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Suppression of Cytokine Signaling: the SOCS perspective

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Abstract

The discovery of the Suppressor Of Cytokine Signaling (SOCS) family of proteins has resulted in a significant body of research dedicated to dissecting their biological functions and the molecular mechanisms by which they achieve potent and specific inhibition of cytokine and growth factor signaling. The Australian contribution to this field has been substantial, with the initial discovery of SOCS1 by Hilton, Starr and colleagues (discovered concurrently by two other groups) and the following work, providing a new perspective on the regulation of JAK/STAT signaling. In this review, we reflect on the critical discoveries that have lead to our current understanding of how SOCS proteins function and discuss what we see as important questions for future research.

Keywords

SOCS; SOCS box; cytokine; receptor; JAK

Preface

Cytokines are important soluble mediators of many physiological processes, including growth and development, and the innate and adaptive immune responses. Cytokine engagement with cognate cell surface receptors initiates intracellular signaling cascades, which culminate in changes to the transcriptional profile of the cell. These cascades are inextricably linked to the JAK protein tyrosine kinases and their substrates, the signal transducers and activators of transcription, or STAT proteins. Study of the JAK/STAT pathway has revealed a tightly regulated system and has highlighted the physiological and pathological consequences of aberrant signaling, which often results in inflammatory and/or tumorigenic disease states. Regulation by SOCS proteins is critical to the normal functioning and cessation of the primary cytokine signal, and is achieved at many levels in the intracellular biochemical cascade.

Since their discovery in 1997, the suppressors of cytokine signaling, or SOCS proteins, have emerged as arguably the most significant class of proteins to negatively regulate signaling following cytokine engagement of the receptor complex.

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Australian scientists have made seminal contributions to the SOCS field and these include the discovery of the SOCS proteins and their non-redundant biological roles, in addition to a detailed understanding of the biochemical mechanisms by which they act. While this review highlights a selection from the contribution of the Australian research community, we acknowledge that many others have made valuable contributions over the years. The SOCS field continues to burgeon and with now over 1700 publications listed in PubMed, the scientific interest in this family of small adaptor proteins shows no sign of waning.

1. Introduction

SOCS1 (also known as SSI-1 and JAB) was discovered simultaneously by three groups, one led by Doug Hilton at the Walter & Eliza Hall Institute in Melbourne [1], another led by Akahiko Yoshimura at the Institute of Life Science in Karume [2], and a third group led by Tadimitsu Kishimoto at the Osaka University Medical School [3].

In Melbourne, Robyn Starr had initiated a retroviral expression screen to search for negative regulators capable of inhibiting the IL-6-induced differentiation of M1 cells into macrophages. One recovered cDNA encoded a small SH2 domain-containing protein with homology to the known cytokine-inducible SH2-containing (CIS) protein [4] and to several related ESTs. This cDNA was named SOCS1 (Suppressor of Cytokine Signaling 1) and led to the identification and cloning of six more SOCS family proteins: SOCS2-7. Like *Cis*, *Socs1*, 2 and 3 were cytokine-inducible genes [1, 5]. Yoshimura's group identified SOCS1 in a yeast 2-hybrid screen using the JAK kinase (JH1) domain as bait; hence the alias JAB, for JAK-binding protein [2], while Kishimoto's group cloned SOCS1 through antigenic similarity with the Stat3-SH2 domain [3]. Collectively, these studies provided the first evidence that SOCS1/JAB/SSI-1 was not only induced in response to cytokine activation of the JAK/STAT pathway, but also then acted in a classic, negative-feedback loop to inhibit JAK signaling.

The sequence comparison of multiple SOCS family members revealed a conserved 40-residue motif located C-terminal to the SH2 domain. Subsequent interrogation of the databases identified a greater family of proteins containing this motif, now commonly referred to as the "SOCS box". As in the canonical SH2-containing proteins, the SOCS box is always found coupled to one or more protein-interaction modules, such as SPRY domains, WD40 or ankyrin repeats [5, 6], with a recent survey identifying over 80 SOCS box-containing genes in the human genome [7].

The presence of the short conserved sequence in proteins of diverse biological function suggested that the SOCS box might be involved in protein interaction/s, and an elegant series of experiments led by Jian-Guo Zhang and Manuel Baca subsequently identified a complex of elongins B and C, which associated with the SOCS box [8], a finding that was concurrently reported by others [9]. At that time, the elongin BC complex was known to bind to the von Hippel-Lindau (VHL) protein via a sequence that aligned with the first half of the SOCS box, and given that the VHL/elongin BC complex had been implicated in the ubiquitination and proteasomal degradation of proteins [10, 11], this raised the possibility that the SOCS box complex was similarly involved in E3 ubiquitin ligase activity [8]. We now know that the first half of the SOCS box constitutes an elongin C binding site, while the second half mediates an interaction with the Cullin 5 scaffold which in turn, recruits a RING protein Rbx2, and it is this elongin/Cullin/Rbx complex which forms an active E3 ubiquitin ligase [7].

There are several important distinctions to be made between the eight SOCS proteins; firstly they can be subdivided on the basis of a short (CIS, SOCS1, 2 and 3) or long, N-terminal

region (270 - 385 residues; SOCS4, 5, 6, 7) and secondly, it is the first group, which are most clearly induced in response to cytokine signaling and act in a classical negative-feedback loop (Figure 1). Indeed, the majority of research has focused on CIS and SOCS1-3, and it is obvious that they have evolved as true negative regulators of the JAK/STAT pathway. In contrast, the precise biological roles of SOCS4-7 are yet to be fully delineated and they are, in some cases, widely and constitutively expressed, with more varied protein targets.

Finally, SOCS1 and SOCS3 are unique in their ability to directly regulate JAK enzymatic activity. We now have a detailed molecular understanding of how SOCS1, and in particular SOCS3, function to regulate JAK/STAT signaling and this will be discussed at length in the following sections.

2. Biological role of the SOCS proteins

Early studies of the SOCS proteins showed that transcription of the *Cis*, *Socs1*, 2 and 3 genes was induced by many different cytokines and conversely, that forced expression of SOCS1 and SOCS3 could inhibit signaling from multiple receptor complexes. The specificity inherent in these biological systems began to emerge with the generation and analysis of genetically targeted mice, which lacked individual *Socs* genes.

Deletion of the *Socs1* gene resulted in mice that died shortly after weaning due to a catastrophic monocytic inflammation of the liver largely caused by excessive interferon (IFN) γ signaling [12-15]. Lethality could be rescued by crossing these mice with IFN γ -[12], Stat1-, Stat6- [14] or IFN alpha receptor 1 [16]-deficient animals and although viable, each of these compound mutant mice eventually develop a severe inflammatory disease. Together with conditional deletion of the *Socs1* gene in T cells [17], these studies revealed a critical role for SOCS1 in regulating responses to type I and type II interferons, and to cytokines which signal through the common gamma chain subunit of the interleukin (IL)-2 receptor [15, 17-20].

Although SOCS2-deficient mice are indistinguishable from their littermates at birth, by six weeks of age they attain a 30-40% growth advantage over wild-type animals, their gigantism resulting from unregulated responses to growth hormone [21-23]. SOCS2 is also thought to have a role in neuronal differentiation, with deletion of *Socs2* resulting in fewer cortical neurons, presumably as a result of enhanced growth hormone signaling [24]. SOCS2 has similarly been shown to modulate prolactin signaling, with deletion of the *Socs2* gene able to rescue the defects in lactation and Stat5 phosphorylation found in mice that are heterozygous for deletion of the prolactin receptor [25].

SOCS3-deficient mice are embryonic lethal at E10-13 due to a defect in placental development, which results from excessive signaling in response to leukemia inhibitory factor (LIF) [26, 27]. Neuronal progenitor cells derived from these mice also show enhanced responses to LIF, resulting in an increased capacity for self-renewal [28]. Conditional deletion of the *Socs3* gene subsequently revealed a role for SOCS3 in regulating other cytokines which signal through the gp130 receptor subunit, such as IL-6 [29, 30], IL-27 [31], CNTF and IL-11 [32, 33]. The deletion of *Socs3* in hemopoietic cells results in defects in granulocyte progenitors and a severe inflammatory pathology, driven by neutrophils, which are hyper-responsive to granulocyte-colony stimulating factor (G-CSF) [34]. SOCS3 appears to not only affect the magnitude of the signaling response, but also the quality of that response, with the enhanced IL-6-mediated Stat1 response observed in *Socs3*-deficient macrophages, skewing towards an IFN γ -like transcriptional profile [29, 30]. A corollary of this is the surprisingly strong overlap in phenotype between the SOCS3 and Stat3

knockouts, suggesting that loss of SOCS3 may contribute to the defects observed in Stat3-deficient cells [30, 35].

The delicate balance inherent in regulating these pathways is further highlighted by the phenotypes associated with mice harboring germ-line “knock-in” mutations in gp130 (discussed in detail elsewhere in this issue). Mice lacking the gp130 Stat3 binding sites (gp130^{DSTAT}) can no longer induce SOCS3 and are characterized by enhanced SHP-2-Ras-ERK signaling emanating from Tyr757 [36], whilst mice in which the SOCS3 and SHP-2-binding site on the gp130 receptor subunit (Tyr757) has been mutated to phenylalanine, display enhanced STAT signaling in the absence of SOCS3 recruitment. The gp130^{757F} mice develop gastric adenomas, a phenotype not observed in mice which lack either IL-6 or LIF [37], while gp130^{DSTAT} mice that can no longer signal through STAT1/3 suffer from degenerative joint disease and gastrointestinal ulceration [36].

As might be predicted, deletion of a negative regulator such as SOCS1 or SOCS3 exacerbates cytokine-driven disease models. For example, in mouse models of IL-1-dependent inflammatory arthritis, deletion of either *Socs1* or *Socs3* resulted in more severe joint inflammation. However, the resulting pathologies were quite distinct, with increased monocytic infiltration into the synovium observed in mice lacking *Socs1*, compared to increased neutrophil infiltrates in mice lacking *Socs3*, accompanied by increased production of, and response to, IL-6 and G-CSF [38, 39]. Consistent with this, aging gp130^{757F} mice are prone to spontaneously develop arthritis [40].

Interestingly, “knock-in” alleles deleting either the SOCS1 or SOCS3-SOCS boxes had a relatively modest effect when compared to deletion of the entire coding regions. *In vivo* deletion of the SOCS1-SOCS box significantly delayed the onset of disease, with the mice eventually succumbing to an IFN γ -driven inflammatory disease [41], while SOCS box-less SOCS3 mice remained viable and free from inflammatory disease. The latter did however, display hyper-responsiveness to G-CSF when challenged [42]. Together these mouse models suggest that although the ubiquitination of SH2 domain substrates certainly comprises one aspect of *in vivo* SOCS1 and SOCS3 function, the ability to directly regulate JAK enzymatic activity is of prime importance.

3. Biochemical mechanism of action

The SOCS proteins have been demonstrated to inhibit signaling through three primary mechanisms, all of which rely in part on their SH2 domains. CIS and SOCS2 have been proposed to act by competing with STAT proteins for binding to phosphorylated tyrosine residues within the receptor cytoplasmic domains, SOCS1 and SOCS3 directly inhibit JAK activity via an interaction that involves the SH2 domain and a short region preceding it, which is known as the kinase inhibitory region (KIR), and through the SOCS box-associated E3 ligase, all have the capacity to ubiquitinate SH2 and N-terminal bound substrates, resulting in the proteasomal degradation of the bound proteins.

The sophisticated biochemical basis for the specificity of SOCS action is only now becoming apparent and is generated by the preference of individual SOCS-SH2 domains for specific phosphorylated tyrosine motifs, and the ability of SOCS1 and SOCS3 to bind to and inhibit different JAK catalytic domains.

3.1 The SOCS-SH2 domain

The first structural insights into the SOCS-SH2 domain came from the solution structure of the SOCS3-SH2 domain [43, 44] and this was quickly followed by the crystal structures of the SOCS2 [45], SOCS3 [46], SOCS4 [47] and more recently, SOCS6 [48] SH2 domains.

Although in general, the SOCS-SH2 structures conform to the classical three-stranded β -sheet flanked by two α -helices, these studies revealed several distinguishing features. Specifically, the SOCS-SH2 domains contain a conserved, short α -helical extension, designated the extended SH2 domain (ESS), which contributes to binding through a number of hydrophobic contacts with the phosphotyrosyl-binding pocket, and stabilizes the modular SH2 domain [43, 45, 46]. The SOCS3 and SOCS6-SH2 domains also display an extended interaction interface, which is reflected in the tighter binding affinities observed for phosphotyrosyl ligands [48, 49] (Table 1). A unique feature of the SOCS3 and CIS-SH2 domains is the presence of a PEST motif inserted between the β helix and the BG loop [44], and this likely contributes to the rapid turnover of SOCS3 *in vivo* [43]. Conversely, deletion of the PEST sequence significantly improved the stability of recombinant SOCS3 protein, facilitating subsequent in-depth biochemical and structural analyses [43, 44].

The SOCS1 and SOCS3-SH2 domains were initially thought to bind to the critical catalytic loop phosphotyrosines in JAK (Y1007, 1008 in JAK2), thus enabling the KIR to act as a pseudo-substrate, blocking further substrate phosphorylation [2, 50, 51]. We now have a much clearer understanding of both the SH2 domain targets and the role of the KIR.

SOCS1 is recruited to the IFN γ receptor (IFNGR) complex via SH2 domain binding to phosphotyrosine 441 within the IFNGR1 subunit [52, 53]. However, in the context of type I IFN signaling, the receptor tyrosine residues seem superfluous, and SOCS1 appears to directly interact with the TYK2/IFN α receptor 1 (IFNAR1) complex [16, 54]. In contrast, the SOCS3-SH2 domain is thought to interact exclusively with phosphotyrosines within the receptor cytoplasmic domains, namely, the IL-6 signaling subunit gp130, erythropoietin (EPO), G-CSF and leptin receptors, and the IL-12 receptor β 2 subunit [49, 55-58] (Table 1).

Binding to receptor phosphotyrosines can serve a dual inhibitory function: not only is SOCS3 brought into the vicinity of the JAKs, but it is also thought to compete with SHP-2 for Tyr757 in gp130 [49]. This latter function may also extend to CIS and SOCS2, which interact with phosphotyrosyl residues within the EPO, prolactin, leptin and growth hormone receptors [22, 59-61] (Table 1).

There is clearly a functional overlap which is yet to be resolved, with for instance, CIS, SOCS2 and SOCS3 able to regulate EPO signaling. Likewise, further analysis of the binding preferences of the less well-defined SOCS-SH2 domains will no doubt yield valuable information as to their physiological roles.

3.2 Kinase inhibitory region (KIR)

Deletion analysis of SOCS1 and SOCS3 identified a 12-residue motif located N-terminal to the ESS-SH2, which was independently required for inhibition of JAK activity (the KIR). Mutation of this region identified key residues that were required for inhibition of JAK (for example Phe56 and Phe59 in SOCS1, and Leu22, Phe25 and Glu30 in SOCS3) [50, 51, 62]. Owing to some sequence similarity between the KIR and the JAK activation loop, a model was proposed whereby the KIR might act as a pseudo-substrate, displacing the JAK activation loop from the catalytic cleft and preventing substrate or ATP binding [50].

Experimental evidence to support or dispute this model of inhibition by SOCS1 and 3 has only recently been published, some 12 years later, reflecting both the difficulties in the production of SOCS and JAK as soluble recombinant protein and the complexities of the interaction. The co-crystal structure of the SOCS3-KIR-SH2 with the JAK2 JH1 domain shows that the KIR does not displace the activation loop but rather binds adjacent to it and blocks substrate access to the active site. The structure also explains why mutation of key KIR residues had such a dramatic effect and for example, the critical Phe25 can now be seen

to sit deep into a hydrophobic pocket in the JAK2 JH1 domain/SOCS3 interface (Fig. 2B) [63].

Intriguingly, the SOCS3-SH2 domain contributes both phosphorylation dependent and independent interactions, simultaneously binding to the gp130 receptor subunit, via a canonical SH2 domain-phosphotyrosine interaction, whilst the opposing face of the SH2 domain, in combination with the ESS and KIR, provides a hydrophobic interface for interaction with the JAK JH1 domain [63]. The SOCS3 interaction with JAK is centered on a 'GQM' motif conserved in JAK1, JAK2 and TYK2, but not JAK3 [64] (Fig. 2C). As JAK is also tethered to the receptor via its FERM domain, the result is a high-affinity tripartite binding complex (Fig. 2A). Thus, the specificity of inhibition by SOCS3 *in vivo* correlates with cytokines whose receptors contain a SOCS3-SH2 binding site and which utilize JAK1, JAK2 or TYK2.

Given the sequence homology and previous mutational analysis, it seems likely that SOCS1 will act in an equivalent way to SOCS3, particularly as, in the context of a hybrid SOCS1-KIR-SOCS3-SH2 domain, the SOCS1-KIR binds and inhibits JAK with greater potency than the SOCS3-KIR [63, 64]. However, it remains to be determined whether the SOCS1-SH2 domain docks via the JAK activation loop tyrosines (possibly in trans) or always requires specific receptor sites.

3.3 SOCS box substrates

The SOCS proteins are now synonymous with the C-terminal SOCS box motif, which classes the greater SOCS family as the substrate recruitment modules of Cullin-RING-E3 ubiquitin ligases. SOCS proteins recruit substrates for lysine-48 linked ubiquitination, targeting those substrates for proteasomal degradation.

The SOCS box forms a three helical bundle with the first helix inserted in a cleft within elongin C, while the loop region between helices 2 and 3 makes limited contacts with elongin B to stabilize the ternary complex [45, 47]. Biochemical studies demonstrated that, in comparison to the other six family members, the SOCS box of SOCS1 and SOCS3 bound with weaker affinity to cullin5 [65]; a phenomenon which is nicely reflected *in vivo* as discussed, with the genetic deletion of the *Socs1* or *Socs3* SOCS boxes resulting in an ameliorated phenotype [8, 42]. By extension, this predicts that the other SOCS family members will rely on the ubiquitination and degradation of SH2- and N-terminally-bound substrates as their major mechanism of action.

Many candidate proteins have been shown to be ubiquitinated and/or regulated by the proteasome when the SOCS proteins or tagged-ubiquitin are exogenously expressed [7]. What has remained elusive however, are definitive biological experiments that confirm specific substrates of the SOCS E3 ligases. This is due in part to the difficulties inherent in assaying fast-moving targets; the SOCS proteins are generally expressed only in response to cytokine or growth factor stimulation, in discrete cellular subsets, after which they participate in transient and dynamic binding interactions with substrates. Perhaps the strongest biological evidence for a *bona fide* SOCS E3 substrate was obtained from ES cells containing a homozygous deletion of the SOCS3-SOCS box, in which phosphorylated JAK1 accumulated following LIF treatment [66].

Identifying SOCS E3 substrates remains a challenge, but is one that we foresee may plausibly be tackled through the use of more sophisticated approaches, which for instance, combine analysis of the endogenous proteins with technological advances in sample preparation and mass spectrometry.

4. SOCS in disease: a case for therapeutic intervention?

Activating mutations in kinases and other components of intracellular signaling cascades are now inextricably linked to a variety of cancers. Given the role of JAK/STAT signaling in hemopoiesis and the immune system, it is perhaps not surprising that disruption of normal JAK activity has been shown in patients with acute lymphoblastic leukemia (ALL) and is particularly commonplace in myeloproliferative disease [67]. For example, mutation of Val617 to Phe in the pseudo-kinase domain of JAK2, results in a constitutively active kinase, which appears to be the driving force in the majority of myeloproliferative neoplasms [68]. Mutations in the IL-7 receptor α -chain, which result in cytokine-independent dimerization and activation of the associated JAK1, are also found in a subgroup of T cell ALL patients [69].

The intrinsic role played by SOCS proteins in regulating cytokine signaling raises the important question of whether or not the SOCS present as viable therapeutic targets. That is, can our knowledge of the SOCS proteins be used to generate clinical tools for the treatment of disease mediated by excessive cytokine signaling?

The simplest paradigm involves therapeutics that lead to increased intracellular levels of a SOCS protein, thus reducing the response of the 'culprit' cytokine, and there is some experimental evidence to support SOCS-targeted immunotherapy. Administration of *Socs1* cDNA reduced the activation of dendritic cells and the subsequent cytokine secretion in an experimental autoimmune myocarditis model in mice [70], whilst injection of an adenoretroviral vector expressing SOCS3, into the joints of mice with experimentally-induced arthritis significantly delayed and reduced the disease state [71]. Additionally, IL-6-mediated STAT3 activation in the colonic crypt is thought to be one of the major factors in the development of colorectal cancer [72], with induction of SOCS3 shown to reduce this STAT3-mediated inflammation [73, 74]. As *Socs1* is epigenetically silenced in various cancer cell lines, ovarian, pancreatic and hepatocellular carcinomas, and leukemias [75-81] and exogenous SOCS1 expression has the capacity to suppress tumor progression [82], this type of therapy might be of benefit in treating human malignancies.

Perhaps the most tantalizing possibility however, is the potential for the design of novel JAK inhibitors, which take advantage of the unique SOCS-JAK interaction [63]. The majority of the current JAK inhibitors are ATP-analogs and thus constantly compete with high intracellular concentrations of ATP, and often have off-target effects on other kinases due to common features of the ATP-binding pockets [83]. Despite this, JAK2 inhibitors are already in use in the clinic, validating this approach for the treatment of JAK2V617F-driven malignancies [84, 85]. Compounds that mimic the molecular action of SOCS3 would be predicted to elicit far greater specificity in controlling aberrant JAK activity; for example a compound that bound the unique 'GQM' binding site of JAK1, JAK2 and TYK2, would be of intense interest in the context of constitutive JAK signaling.

5. Concluding remarks

It is becoming increasingly clear that the SOCS proteins can interact independently of the canonical SH2-phosphotyrosine interactions; in particular the long N-termini of SOCS4-7 are predicted to mediate protein interactions [86]. Key questions remain for these lesser-studied family members, including whether they act to inhibit JAK/STAT signaling, or contribute to the negative regulation of other signaling cascades.

The SOCS protein family has been extensively studied in Australia, due in no small part to the initial discovery of SOCS1 as a suppressor of IL-6 signaling. Investigating these negative regulators of cytokine signaling has provided a fascinating insight into the exquisite

molecular mechanisms that have evolved to co-ordinate the complexities of the immune system. This level of detail and understanding will be critical to the design and development of novel JAK inhibitors, which can mimic the physiological role performed by the SOCS proteins in regulating pro-inflammatory and oncogenic signaling cascades.

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Biography

Sandra Nicholson currently heads a laboratory in the Inflammation division at the Walter & Eliza Hall Institute in Melbourne, Australia. Born in 1965, her expertise is in protein structure-function relationships and JAK/STAT signaling. **Postdoctoral training:** After completing a PhD at the Ludwig Institute for Tumour Biology in Melbourne (1996), Sandra Nicholson undertook postdoctoral training in the Cancer & Haematology division at the Walter & Eliza Hall Institute (1997-2006). **Academic appointments:** Sandra Nicholson has been supported by an NHMRC career development award (2004-7) and Senior Research Fellowship (2008-current), and following post-doctoral training was appointed as Laboratory head in the Cancer & Haematology division (2006-2011). She holds an honorary appointment at the University of Melbourne. **Major research interests:** Sandra Nicholson is interested in understanding how SOCS box proteins negatively regulate cytokine and growth factor signaling. Her interest extends to the molecular structure of the protein complexes and how these relationships may be perturbed in inflammatory and infectious disease.

Edmond Linossi is a postgraduate student at the Walter and Eliza Hall Institute in the Inflammation Division. He graduated from the University of Melbourne in 2011 and was awarded an Australian Postgraduate Award. His current work aims to investigate the role of the lesser known SOCS4 and SOCS5.

Professor Douglas Hilton, PhD FAA FTSE

Prof. Doug Hilton, born in 1964, is the 6th Director of the Walter and Eliza Hall Institute (WEHI), Australia, Head of its division of Molecular Medicine and head of the Department of Medical Biology in the Faculty of Medicine, Dentistry and Health Sciences at the University of Melbourne. **Postdoctoral training:** 1991-93 The Whitehead Institute, MIT in Cambridge working on the structure/function relationship of the erythropoietin receptor; 1993-95 Cancer and Haematology Division, WEHI, Australia. **Academic appointments:** Hilton became a laboratory head at WEHI in 1996, was appointed Director of Cooperative Research Centre for Cellular Growth Factors (1997-2001), Head of Molecular Medicine Division at WEHI (since 2006), and since 2009 Professor and Head of Department of Medical Biology at the University of Melbourne, and Director of WEHI. **Awards and Honours:** Hilton has received many prizes and awards for his research into how blood cells communicate, including the Amgen Medical Researcher Award, the inaugural Commonwealth Health Minister's Award for Excellence in Health and Medical Research, the GSK Australia Award for Research Excellence, Milstein Award from the International Society of Interferon and Cytokine Research, Lemberg Medal from the Australian Society of Biochemistry and Molecular Biology, was elected a Fellow of the Australian Academy of Science in 2004, and a Fellow of the Academy of Technological Sciences and Engineering in 2010. **Major research interests:** Hilton is best known for his discoveries in the area of cytokine signalling, particularly the isolation and cloning of an entirely novel family of negative regulators of cytokine signalling, the SOCS proteins. The Hilton lab aims to understand which of the 30,000 genes are important in the production and function of blood cells, and how this information can be used to better prevent, diagnose and treat blood cell diseases such as leukaemia, arthritis and asthma.

Jeff Babon, Laboratory Head at the Walter and Eliza Hall Institute of Medical Research (Australia). He was born in 1972 and is a specialist in the field of structural biology and biochemistry. He is a graduate of Melbourne University and obtained his Ph.D at the Murdoch Institute. **Postdoctoral training:** Jeff Babon undertook postdoctoral training at the National Institute of Medical Research (London, UK), 2000-2003, in the division of Molecular Structure and then returned to Australia in 2003 to take a postdoctoral position in the Structural Biology Division at the Walter and Eliza Hall Institute. **Major Research Interest** The group of Dr. Babon focusses on the regulation of Cytokine Signalling, in

particular the inhibition of JAK/STAT signalling via the SOCS (Suppressor of Cytokine Signalling) family of proteins. He uses structural biology and biochemistry to study mechanism within these pathways to understand the role they play in haematological disease.

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Fig. 1. Domain architecture of the SOCS family of proteins

The SOCS proteins contain a C-terminal SOCS box motif, a central SH2-domain and an N-terminal region of varied length and amino acid composition. The SOCS are shown in pairs based on the amino acid sequence homology between their SH2 domains. Key features are distinguished by color and are enumerated in the legend, including a recently identified conserved region in the N-termini of SOCS4 and SOCS5 [86]. Abbreviations: kinase inhibitory region (KIR), extended SH2 domain (ESS), proline, glutamic acid, serine and threonine (PEST) rich region.

Fig. 2. Structural analysis of the SOCS3/JAK2/gp130 complex

A) Schematic representation of the JAK/gp130/SOCS3 tripartite complex. JAK2 and SOCS3 can bind independently to the gp130 receptor subunit via their FERM and SH2 domains, respectively. A second interaction interface between SOCS3 (KIR-ESS-SH2) and JAK2 (JH1) completes the high-affinity ternary complex and facilitates the inhibition of JAK. **B)** *The JAK2/SOCS3 interaction.* The JAK2 binding epitope, centered on the GQM motif, is shown as an electrostatic surface, with SOCS3 shown as a ribbon diagram (green). Residues from the SOCS3-SH2 domain (BC loop), ESS and KIR all contribute to the binding interface. The critical phenylalanine (Phe25) of the SOCS3 KIR sits in a hydrophobic pocket between the JAK2 activation loop and the GQM motif. **C)** Ribbon diagram of the SOCS3-SH2 domain (green) bound simultaneously to gp130Tyr757 (black) and the JH1 domain of JAK2 (beige). The SOCS3/JAK interaction occurs on the opposite face to the SOCS3 /gp130pY757 tyrosine. (B) & (C) are reproduced with permission from Kershaw et al., [63].

Table 1

SOCS regulation of signaling – cytokines and SH2 domain targets

	Cytokine [*]	Receptor	Tyrosine	K _D	References
CIS	IL-2	IL-2-R	N/D ²		[87, 88]
	IL-3, IL-5, GM-CSF	IL-3-R ^c	N/D		[4]
	EPO	EPO-R	N/D		[4]
	Prolactin	Prolactin-R	N/D		[89, 90]
SOCS1	IFN	IFNGR1	Y441		[52, 53]
	IFN /	IFNAR1	N/D		[16, 54]
	IL-2, IL-4, IL-7, IL-15	IL-2-R ¹	N/D		[15, 17, 18, 20, 91, 92]
	IL-12	IL-12-R	N/D		[93]
SOCS2	Growth hormone	GH-R	Y595	1.6 μM	[45, 94]
	Prolactin	Prolactin-R	N/D		[25, 95]
	EPO	EPO-R	Y401	7.1 μM	[45, 96]
SOCS3	IL-6/LIF/IL-11/IL-27	gp130	Y757	0.042 μM	[30, 31, 49]
	G-CSF	G-CSF-R	Y729	2.8 μM	[56]
	Leptin	LRb1	Y1138	N/D	[58]
	EPO	EPO-R	Y401	9.5 μM	[55, 97]
		EPO-R	Y429/431	1.1 μM	[55]
	IL-12	IL-12R ²	Y800	N/D	[57]

^{*} The relevant biological cytokine, receptor subunit, and where known, the phosphotyrosyl residues targeted by the SOCS-SH2 domain together with the reported affinities for SH2/phosphopeptide binding.

¹ LRb, long form of the leptin receptor;

² N/D, not determined.