2, SOCS3 and CIS are often present in cells at low levels, but can be induced within 15-30 minutes by a wide variety of cytokines, hormones or growth factors. Indeed, expression of SOCS1, SOCS2, SOCS3 and CIS mRNAs can all be induced by cytokines such as interferon γ (IFN γ), Epo, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-2 (IL-2), IL-3, IL-4, IL-6, leukemia inhibitory factor (LIF), growth hormone (GH) and prolactin. At present, there is little evidence that expression of SOCS4,

SOCS5, SOCS6, SOCS7, ASB or SSB mRNAs is induced by cytokines. However, the expression of WSB mRNA is induced by sonic hedgehog (Shh) in explant cultures of somitic mesoderm and in the chick limb bud (Vasiliauskas et al., 1999). The few studies investigating whether the synthesis of SOCS proteins is stimulated by cytokines show that SOCS protein synthesis correlates with induction of the corresponding mRNA (Bousquet et al., 1999; Stoiber et al., 1999; Cohnney et al., 1999).

In most cases, there is no correlation between particular cytokines and the SOCS mRNAs they can induce. Indeed, cytokine induction of *Socs* genes often varies with respect to the cell line or tissue being examined. For example, injection of GH induces the expression of CIS, SOCS2 and SOCS3 mRNAs in murine liver, but only CIS and SOCS2 mRNAs are induced in mammary gland (Davey et al., 1999). However, in some cases, there does appear to be specificity with respect to the SOCS mRNA induced by a particular cytokine. For example, injection of either leptin or ciliary neurotrophic factor (CNTF) induces the expression of SOCS3 but not SOCS1, SOCS2 or CIS mRNA in the mouse hypothalamus (Bjorbaek, 1998, 1999a,b). Similarly, IL-10 and LPS both induce expression of SOCS3 but not SOCS1, SOCS2 or CIS mRNA in monocytes and macrophages, respectively (Ito et al., 1999; Stoiber et al., 1999).

STATs MEDIATE INDUCTION OF SOCS TRANSCRIPTION

Members of the STAT family of transcription factors are activated by cytokines and in many cases the transcriptional upregulation of *Socs* genes is mediated by the STATs. For example, the proximal promoter of the *Cis* gene contains four STAT5-binding sites, all of which are necessary for Epo-dependent activation of the *Cis* promoter in reporter assays (Matsumoto et al., 1997). Furthermore, the induction of CIS mRNA in response to IL-3 is inhibited in cell lines expressing a dominant negative version of STAT5 (Mui et al., 1996) and CIS expression in mouse ovary is eliminated in mice lacking both STAT5a and STAT5b (Teglund et al., 1998). Expression of other *Socs* genes may also be induced by the STATs. The promoter of the *Socs1* gene contains putative STAT3- and STAT6-binding sites as well as a potential GAS motif, which mediates STAT1 binding. In addition, a dominant negative version of STAT3 inhibits the induction of SOCS1 mRNA in response to IL-6 or LIF in M1 cells (Naka et al., 1997). Thus, STATs can form a direct link between the activated cytokine receptor and the induction of *Socs* genes in the nucleus.

THE ACTION OF SOCS FAMILY MEMBERS IN VITRO

As discussed above, *Socs1* was cloned during a functional screen for negative regulators of cytokine signaling. It is now clear that expression of *Cis*, *Socs1*, *Socs2* or *Socs3* results in the attenuation of signaling by many different cytokines, growth factors and hormones (Fig. 1). For example, CIS, SOCS1 and SOCS3 all inhibit signaling by Epo, prolactin, GH, IL-2 and IL-3 (Matsumoto et al., 1997; Endo et al., 1997; Marine et al., 1999b; Matsumoto et al., 1999; Pezet et al., 1999;

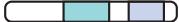
<u>NAME</u>	<u>STRUCTURE</u>	<u>INHIBITS SIGNALING BY</u>
CIS / CIS1		IL-2 (1), IL-3 (2), GH (1), prolactin (1), Epo (2)
SOCS1 / JAB / SSI1		IL-2 (3), IL-3 (3), IL-4 (4), IL-6 (5), GH (6), prolactin (7), Epo (3), LIF (5), IFN γ (5), IFN α (8), OSM (5), TSLP (9), Tpo (5)
SOCS2 / CIS2 / SSI2		GH (10), IL-6 (12), LIF (11)
SOCS3 / CIS3 / SSI3		IL-2 (13), IL-3 (13), IL-4 (4), IL-6 (12), IL-11 (14), GH (6), prolactin (7), Epo (15), LIF (12), IFN γ (8), IFN α (8), CNTF (16), leptin (17), OSM (8)
SOCS4		not tested
SOCS5 / CIS6		IL-6 (12)
SOCS6 / CIS4		does not inhibit in systems tested
SOCS7 / CIS5		not tested

Fig. 1. SOCS family members negatively regulate signaling by a variety of cytokines. The domain structure and alternative names for each SOCS family member are shown. In addition, the cytokines that each SOCS protein negatively regulates *in vitro* are listed. OSM, oncostatin M; TSLP, thymic stromal lymphopoietin; Tpo, thrombopoietin; CNTF, ciliary neurotrophic factor. References: 1. Matsumoto et al. (1999); 2. Matsumoto et al. (1997); 3. Endo et al. (1997); 4. Losman et al. (1999); 5. Starr et al. (1997); 6. Hansen et al. (1999); 7. Pezet et al. (1999); 8. Song and Shuai (1998); 9. Isaksen et al. (1999); 10. Ram and Waxman (1999); 11. Minamoto et al. (1997); 12. Nicholson et al. (1999); 13. Cohny et al. (1999); 14. Auernhammer and Melmed (1999); 15. Marine et al. (1999b); 16. Bjorbaek et al. (1999a) 17. Bjorbaek et al. (1999b).

Cohny et al., 1999). Similarly, SOCS2 inhibits signaling by IL-6 and GH (Nicholson et al., 1999; Ram and Waxman, 1999). In some cases there may be specificity with respect to the SOCS protein involved in inhibition. For example, SOCS3 but not SOCS2 or CIS inhibits signaling by leptin, a hormone that functions in the brain to regulate food intake (Bjorbaek et al., 1999b). Interestingly, Bjorbaek et al. (1998) observed an elevated level of SOCS3 mRNA in the hypothalamus of the Agouti mouse, which exhibits leptin resistance and obesity. Currently, there is no evidence that SOCS4, SOCS6 or SOCS7 regulates cytokine signaling; however, SOCS5 weakly inhibits the induction of a STAT3-dependent reporter gene in response to LIF, which suggests that it has a potential inhibitory role (Nicholson et al., 1999).

Because *Socs* genes are induced by cytokines, and the corresponding SOCS proteins inhibit cytokine-induced signaling pathways, SOCS proteins are believed to form part of a classical negative feedback loop (Fig. 2). For example, the *Cis* gene is induced by STAT5 in response to Epo stimulation, and the CIS protein can inhibit the activation of STAT5 by Epo (Matsumoto et al., 1997). In some cases, induction of SOCS proteins by one cytokine might inhibit signaling by another. Indeed, pretreatment of monocytes with IL-10 inhibits subsequent signaling by IFN γ or IFN α , and this has been attributed to the induction of SOCS3 protein (Ito et al., 1999). Thus, SOCS proteins might mediate cross-talk between signaling pathways.

In addition to negatively regulating the JAK-STAT pathway, SOCS proteins might have a broader function. De Sepulveda et al. (1999) found that SOCS1 associates with the tyrosine-phosphorylated KIT and FLT3 receptors and inhibits mitogenic signaling by these receptors. Furthermore, SOCS1 associates with TEC, GRB2, VAV, the FGF receptor and PYK2 (Masuhara et al., 1997; De Sepulveda et al., 1999; Ohya et al., 1997). In addition, SOCS2 associates with the tyrosine-phosphorylated insulin-like growth factor 1 receptor (IGF-1R; Dey et al., 1998), and SOCS3 associates with LCK, the FGF receptor and PYK2 (Masuhara et al., 1997). Future research

is necessary to clarify the significance of these protein interactions.

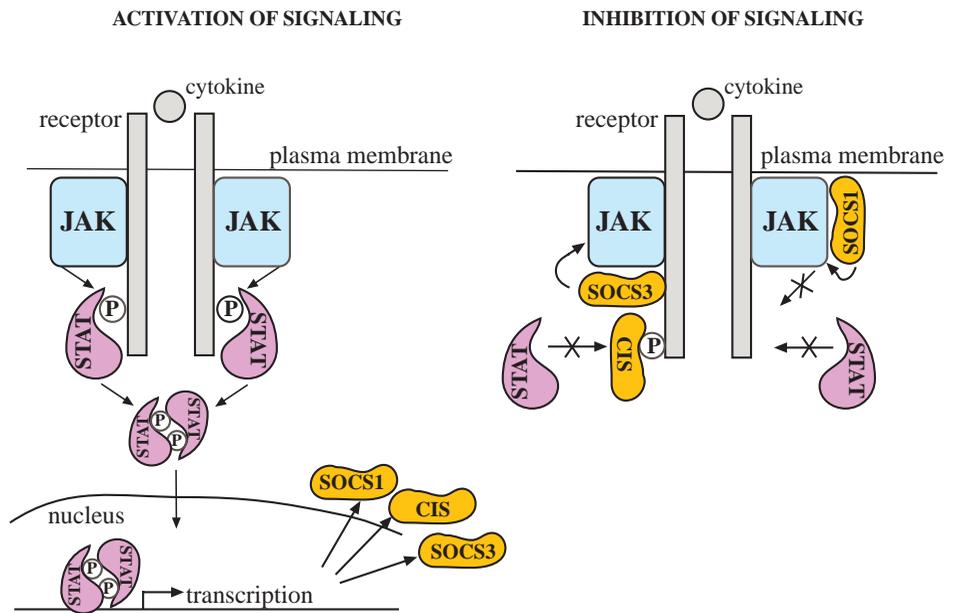
THE PHYSIOLOGICAL FUNCTION OF SOCS PROTEINS

In vitro studies suggest that SOCS proteins can inhibit signaling by a wide variety of cytokines; however, because these studies involve the ectopic expression of SOCS proteins in cell lines, the results could overestimate the range of SOCS action. It is perhaps not surprising that research using physiological models has provided new and illuminating information with respect to SOCS function.

To study the *in vivo* function of SOCS1, three groups have used gene targeting to generate mice lacking SOCS1 (Starr et al., 1998; Naka et al., 1998; Alexander et al., 1999; Marine et al., 1999a). Phenotypically, *Socs1*^{-/-} mice are runted and die at three weeks of age; they have a pathology characterized by severe lymphopenia, fatty degeneration of the liver and macrophage infiltration of major organs. In one report, lymphopenia in *Socs1*^{-/-} mice was attributed to increased lymphocyte apoptosis due to upregulation of the proapoptotic protein Bax. This suggested that SOCS1 inhibits apoptosis in this population of cells by downregulating Bax expression (Naka et al., 1998). The mechanism by which this might occur has not been clarified.

Two recent studies have provided insight into the physiological function of SOCS1 (Alexander et al., 1999; Marine et al., 1999a). Strikingly, the complex phenotype of *Socs1*^{-/-} mice appears to be a consequence of augmented signaling by IFN γ . Consistent with this, a phenotype similar to that observed in *Socs1*^{-/-} mice can be induced in wild-type neonatal mice by administration of IFN γ (Gresser et al., 1981; Alexander et al., 1999). Furthermore, the disease in *Socs1*^{-/-} mice can be prevented by injection of neutralizing anti-IFN γ antibodies or by crossing the mice on to an IFN γ -negative background (Alexander et al., 1999; Marine et al., 1999a). In

Fig. 2. The mechanism by which SOCS proteins negatively regulate cytokine signaling. Cytokine stimulation activates the JAK-STAT pathway, leading to the induction of CIS, SOCS1 and/or SOCS3. These SOCS proteins then negatively regulate cytokine signaling, thus forming part of a negative feedback loop. CIS, SOCS1 and SOCS3 appear to inhibit signaling by different mechanisms. SOCS1 associates with JAKs and inhibits catalytic activity, CIS inhibits the binding of STATs to activated cytokine receptors, and SOCS3 might inhibit JAK activity through a mechanism involving receptor association.



light of the fact that overexpressed SOCS1 inhibits IFN γ signaling in cell lines, it was hypothesized that the disease in *Socs1*^{-/-} mice is due to a hypersensitivity to IFN γ . The observation that *Socs1*^{-/-} bone-marrow-derived macrophages clear a *Leishmania* major parasite infection in response to 100-fold less IFN γ than do wild-type macrophages supports this (Alexander et al., 1999). SOCS1 therefore seems to modulate IFN γ signaling in vivo such that the detrimental effects of uncontrolled IFN γ signaling are held in check.

Marine et al. (1999a) characterized the phenotype of the *Socs1*^{-/-} mice further, finding that thymic T cell development is perturbed and peripheral T cells exhibit an activated phenotype in these animals. These findings, together with the observation that *Socs1* is expressed primarily in the thymocytes of normal mice, suggest that SOCS1 can regulate T cell differentiation. Furthermore, the authors found detectable levels of IFN γ in the serum of *Socs1*^{-/-} but not wild-type mice. Thus, the intrinsic hypersensitivity of *Socs1*^{-/-} tissues to IFN γ may be aggravated by an elevated level of IFN γ in the serum. Because T cells are the primary source of IFN γ , Marine et al. (1999a) hypothesized that the population of activated T cells in *Socs1*^{-/-} mice is the source of the elevated level of IFN γ in the serum. Consistent with this, *Socs1*^{-/-} mice that also lack RAG2, a key gene in lymphoid maturation, exhibit normal levels of IFN γ and survive. Thus, the authors concluded that, in addition to negatively regulating IFN γ signaling, SOCS1 regulates T cell differentiation and prevents the emergence of a population of T cells that secrete high levels of IFN γ .

The physiological function of SOCS3 has also been recently investigated. Marine et al. (1999b) examined the expression pattern of *Socs3* in vivo and found that although *Socs3* is expressed at low levels in adult tissues, it is highly expressed in fetal liver erythroid progenitors. Furthermore, *Socs3* expression is specifically induced during a stage of erythropoiesis characterized by an explosive Epo-dependent expansion of cells of the erythroid lineage. Thus, Marine et al. (1999b) hypothesized that SOCS3 plays a role in erythropoiesis. To test this hypothesis, the authors generated

mice lacking *Socs3* as well as transgenic mice overexpressing *Socs3*. Neither *Socs3*^{-/-} mice nor *Socs3* transgenic mice survived to birth. Strikingly, transgenic embryos that constitutively overexpressed *Socs3* exhibited no detectable fetal liver erythropoiesis, whereas a proportion of *Socs3*^{-/-} embryos died, exhibiting a pathology characterized by massive erythrocytosis throughout the embryo. Together, these experiments suggest that SOCS3 negatively regulates fetal liver erythropoiesis. Since Epo signaling via JAK2 is required for erythropoiesis, SOCS3 might regulate erythropoiesis by modulating JAK2 activity. In keeping with this, *Socs3* transgenic mice are phenotypically similar to *Jak2*^{-/-} mice (Neubauer et al., 1998; Parganas et al., 1998).

Interestingly, recent studies by Marine et al. (1999a,b) suggest that the in vivo expression of SOCS proteins can occur in a manner independent of cytokine signaling. For example, *Socs3* is expressed in fetal liver erythroid cells in mice in which Epo signaling is defective owing to a lack of either the Epo receptor, JAK2 or STAT5a/b. Similarly, *Socs1* is expressed in thymocytes from mice in which cytokine signaling is defective owing to a deficiency in the IL-7 receptor α chain, JAK3 or STAT5a/b, as well as in thymocytes from RAG2-deficient mice lacking the T cell receptor. Furthermore, expression of both *Socs1* and *Socs3* appears to be induced during a specific stage of hematopoiesis. Thus, Marine et al. (1999a,b) suggest that expression of *Socs1* and *Socs3* can be developmentally regulated and serves to inhibit cytokine signaling at key periods of embryonic and post-natal life.

HOW DO SOCS FAMILY MEMBERS NEGATIVELY REGULATE CYTOKINE SIGNALING?

Inhibition of the intrinsic kinase activity of JAKs

SOCS family members inhibit cytokine signaling by several mechanisms that are not mutually exclusive. Many studies suggest that SOCS1 inhibits cytokine signaling by associating with JAK1, JAK2, JAK3 and TYK2 and inhibiting their catalytic activity (Naka et al., 1997; Endo et al., 1997;

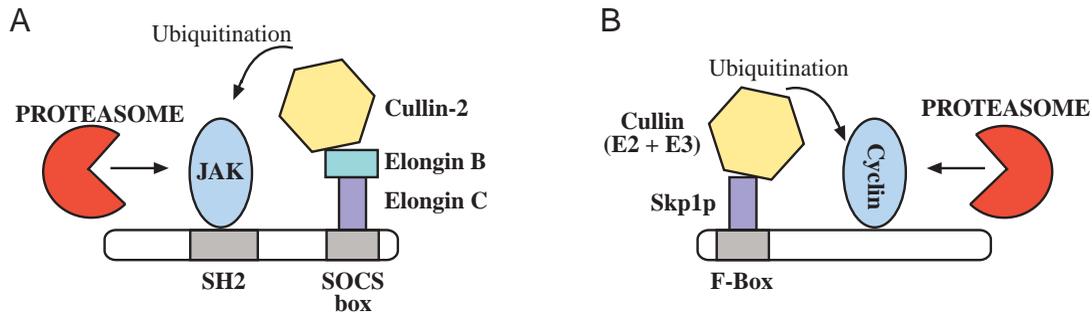


Fig. 3. SOCS family members might target signaling proteins for degradation by the proteasome: similarity between SOCS proteins and F-box proteins. (A) All SOCS proteins tested bind the elongin BC complex through their SOCS box. In turn, the elongin BC complex associates with the putative ubiquitin ligase cullin-2. Signaling proteins associated with the N-terminal or SH2 domains of SOCS proteins could be ubiquitinated by cullin-2, targeting them for degradation by the proteasome. (B) SOCS proteins may be analogous to F-box proteins that operate in the yeast phosphoprotein ubiquitin ligase complex (PULC). F-box proteins contain an N-terminal F-box motif that associates with the elongin C homolog Skp1p and the cullin-2 ubiquitin ligase homologs E2 and E3. Yeast F-box family members bind cyclins in response to serine phosphorylation, thus targeting them for ubiquitination and degradation by the proteasome. This permits cell cycle progression from G₁ to S.

Yasukawa et al., 1999; Nicholson et al., 1999). SOCS3 might also inhibit the activity of JAKs; however, compared with SOCS1, SOCS3 binds JAK2 with a lower affinity and must be expressed at a significantly higher level than SOCS1 for equivalent inhibition of kinase activity (Masuhara et al., 1997; Suzuki et al., 1998; Pezet et al., 1999). In contrast, neither CIS nor SOCS2 inhibits the kinase activity of JAK2 (Yasukawa et al., 1999; Pezet et al., 1999).

Structure-function studies using truncated or chimeric versions of SOCS1 have revealed the mechanism by which SOCS proteins bind to and inhibit the JAKs. Interestingly, although the SOCS1 SH2 domain is sufficient to mediate the association between SOCS1 and JAK2, both the SH2 domain and 24 residues immediately N-terminal to the SH2 domain are necessary for inhibition of JAK2 activity. Thus, the region immediately N-terminal to the SOCS1 SH2 domain appears to have a kinase-inhibitory function (Narazaki et al., 1998; Nicholson et al., 1999; Yasukawa et al., 1999). Within this N-terminal region, both SOCS1 and SOCS3 contain a sequence of 12 residues that resembles the JAK activation loop (a JAK substrate). Thus, Yasukawa et al. (1999) hypothesized that this region inhibits JAK activity by acting as a pseudosubstrate that prevents access of legitimate kinase substrates.

Association with cytokine receptors

Another way in which SOCS proteins might function is by directly interacting with activated cytokine receptors. For example, CIS associates with the tyrosine-phosphorylated Epo and IL-3 receptors and inhibits signaling by these cytokines (Yoshimura et al., 1995). CIS associates with phosphorylated Y401 of the Epo receptor, which is one of the two STAT5-binding sites in this receptor (Verdier et al., 1998). Furthermore, high levels of STAT5 expression overcome the inhibitory action of CIS which suggests that CIS competes with STAT5 for receptor binding (Matsumoto et al., 1999). Thus, Matsumoto et al. (1999) proposed that CIS could inhibit cytokine signaling by blocking access of STAT5 to tyrosine-phosphorylated receptors. However, note that Epo-induced STAT5 activation still occurs when Y401 on the Epo receptor is mutated to phenylalanine. This could be because the binding of STAT5 to Y343 on the Epo receptor is sufficient for

activation (Gobert et al., 1996; Klingmuller et al., 1996). Thus, the fact that CIS interrupts the binding of STAT5 to Y401 on the Epo receptor does not fully explain the mechanism by which CIS inhibits Epo signaling.

SOCS3 also associates with activated cytokine receptors, but its mode of inhibition appears to differ from that of CIS. Although SOCS3 associates with the tyrosine-phosphorylated GH receptor and inhibits GH signaling, it does not associate at the STAT5-binding site and excess SOCS3 cannot compete with STAT5 for receptor binding. This suggests that SOCS3 does not function by blocking access of STAT5 to the receptor. Interestingly, a chimeric protein containing the CIS N-terminal domain fused to the SOCS3 SH2 domain and SOCS box cannot inhibit GH signaling, which suggests that the SOCS3 N-terminal domain is required for inhibition (Hansen et al., 1999).

Although SOCS3 cannot significantly inhibit the intrinsic kinase activity of JAK2, SOCS3 inhibits the activation of JAK2 in response to GH stimulation when both SOCS3 and the GH receptor are co-expressed in 293 cells. Furthermore, the inhibitory action of SOCS3 increases with increased expression of the GH receptor, which suggests that optimal inhibition of JAK2 occurs when SOCS3 is bound to the GH receptor (Hansen et al., 1999). A similar mode of inhibition by SOCS3 was observed in the case of IL-2 signaling. Indeed, SOCS3 both inhibits IL-2 signaling and associates with the activated IL-2R β chain. Furthermore, the ability of SOCS3 to inhibit JAK1 activity is significantly enhanced in the presence of the IL2R β chain which suggests that receptor association is necessary for maximal inhibition (Cohney et al., 1999). Thus, although both CIS and SOCS3 can bind to activated cytokine receptors and inhibit cytokine signaling, they appear to function in different ways. CIS associates with activated receptors but neither interacts with nor inhibits the JAKs. SOCS3 binds weakly to the JAKs and, when associated with activated receptors, SOCS3 can inhibit JAK activity through its N-terminal domain.

SOCS proteins might target signaling proteins for degradation by the proteasome

Another model for SOCS function has recently been put

forward by Zhang et al. (1999). In this model, SOCS proteins target activated signaling proteins for degradation by the proteasome. All SOCS proteins tested associate with a complex containing elongins B and C (elongin BC) through their SOCS box (Kamura et al., 1998; Zhang et al., 1999). In turn, the elongin BC complex binds to a putative E3 ubiquitin ligase termed cullin-2. Given that SOCS proteins contain SH2 domains that bind tyrosine-phosphorylated signaling proteins, they can thus act as adapters that bring ubiquitin ligases into the vicinity of activated signaling proteins and cause their ubiquitination and degradation. The SOCS proteins could themselves be ubiquitinated and degraded in this process.

The above-mentioned function of SOCS proteins is analogous to the function of F-box proteins that operate in the yeast phosphoprotein ubiquitin ligase complex (PULC). F-box proteins contain a conserved N-terminal F-box motif that associates with the ubiquitination machinery (reviewed by Hershko and Ciechanover, 1998). The SOCS box associates with elongin BC and cullin-2 (an E3 ubiquitin ligase); the F box associates with the elongin C homolog Skp1p and the cullin-2 ubiquitin ligase homologs E2 and E3. Yeast F-box family members, such as Grr1p, function by binding to cyclins such as Cln1p and Cln2p in response to serine phosphorylation, thus targeting them for ubiquitination and degradation. This permits the cell to progress from G₁ to S phase of the cell cycle. The similarity between the F-box and the SOCS box suggests that these motifs could have analogous functions (Fig. 3).

The model outlined above predicts that SOCS-associated proteins, and in some cases the SOCS proteins themselves, are ubiquitinated and degraded by the proteasome. The observation that CIS can be monoubiquitinated and that the monoubiquitinated form of CIS accumulates in the presence of proteasome inhibitors is consistent with this (Verdier et al., 1998). Similarly, the IL-6 or LIF-induced expression of SOCS3 is sustained in the presence of proteasome inhibitors (Zhang et al., 1999; Bousquet et al., 1999). Moreover, Epo and IL-2-induced signaling through STAT5, as well as the association between STAT5 and the Epo receptor, is prolonged when proteasome inhibitors are present, which also supports the model that the proteasome negatively regulates these signaling pathways (Yu and Burakoff, 1997; Verdier et al., 1998).

CONCLUSIONS

In vivo studies highlight the role specific SOCS proteins play in the physiological regulation of cytokine action. SOCS1 is intimately involved in regulating T cell activation and IFN γ signaling, and SOCS3 regulates erythropoiesis and Epo signaling. The challenge ahead remains to determine whether there are overlapping actions of SOCS1, SOCS3 and CIS not revealed by single knockouts and to reveal the function of SOCS2, SOCS4, SOCS5, SOCS6 and SOCS7.

Biochemical studies reveal that the SH2 domains of SOCS1 and SOCS3 recognize different targets: JAKs in the case of SOCS1, and receptor cytoplasmic domains in the case of SOCS3. In both cases, however, the N-terminal domains are required for activity and are functionally interchangeable (Nicholson et al., 1999; Hansen et al., 1999). A motif within the N-terminal domain of SOCS1 and SOCS3 resembles the activation loop region of JAKs and might act as a JAK

pseudosubstrate, thereby inhibiting kinase activity. Taken together, these results suggest that SOCS1 and SOCS3 both act as JAK inhibitors but become localized to the signaling complex in distinct ways: SOCS1 through direct interaction with JAKs, and SOCS3 through interaction with the receptor cytoplasmic domain.

Once associated with a signaling complex, SOCS proteins probably recruit ubiquitin ligases, causing ubiquitination of proximal signaling proteins and their destruction by the proteasome. It is likely that physical interaction between SOCS and their targets, in addition to promoting degradation, contributes to negative regulation of cytokine signaling.

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