REVIEW ARTICLE

Principles of interleukin (IL)-6-type cytokine signalling and its regulation[1]

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The IL (interleukin)-6-type cytokines IL-6, IL-11, LIF (leukaemia inhibitory factor), OSM (oncostatin M), ciliary neurotrophic factor, cardiotrophin-1 and cardiotrophin-like cytokine are an important family of mediators involved in the regulation of the acute-phase response to injury and infection. Besides their functions in inflammation and the immune response, these cytokines play also a crucial role in haematopoiesis, liver and neuronal regeneration, embryonal development and fertility. Dysregulation of IL-6-type cytokine signalling contributes to the onset and maintenance of several diseases, such as rheumatoid arthritis, inflammatory bowel disease, osteoporosis, multiple sclerosis and various types of cancer (e.g. multiple myeloma and prostate cancer). IL-6-type cytokines exert their action via the signal transducers gp (glycoprotein) 130, LIF receptor and OSM receptor leading to the activation of the JAK/STAT (Janus kinase/signal transducer and activator of transcription) and MAPK (mitogen-activated protein kinase) cascades. This review focuses on recent progress in the understanding of the molecular mechanisms of IL-6-type cytokine signal transduction. Emphasis is put on the termination and modulation of the JAK/STAT signalling pathway mediated by tyrosine phosphatases, the SOCS (suppressor of cytokine signalling) feedback inhibitors and PIAS (protein inhibitor of activated STAT) proteins. Also the cross-talk between the JAK/STAT pathway with other signalling cascades is discussed.

Key words: cytokine signalling, glycoprotein 130 (gp 130) interleukin-6 (IL-6), Janus kinase (JAK), mitogen-activated protein kinase (MAPK), signal transducer and activator of transcription (STAT).

INTRODUCTION

The family of IL (interleukin)-6-type cytokines comprises IL-6, IL-11, LIF (leukaemia inhibitory factor), OSM (oncostatin M), CNTF (ciliary neurotrophic factor), CT-1 (cardiotrophin-1) and CLC (cardiotrophin-like cytokine). They activate target genes involved in differentiation, survival, apoptosis and proliferation. The members of this cytokine family have pro- as well as anti-inflammatory properties and are major players in haematopoiesis, as well as in acute-phase and immune responses of the organism. IL-6-type cytokines bind to plasma membrane receptor complexes containing the common signal transducing receptor chain gp 130 (glycoprotein 130). Signal transduction involves the activation of JAK (Janus kinase) tyrosine kinase family members, leading to the activation of transcription factors of the STAT (signal transducers and activators of transcription) family. Another major signalling pathway for IL-6-type cytokines is the MAPK (mitogen-activated protein kinase) cascade (Figure 1).

Recent reviews on the subject of signal transduction via the JAK/STAT pathway have been published [1–3]. The present review focuses on advances made during the last 5 years in structural/functional aspects of IL-6-type cytokine receptor activation, JAK–receptor interactions, STAT activation, signal modulation and, in particular, on mechanisms of the negative regulation in the field of IL-6-type cytokine signalling. In contrast with our previous review [4], we do not refer to the numerous studies applying gene targeting in mice. Instead, we put emphasis on mechanistic aspects of IL-6-type cytokine signalling.

GENERAL MECHANISMS

Receptor complexes formed by IL-6-type cytokines: gp130 is the central player

Receptors involved in recognition of the IL-6-type cytokines can be subdivided in the non-signalling α-receptors (IL-6Rα, IL-11Rα, and CNTFRα, where R refers to receptor) and the signal transducing receptors (gp130, LIFR, and OSMR). The latter associate with JAKs and become tyrosine phosphorylated in response to cytokine stimulation. Each of the IL-6-type cytokines is characterized by a certain profile of receptor recruitment that in all cases involves at least one molecule of gp130.

IL-6, IL-11 and CNTF first bind specifically to their respective α-receptor subunits. Here, only the complex of cytokine and α-receptor efficiently recruits the signalling receptor subunits. Also,

Abbreviations used: α_2M, α_2-macroglobulin; CBM, cytokine-binding module; CIS, cytokine-inducible SH2 protein; CLC, cardiotrophin-like cytokine; CLF, cytokine-like factor; CNTF, ciliary neurotrophic factor; CT, cardiotrophin; EGF, epidermal growth factor; Epo, erythropoietin; ERK, extracellular-regulated kinase; EZI, endothelial cell-derived zinc-finger protein; Fab, fragment antigen binding; FERM, four-point-one, ezrin, radixin, moesin; FKHR, forkhead-related transcription factor; FN, fibronectin; Gab, Grb-associated binder; gp, glycoprotein; Grb, growth-factor-receptor-bound protein; Hck, haematopoietic cell kinase; IFN, interferon; IL, interleukin; IRS, insulin receptor substrate; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; KIR, kinase inhibitory region; KSHV, Kaposi's sarcoma-associated herpes virus; LIF, leukaemia inhibitory factor; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MK2, MAPK-activated protein kinase 2; NES, nuclear export signal; NF-κB, nuclear factor κB; NLS, nuclear localization signal; Nmi, N-Myc-interactor; OSM, oncostatin M; p, phospho-; PDGF, platelet-derived growth factor; PH, pleckstrin homology; PKC, protein kinase C; PI3K, phosphoinositide 3-kinase; PIAS, protein inhibitor of activated STAT; PRMT, protein arginine methyltransferase; PTP, protein tyrosine phosphatase; R, receptor; s, soluble; SH2, Src homology 2; SHC, SH2 and collagen homology domain containing protein; SHP, SH2-domain-containing tyrosine phosphatase; SMRT, silencing mediator of retinoic and thyroid hormone receptors; SOCS, suppressor of cytokine signalling; SOS, Son of Sevenless; SSI, STAT-induced STAT inhibitor; STAT, signal transducer and activator of transcription; TNF, tumour necrosis factor.

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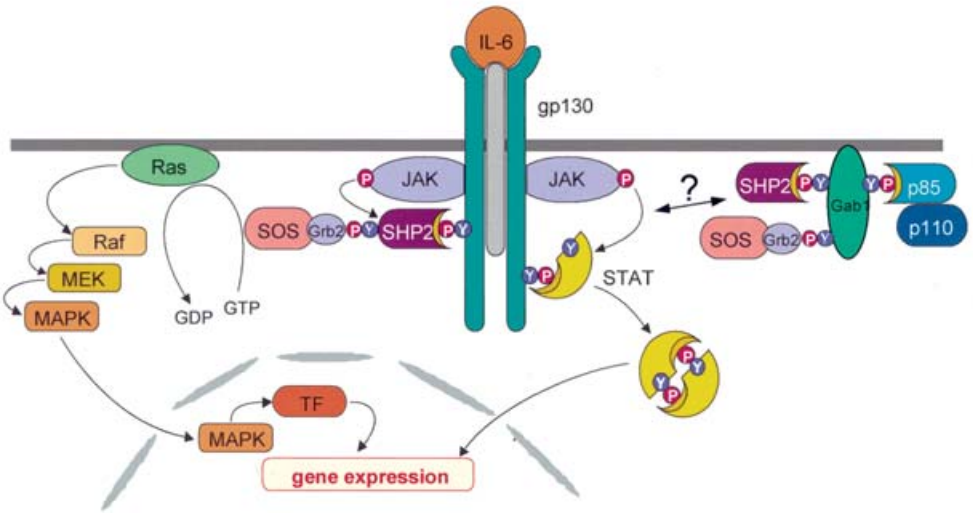

Figure 1 IL-6 activates the JAK/STAT pathway and the MAPK cascade

Representation of the two major pathways activated by IL-6-type cytokines. TF, transcription factor.

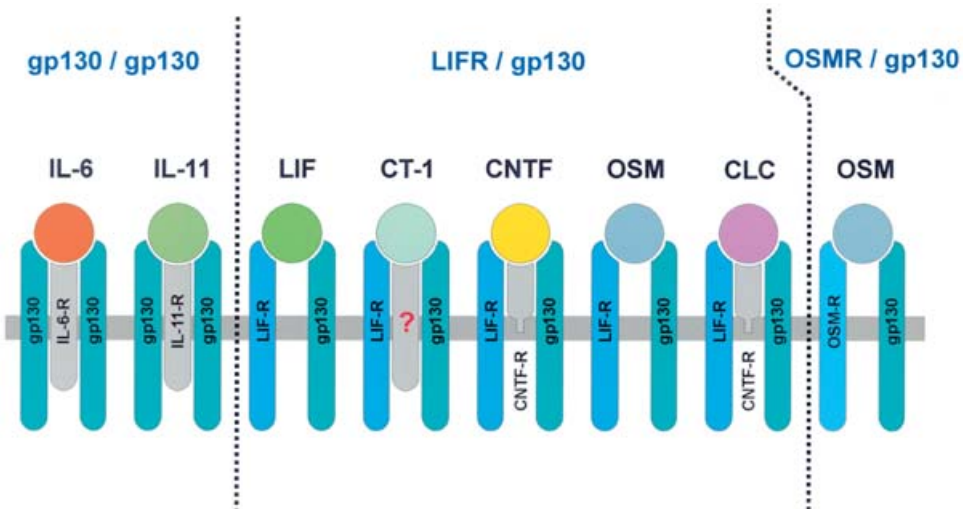

Figure 2 Receptor complexes of IL-6-type cytokines

IL-6-type cytokine receptor complexes signal through different combinations of the signalling receptor subunits gp130, LIFR and OSMR, with gp130 being used by all the family members.

an α-receptor subunit has been postulated for CT-1 [5], but since this putative receptor protein has not been cloned yet its existence is questionable. IL-6 and IL-11 are the only IL-6-type cytokines that signal via gp130 homodimers. The remaining IL-6 type cytokines signal via heterodimers of either gp130 and the LIFR (LIF, CNTF, CT-1 and CLC) or gp130 and the OSMR (OSM). Human OSM has the exceptional capability to recruit two different receptor complexes. It forms both LIFR–gp130 and OSMR–gp130 heterodimers. LIF and OSM directly engage their signalling receptor subunits without requirement for additional α-receptor subunits (Figure 2) [4,6].

Although gp130 is ubiquitously expressed, the number of cells that respond to a certain IL-6-type cytokine is limited, since the expression of the other receptor subunits, especially of the α-receptors, is more restricted and tightly regulated. The function of the α-receptors to render cells sensitive to the respective cytokine, however, can also be taken over by the soluble form of the α-receptors lacking the transmembrane and cytoplasmic parts. This is one of the rare situations in which a complex of cytokine and soluble receptor can act agonistically instead of antagonistically. Soluble forms of cytokine receptors *in vivo* are formed either by limited proteolysis (shedding) of membrane-bound receptors or by translation from an alternatively spliced mRNA [4]. In the case of IL-6, the scenario is more complex, since soluble forms for IL-6Rα (sIL-6Rα) and gp130 (sgp130) are both present in human serum. It has been demonstrated that sIL-6Rα potentiates the antagonistic activity of sgp130. Thus the naturally occuring combination of sIL-6Rα and sgp130 might act as a kind of buffer to modulate systemic responses to circulating IL-6 [7].

Although the cytoplasmic part of the IL-6Rα is dispensable for receptor complex formation and signal transduction, a function has recently been assigned to this part of the protein [8]: it contains

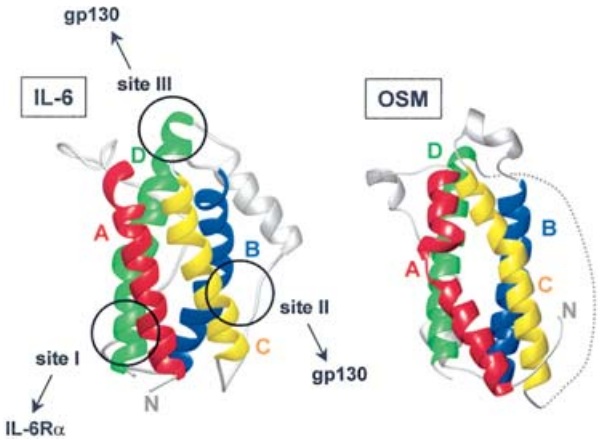

Figure 3 Structures of IL-6 and OSM

The four long helices A, B, C and D are highlighted in different colours. Receptor-binding sites I, II, and III of IL-6 are indicated by circles (Brookhaven Databank accession numbers for IL-6 and OSM are 1IL6 and 1EVS respectively).

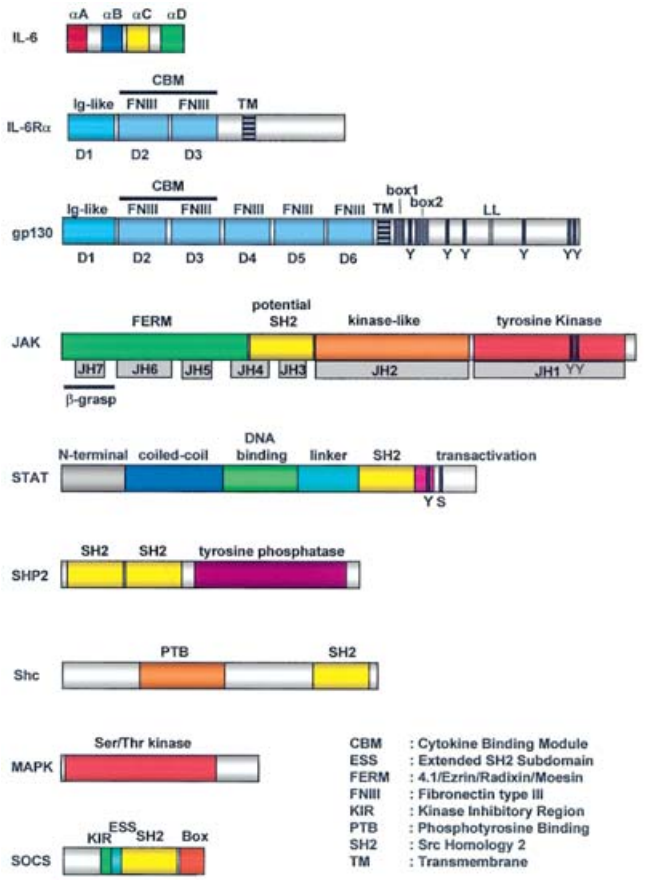

Figure 4 Structural organization of various IL-6-type cytokine signalling components

Relevant tyrosine (Y) and serine (S) residues of gp130, JAK and STAT proteins that become phosphorylated are indicated. For gp130 the box1 and box2 regions, as well as the dileucine motif (LL, Leu^{786}-Leu^{787}), are highlighted. JH, JAK homology domain.

a tyrosine-based and a dileucine-type motif which direct sorting of IL-6Rα to the basolateral membrane of polarized cells. Similarly, also targeting of gp130 to the basolateral membrane is dependent on a sorting motif within its cytoplasmic part. In certain cell types, a localization of gp130 in plasma membrane microdomains, such as lipid rafts and caveolae, has been observed [9–11]. This might be a prerequisite for special signalling functions of the receptor.

For CLC, the most recently discovered IL-6-type cytokine [6], a very special mechanism of secretion and receptor recruitment has been described [12,13]. To become secreted, CLC must be co-expressed with either CLF-1 (cytokine-like factor-1) or CNTFRα. CLF-1 resembles a soluble cytokine receptor and specifically binds CLC. After secretion of the CLC–CLF-1 or CLC–CNTFRα complexes, signalling is dependent on CNTFRα and occurs via gp130–LIFR heterodimers.

KSHV-IL-6 (Kaposi's sarcoma-associated herpes virus IL-6) [14] and Rhesus macaque rhadinovirus IL-6 [15] are viral variants of the IL-6-type cytokines that exhibit low similarity to IL-6 and signal by recruitment of gp130. Interestingly, KSHV-IL-6 binds the gp130 homodimer in the absence of any α-receptor [16], but with lower affinity as compared with IL-6–IL-6Rα–gp130 ternary complexes.

Structure and function of IL-6-type cytokines and their receptors: α-helices meet β-sheets

IL-6-type cytokines form a subfamily of the helix bundle cytokines. All IL-6-type cytokines comprise four long α-helices termed A, B, C and D, which are arranged in a way that leads to an up-up-down-down topology (Figure 3). In contrast with IL-6, and presumably also IL-11, where all the helices are straight, the A helix of LIF, OSM and CNTF is kinked [17,18]. This structural divergence might reflect differences in the mechanisms of receptor recruitment, since the straight cytokines signal via gp130 homodimers, whereas the kinked cytokines signal via LIFR–gp130 or OSMR–gp130 heterodimers.

The ectodomains of the receptors involved in IL-6-type cytokine signalling comprise an array of FNIII (fibronectin type III)-like and Ig-like domains (Figure 4). Each receptor contains at least one cytokine-binding module (CBM) that comprises two FNIII-domains. A CBM is characterized by conserved structural features, such as a distinct pattern of cysteine residues in the N-terminal domain and a WSXWS motif in the C-terminal domain. In each receptor an Ig-like domain is located N-terminally to the membrane-proximal CBM. In contrast with the α-receptors, the receptors that initiate signal transduction have three additional membrane-proximal FNIII domains [4].

How the different IL-6-type cytokines bind specifically to their receptors has been intensely investigated during recent years. Mutagenesis studies have identified distinct areas on the surface of the cytokines (termed 'sites') which specifically interact with the respective receptors. Common to all IL-6-type cytokines is site II that interacts with the CBM of gp130. The second signalling receptor, either a second gp130 or LIFR or OSMR, is recruited to site III [4]. A surprise was the discovery that site III is recognized by the Ig-like domain of gp130, LIFR or OSMR [19–22]. Thus in the homodimer two different binding epitopes of gp130 are involved in ligand recognition [23]. When a non-signalling α-receptor is involved in the receptor complex, it binds with its CBM to site I [4]. The interaction sites predicted by mutagenesis studies have recently been confirmed by the X-ray structure of KSHV-IL-6 bound to a soluble gp130 fragment comprising the CBM and the Ig-like domain [24]. When the recently solved structure of the IL-6Rα ectodomain [25] is accommodated into this structure, a reliable model of the membrane-distal part of

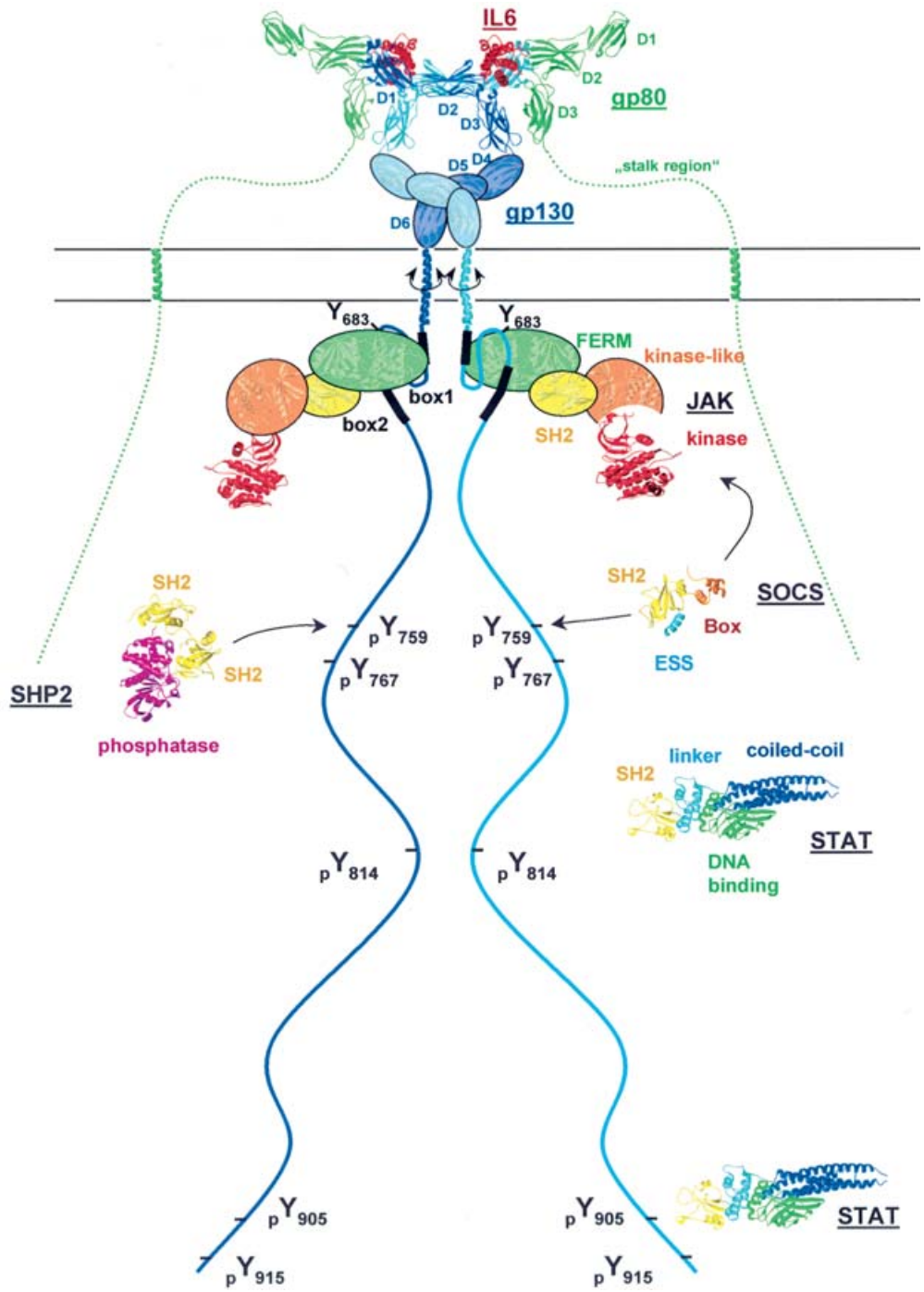

Figure 5 Structural model of the signalling IL-6 receptor complex

Solved structures for vIL-6/gp130, gp80, STAT3 and SHP2 (Brookhaven Databank accession numbers 1I1R, 1N26, 1BG1 and 2SHP respectively), as well as molecular models of a JAK2 kinase domain and SOCS1 (amino acids 65–212), are represented. In the extracellular part, IL-6 is shown in red, IL-6Rα in green and the two gp130 molecules of the homodimer in cyan and blue. The domains D4–D6 of gp130, as well as the FERM, SH2 and kinase-like domains of the JAKs, are depicted as coloured ovals with the sizes corresponding to the tenascin FNIII, moesin FERM, SH2 and insulin receptor kinase domains. Arrangement of D4–D6 of gp130 was proposed by Kurth et al. [36]. The cytoplasmic parts of gp130 and gp80, as well as the non-structured extracellular 'stalk' region of gp80 [25], are represented as blue and green lines with lengths corresponding to non-structured polypeptides. The positions of the six tyrosine residues of gp130 are indicated and the box1 and box2 regions are drawn as black lines. The cytoplasmatically associated proteins are depicted in the colour code corresponding to Figure 3. Note that in the case of STATs, the N-terminal domain, as well as the C-terminal transactivation domain, are not represented. Re-orientation of the transmembrane region in response to ligand binding is indicated by circular arrows.

the hexameric IL-6–sIL-6Rα–sgp130(D1–D3) complex can be constructed (Figure 5).

The binding of IL-6-type cytokines to their receptors leads to a rapid internalization of the ligand. So far, no internalization signals have been identified in the α-receptors. Thus internalization is mediated by the signal transducing receptor subunits [4]. Indeed, in gp130 [26] and the LIFR [27] dileucine-like motifs have been identified that are required for receptor-mediated

ligand internalization. Internalization of gp130 seems to be independent from ligand binding [28], and is modulated by phosphorylation at Ser^{782} [29] which is located adjacent to the dileucine motif. Some evidence has been provided that under certain circumstances gp130 and LIFR are degraded after internalization [30–32].

There are several reports showing that unrelated receptors are involved in signal transduction of IL-6-type cytokines: stimulation of fibrosarcoma cells with OSM leads to a pronounced phosphorylation of the IFN (interferon) receptor chain 1 [33], and in murine embryonic fibroblasts lacking the IFNα–IFNβ signalling complex IL-6 signals are significantly reduced [34]. Moreover, it was reported that ErbB2 forms a complex with gp130 in an IL-6-dependent manner in prostate carcinoma cells [35].

Receptors at work: let's do the twist

Why do the signalling receptors of the IL-6-type cytokines contain additional membrane-proximal FNIII domains that are not involved in ligand binding? It has been shown that these domains are necessary for coupling ligand binding and signal transduction, since deletion of these domains leads to signalling incompetent receptors [36,37]. A domain model of the IL-6 receptor complex [36], as well as the recent X-ray data [24], show that the C-termini of the two CBMs in the gp130 homodimer are separated by a distance of about 9 nm. The membrane-proximal FNIII domains might bridge this distance so that the cytoplasmic parts are in close enough proximity to become activated by the associated JAKs (Figure 5). This assumption is supported by a recent study on the role of the gp130 FNIII domains for heterodimerization with the LIFR [38].

What are the prerequisites for cytokine receptor activation? Is it sufficient to bring two receptors into close proximity or has a well defined conformation to be adjusted for signalling to occur? For the gp130 homodimer, studies using agonistic monoclonal antibodies have contributed to clarify this issue. Unlike many other receptors, efficient gp130 activation could not be achieved by a single antibody, but requires the action of two distinct monoclonal antibodies [39,40]. The minimal requirement for receptor activation is one intact antibody and the Fab (fragment antigen binding) of the second antibody. This finding has been interpreted in a way that the bivalent intact antibody enforces receptor dimerization and the monovalent Fab adjusts the conformation required for JAK activation and downstream signalling [40].

For some receptors, including cytokine receptors, pre-dimerization or pre-oligomerization is discussed [41–44]. In these cases, the ligand does not actively induce receptor dimerization, but stabilizes a preformed receptor complex and initiates receptor activation by additionally inducing a conformational change. In the case of gp130, this concept bears some intrinsic problems since gp130 interacts with several different receptors. Therefore, the requirement for pre-dimerization with another signal transducing receptor or an α-receptor would predetermine gp130 to interact with certain, but not all, IL-6-type cytokines.

In order to transfer the information of a ligand-adjusted conformation from the outside to the inside of the cell, it seems conceivable that the transmembrane domain of cytokine receptors adopts a rigid structure. Indeed, recent studies have suggested that the transmembrane and membrane-proximal intracellular regions of cytokine receptors adopt an α-helical conformation. Interestingly, insertion of one to four alanine residues into the membrane-proximal intracellular region of gp130 demonstrated that full activation of the receptor (i.e. induction of target genes) was only obtained with the wild-type and the + 3A-mutant [45]. An independent study on the EpoR (erythropoietin receptor) juxtamembrane intracellular region also described an α-helical dependency of Epo-induced signal transduction [46]. These results were explained by the hypothesis that insertion of one alanine residue twists the receptor by approx. 110°, resulting in a position very different from the wild-type receptor state which might prevent efficient signal transduction. In contrast, insertion of three extra alanine residues into an α-helix should lead to a rotation of approx. 330°, twisting the receptor back, close to its native position. Interestingly, within the cascade of signalling events cytokine receptors seem to differ in the step that is sensitive towards orientation. While for gp130, the STATs were found to depend on a specific conformation in order to become phosphorylated, for the EpoR the receptor phosphorylation seems to be sensitive to changes in orientation. Constantinescu et al. [46] identified a hydrophobic motif in the EpoR membrane-proximal domain (Leu^{253}, Ile^{257} and Trp^{258}) which is conserved in many cytokine receptors and needs to be precisely orientated in order to promote signalling.

Receptor–JAK interaction: more than just a rendez-vous

As found for many other cytokines, IL-6-type cytokines also rapidly induce the activation of tyrosine kinases of the JAK family. The signal transducing chains gp130, LIFR and OSMR bind to JAK1, JAK2 and TYK2 [47–50]. Of these, JAK1 plays an essential role, because in cells lacking JAK1 IL-6 signal transduction is greatly impaired [51,52,134].

The interaction between gp130 and JAK1 is very tight and long-lasting: a recent FRAP (fluorescence recovery after photobleaching) analysis with fluorescent fusion proteins revealed that JAK1 does not diffuse like a typical cytoplasmic protein. Instead, its mobility is approx. 100-fold lower and similar to the one of the transmembrane protein gp130. Interestingly, immobilization of gp130 by antibodies leads to a concomitant immobilization of JAK1, indicating that there is no rapid exchange of JAK1 between different receptors (B. Giese and G. Müller-Newen, unpublished work).

JAKs bind to the membrane-proximal region of cytokine receptors, which contains conserved so-called box1 and box2 motifs. Deletion of the proline-rich box1 or mutation of two critical proline residues within box1 abrogates receptor binding of JAKs to gp130, OSMR or LIFR [49,53,54]. Box2 of gp130, a sequence dominated by hydrophobic amino acids followed by charged ones, contributes to JAK binding, presumably by increasing the affinity; a gp130 construct lacking box2 co-precipitates with JAK1 only when the kinase is over-expressed [55]. This demonstrates the sensitivity of the experimental read-out to variations in expression levels of the interaction partners and helps to explain previous somewhat conflicting results on the importance of the gp130 box2 region [56–58]. Finally, also the interbox1–2 region of gp130 is critical, e.g. mutation of a single amino acid (Trp^{666}) abrogates JAK binding and thereby leads to inactivation of the receptor complex [54]. Thus the interaction surface with JAKs may involve multiple contact sites within the receptor.

In contrast with the modular character of STAT recruitment motifs, the JAK-recruiting region loses its functionality upon transfer within the cytokine receptor chain; addition of the box1–2 region to the C-terminal part of a gp130 mutant, which was unable to associate with JAKs in the membrane-proximal region, did not restore JAK association [45]. This may point at the possibility that membrane proximity is crucial for receptor–JAK interaction to occur. However, it cannot be excluded that the structural integrity of the JAK interaction interface within the receptor depends on N-terminally adjacent sequences, i.e. the helix

spanning the membrane which probably extends into the cytoplasmic region [45,46]. It would be a great achievement to determine the three-dimensional structure of the membrane-proximal region of a cytokine receptor, possibly in conjunction with a JAK or a fragment thereof.

The receptor not only serves as a docking site for JAKs. In addition, certain residues within gp130 have been identified to be crucial for JAK activation, e.g. substitution of Trp^{652} with alanine (W652A) within the box1 region has no effect on JAK1 association but abrogates JAK1 activation. Signal transduction is even drastically impaired if only one chain within a gp130 dimer carries the W652A mutation [55]. Mutations with similar consequences (no JAK activation in spite of JAK association) have been described for the EpoR [59], indicating that cytokine receptors in general contribute to the JAK-activation process.

The general structure of JAKs is shown in Figure 4. The C-terminal tyrosine kinase domain is preceded by a pseudokinase domain, which itself is devoid of catalytic activity, but regulates the activity of the kinase domain [3]. JAKs also contain a predicted SH2 (Src homology 2) domain. It will be interesting to find out the significance of this domain for JAK function and to identify potential interaction partners.

The N-terminal region of JAKs comprises a FERM (four-point-one, ezrin, radixin and moesin) domain which is crucial for receptor association. FERM domains comprise three subdomains: subdomain F1 with a ubiquitin-like β-grasp fold, F2 with an acyl-CoA-binding-protein-like fold, and F3 which shares the fold of phosphotyrosine binding or PH (pleckstrin homology) domains. F1, F2 and F3 together form a compact clover-shaped structure [60–62]. A recent mutagenesis study has highlighted the importance of the F1 subdomain of JAK1 for the interaction with gp130 [63].

Although the catalytic activity of JAKs is dispensible for receptor recruitment, it was described that alteration of the kinase domain structure of JAK3 by the kinase inhibitor staurosporine decreased the ability to bind to the common γ chain [64]. This indicates a potential interaction between the kinase domain and the FERM domain.

There are several reports describing other protein kinases (such as Src and Tec family kinases) that are associated with signal transduction of IL-6-type cytokines (for older references see [4]). The Src family kinase Hck has recently been shown to associate with an acidic region (amino acids 771–811) of gp130 [65]. Deletion of these amino acids reduces IL-6-induced Hck kinase activity, ERK (extracellular-regulated kinase) activation, dephosphorylation of Pyk2 and proliferation of transfected pro-B Ba/F3 cells [65]. Cdk9 (cyclin-dependent kinase 9) was also found to bind to gp130, and the association increased upon IL-6 stimulation of HEK 293 cells over-expressing gp130 and Cdk9 [66]. PKCδ (protein kinase Cδ), a kinase implicated in serine phosphorylation of STAT3 (see below), has been found in a complex with gp130 upon IL-6 stimulation. PKCδ enhances the association of STAT3 with the receptor, which possibly involves the phosphorylation of Thr^{890} of gp130 [67]. It will be interesting to define the respective contribution of these 'non-JAK' kinases to IL-6 signal transduction.

Another face of JAKs: determination of the receptor's fate

Apart from their role in signal transduction JAKs are important for the regulation of surface expression of at least some cytokine receptors.

Co-expression of JAK1, JAK2 and TYK2 substantially enhances the surface expression of the human OSMR. While kinase activity is dispensible for this effect, association of the JAKs to the box1/box2 region leads to the masking of a negative regulatory signal, potentially a previously uncharacterized endoplasmic reticulum retention/retrieval signal, that prevents efficient surface expression. This effect is also observed in cells with endogenous expression levels of the OSMR and JAKs: human fibrosarcoma cells lacking JAK1 express less OSMR at their surface compared with the parental cells, but transient transfection of JAK1 can again increase the amount of surface-expressed receptors [49].

Similarly, JAK2 is crucial for EpoR surface expression. In this case it has been hypothesized that JAK2 supports the proper folding of the receptor [59].

TYK2 is important for surface expression of the IFNα receptor 1 chain [68]. Recently, it has been found that TYK2 prevents receptor internalization [69]. Thus, the mechanisms how JAKs are involved in the regulation of surface expression seem to vary between receptor systems.

The STAT transcription factors: more nuts to crack?

Extensive studies have established the central role of STATs in IL-6-type cytokine signalling. The STAT family of transcription factors encompasses seven mammalian members, designated STAT1, -2, -3, -4, -5a, -5b and -6. The domain structure of STAT proteins comprises from N- to C-terminus an oligomerization domain, the so-called coiled-coil domain, the DNA-binding domain, the linker domain, the SH2 domain and the transactivation domain (Figure 4). An alternative denomination for the coiled-coil domain, the DNA-binding domain and the linker domain is 4-helix bundle, β-barrel and connector domain respectively. This knowledge is derived from the solved partial crystal structures of STAT4, as well as STAT1 and STAT3 [70–72].

Activation of the STAT family members requires the transient association of the STATs with cytokine receptors [73,74]. The classical view favours the recruitment of monomeric STAT proteins to the activated receptors, but there is evidence that pre-associated STAT factors exist in higher molecular mass complexes prior to stimulation [75–78]. Although the exact nature and role of the higher molecular mass complexes is not yet established and their presence does not exclude the recruitment of STAT factors in a monomeric state, their mere existence, however, suggests that STAT activation may be more complex than previously assumed. Recently, non-phosphorylated, as well as IL-6 activated phosphorylated, STAT3 and STAT1 pools were found to be present in plasma membrane rafts [11]. In addition, STAT3 was reported to be associated with both caveolin-1 and heat-shock protein-90 in these rafts, as well as in the cytosol [79].

All IL-6-type cytokines potently activate STAT3, and to a minor extent STAT1 through their common receptor subunit gp130 [4]. In the cases of LIFR and OSMR, STAT3 and STAT1, as well as STAT5, activation has been observed, with OSMR being the most potent activator of STAT5 [56]. This activation may not require receptor tyrosine phosphorylation, but result from a direct interaction of STAT5 with JAKs [80]. For OSMR-mediated STAT5 activation both mechanisms have been observed (C. Evers and H. M. Hermanns, unpublished work). The recruitment of STATs to the activated receptors has been shown to be mediated by their SH2 domain and requires the phosphorylation of receptor tyrosine motifs [74,81–83]. Whereas STAT3 binds to phospho (p)YXXQ motifs (Y^{767}RHQ, Y^{814}FKQ, Y^{905}LPQ and Y^{915}MPQ in gp130; Y^{981}QPQ, Y^{1001}KPQ and Y^{1028}RPQ in LIFR) [81,84–86], STAT1 is recruited to the more restricted consensus sequence pYXPQ (Y^{905}LPQ and Y^{915}MPQ in gp130) [84]. Although several gp130 motifs mediate STAT3 activation, they are not equivalent with respect to their potential to activate STAT factors and acute-phase protein gene promoters

[87]. Subsequent to receptor binding, the STAT factors are phosphorylated on a single tyrosine residue (Tyr^{701} in STAT1 and Tyr^{705} in STAT3) [88,89]. This leads to the formation of active STAT dimers, also shown to be mediated by their SH2 domains [90]. Interestingly, the STAT1 and STAT3 phosphotyrosine motifs (STAT1, pY^{701}IKT; STAT3, pY^{705}LKT) do not agree with the STAT3 consensus sequences deduced from the gp130 and LIFR recruitment sites [81,84–86]. A conformational change in the SH2 domain may account for this dual specificity [91].

An additional STAT3 tyrosine phosphorylation site (Tyr^{657}) was reported by Pfeffer et al. [92] as a binding site for PI3K (phosphoinositide 3-kinase). However, the location of Tyr^{657} in the solved structure of STAT3β bound to DNA questions that Tyr^{657} is able to recruit the SH2 domain of PI3K. Although Tyr^{657} is partially exposed at the surface of the SH2 domain of STAT3, it builds up hydrophobic contacts with a number of hydrophobic amino acid side chains and is part of the hydrophobic core of the STAT3 SH2 domain. Phosphorylation of this Tyr^{657} would severely impair the function of the SH2 domain, as it is located in the centre of the binding pocket responsible for specific recognition of phosphotyrosine motifs. In addition, the partially buried side chain of Tyr^{657} would not be able to bind into the phosphotyrosine binding pocket of PI3K. This, together with the lack of reports by other groups confirming the phosphorylation of Tyr^{657}, questions the relevance of this tyrosine phosphorylation site for STAT3 function.

Serine phosphorylation and methylation of STATs: making the nutshell even harder

In addition to tyrosine phosphorylation, other post-translational modifications were reported to affect STAT function.

Serine phosphorylation

Serine phosphorylation has been described for STAT1, -3, -5a and -5b. Although the site of serine phosphorylation in STAT1 and STAT3 has been identified as Ser^{727}, there is presently no clear picture on the nature of the involved serine kinase(s).

In most studies small molecular mass inhibitors, as well as dominant negative kinases, have been used to identify the serine/threonine kinases. Depending on the experimental system, i.e. the cell type and the cytokine/growth factor investigated, evidence for the involvement of PKCδ [93–96], p38 MAPK [93], MEKK1 (MAPK/ERK kinase kinase 1) [97], ERK [98], JNK (c-Jun N-terminal kinase) and, most recently, the Ca^{2+}/calmodulin-dependent kinase II [99] has been obtained.

Most investigators have found an increase in transcription of target genes upon cytokine-induced serine phosphorylation of STAT1, -3 and -5 [93,98–102]. In prolactin-stimulated mammary epithelial cells serine phosphorylation has an impact on signal duration [101].

In the case of STAT3 activation after IL-6 stimulation of HepG2 cells, two distinct pathways for the serine phosphorylation have been identified: one sensitive and the other insensitive to the serine/threonine kinase inhibitor H7 [98]. Earlier studies [103,104] had already shown: (i) Ser^{727} phosphorylation of STAT3 to occur slower than the phosphorylation of Tyr^{705}; (ii) dominant-negative Ras to have no effect on STAT3 Ser^{727} phosphorylation; and (iii) the existence of an H7-sensitive serine/threonine kinase indicating that the MAPK pathway is not involved. These results were confirmed by Chung et al. [105], who also observed that IL-6-induced STAT3 activation is MAPK independent, but sensitive to H7.

For several cell lines it has been reported that PKCδ associates IL-6 dependently with STAT3 and phosphorylates it on Ser^{727}, leading to an inhibition of STAT3 DNA binding and transcriptional activity [94]. A sequential activation of Vav, Rac-1, MKK-4 (MAP kinase kinase 4) and PKCδ is necessary for the IL-6-mediated STAT3 Ser^{727} phosphorylation and transactivation in HepG2 cells. Moreover, there is evidence that the PKCδ-mediated STAT3 Ser^{727} phosphorylation occurs in the nucleus [95]. It is presently not clear how PKCδ is activated upon IL-6 stimulation.

Methylation of STATs

Another post-translational modification has only recently been recognized to play an important role in STAT function; Arg^{31} of STAT1 was found to be specifically methylated by PRMT-1 (protein arginine methyltransferase-1). There was earlier evidence for a link between PRMT and the JAK/STAT pathway: PRMT-1 was found to associate with the IFNα/β receptor 1 [105a]; and also a JAK-associated protein was identified as PRMT-5 [105b].

STAT1 methylation is observed in the absence of cytokine stimulation, independent of tyrosine or serine phosphorylation, but requires the intact STAT1 SH2 domain. Compared with the unmethylated protein, methylated STAT1 has a higher tendency to associate with DNA, since its interaction with PIAS (protein inhibitor of activated STATs) 1 (see the subsection below entitled 'PIAS – more than inhibitors of activated STATs?') seems to be weaker [106]. Arg^{31} is conserved among the STATs and it will be interesting to find out whether other STATs are also subject to methylation.

STAT nuclear translocation: not a one way ticket

In response to IL-6 stimulation, cytoplasmic STAT3 rapidly accumulates in the nucleus. Because of their size of 90 kDa that is far beyond the exclusion limit of the nuclear pore, STATs need to be actively translocated into the nucleus. Indeed, extracellular-signal-dependent translocation of STAT1 in response to IFN requires the nuclear import receptor NPI-1/importin-α5, which mediates translocation via a Ran-dependent mechanism [107]. Sensitivity to leptomycin suggests that nuclear export of STAT1 involves the nuclear export receptor CRM-1 that also acts in a Ran-dependent manner [108,109]. Nuclear accumulation is triggered by STAT dimerization in response to tyrosine phosphorylation. Tyrosine phosphorylation itself is not necessarily required for STAT nuclear translocation. Artificially dimerized STAT proteins that are not phosphorylated also accumulate in the nucleus [110,111].

A recent study [112] on STAT3 activation in response to EGF (epidermal growth factor) suggests that receptor-mediated endocytosis is required for shuttling of STAT3 from the plasma membrane to the perinuclear region. According to this study, STAT3 is associated with endocytotic vesicles during directed transport through the cytosol [112]. However, from an earlier study [113], it was concluded that nuclear translocation of STAT1 in response to IFNγ does not require cytoskeletal structures, such as actin filaments or microtubules. There, a non-directional random-walk model was proposed for the cytoplasmic passage of STAT1. Furthermore, nuclear accumulation of constitutively active STAT proteins is independent from endocytosis, because in these cases the STATs are not recruited to any plasma membrane receptor [110,114]. Also microinjected phosphorylated STAT1 protein readily concentrates in the nucleus without obvious involvement of endocytotic vesicles (U. Vinkemeier, personal communication). Thus there seems to exist no strict requirement for

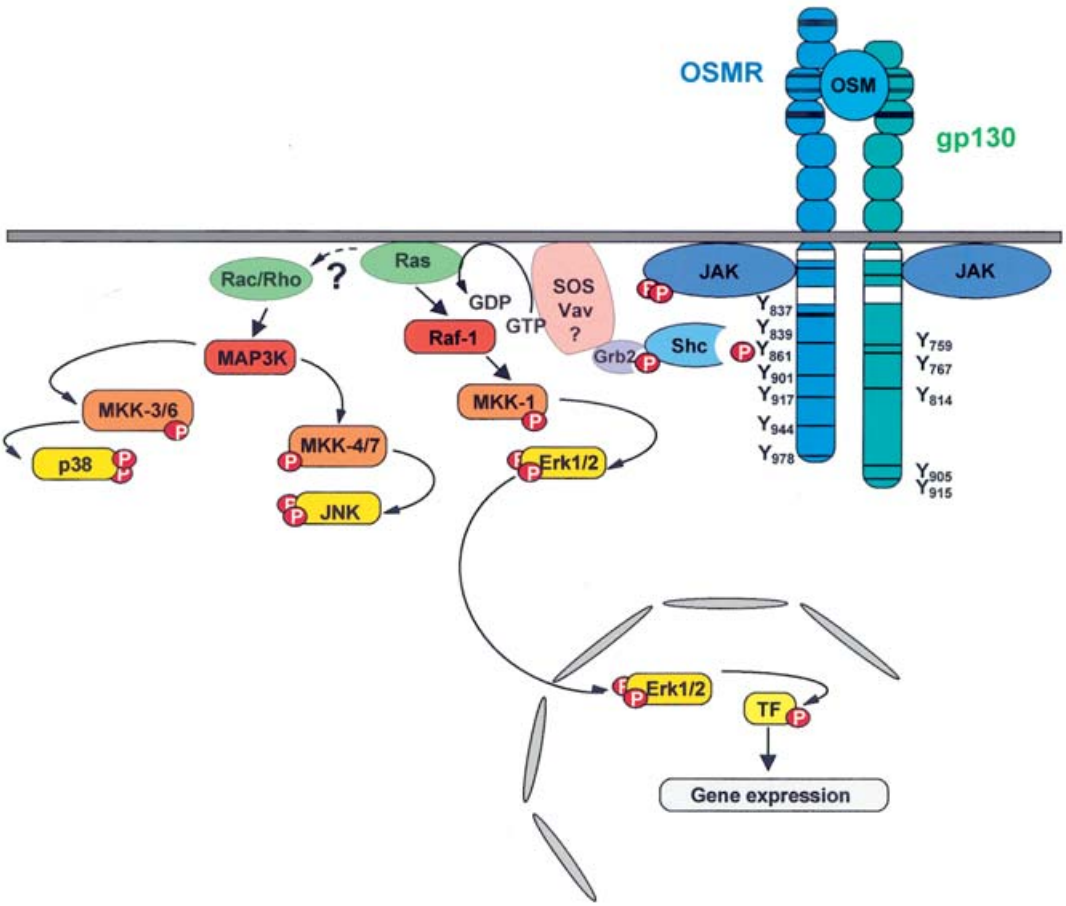

Figure 6 Activation of the MAPK cascade via the OSM receptor

OSMR-mediated activation of the MAPK pathways via the adaptor molecule Shc. TF, transcription factor.

the targeted movement of endocytotic vesicles for STAT nuclear translocation to occur.

Most studies aimed at the identification of putative nuclear import signals [NLSs (nuclear localization signals)] or export signals [NESs (nuclear export signals)] were performed with STAT1. Nevertheless, mutagenesis studies on STAT5 provided the first evidence for a functional role of the DNA-binding domain in nuclear translocation [115]. In the DNA-binding domain of STAT1, initially an NES was postulated that comprises residues 399–410 [108]. Later it turned out that residues located within this short sequence stretch are important for the interaction of STAT1 with the import receptor importin-α5 and therefore for nuclear import of STAT1 [116,117]. Adjacent to this sequence, basic residues (Lys^{410} and Arg^{413}) were identified that also contribute to the NLS of dimeric STAT1 [117–119]. Two other NESs were characterized in the coiled-coil region of STAT1 (residues 302–314), both containing several leucine residues that are critical for their function [109,120]. The functional overlap of DNA-binding, NLS and NES sequences points to a role for DNA binding in the regulation of nucleocytoplasmic shuttling of STAT proteins.

Previous studies suggested that tyrosine phosphorylation of STATs results in dimerization and nuclear translocation. Dephosphorylation of STAT within the nucleus leads to export from the nucleus [121]. Our recent investigations and work of others [117,122,123] led to the establishment of continous nucleocytoplasmic shuttling of STAT proteins that is independent from extracellular stimulation and phosphorylation. The finding of unphosphorylated STAT proteins within the nucleus [122] is consistent with the well-established role of unphosphorylated STATs as transcriptional coactivators [124,125]. NLSs for this phosphorylation-independent shuttling are different from those for phosphorylation-induced nuclear accumulation, but are not yet identified [117]. Further evidence suggests that continuous nucleocytoplasmic shuttling of STAT proteins even occurs after stimulation and nuclear accumulation [126].

Activation of MAPK cascades: one tyrosine to make things more complicated

Dimerization of IL-6-type cytokine receptors does not only lead to activation of the JAK/STAT-signalling pathway, but also to the induction of the MAPK cascade. Initial analyses concerning gp130- and LIFR-mediated activation of MAPKs identified the SHP2 (SH2-domain-containing tyrosine phosphatase)-binding site Tyr^{759} of gp130 [81] and Tyr^{974} of LIFR to be crucial for the activation of the MAPK cascade [127]. In contrast with gp130 and LIFR, the OSMR does not recruit SHP2. Nevertheless, the OSMR is also able to induce activation of the Ras–Raf–MAPK pathway. It has been demonstrated that the adaptor protein Shc (SH2- and collagen-homology-domain-containing protein) (Figure 4) is recruited to the receptor via Tyr^{861} in the cytoplasmic region of the OSMR [128] (Figure 6).

According to the current view of the SHP2-dependent activation of MAPKs adopted from EGF and PDGF (platelet-derived growth factor) signal transduction, SHP2 links the Grb2–SOS (growth-factor-receptor-bound protein/Son of Sevenless) complex and/or Gab1 (Grb2-associated binder-1) to gp130 [127,129,130]. SHP2 is rapidly recruited to tyrosine-phosphorylated gp130 and becomes also phosphorylated in a JAK1-dependent manner [131]. Subsequently, tyrosine-phosphorylated SHP2 interacts with Grb2 [132]. Two C-terminal tyrosine residues within SHP2 (Tyr^{542} and Tyr^{580}) are believed to interact with the Grb2–SOS complex. The alternative Shc-mediated pathway mediated by OSM also involves the adaptor protein Grb2 which is recruited to Tyr^{317}-phosphorylated Shc. Finally, recruitment of SOS to the receptor complex at the membrane allows Ras activation, which in turn leads to the activation of the Ras–Raf–MAPK cascade. Thus the OSMR, unlike gp130 or the LIFR, uses the Shc–Grb2–SOS route for activation of MAPKs [128].

Another degree of complexity is added by the fact that Gab1 is also involved in the activation of the Ras–Raf–MAPK cascade. Gab1 is a scaffolding adaptor protein which is targeted via a PH domain to the plasma membrane. Furthermore, it contains binding sites for Grb2, SHP2, PI3K, Crk, phospholipase Cγ and the c-Met receptor [129,133,134]. In response to IL-6, Gab1 is tyrosine phosphorylated and interacts subsequently with SHP2 and PI3K. Interestingly, no direct interaction of Gab1 and gp130 is required for its tyrosine phosphorylation. Nevertheless, mutation of Tyr^{759}, the SHP2 recruitment site within gp130, impairs the interaction of Gab1 with SHP2 and PI3K, suggesting that binding of SHP2 to gp130 and its subsequent phosphorylation is a prerequisite for the interaction with Gab1. Finally, IL-6-induced association of Gab1 with SHP2 leads to activation of ERK2 [135]. As expected, Gab1-deficient fibroblasts show a markedly reduced MAPK activity in response to IL-6 [136].

Although Gab1 integrates several signal transduction pathways, translocation of SHP2 to the membrane, in proximity to membrane anchored Ras, seems to be sufficient to mediate ERK activation, since expression of a fusion protein comprising the PH domain of Gab1 and active SHP2 induces constitutive MEK1 (MAPK/ERK kinase) and ERK2 activation [137].

After EGF stimulation, SHP2 associated with Gab1 also regulates and counteracts PI3K binding to Gab1 [138]. Analogous mechanisms in respect to IL-6-type cytokine signalling should not automatically be expected, since even PDGF- and insulin-like growth factor 1-dependent PI3K activation is not affected by SHP2 [138], suggesting that SHP2 acts in a strictly receptor specific manner.

Recently, a further mechanism for the regulation of the MAPK cascade by cytokines has been found by Cacalano et al. [139]. In response to IL-2, Epo, EGF, and PDGF, SOCS3 (suppressor of cytokine signalling 3) becomes tyrosine phosphorylated, and subsequently binds and inactivates Ras/GTPase-activating protein. This results in the inhibition of the GTPase activity of Ras, leading to a sustained Ras/GTP and MAPK activity [139]. Analogous observations for IL-6-type cytokines have not been reported.

The family of IL-6-type cytokines not only activates ERK1/2, a MAPK known for cellular processes that maintain cell survival, but also the stress-activated members of the MAPK family: p38 and JNK [140–142] (Figure 6). The signal transduction pathways resulting in their activation, however, remain poorly understood. A recent study by Schuringa et al. [95] postulated that the activation of JNK is involved in the serine phosphorylation of STAT3 after stimulation of hepatoma cells with IL-6. Using constitutive-active and dominant-negative variants of the signalling components, they delineated the signalling pathway leading to JNK activation and suggest the involvement of the GTP-exchange factor Vav, the small G-protein Rac and the MAPK kinase, MKK6. Their study is in contrast with the finding of Zauberman et al. [142], which demonstrated an activation of p38 MAPK, but not JNK, in hepatoma cells and found an involvement of p38 MAPK in STAT3-mediated transcriptional activation of the acute-phase protein haptoglobin.

Activation of the PI3K cascade: the lipid connection

IL-6-type cytokines can lead to the activation of yet an additional signalling cascade involving PI3K. This enzyme modifies certain phosphatidylinositides, so that the serine/threonine kinase protein kinase B/Akt is recruited to the plasma membrane, where it becomes activated through phosphorylation by PDK1 (phosphoinositide-dependent kinase-1). Substrates of Akt include the forkhead transcription factor FKHR and the pro-apoptotic factor Bad (Bcl-2/Bcl-X_L-antagonist, causing cell death), whose phosphorylation is associated with increased survival or cell growth. In cardiac myocytes gp130 conveys signals through this pathway which lead to prevention of doxorubicin-induced apoptosis [143]. Also, in basal cell carcinoma cells the PI3K pathway is crucially involved in the IL-6-mediated prevention of apoptosis which coincides with the up-regulation of the anti-apoptotic protein Mcl-1 [144]. IL-6-induced activation of the PI3K/Akt pathway is involved in protection against apoptosis, as well as in enhanced proliferation of multiple myeloma cells [145–147]. Moreover, in human Hep3B hepatoma cells IL-6 leads to activation of the PI3K/Akt pathway necessary for the anti-apoptotic effect of IL-6 during transforming growth factor β treatment [148]. It should be noted, however, that PI3K activation upon IL-6 treatment is observed in a cell-type specific manner; e.g. no significant Akt activation could be observed in IL-6-treated HepG2 hepatoma cells [149]. The molecular mechanism linking gp130 engagement to the activation of the PI3K/Akt pathway is not well understood. After IL-6 stimulation the adaptor protein Gab1 (see the subsection above entitled 'Activation of MAPK cascades: one tyrosine to make things more complicated') interacts with PI3K [135]. Similarly, PI3K associates with the IRS-1 (insulin receptor substrate-1) adaptor in response to OSM [150], suggesting that both IRS-1 and Gab1 may couple gp130 to PI3K activation.

MECHANISMS OF SIGNAL TERMINATION

To prevent overstimulation, Nature has invented sophisticated mechanisms to turn off cytokine-mediated signal transduction (see Figure 8).

Protein tyrosine phosphatases (PTPs): what goes up must come down

A key event in signal transduction of IL-6-type cytokines is the phosphorylation of components of the signal transduction cascade. Thus it is obvious that, besides kinases, phosphatases also have to be involved in proper signal transmission. First evidence for the contribution of a phosphatase in IL-6 signalling was obtained by the finding that the PTP SHP2 is recruited to the cytoplasmic Tyr^{759} of activated gp130 [81,151].

SHP2 is a ubiquitously expressed cytoplasmic PTP containing two N-terminal SH2 domains and a catalytic phosphatase domain in the C-terminal half of the protein (Figure 4). The crystal structure of SHP2 suggests that, in the absence of a tyrosine-phosphorylated binding partner, the N-terminal SH2 domain

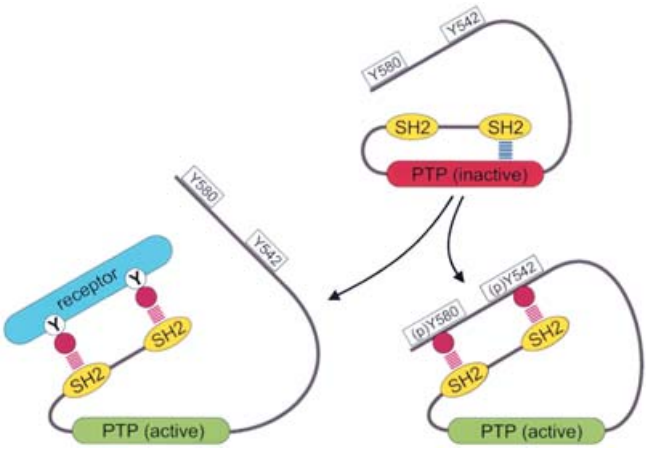

Figure 7 Regulation of SHP2

In the inactive state, the N-terminal SH2 domain of SHP2 sterically hinders access of phosphotyrosine substrates to the PTP domain and thus blocks PTPase activity. Binding of the N- or C-terminal SH2 domain by specific phosphotyrosine-containing motifs releases the block. These phosphotyrosine residues may be part of an activated receptor or the tyrosine-phosphorylated C-terminal tail of SHP2 (from [156]). Reprinted from Molecular Cell, vol. 8, W. Lu, D. Gong, D. Bar-Sagi and P. A. Cole, Site-specific incorporation of a phosphotyrosine mimetic reveals a role for tyrosine phosphorylation of SHP-2 in cell signaling, pp. 759–769, Copyright 2001, with permission from Elsevier.

covers the active site and thereby inhibits the enzymic activity [152]. Binding of the SH2 domains to phosphotyrosine motifs of receptors or adapters unfolds the protein, leading to enzymic activity [153–155]. SHP2 also becomes activated by the phosphorylation of tyrosine residues 542 or 580 within the C-terminal part of the enzyme. Subsequently, these phosphotyrosines interact with the N- and C-terminal SH2-domains respectively, relieving the PTP domain from the N-terminal SH2-domain-mediated inhibition [156] (Figure 7).

Tyr^{759} of gp130 appears to have multivalent functions for signalling; substitution with phenylalanine impairs SHP2 recruitment and phosphorylation [81] and leads to enhanced IL-6 [131,157,158], as well as LIF and OSM, signal transduction [159]. In addition to SHP2, the feedback inhibitor SOCS3 also contributes to pTyr^{759}-mediated inhibition (see the subsection below entitled 'The SOCS family of feedback inhibitors: natural born terminators', and Figure 8). On the other hand, the IL-6-induced activation of the MAPK cascade is impaired by mutation of Tyr^{759} within gp130, indicating that SHP2, as an adaptor, has a positive function on the activation of the MAPK cascade (as shown in Figure 1). However, SHP2 also plays a negative regulatory role as a tyrosine phosphatase in the Jak/STAT pathway [160] (as indicated in Figure 8). Experiments *in vivo* underscore the *in vitro* findings in demonstrating that mice expressing gp130 lacking Tyr^{759} display splenomegaly, lymphadenopathy and an enhanced acute-phase reaction [161].

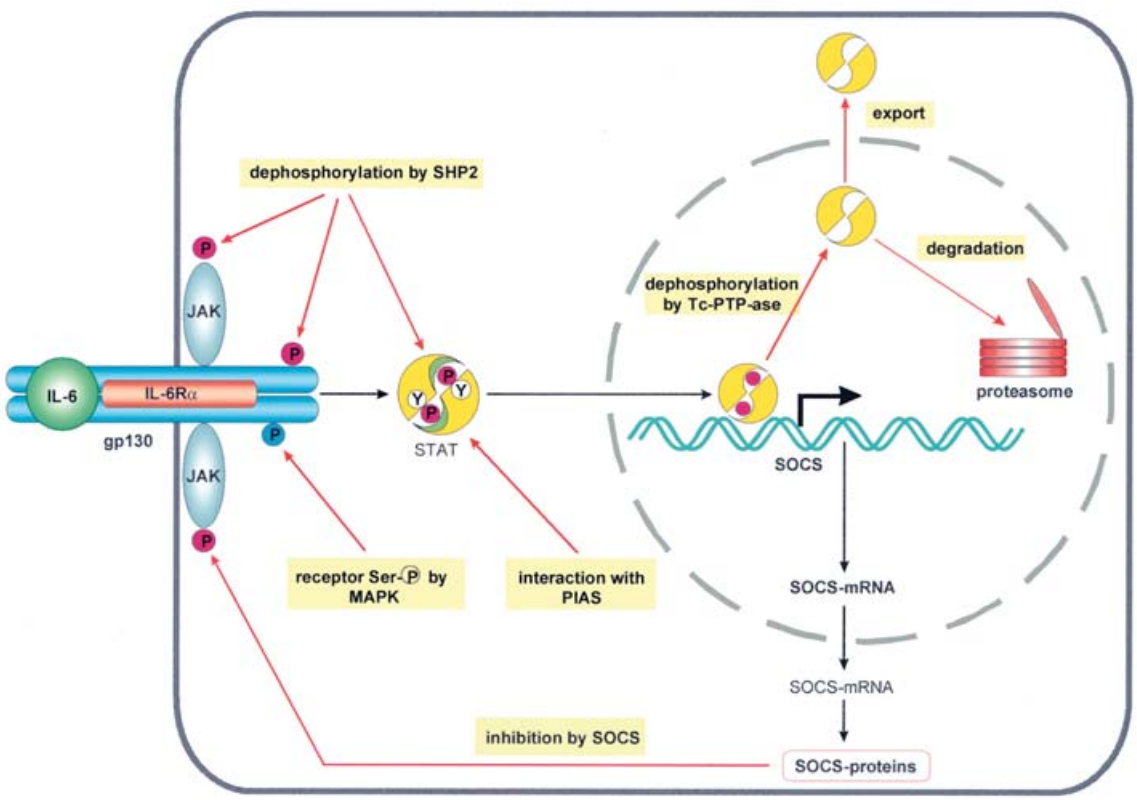

Figure 8 Negative regulatory mechanisms in IL-6-type cytokine signalling

Representation of the negative regulators and their sites of action (red arrows).

All tyrosine-phosphorylated signalling components are potential substrates for the SHP2 phosphatase. Overexpression of dominant-negative SHP2 mutants leads to enhanced receptor, JAK, STAT and SHP2 phosphorylation [160]. Since increased JAK activity also enhances activation of signalling molecules downstream of JAK, it is difficult to establish which proteins are direct substrates for SHP2 *in vivo*. Interaction of SHP2 with JAK1, JAK2 and TYK2, and phosphorylation of SHP2 by JAK1 and JAK2, have at least been shown to occur *in vitro* [131,162]. Further evidence for STAT3 as a direct substrate of SHP2 comes from the detection of STAT3–SHP2 complexes [163]. In the case of STAT1, SHP2 has been described as a dual-specificity phosphatase that dephosphorylates pTyr^{701} and pSer^{727} [164]. Furthermore, the highly conserved N-terminal part of STAT1 and STAT3 appears to mediate sensitivity towards phosphatases, since STAT proteins lacking this domain are found to be hyperphosphorylated [165–167].

Besides SHP2, further phosphatases are suggested to affect IL-6 signalling. The cytoplasmic variant of PTPε, PTPεC, is expressed in haematopoietic cells and has been shown to selectively inhibit IL-6-induced gp130, JAK and STAT activation [168]. Whereas SHP2 modulates the amount and amplitude of STAT3 activity [131,158], PTPεC affects the kinetics of the onset of STAT3 activation [169].

Several other PTPs, such as PTP1B, CD45 and SHP1, have also been reported to be involved in JAK/STAT signalling [170–172]. The function of these phosphatases in the signal transduction of IL-6-type cytokines remains to be analysed in detail.

Early results suggested the presence of a nuclear phosphatase which counteracts IFNγ-activated STAT1 and contributes to its nuclear export [121,173]. Meanwhile, this phosphatase has been identified as the nuclear isoform of the T-cell PTP TC45 [174,175]. Interestingly, STAT1 methylated at Arg^{31} is a better substrate for T-cell PTP than non-methylated STAT1. Since both T-cell PTP and PIAS1 associate with the N-terminus of STAT1 (see below), PIAS1 may prevent dephosphorylation by preferentially masking the T-cell PTP binding region of non-methylated STAT1 [176]. Thus methylation not only supports STAT activity (by preventing PIAS association), but also promotes its inactivation by dephosphorylation.

PIAS – more than inhibitors of activated STATs?

The family of PIAS comprises five mammalian members: PIAS1, PIAS3, PIASxα, PIASxβ, and PIASy. Initially identified as Gu/RNA helicase II-binding protein (PIAS1) [177], it rapidly became clear that PIAS proteins are important transcriptional co-regulators of the JAK/STAT pathway [178,179]. PIAS1 inhibits STAT1 signalling [179], but conversely enhances the transcriptional activity of nuclear hormone receptors [180]. Whereas PIAS1 specifically inhibits DNA binding of activated STAT1 and thus STAT1-mediated gene induction (after IFN stimulation) [179], PIAS3 was found to be specific for the inhibition of STAT3-mediated gene expression (after IL-6 stimulation) [178]. The interaction of PIAS proteins with STAT factors seems to require tyrosine phosphorylation of the STAT proteins. As mentioned above, PIAS1 preferentially associates with unmethylated STAT1 [106]. However, the exact molecular mechanism of how PIAS negatively regulates STAT transcriptional activity needs to be elucidated. Recently, PIASy was found to also inhibit STAT1-mediated gene activation [181]. In contrast with PIAS1, however, it did not affect STAT1 DNA-binding activity. PIASy rather appears to act as a transcriptional co-repressor of STAT1.

While earlier studies suggested that PIAS proteins might act in the cytoplasm, the picture that emerges from recently published work is that at least some members of this family display their activity in the nucleus. Here, they are located in specific nuclear bodies [182,183] attached to the nuclear scaffold [184]. With respect to this finding, it is of interest that PIAS proteins exhibit E3-SUMO (small ubiquitin-related modifier)-ligase activity [185, 186]. Sumoylation may play a critical role in targeting transcription factors to nuclear bodies, where they bind to other sumoylated proteins, such as PML (promyelocytic leukaemia) or the co-repressor Sp100, and thereby become transcriptionally active or inactive [186,187].

The SOCS family of feedback inhibitors: natural born terminators

With the discovery of the SOCS proteins several years ago, a new mechanism of inhibition emerged [188–192]. These inhibitors have also been designated CISs (cytokine-inducible SH2 proteins) or SSIs (STAT-induced STAT inhibitors). The eight members of this family (CIS and SOCS1–SOCS7) contain a central SH2 domain, as well as a C-terminal domain called a SOCS box (Figure 4). Expression of SOCS proteins was found to be rapidly up-regulated by IL-6 (CIS, SOCS1, SOCS2 and SOCS3), LIF (CIS, SOCS1, SOCS2 and SOCS3), IL-11 (SOCS3) and OSM (CIS, SOCS1 and SOCS3) [32,190,192,193]. As SOCS proteins are induced via the JAK/STAT pathway and subsequently inhibit STAT-mediated signal transduction, they are acting as classical feedback inhibitors. The detailed mechanism of inhibition, however, seems to differ between the various family members. CIS, which was the first member to be identified, was shown to compete with STAT5 for recruitment sites within the EpoR and to thereby modulate the JAK2/STAT5 pathway [189,194]. SOCS1 and SOCS3 are functionally most related and potently inhibit IL-6-type cytokine signalling. Both have been shown to act on the JAKs, and thereby inhibit the phosphorylation of gp130, STATs and the JAKs themselves [190,192]. As SOCS1 and SOCS3 act at the level of the JAKs they can affect the activation of different STAT factors depending on the receptor system.

SOCS1 inhibits signal transduction by binding to the activation loop of the JAKs via its SH2 domain. The so-called extended SH2 subdomain and the KIR (kinase inhibitory region) of SOCS1, located N-terminally to the SH2 domain (Figure 4), also take part in the inhibition of JAKs. The KIR region is thought to bind to the substrate binding site of the kinase domain and thereby to inhibit its catalytic activity [195]. In contrast with SOCS1, despite the fact that it can also bind to JAKs and that it contains a KIR region [196], SOCS3 associates with specific phosphotyrosine motifs within activated cytokine receptors, such as gp130, EpoR, leptin receptor and the granulocyte colony-stimulating factor receptor [197–203].

For gp130, SOCS3 has been found to bind to the phosphotyrosine motif 759 [197,198], which is also the binding site for SHP2. In fact, the affinity of SOCS3 to bind to gp130 peptides is even slightly higher than that of SHP2 [204]. Thus the involvement of SHP2 and SOCS3 in pTyr^{759}-mediated attenuation has been re-analysed to determine the individual contributions of both proteins. Although both SOCS3 and SHP2 are recruited to the same site within the native gp130, it is suggested that there are two largely distinct modes of negative regulation of gp130 activity: through the feedback inhibition by SOCS3 and/or the dephosphorylation of phosphorylated JAKs, receptors and STATs [160].

The exact mechanism by which SOCS3 functions is not entirely clear, as it was also reported to bind to the activation loop of JAK2 via its SH2 domain [196,199]. One possible explanation is

that receptor recruitment of SOCS3 is a prerequisite for subsequent JAK binding. Binding studies with phosphopeptides suggest that SOCS3 binds pTyr^{759} of gp130 with much higher affinity than pTyr^{1007} within the activation loop of JAK2 [198]. However, the affinity for the double phosphorylated activation loop peptide (pTyr^{1007}–pTyr^{1008}) of JAK2 is comparable with the affinities determined for SOCS3 recruiting receptor motifs like pTyr^{401} of the EpoR (M. Hörtner, S. Haan and P. C. Heinrich, unpublished work). The phosphorylation status of the JAKs (single tyrosine phosphorylation of the activation loop versus double tyrosine phosphorylation) may thus influence the mode of inhibition by which SOCS3 acts. This difference in the inhibitory mechanisms of SOCS1 and SOCS3 is also reflected in the fact that SOCS1 blocks JAK activity more efficiently than SOCS3 [205].

Another degree of complexity is added to the inhibitory mechanism of SOCS proteins by reports that they are also involved in the degradation of some of their binding partners. Recent work suggests that SOCS proteins may be part of E3-ubiquitin ligases in complex with elongins B and C, as well as Rbx1 (RING box protein 1) and Cul5 (cullin 5) [206,207]. In this complex, the SOCS proteins interact with elongin C via the SOCS box. Consistent with the postulated E3-ligase function are new findings that SOCS1 targets JAK2, Tel-JAK2 and IRS1/2, as well as Vav, to degradation [208–212]. However, the implications of this interaction for the SOCS proteins themselves are not clear at present. Mutations within the box region of SOCS1, as well as serine phosphorylation, which disrupt elongin C binding, have been shown to stabilize SOCS1 [213,214]. Other reports, however, suggest that elongin C binding can stabilize SOCS1 and that the disruption of the interaction leads to proteasomal degradation of SOCS1 [206,215]. In the case of SOCS3, tyrosine phosphorylation in response to IL-2, Epo, EGF and PDGF was reported [139,216]. Phosphorylation occurs at two tyrosine residues (Tyr^{204} and Tyr^{221}) within the SOCS box and phosphorylation of Tyr^{221} recruits p120 RasGAP leading to sustained ERK activation [139]. As elongin C binds to the SOCS box, it is conceivable that tyrosine phosphorylation of SOCS3 also affects elongin C binding and SOCS3 stability.

MODULATION OF SIGNAL TRANSDUCTION

The topics covered in the previous section on signal termination represent rather linear types of regulation. However, a more complex network of regulatory mechanisms for the modulation of cytokine signalling exists.

Regulation of cytokine availability: life and death of the party

An important way to modulate the availability of cytokines is by regulating the half-life of their corresponding mRNAs, which are generally short-lived due to AU-rich sequences in their 3′-untranslated regions. The stress-activated p38 MAPK has been shown to play an important role in stabilizing the mRNA of many cytokines, including IL-6, by activation of MK2 (MAPKAPK2, MAPK-activated protein kinase 2) [217]. More recently this finding was further corroborated. It was shown in MK2-deficient macrophages that the half-life of IL-6 mRNA was reduced by more than 10-fold. Deletion of the AU-rich element of the TNFα (tumour necrosis factor-α) mRNA abrogates the effect of MK-2 [218]. Other signalling pathways have been shown to induce stabilization of cytokine mRNAs as well: the JNK pathway induces stabilization of the short-lived IL-2 mRNA [219], and PI3K activation induces stabilization of IL-3 mRNA [220]. The exact mechanism by which signalling influences the stability of cytokine mRNAs still needs to be elucidated, but several reports implicate a function of AU-rich-element-binding regulators, such as tristetraproline and its homologue butyrate-response factor-1, Hu antigen R, heterogeneous nuclear ribonucleoprotein-D0/AUF1 and others.

IL-6 protein availability might also be regulated by cytokine proteolysis. At sites of inflammation IL-6 induces the release of the serine proteases elastase, proteinase 3 and cathepsin G from neutrophils, which in turn degrade IL-6 [221].

Table 1 Half-lives of IL-6-type cytokine signalling components [222]

Protein	Half-life (h)
SOCS1	1.5
SOCS2	1
SOCS3	1.6
IL-6Rα	2–3
gp130	2.5
JAK1	3.2
JAK2	1.9
TYK2	2
STAT3α	8.5
STAT3β	4.5
STAT1	16
SHP-2	18–20

Half-lives of signalling components: party as long as you live

Of course, signal transduction by different players involved in a pathway depends on the availability of these proteins. A critical balance of protein synthesis and degradation also affects IL-6-type cytokine signal transduction. It has been reported that the half-lives of the various players involved in IL-6 signalling differ substantially (Table 1) [222,223]. Whereas the feedback inhibitors SOCS1, SOCS2 and SOCS3 are very short-lived, STAT1, STAT3α and SHP2 have slow turnover rates. Interestingly, the half-life of STAT3β, a splice variant of STAT3α, is reduced by almost 50% compared with the half-life of STAT3α. The Janus kinases JAK1, JAK2, TYK2 and gp130 show intermediate half-lives. These differences suggest that signalling components requiring post-translational modifications for their activation are long-lived, whereas the activity of short-lived proteins is regulated mainly at the transcriptional level. It makes sense that very potent inhibitors like the SOCS proteins undergo a rapid turnover. New data suggest that post-translational modifications of these inhibitors may be a means to further regulate their activity by prolonging or shortening their half-lives (see earlier section entitled The SOCS family of feedback inhibitors: natural born terminators).

The difference in half-lives between STAT3α and STAT3β is possibly one feature contributing to the functional differences between the two transcription factors. For STAT3β both positive and negative regulatory functions have been reported [224–228]. Further studies are required to clarify these conflicting results.

Cross-talk of inflammatory cytokines: how MAPKs and NF-κB (nuclear factor κB) talk to STATs

The inflammatory cascade underlying the acute-phase response is largely controlled by the action of different mediators released under inflammatory conditions. Upon activation, blood monocytes and tissue macrophages release a set of primary inflammatory mediators such as IL-1β and TNFα.

There is strong evidence indicating that IL-6-induced signalling and transcriptional activation are modulated at levels of signal transduction upstream from gene transcription. For example, LPS (lipopolysaccharide) and the pro-inflammatory cytokine TNFα have been shown to inhibit IL-6-mediated STAT3 activation in macrophages. This inhibition is most likely due to induction of the *de novo* synthesis of the JAK-inhibitor SOCS3 [229]. A similar mechanism has been discovered for IFNγ signalling, where LPS inhibits IFNγ-dependent STAT1 activation also via the induction of SOCS3 [230]. Additional mechanisms for inhibiting IL-6 signalling by pro-inflammatory cytokines may exist, since other data show that IL-1, TNF-α and LPS inhibit IL-6-dependent STAT activation in macrophages through mechanisms which do not depend on *de novo* protein synthesis [231,232]. Interestingly, both SOCS3-dependent and SOCS3-independent inhibitory mechanisms of pro-inflammatory stimuli depend on the p38 stress kinase [229,231,233]. Similarly other MAPKs down-regulate IL-6-induced STAT3 activation. For example, the inhibitory effect of ionomycin or the phorbol ester PMA on IL-6-mediated STAT activation occurs rapidly and does not require *de novo* protein synthesis. Instead ERK1/2 MAPK were found to play a crucial role [234].

IL-1β is also known to inhibit IL-6-induced acute-phase protein synthesis in hepatocytes [235]. However, no SOCS3 induction is observed upon IL-1β stimulation of human hepatoma cells. Instead, NF-κB was identified as a mediator for IL-1β-dependent suppression of IL-6-induced α_2M (α_2- macroglobulin) expression in liver cells [236].

STAT-binding sites are often in close proximity to binding sites for other transcription factors, such as nuclear factor-IL6 [237], NF-κB [238], activator protein-1 [224,239–241] and glucocorticoid receptor [242], making a co-operative action of these factors with STATs in gene regulation most likely. Moreover, in the promoters of the rat α_2M and the human α_1-antichymotrypsin genes, STAT3-binding sites are arranged as a tandem [243,244], suggesting that formation of multimers on clustered binding sites also represents a regulatory step in STAT-dependent gene activation. This tandem organization of STAT-binding sites has also been emphasized to be essential for binding of tetrameric STAT3 to the α_2M promoter [245].

The first evidence for a competition of STAT3 and NF-κB for the α_2M promoter binding was provided by Zhang and Fuller [246] on an isolated binding site within the α_2M promoter. Interestingly, although NF-κB acts as a competitive inhibitor for STAT3 DNA-binding, at least one intact NF-κB and one intact STAT3 consensus site is crucial for the STAT3-dependent activation of the α_2M gene promoter [236], suggesting that a balanced ratio of NF-κB and STAT3 is essential for activating the α_2M promoter. This indicates a dual, negative as well as positive, regulatory function of NF-κB in acute-phase protein gene induction.

A similar contribution of NF-κB to the regulation of other acute-phase proteins, such as α_1-antichymotrypsin [236] and fibrinogen [247], may indicate the general relevance of this new mechanism for controlling acute-phase protein gene expression. A recent report [248] describing a CBP [CREB (cAMP response element binding factor)-binding protein]-mediated synergistic transcriptional activation of STAT1 and NF-κB of the CXC9 gene further supports this idea.

STAT action within the transcriptosome: where transcription factors gather

Most eukaryotic transcription factors interact with histone acetyltransferases and transcriptional co-activators such as CBP/p300, although the composition of the individual co-activator complexes may vary [249]. The C-terminal transactivation domains of STAT1, STAT3 and STAT5 are known to interact with CBP/p300 [239,250–252]. This interaction is further modulated by Nmi (N-Myc interactor) which augments CBP recruitment to STAT1 and STAT5, and potentiates IL-2 and IFNγ signalling [253]. Interestingly, Nmi itself is induced by IL-2 and IFNγ, indicating a positive feedback loop within this signal transduction. Whether Nmi also influences IL-6-type cytokine signalling remains speculation.

CBP/STAT interactions also contribute to negative regulation of gene expression by competition of transcription factors for a limited amount of CBP. Such a regulatory function has been shown for STAT1 which abolishes activator protein-1/ets transcriptional activity by competing for CBP binding [239]. By a similar mechanism STAT5B limits NF-κB-mediated signalling [254].

Not only the lack of histone actetyltransferase activity may counteract STAT-mediated gene induction. Also, a contribution of histone deacetylases to the inhibition of STAT5-dependent promoter activation has been suggested. The nuclear receptor co-repressor SMRT (silencing mediator of retinoic and thyroid hormone receptors) is a potential binding partner for STAT5 and represses STAT5-dependent transcription [255]. Since both Nmi and SMRT interact with the coiled-coil domain of STAT5, the balance between bound co-activators and co-repressors may determine the activity of the transcription factor.

STAT3 transcriptional activity can also be enhanced by interaction with other transcription factors. Interesting examples are the forkhead transcription factor FKHR and EZI (endothelial cell-derived zinc-finger protein). FKHR specifically enhanced the activity of STAT3-dependent promoters, such as the α_2M promoter, but not that of a STAT5-responsive promoter. Furthermore, FKHR and STAT3 can be co-immunoprecipitated and co-localize in the nucleus of IL-6-treated HepG2 cells [149]. These results indicate that FKHR can modulate the IL-6-induced transcriptional activity by enhancing STAT3 action. FKHR is inactivated by Akt/protein kinase B-mediated serine/threonine phosphorylation. This may explain previous findings describing an attenuation of the IL-6-mediated stimulation of acute-phase-protein synthesis by insulin and other growth factors which activate the PI3K/Akt pathway [256,257].

The nuclear zinc-finger protein EZI was first recognized as an OSM-inducible gene product, but is expressed rather ubiquitously [258]. STAT3 and EZI physically interact as demonstrated by co-immunoprecipitation. Interestingly, an EZI mutant predominantly localized in the cytoplasm inhibited nuclear localization of STAT3, as well as STAT3-mediated transactivation. Thus, EZI may augment STAT3 activity by keeping it in the nucleus.

PERSPECTIVES

With more and more structural data on signalling components becoming available and allowing a better evaluation (or re-evaluation) of previously performed mutagenesis studies, it is tempting to believe that signalling pathways will soon be completely understood. However, for some key components such data are still missing. For example, no data on the structure and conformational states of the cytoplasmic parts of cytokine receptors are available. Are they without structure at all or is there some kind of tight or loose structure that is induced or modified by the recruitment of signalling components (see Figure 5)? In addition, insight into the three-dimensional structures of JAKs and SOCS proteins would be extremely valuable.

Even if all interactions between the components of the Jak/STAT cascade were known, the signalling pathway would be still far from being fully understood. Additional complexity arises from newly discovered functions for the players involved and from the existence of a multitude of cross-talk mechanisms. Yet again, the cross-talk between the signalling pathways depends on the cell type. Furthermore, the biological outcome of a cytokine signal varies between different cells and their states of differentiation.

There are rare attempts to represent this emerging complexity in mathematical models. In most cases the quality of the models suffers from the lack of sufficient quantitative experimental data. Thus many ambitious efforts still have to be made to fill these gaps.

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REFERENCES

1 Levy, D. E. and Darnell, Jr, J. E. (2002) STATs: transcriptional control and biological impact. Nat. Rev. Mol. Cell Biol. **3**, 656–662
2 Brivanlou, A. H. and Darnell, Jr, J. E. (2002) Signal transduction and the control of gene expression. Science (Washington, D.C.) **295**, 813–818
3 O'Shea, J. J., Gadina, M. and Schreiber, R. D. (2002) Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. Cell (Cambridge, Mass.) **109** (suppl.), S121–S131
4 Heinrich, P. C., Behrmann, I., Müller-Newen, G., Schaper, F. and Graeve, L. (1998) IL-6-type cytokine signalling through the gp130/JAK/STAT pathway. Biochem. J. **334**, 297–314
5 Robledo, O., Fourcin, M., Chevalier, S., Guillet, C., Auguste, P., Pouplard-Barthelaix, A., Pennica, D. and Gascan, H. (1997) Signaling of the cardiotrophin-1 receptor. Evidence for a third receptor component. J. Biol. Chem. **272**, 4855–4863
6 Senaldi, G., Varnum, B. C., Sarmiento, U., Starnes, C., Lile, J., Scully, S., Guo, J., Elliott, G., McNinch, J., Shaklee, C. L. et al. (1999) Novel neurotrophin-1/B cell-stimulating factor-3: a cytokine of the IL-6 family. Proc. Natl. Acad. Sci. U.S.A. **96**, 11458–11463
7 Müller-Newen, G., Küster, A., Hemmann, U., Keul, R., Horsten, U., Martens, A., Graeve, L., Wijdenes, J. and Heinrich, P. C. (1998) Soluble interleukin-6 receptor potentiates the antagonistic activity of soluble gp130 on interleukin-6 responses. J. Immunol. **161**, 6347–6355
8 Martens, A. S., Bode, J. G., Heinrich, P. C. and Graeve, L. (2000) The cytoplasmic domain of the interleukin-6 receptor gp80 mediates its basolateral sorting in polarized madin-darby canine kidney cells. J. Cell Sci. **113**, 3593–3602
9 Koshelnick, Y., Ehart, M., Hufnagl, P., Heinrich, P. C. and Binder, B. R. (1997) Urokinase receptor is associated with the components of the JAK1/STAT1 signaling pathway and leads to activation of this pathway upon receptor clustering in the human kidney epithelial tumor cell line TCL-598. J. Biol. Chem. **272**, 28563–28567
10 Podar, K., Tai, Y. T., Cole, C. E., Hideshima, T., Sattler, M., Hamblin, A., Mitsiades, N., Schlossman, R. L., Davies, F. E., Morgan, G. J., Munshi, N. C., Chauhan, D. and Anderson, K. C. (2002) Essential role of caveolae in IL-6- and IGF-I- triggered Akt-1- mediated survival of multiple myeloma cells. J. Biol. Chem. **278**, 5794–5801
11 Sehgal, P. B., Guo, G. G., Shah, M., Kumar, V. and Patel, K. (2002) Cytokine signaling: STATS in plasma membrane rafts. J. Biol. Chem. **277**, 12067–12074
12 Elson, G. C. A., Lelièvre, E., Guillet, C., Chevalier, S., Plun-Favreau, H., Froger, J., Suard, I., Benoit de Coignac, A., Delneste, Y., Bonnefoy, J.-F. et al. (2000) CLF associates with CLC to form a functional heteromeric ligand for the CNTF receptor complex. Nat. Neurosci. **3**, 867–872
13 Plun-Favreau, H., Elson, G., Chabbert, M., Froger, J., deLapeyriere, O., Lelievre, E., Guillet, C., Hermann, J., Gauchat, J. F., Gascan, H. and Chevalier, S. (2001) The ciliary neurotrophic factor receptor α component induces the secretion of and is required for functional responses to cardiotrophin-like cytokine. EMBO J. **20**, 1692–1703
14 Moore, P. S., Boshoff, C., Weiss, R. A. and Chang, Y. (1996) Molecular mimicry of human cytokine and cytokine response pathway genes by KSHV. Science (Washington, D.C.) **274**, 1739–1744
15 Kaleeba, J. A., Bergquam, E. P. and Wong, S. W. (1999) A rhesus macaque rhadinovirus related to Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8 encodes a functional homologue of interleukin-6. J. Virol. **73**, 6177–6181
16 Hoischen, S. H., Vollmer, P., Marz, P., Ozbek, S., Gotze, K. S., Peschel, C., Jostock, T., Geib, T., Müllberg, J., Mechtersheimer, S. et al. (2000) Human herpes virus 8 interleukin-6 homologue triggers gp130 on neuronal and hematopoietic cells. Eur. J. Biochem. **267**, 3604–3612
17 Bravo, J. and Heath, J. K. (2000) Receptor recognition by gp130 cytokines. EMBO J. **19**, 2399–2411
18 Deller, M. C., Hudson, K. R., Ikemizu, S., Bravo, J., Jones, E. Y. and Heath, J. K. (2000) Crystal structure and functional dissection of the cytostatic cytokine oncostatin M. Structure Fold. Des. **8**, 863–874
19 Owczarek, C. M., Zhang, Y., Layton, M. J., Metcalf, D., Roberts, B. and Nicola, N. A. (1997) The unusual species cross-reactivity of the leukemia inhibitory factor receptor α-chain is determined primarily by the immunoglobulin-like domain. J. Biol. Chem. **272**, 23976–23985
20 Hammacher, A., Richardson, R. T., Layton, J. E., Smith, D. K., Angus, L. J. L., Hilton, D. J., Nicola, N. A., Wijdenes, J. and Simpson, R. J. (1998) The immunoglobulin-like module of gp130 is required for signaling by interleukin-6 but not by leukemia inhibitory factor. J. Biol. Chem. **273**, 22701–22707
21 Kurth, I., Horsten, U., Pflanz, S., Dahmen, H., Küster, A., Grötzinger, J., Heinrich, P. C. and Müller-Newen, G. (1999) Activation of the signal transducer gp130 by both interleukin-6 and interleukin-11 requires two distinct binding epitopes. J. Immunol. **162**, 1480–1487
22 Timmermann, A., Pflanz, S., Grötzinger, J., Küster, A., Kurth, I., Pitard, V., Heinrich, P. C. and Müller-Newen, G. (2000) Different epitopes are required for gp130 activation by interleukin-6, oncostatin M and leukemia inhibitory factor. FEBS Lett. **468**, 120–124
23 Pflanz, S., Kurth, I., Grötzinger, J., Heinrich, P. C. and Müller-Newen, G. (2000) Two different epitopes of the signal transducer gp130 sequentially cooperate on IL-6-induced receptor activation. J. Immunol. **165**, 7042–7049
24 Chow, D., He, X., Snow, A. L., Rose-John, S. and Garcia, K. C. (2001) Structure of an extracellular gp130 cytokine receptor complex. Science (Washington, D.C.) **291**, 2150–2155
25 Varghese, J. N., Moritz, R. L., Lou, M. Z., Van Donkelaar, A., Ji, H., Ivancic, N., Branson, K. M., Hall, N. E. and Simpson, R. J. (2002) Structure of the extracellular domains of the human interleukin-6 receptor α-chain. Proc. Natl. Acad. Sci. U.S.A. **99**, 15959–15964
26 Dittrich, E., Renfrew Haft, C., Muys, L., Heinrich, P. C. and Graeve, L. (1996) A di-leucine motif and an upstream serine in the interleukin-6 (IL-6) signal transducer gp130 mediate ligand-induced endocytosis and down-regulation of the IL-6 receptor. J. Biol. Chem. **271**, 5487–5494
27 Thiel, S., Behrmann, I., Timmermann, A., Dahmen, H., Müller-Newen, G., Schaper, F., Tavernier, J., Pitard, V., Heinrich, P. C. and Graeve, L. (1999) Identification of a Leu–Ile internalization motif within the cytoplasmic domain of the leukaemia inhibitory factor receptor. Biochem. J. **339**, 15–19
28 Thiel, S., Dahmen, H., Martens, A., Müller-Newen, G., Schaper, F., Heinrich, P. C. and Graeve, L. (1998) Constitutive internalization and association with adaptor protein-2 of the interleukin-6 signal transducer gp130. FEBS Lett. **441**, 231–234
29 Gibson, R. M., Schiemann, W. P., Prichard, L. B., Reno, J. M., Ericsson, L. H. and Nathanson, N. M. (2000) Phosphorylation of human gp130 at Ser-782 adjacent to the di-leucine internalization motif. Effects on expression and signaling. J. Biol. Chem. **275**, 22574–22582
30 Wang, Y. and Fuller, G. M. (1994) Phosphorylation and internalization of gp130 occur after IL-6 activation of Jak2 kinase in hepatocytes. Mol. Biol. Cell **5**, 819–828
31 Blanchard, F., Duplomb, L., Wang, Y., Robledo, O., Kinzie, E., Pitard, V., Godard, A., Jacques, Y. and Baumann, H. (2000) Stimulation of leukemia inhibitory factor receptor degradation by extracellular signal-regulated kinase. J. Biol. Chem. **275**, 28793–28801
32 Blanchard, F., Wang, Y., Kinzie, E., Duplomb, L., Godard, A. and Baumann, H. (2001) Oncostatin M regulates the synthesis and turnover of gp130, leukemia inhibitory factor receptor α, and oncostatin M receptor β by distinct mechanisms. J. Biol. Chem. **276**, 47038–47045
33 Strobl, B., Arulampalam, V., Is'harc, H., Newman, S. J., Schlaak, J. F., Watling, D., Costa-Pereira, A. P., Schaper, F., Behrmann, I., Sheehan, K. C. et al. (2001) A completely foreign receptor can mediate an interferon-γ-like response. EMBO J. **20**, 5431–5442
34 Mitani, Y., Takaoka, A., Kim, S. H., Kato, Y., Yokochi, T., Tanaka, N. and Taniguchi, T. (2001) Cross talk of the interferon-α/β signalling complex with gp130 for effective interleukin-6 signalling. Genes Cells **6**, 631–640
35 Qiu, Y., Ravi, L. and Kung, H.-J. (1998) Requirement of ErbB2 for signalling by interleukin-6 in prostate carcinoma cells. Nature (London) **393**, 83–85

36 Kurth, I., Horsten, U., Pflanz, S., Timmermann, A., Küster, A., Dahmen, H., Tacken, I., Heinrich, P. C. and Müller-Newen, G. (2000) Importance of the membrane-proximal extracellular domains for activation of the signal transducer gp130. J. Immunol. **164**, 273–282

37 Hammacher, A., Wijdenes, J., Hilton, D. J., Nicola, N. A., Simpson, R. J. and Layton, J. E. (2000) Ligand-specific utilization of the extracellular membrane-proximal region of the gp130-related signalling receptors. Biochem. J. **345**, 25–32

38 Timmermann, A., Küster, A., Kurth, I., Heinrich, P. C. and Müller-Newen, G. (2002) A functional role of the membrane-proximal extracellular domains of the signal transducer gp130 in heterodimerization with the leukemia inhibitory factor receptor. Eur. J. Biochem. **269**, 2716–2726

39 Autissier, P., De Vos, J., Liautard, J., Tupitsyn, N., Jacquet, C., Chavdia, N., Klein, B., Brochier, J. and Gaillard, J. P. (1998) Dimerization and activation of the common transducing chain (gp130) of the cytokines of the IL-6 family by mAb. Int. Immunol. **10**, 1881–1889

40 Müller-Newen, G., Küster, A., Wijdenes, J., Schaper, F. and Heinrich, P. C. (2000) Studies on the IL-6-type cytokine signal transducer gp130 reveal a novel mechanism of receptor activation by monoclonal antibodies. J. Biol. Chem. **275**, 4579–4586

41 Livnah, O., Stura, E. A., Middleton, S. A., Johnson, D. L., Jolliffe, L. K. and Wilson, I. A. (1999) Crystallographic evidence for preformed dimers of erythropoietin receptor before ligand activation. Science (Washington, D.C.) **283**, 987–990

42 Chan, F. K., Chun, H. J., Zheng, L., Siegel, R. M., Bui, K. L. and Lenardo, M. J. (2000) A domain in TNF receptors that mediates ligand-independent receptor assembly and signaling. Science (Washington, D.C.) **288**, 2351–2354

43 Ballinger, M. D. and Wells, J. A. (1998) Will any dimer do? Nat. Struct. Biol. **5**, 938–940

44 Jiang, G. and Hunter, T. (1999) Receptor signaling: When dimerization is not enough. Curr. Biol. **9**, R568–R571

45 Greiser, J. S., Stross, C., Heinrich, P. C., Behrmann, I. and Hermanns, H. M. (2002) Orientational constraints of the gp130 intracellular juxtamembrane domain for signaling. J. Biol. Chem. **277**, 26959–26965

46 Constantinescu, S. N., Huang, L. J., Nam, H. and Lodish, H. F. (2001) The erythropoietin receptor cytosolic juxtamembrane domain contains an essential, precisely oriented, hydrophobic motif. Mol. Cell **7**, 377–385

47 Lütticken, C., Wegenka, U. M., Yuan, J., Buschmann, J., Schindler, C., Ziemiecki, A., Harpur, A. G., Wilks, A. F., Yasukawa, K., Taga, T. et al. (1994) Association of transcription factor APRF and protein kinase JAK1 with the interleukin-6 signal transducer gp130. Science (Washington, D.C.) **263**, 89–92

48 Stahl, N., Boulton, T. G., Farruggella, T., Ip, N. Y., Davis, S., Witthuhn, B. A., Quelle, F. W., Silvennoinen, O., Barbieri, G., Pellegrini, S. et al. (1994) Association and activation of Jak-Tyk kinases by CNTF–LIF–OSM–IL-6β receptor components. Science (Washington, D.C.) **263**, 92–95

49 Radtke, S., Hermanns, H. M., Haan, C., Schmitz-Van De Leur, H., Gascan, H., Heinrich, P. C. and Behrmann, I. (2002) Novel role of Janus kinase 1 in the regulation of oncostatin M receptor surface expression. J. Biol. Chem. **277**, 11297–11305

50 Hermanns, H. M., Radtke, S., Haan, C., Schmitz-Van de Leur, H., Tavernier, J., Heinrich, P. C. and Behrmann, I. (1999) Contributions of leukemia inhibitory factor receptor and oncostatin M receptor to signal transduction in heterodimeric complexes with glycoprotein 130. J. Immunol. **163**, 6651–6658

51 Guschin, D., Rogers, N., Briscoe, J., Witthuhn, B., Watling, D., Horn, F., Pellegrini, S., Yasukawa, K., Heinrich, P., Stark, G. R. and Kerr, I. M. (1995) A major role for the protein tyrosine kinase JAK1 in the JAK/STAT signal transduction pathway in response to interleukin-6. EMBO J. **14**, 1421–1429

52 Rodig, S. J., Meraz, M. A., White, J. M., Lampe, P. A., Riley, J. K., Arthur, C. D., King, K. L., Sheehan, K. C., Yin, L., Pennica, D., Johnson, E. M., Jr. and Schreiber, R. D. (1998) Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. Cell (Cambridge, Mass.) **93**, 373–383

53 Murakami, M., Narazaki, M., Hibi, M., Yawata, H., Yasukawa, K., Hamaguchi, M., Taga, T. and Kishimoto, T. (1991) Critical cytoplasmic region of the interleukin 6 signal transducer gp130 is conserved in the cytokine receptor family. Proc. Natl. Acad. Sci. U.S.A. **88**, 11349–11353

54 Haan, C., Hermanns, H. M., Heinrich, P. C. and Behrmann, I. (2000) A single amino acid substitution ($Trp^{666} \rightarrow Ala$) in the interbox1/2 region of the interleukin-6 signal transducer gp130 abrogates binding of JAK1, and dominantly impairs signal transduction. Biochem. J. **349**, 261–266

55 Haan, C., Heinrich, P. C. and Behrmann, I. (2002) Structural requirements of the interleukin-6 signal transducer gp130 for its interaction with Janus kinase 1: the receptor is crucial for kinase activation. Biochem. J. **361**, 105–111

56 Lai, C. F., Ripperger, J., Morella, K. K., Wang, Y., Gearing, D. P., Fey, G. H. and Baumann, H. (1995) Separate signaling mechanisms are involved in the control of STAT protein activation and gene regulation via the interleukin 6 response element by the box 3 motif of gp130. J. Biol. Chem. **270**, 14847–14850

57 Tanner, J. W., Chen, W., Young, R. L., Longmore, G. D. and Shaw, A. S. (1995) The conserved box 1 motif of cytokine receptors is required for association with JAK kinases. J. Biol. Chem. **270**, 6523–6530

58 Kishimoto, T., Akira, S., Narazaki, M. and Taga, T. (1995) Interleukin-6 family of cytokines and gp130. Blood **86**, 1243–1254

59 Huang, L. J., Constantinescu, S. N. and Lodish, H. F. (2001) The N-terminal domain of Janus kinase 2 is required for Golgi processing and cell surface expression of erythropoietin receptor. Mol. Cell **8**, 1327–1338

60 Pearson, M. A., Reczek, D., Bretscher, A. and Karplus, P. A. (2000) Structure of the ERM protein moesin reveals the FERM domain fold masked by an extended actin binding tail domain. Cell (Cambridge, Mass.) **101**, 259–270

61 Hamada, K., Shimizu, T., Matsui, T., Tsukita, S. and Hakoshima, T. (2000) Structural basis of the membrane-targeting and unmasking mechanisms of the radixin FERM domain. EMBO J. **19**, 4449–4462

62 Han, B. G., Nunomura, W., Takakuwa, Y., Mohandas, N. and Jap, B. K. (2000) Protein 4.1R core domain structure and insights into regulation of cytoskeletal organization. Nat. Struct. Biol. **7**, 871–875

63 Haan, C., Is'harc, H., Hermanns, H. M., Schmitz-Van De Leur, H., Kerr, I. M., Heinrich, P. C., Grötzinger, J. and Behrmann, I. (2001) Mapping of a region within the N-terminus of Jak1 involved in cytokine receptor interaction. J. Biol. Chem. **276**, 37451–37458

64 Zhou, Y. J., Chen, M., Cusack, N. A., Kimmel, L. H., Magnuson, K. S., Boyd, J. G., Lin, W., Roberts, J. L., Lengi, A., Buckley, R. H. et al. (2001) Unexpected effects of FERM domain mutations on catalytic activity of Jak3: structural implication for Janus kinases. Mol. Cell **8**, 959–969

65 Schaeffer, M., Schneiderbauer, M., Weidler, S., Tavares, R., Warmuth, M., de Vos, G. and Hallek, M. (2001) Signaling through a novel domain of gp130 mediates cell proliferation and activation of Hck and Erk kinases. Mol. Cell. Biol. **21**, 8068–8081

66 De Falco, G., Neri, L. M., Falco, M. D., Bellan, C., Yu, Z., Luca, A. D., Leoncini, L. and Giordano, A. (2002) Cdk9, a member of the cdc2-like family of kinases, binds to gp130, the receptor of the IL-6 family of cytokines. Oncogene **21**, 7464–7470

67 Novotny-Diermayr, V., Zhang, T., Gu, L. and Cao, X. (2002) Protein kinase Cδ associates with the interleukin-6 receptor subunit glycoprotein (gp) 130 via Stat3 and enhances STAT3–gp130 interaction. J. Biol. Chem. **277**, 49134–49142

68 Gauzzi, M. C., Barbieri, G., Richter, M. F., Uze, G., Ling, L., Fellous, M. and Pellegrini, S. (1997) The N-terminal region of Tyk2 sustains the level of interferon α receptor 1, a component of the interferon α/β receptor. Proc. Natl. Acad. Sci. U.S.A. **94**, 11839–11844

69 Ragimbeau, J., Dondi, E., Alcover, A., Eid, P., Uze, G. and Pellegrini, S. (2003) The tyrosine kinase Tyk2 controls IFNAR1 cell surface expression. EMBO J. **22**, 537–547

70 Vinkemeier, U., Moarefi, I., Darnell, Jr, J. E. and Kuriyan, J. (1998) Structure of the N-terminal protein interaction domain of STAT4. Science (Washington, D.C.) **279**, 1048–1052

71 Chen, X., Vinkemeier, U., Zhao, Y., Jeruzalmi, D., Darnell, Jr, J. E. and Kuriyan, J. (1998) Crystal structure of a tyrosine phosphorylated STAT-1 dimer bound to DNA. Cell (Cambridge, Mass.) **93**, 827–839

72 Becker, S., Groner, B. and Müller, C. W. (1998) Three-dimensional structure of the Stat3β homodimer bound to DNA. Nature (London) **394**, 145–151

73 Fu, X. Y. and Zhang, J. J. (1993) Transcription factor p91 interacts with the epidermal growth factor receptor and mediates activation of the c-fos gene promoter. Cell (Cambridge, Mass.) **74**, 1135–1145

74 Greenlund, A. C., Farrar, M. A., Viviano, B. L. and Schreiber, R. D. (1994) Ligand-induced IFN gamma receptor tyrosine phosphorylation couples the receptor to its signal transduction system (p91). EMBO J. **13**, 1591–1600

75 Stancato, L. F., David, M., Carter-Su, C., Larner, A. C. and Pratt, W. B. (1996) Preassociation of STAT1 with STAT2 and STAT3 in separate signalling complexes prior to cytokine stimulation. J. Biol. Chem. **271**, 4134–4137

76 Novak, U., Ji, H., Kanagasundaram, V., Simpson, R. and Paradiso, L. (1998) STAT3 forms stable homodimers in the presence of divalent cations prior to activation. Biochem. Biophys. Res. Commun. **247**, 558–563

77 Ndubuisi, M. I., Guo, G. G., Fried, V. A., Etlinger, J. D. and Sehgal, P. B. (1999) Cellular physiology of STAT3: Where's the cytoplasmic monomer? J. Biol. Chem. **274**, 25499–25509

78 Haan, S., Kortylewski, M., Behrmann, I., Müller-Esterl, W., Heinrich, P. C. and Schaper, F. (2000) Cytoplasmic STAT proteins associate prior to activation. Biochem. J. **345**, 417–421

79 Shah, M., Patel, K., Fried, V. A. and Sehgal, P. B. (2002) Interactions of STAT3 with caveolin-1 and heat shock protein 90 in plasma membrane raft and cytosolic complexes. Preservation of cytokine signaling during fever. J. Biol. Chem. **277**, 45662–45669

80 Fujitani, Y., Hibi, M., Fukada, T., Takahashi-Tezuka, M., Yoshida, H., Yamaguchi, T., Sugiyama, K., Yamanaka, Y., Nakajima, K. and Hirano, T. (1997) An alternative pathway for STAT activation that is mediated by the direct interaction between JAK and STAT. Oncogene **14**, 751–761

81 Stahl, N., Farruggella, T. J., Boulton, T. G., Zhong, Z., Darnell, Jr, J. E. and Yancopoulos, G. D. (1995) Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors. Science (Washington, D.C.) **267**, 1349–1353

82 Heim, M. H., Kerr, I. M., Stark, G. R. and Darnell, Jr, J. E. (1995) Contribution of STAT SH2 groups to specific interferon signaling by the Jak–STAT pathway. Science (Washington, D.C.) **267**, 1347–1349

83 Hemmann, U., Gerhartz, C., Heesel, B., Sasse, J., Kurapkat, G., Grötzinger, J., Wollmer, A., Zhong, Z., Darnell, Jr, J. E., Graeve, L. et al. (1996) Differential activation of acute phase response factor/Stat3 and Stat1 via the cytoplasmic domain of the interleukin 6 signal transducer gp130. II. Src homology SH2 domains define the specificity of Stat factor activation. J. Biol. Chem. **271**, 12999–13007

84 Gerhartz, C., Heesel, B., Sasse, J., Hemmann, U., Landgraf, C., Schneider-Mergener, J., Horn, F., Heinrich, P. C. and Graeve, L. (1996) Differential activation of acute phase response factor/STAT3 and STAT1 via the cytoplasmic domain of the interleukin-6 signal transducer gp130. 1. Definition of a novel phosphotyrosine motif mediating STAT1 activation. J. Biol. Chem. **271**, 12991–12998

85 Kuropatwinski, K. K., De Imus, C., Gearing, D., Baumann, H. and Mosley, B. (1997) Influence of subunit combinations on signaling by receptors for oncostatin M, leukemia inhibitory factor, and interleukin-6. J. Biol. Chem. **272**, 15135–15144

86 Tomida, M., Heike, T. and Yokota, T. (1999) Cytoplasmic domains of the leukemia inhibitory factor receptor required for STAT3 activation, differentiation, and growth arrest of myeloid leukemic cells. Blood **93**, 1934–1941

87 Schmitz, J., Dahmen, H., Grimm, C., Gendo, C., Müller-Newen, G., Heinrich, P. C. and Schaper, F. (2000) The cytoplasmic tyrosine motifs in full-length gp130 have different roles in IL-6 signal transduction. J. Immunol. **164**, 848–854

88 Shuai, K., Stark, G. R., Kerr, I. M. and Darnell, Jr, J. E. (1993) A single phosphotyrosine residue of Stat91 required for gene activation by interferon-γ. Science (Washington, D.C.) **261**, 1744–1746

89 Kaptein, A., Paillard, V. and Saunders, M. (1996) Dominant negative stat3 mutant inhibits interleukin-6-induced Jak–STAT signal transduction. J. Biol. Chem. **271**, 5961–5964

90 Shuai, K., Horvath, C. M., Huang, L. H., Qureshi, S. A., Cowburn, D. and Darnell, Jr, J. E. (1994) Interferon activation of the transcription factor Stat91 involves dimerization through SH2-phosphotyrosyl peptide interactions. Cell (Cambridge, Mass.) **76**, 821–828

91 Haan, S., Hemmann, U., Hassiepen, U., Schaper, F., Schneider-Mergener, J., Wollmer, A., Heinrich, P. C. and Grotzinger, J. (1999) Characterization and binding specificity of the monomeric STAT3-SH2 domain. J. Biol. Chem. **274**, 1342–1348

92 Pfeffer, L. M., Mullersman, J. E., Pfeffer, S. R., Murti, A., Shi, W. and Yang, C. H. (1997) STAT3 as an adapter to couple phosphatidylinositol 3-kinase to the IFNAR1 chain of the type I interferon receptor. Science (Washington, D.C.) **276**, 1418–1420

93 Uddin, S., Sassano, A., Deb, D. K., Verma, A., Majchrzak, B., Rahman, A., Malik, A. B., Fish, E. N. and Platanias, L. C. (2002) Protein kinase C-δ (PKC-δ) is activated by type I interferons and mediates phosphorylation of Stat1 on serine 727. J. Biol. Chem. **277**, 14408–14416

94 Jain, N., Zhang, T., Kee, W. H., Li, W. and Cao, X. (1999) Protein kinase Cδ associates with and phosphorylates Stat3 in an interleukin-6-dependent manner. J. Biol. Chem. **274**, 24392–24400

95 Schuringa, J. J., Dekker, L. V., Vellenga, E. and Kruijer, W. (2001) Sequential activation of Rac-1, SEK-1/MKK-4, and protein kinase Cδ is required for interleukin-6-induced STAT3 Ser-727 phosphorylation and transactivation. J. Biol. Chem. **276**, 27709–27715

96 Schuringa, J. J., Jonk, L. J., Dokter, W. H., Vellenga, E. and Kruijer, W. (2000) Interleukin-6-induced STAT3 transactivation and Ser727 phosphorylation involves Vav, Rac-1 and the kinase SEK-1/MKK-4 as signal transduction components. Biochem. J. **347**, 89–96

97 Lim, C. P. and Cao, X. (2001) Regulation of Stat3 activation by MEK kinase 1. J. Biol. Chem. **276**, 21004–21011

98 Abe, K., Hirai, M., Mizuno, K., Higashi, N., Sekimoto, T., Miki, T., Hirano, T. and Nakajima, K. (2001) The YXXQ motif in gp 130 is crucial for STAT3 phosphorylation at Ser^{727} through an H7-sensitive kinase pathway. Oncogene **20**, 3464–3474

99 Nair, J. S., DaFonseca, C. J., Tjernberg, A., Sun, W., Darnell, Jr, J. E., Chait, B. T. and Zhang, J. J. (2002) Requirement of Ca^{2+} and CaMKII for Stat1 Ser-727 phosphorylation in response to IFN-γ. Proc. Natl. Acad. Sci. U.S.A. **99**, 5971–5976

100 Haq, R., Halupa, A., Beattie, B. K., Mason, J. M., Zanke, B. W. and Barber, D. L. (2002) Regulation of erythropoietin-induced STAT serine phosphorylation by distinct mitogen-activated protein kinases. J. Biol. Chem. **277**, 17359–17366

101 Beuvink, I., Hess, D., Flotow, H., Hofsteenge, J., Groner, B. and Hynes, N. E. (2000) Stat5a serine phosphorylation. Serine 779 is constitutively phosphorylated in the mammary gland, and serine 725 phosphorylation influences prolactin-stimulated *in vitro* DNA binding activity. J. Biol. Chem. **275**, 10247–10255

102 Sanceau, J., Hiscott, J., Delattre, O. and Wietzerbin, J. (2000) IFN-β induces serine phosphorylation of Stat-1 in Ewing's sarcoma cells and mediates apoptosis via induction of IRF-1 and activation of caspase-7. Oncogene **19**, 3372–3383

103 Lütticken, C., Coffer, P., Yuan, J., Schwartz, C., Caldenhoven, E., Schindler, C., Kruijer, W., Heinrich, P. C. and Horn, F. (1995) Interleukin-6-induced serine phosphorylation of transcription factor APRF: evidence for a role in interleukin-6 target gene induction. FEBS Lett. **360**, 137–143

104 Boulton, T. G., Zhong, Z., Wen, Z., Darnell, Jr, J. E., Stahl, N. and Yancopoulos, G. D. (1995) STAT3 activation by cytokines utilizing gp130 and related transducers involves a secondary modification requiring an H7-sensitive kinase. Proc. Natl. Acad. Sci. U.S.A. **92**, 6915–6919

105 Chung, J., Uchida, E., Grammer, T. C. and Blenis, J. (1997) STAT3 serine phosphorylation by ERK-dependent and -independent pathways negatively modulates its tyrosine phosphorylation. Mol. Cell. Biol. **17**, 6508–6516

105a Abramovich, C., Yakobson, B., Chebath, J. and Revel, M. (1997) A protein-arginine methyltransferase binds to the intracytoplasmic domain of the IFNAR1 chain in the type I interferon receptor. EMBO J. **16**, 260–266

105b Pollack, B. P., Kotenko, S. V., He, W., Izotova, L. S., Barnoski, B. L. and Pestka, S. (1999) The human homologue of the yeast proteins Skb1 and Hsl7p interacts with Jak kinases and contains protein methyltransferase activity. J. Biol. Chem. **274**, 31531–31542

106 Mowen, K. A., Tang, J., Zhu, W., Schurter, B. T., Shuai, K., Herschman, H. R. and David, M. (2001) Arginine methylation of STAT1 modulates IFNα/β-induced transcription. Cell (Cambridge, Mass.) **104**, 731–741

107 Sekimoto, T., Imamoto, N., Nakajima, K., Hirano, T. and Yoneda, Y. (1997) Extracellular signal-dependent nuclear import of Stat1 is mediated by nuclear pore-targeting complex formation with NPI-1, but not Rch1. EMBO J. **16**, 7067–7077

108 McBride, K. M., McDonald, C. and Reich, N. C. (2000) Nuclear export signal located within the DNA-binding domain of the STAT1 transcription factor. EMBO J. **19**, 6196–6206

109 Begitt, A., Meyer, T., van Rossum, M. and Vinkemeier, U. (2000) Nucleocytoplasmic translocation of Stat1 is regulated by a leucine-rich export signal in the coiled-coil domain. Proc. Natl. Acad. Sci. U.S.A. **97**, 10418–10423

110 Bromberg, J. F., Wrzeszczynska, M. H., Devgan, G., Zhao, Y., Pestell, R. G., Albanese, C. and Darnell, Jr, J. E. (1999) Stat3 as an oncogene. Cell (Cambridge, Mass.) **98**, 295–303

111 Milocco, L. H., Haslam, J. A., Rosen, J. and Seidel, H. M. (1999) Design of conditionally active STATs: insights into STAT activation and gene regulatory function. Mol. Cell. Biol. **19**, 2913–2920

112 Bild, A. H., Turkson, J. and Jove, R. (2002) Cytoplasmic transport of Stat3 by receptor-mediated endocytosis. EMBO J. **21**, 3255–3263

113 Lillemeier, B. F., Köster, M. and Kerr, I. M. (2001) STAT1 from the cell membrane to the DNA. EMBO J. **20**, 2508–2517

114 Spiekermann, K., Pau, M., Schwab, R., Schmieja, K., Franzrahe, S. and Hiddemann, W. (2002) Constitutive activation of STAT3 and STAT5 is induced by leukemic fusion proteins with protein tyrosine kinase activity and is sufficient for transformation of hematopoietic precursor cells. Exp. Hematol. **30**, 262–271

115 Herrington, J., Rui, L., Luo, G., Yu-Lee, L. Y. and Carter-Su, C. (1999) A functional DNA binding domain is required for growth hormone-induced nuclear accumulation of Stat5B. J. Biol. Chem. **274**, 5138–5145

116 McBride, K. M., Banninger, G., McDonald, C. and Reich, N. C. (2002) Regulated nuclear import of the STAT1 transcription factor by direct binding of importin-α. EMBO J. **21**, 1754–1763

117 Meyer, T., Begitt, A., Lodige, I., van Rossum, M. and Vinkemeier, U. (2002) Constitutive and IFN-γ-induced nuclear import of STAT1 proceed through independent pathways. EMBO J. **21**, 344–354

118 Melen, K., Kinnunen, L. and Julkunen, I. (2001) Arginine/lysine-rich structural element is involved in interferon-induced nuclear import of STATs. J. Biol. Chem. **276**, 16447–16455

119 Fagerlund, R., Melen, K., Kinnunen, L. and Julkunen, I. (2002) Arginine/lysine-rich nuclear localization signals mediate interactions between dimeric STATs and importin α5. J. Biol. Chem. **277**, 30072–30078.

120 Mowen, K. and David, M. (2000) Regulation of STAT1 nuclear export by Jak1. Mol. Cell. Biol. **20**, 7273–7281

121 Haspel, R. L. and Darnell, Jr, J. E. (1999) A nuclear protein tyrosine phosphatase is required for the inactivation of Stat1. Proc. Natl. Acad. Sci. U.S.A. **96**, 10188–10193

122 Meyer, T., Gavenis, K. and Vinkemeier, U. (2002) Cell type-specific and tyrosine phosphorylation-independent nuclear presence of STAT1 and STAT3. Exp. Cell Res. **272**, 45–55

123 Zeng, R., Aoki, Y., Yoshida, M., Arai, K. and Watanabe, S. (2002) Stat5B shuttles between cytoplasm and nucleus in a cytokine-dependent and -independent manner. J. Immunol. **168**, 4567–4575

124 Kumar, A., Commane, M., Flickinger, T. W., Horvath, C. M. and Stark, G. R. (1997) Defective TNF-α-induced apoptosis in STAT1-null cells due to low constitutive levels of caspases. Science (Washington, D.C.) **278**, 1630–1632

125 Chatterjee-Kishore, M., Wright, K. L., Ting, J. P. and Stark, G. R. (2000) How Stat1 mediates constitutive gene expression: a complex of unphosphorylated Stat1 and IRF1 supports transcription of the LMP2 gene. EMBO J. **19**, 4111–4122

126 Andrews, R. P., Ericksen, M. B., Cunningham, C. M., Daines, M. O. and Hershey, G. K. (2002) Analysis of the life cycle of STAT6. Continuous cycling of STAT6 is required for IL-4 signaling. J. Biol. Chem. **277**, 36563–36569

127 Schiemann, W. P., Bartoe, J. L. and Nathanson, N. M. (1997) Box 3-independent signaling mechanisms are involved in leukemia inhibitory factor receptor α- and gp130-mediated stimulation of mitogen-activated protein kinase. Evidence for participation of multiple signaling pathways which converge at Ras. J. Biol. Chem. **272**, 16631–16636

128 Hermanns, H. M., Radtke, S., Schaper, F., Heinrich, P. C. and Behrmann, I. (2000) Non-redundant signal transduction of interleukin-6-type cytokines. The adapter protein Shc is specifically recruited to rhe oncostatin M receptor. J. Biol. Chem. **275**, 40742–40748

129 Holgado-Madruga, M., Emlet, D. R., Moscatello, D. K., Godwin, A. K. and Wong, A. J. (1996) A Grb2-associated docking protein in EGF- and insulin-receptor signalling. Nature (London) **379**, 560–564

130 Gu, H., Pratt, J. C., Burakoff, S. J. and Neel, B. G. (1998) Cloning of p97/Gab2, the major SHP2-binding protein in hematopoietic cells, reveals a novel pathway for cytokine-induced gene activation. Mol Cell **2**, 729–740

131 Schaper, F., Gendo, C., Eck, M., Schmitz, J., Grimm, C., Anhuf, D., Kerr, I. M. and Heinrich, P. C. (1998) Activation of the protein tyrosine phosphatase SHP2 via the interleukin-6 signal transducing receptor protein gp130 requires tyrosine kinase Jak1 and limits acute-phase protein expression. Biochem. J. **335**, 557–565

132 Fukada, T., Hibi, M., Yamanaka, Y., Takahashi-Tezuka, M., Fujitani, Y., Yamaguchi, T., Nakajima, K. and Hirano, T. (1996) Two signals are necessary for cell proliferation induced by a cytokine receptor gp130: involvement of STAT3 in anti-apoptosis. Immunity **5**, 449–460

133 Schaeper, U., Gehring, N. H., Fuchs, K. P., Sachs, M., Kempkes, B. and Birchmeier, W. (2000) Coupling of Gab1 to c-Met, Grb2, and Shp2 mediates biological responses. J. Cell Biol. **149**, 1419–1432

134 Liu, Y. and Rohrschneider, L. R. (2002) The gift of Gab. FEBS Lett. **515**, 1–7

135 Takahashi-Tezuka, M., Yoshida, Y., Fukada, T., Ohtani, T., Yamanaka, Y., Nishida, K., Nakajima, K., Hibi, M. and Hirano, T. (1998) Gab1 acts as an adapter molecule linking the cytokine receptor gp130 to ERK mitogen-activated protein kinase. Mol. Cell. Biol. **18**, 4109–4117

136 Itoh, M., Yoshida, Y., Nishida, K., Narimatsu, M., Hibi, M. and Hirano, T. (2000) Role of Gab1 in heart, placenta, and skin development and growth factor- and cytokine-induced extracellular signal-regulated kinase mitogen-activated protein kinase activation. Mol. Cell. Biol. **20**, 3695–3704

137 Cunnick, J. M., Meng, S., Ren, Y., Desponts, C., Wang, H. G., Djeu, J. Y. and Wu, J. (2002) Regulation of the mitogen-activated protein kinase signaling pathway by SHP2. J. Biol. Chem. **277**, 9498–9504

138 Zhang, S. Q., Tsiaras, W. G., Araki, T., Wen, G., Minichiello, L., Klein, R. and Neel, B. G. (2002) Receptor-specific regulation of phosphatidylinositol 3-kinase activation by the protein tyrosine phosphatase Shp2. Mol. Cell. Biol. **22**, 4062–4072

139 Cacalano, N. A., Sanden, D. and Johnston, J. A. (2001) Tyrosine-phosphorylated SOCS-3 inhibits STAT activation but binds to p120 RasGAP and activates Ras. Nat. Cell Biol. **3**, 460–465

140 Wang, Y., Robledo, O., Kinzie, E., Blanchard, F., Richards, C., Miyajima, A. and Baumann, H. (2000) Receptor subunit-specific action of oncostatin M in hepatic cells and its modulation by leukemia inhibitory factor. J. Biol. Chem. **275**, 25273–25285

141 Bode, J. G., Ludwig, S., Freitas, C. A., Schaper, F., Ruhl, M., Melmed, S., Heinrich, P. C. and Häussinger, D. (2001) The MKK6/p38 mitogen-activated protein kinase pathway is capable of inducing SOCS3 gene expression and inhibits IL-6-induced transcription. Biol. Chem. **382**, 1447–1453

142 Zauberman, A., Zipori, D., Krupsky, M. and Ben-Levy, R. (1999) Stress activated protein kinase p38 is involved in IL-6 induced transcriptional activation of STAT3. Oncogene **18**, 3886–3893

143 Negoro, S., Oh, H., Tone, E., Kunisada, K., Fujio, Y., Walsh, K., Kishimoto, T. and Yamauchi-Takihara, K. (2001) Glycoprotein 130 regulates cardiac myocyte survival in doxorubicin-induced apoptosis through phosphatidylinositol 3-kinase/Akt phosphorylation and Bcl-xL/caspase-3 interaction. Circulation **103**, 555–561

144 Jee, S. H., Chiu, H. C., Tsai, T. F., Tsai, W. L., Liao, Y. H., Chu, C. Y. and Kuo, M. L. (2002) The phosphatidyl inositol 3-kinase/Akt signal pathway is involved in interleukin-6-mediated Mcl-1 upregulation and anti-apoptosis activity in basal cell carcinoma cells. J. Invest. Dermatol. **119**, 1121–1127

145 Shi, Y., Hsu, J. H., Hu, L., Gera, J. and Lichtenstein, A. (2002) Signal pathways involved in activation of p70S6K and phosphorylation of 4E-BP1 following exposure of multiple myeloma tumor cells to interleukin-6. J. Biol. Chem. **277**, 15712–15720

146 Hsu, J. H., Shi, Y., Hu, L., Fisher, M., Franke, T. F. and Lichtenstein, A. (2002) Role of the AKT kinase in expansion of multiple myeloma clones: effects on cytokine-dependent proliferative and survival responses. Oncogene **21**, 1391–1400

147 Hideshima, T., Nakamura, N., Chauhan, D. and Anderson, K. C. (2001) Biologic sequelae of interleukin-6 induced PI3-K/Akt signaling in multiple myeloma. Oncogene **20**, 5991–6000

148 Chen, R. H., Chang, M. C., Su, Y. H., Tsai, Y. T. and Kuo, M. L. (1999) Interleukin-6 inhibits transforming growth factor-β-induced apoptosis through the phosphatidylinositol 3-kinase/Akt and signal transducers and activators of transcription 3 pathways. J. Biol. Chem. **274**, 23013–23019

149 Kortylewski, M., Feld, F., Krüger, K. D., Bahrenberg, G., Roth, R. A., Joost, H. G., Heinrich, P. C., Behrmann, I. and Barthel, A. (2003) Akt modulates STAT3-mediated gene expression through a FKHR (FOXO1a)-dependent mechanism. J. Biol. Chem. **278**, 5242–5249

150 Burfoot, M. S., Rogers, N. C., Watling, D., Smith, J. M., Pons, S., Paonessaw, G., Pellegrini, S., White, M. F. and Kerr, I. M. (1997) Janus kinase-dependent activation of insulin receptor substrate 1 in response to interleukin-4, oncostatin M, and the interferons. J. Biol. Chem. **272**, 24183–24190

151 Boulton, T. G., Stahl, N. and Yancopoulos, G. D. (1994) Ciliary neurotrophic factor/leukemia inhibitory factor/interleukin 6/oncostatin M family of cytokines induces tyrosine phosphorylation of a common set of proteins overlapping those induced by other cytokines and growth factors. J. Biol. Chem. **269**, 11648–11655

152 Hof, P., Pluskey, S., Dhepaganon, S., Eck, M. J. and Shoelson, S. E. (1998) Crystal structure of the tyrosine phosphatase SHP-2. Cell (Cambridge, Mass.) **92**, 441–450

153 Lechleider, R. J., Sugimoto, S., Bennett, A. M., Kashishian, A. S., Cooper, J. A., Shoelson, S. E., Walsh, C. T. and Neel, B. G. (1993) Activation of the SH2-containing phosphotyrosine phosphatase SH-PTP2 by its binding site, phosphotyrosine 1009, on the human plateled-derived growth factor receptor. J. Biol. Chem. **268**, 21478–21481

154 Sugimoto, S., Wandless, T. J., Shoelson, S. E., Neel, B. G. and Walsh, C. T. (1994) Activation of the SH2-containing protein tyrosine phosphatase, SH-PTP2, by phosphotyrosine-containing peptides derived from insulin receptor substrate-1. J. Biol. Chem. **269**, 13614–13622

155 Pluskey, S., Wandless, T. J., Walsh, C. T. and Shoelson, S. E. (1995) Potent stimulation of SH-PTP2 phosphatase activity by simultaneous occupancy of both SH2 domains. J. Biol. Chem. **270**, 2897–2900

156 Lu, W., Gong, D., Bar-Sagi, D. and Cole, P. A. (2001) Site-specific incorporation of a phosphotyrosine mimetic reveals a role for tyrosine phosphorylation of SHP-2 in cell signaling. Mol. Cell **8**, 759–769

157 Symes, A., Stahl, N., Reeves, S. A., Farruggella, T., Servidei, T., Gearan, T., Yancopoulos, G. and Fink, J. S. (1997) The protein tyrosine phosphatase SHP-2 negatively regulates ciliary neurotrophic factor induction of gene expression. Curr. Biol. **7**, 697–700

158 Kim, H. K., Hawley, T. S., Hawley, R. G. and Baumann, H. (1998) Protein tyrosine phosphatase 2 (SHP-2) moderates signaling by gp130 but is not required for the induction of acute-phase plasma protein genes in hepatic cells. Mol. Cell. Biol. **18**, 1525–1533

159 Anhuf, D., Weissenbach, M., Schmitz, J., Sobota, R., Hermanns, H. M., Radtke, S., Linnemann, S., Behrmann, I., Heinrich, P. C. and Schaper, F. (2000) Signal transduction of IL-6, leukemia-inhibitory factor, and oncostatin M: structural receptor requirements for signal attenuation. J. Immunol. **165**, 2535–2543

160 Lehmann, U., Schmitz, J., Weissenbach, M., Sobota, R. M., Hörtner, M., Friederichs, K., Behrmann, I., Tsiaris, W., Sasaki, A., Schneider-Mergener, J. et al. (2003) SHP2 and SOCS3 contribute to Tyr-759-dependent attenuation of interleukin-6 signaling through gp130. J. Biol. Chem. **278**, 661–671

161 Ohtani, T., Ishihara, K., Atsumi, T., Nishida, K., Kaneko, Y., Miyata, T., Itoh, S., Narimatsu, M., Maeda, H., Fukada, T., et al. (2000) Dissection of signaling cascades through gp130 *in vivo*: reciprocal roles for STAT3- and SHP2-mediated signals in immune responses. Immunity **12**, 95–105

162 Yin, T. G., Shen, R., Feng, G. S. and Yang, Y. C. (1997) Molecular characterization of specific interactions between SHP-2 phosphatase and JAK tyrosine kinases. J. Biol. Chem. **272**, 1032–1037

163 Gunaje, J. J. and Bhat, G. J. (2001) Involvement of tyrosine phosphatase PTP1D in the inhibition of interleukin-6-induced Stat3 signaling by α-thrombin. Biochem. Biophys. Res. Commun. **288**, 252–257

164 Wu, T. R., Hong, Y. K., Wang, X. D., Ling, M. Y., Dragoi, A. M., Chung, A. S., Campbell, A. G., Han, Z. Y., Feng, G. S. and Chin, Y. E. (2002) SHP-2 is a dual-specificity phosphatase involved in Stat1 dephosphorylation at both tyrosine and serine residues in nuclei. J. Biol. Chem. **277**, 47572–47580

165 Shuai, K., Liao, J. and Song, M. M. (1996) Enhancement of antiproliferative activity of γ interferon by the specific inhibition of tyrosine dephosphorylation of Stat1. Mol. Cell. Biol. **16**, 4932–4941

166 Sasse, J., Hemmann, U., Schwartz, C., Schniertshauer, U., Heesel, B., Landgraf, C., Schneider-Mergener, J., Heinrich, P. C. and Horn, F. (1997) Mutational analysis of acute-phase response factor Stat3 activation and dimerization. Mol. Cell. Biol. **17**, 4677–4686

167 Strehlow, I. and Schindler, C. (1998) N-terminal signal transducer and activator of transcription (STAT) domains regulate nuclear translocation and STAT deactivation. J. Biol. Chem. **273**, 28049–28056.

168 Tanuma, N., Nakamura, K., Shima, H. and Kikuchi, K. (2000) Protein-tyrosine phosphatase PTPε C inhibits Jak–STAT signaling and differentiation induced by interleukin-6 and leukemia inhibitory factor in M1 leukemia cells. J. Biol. Chem. **275**, 28216–28221

169 Tanuma, N., Shima, H., Nakamura, K. and Kikuchi, K. (2001) Protein tyrosine phosphatase εC selectively inhibits interleukin-6- and interleukin-10-induced JAK–STAT signaling. Blood **98**, 3030–3034

170 Myers, M. P., Andersen, J. N., Cheng, A., Tremblay, M. L., Horvath, C. M., Parisien, J. P., Salmeen, A., Barford, D. and Tonks, N. K. (2001) TYK2 and JAK2 are substrates of protein-tyrosine phosphatase 1B. J. Biol. Chem. **276**, 47771–47774

171 Irie-Sasaki, J., Sasaki, T., Matsumoto, W., Opavsky, A., Cheng, M., Welstead, G., Griffiths, E., Krawczyk, C., Richardson, C. D., Aitken, K. et al. (2001) CD45 is a JAK phosphatase and negatively regulates cytokine receptor signalling. Nature (London) **409**, 349–354

172 Bousquet, C., Susini, C. and Melmed, S. (1999) Inhibitory roles for SHP-1 and SOCS-3 following pituitary proopiomelanocortin induction by leukemia inhibitory factor. J. Clin. Invest. **104**, 1277–1285

173 Haspel, R. L., Salditt-Georgieff, M. and Darnell, Jr, J. E. (1996) The rapid inactivation of nuclear tyrosine phosphorylated Stat1 depends upon a protein tyrosine phosphatase. EMBO J. **15**, 6262–6268

174 ten Hoeve, J., de Jesus Ibarra-Sanchez, M., Fu, Y., Zhu, W., Tremblay, M., David, M. and Shuai, K. (2002) Identification of a nuclear Stat1 protein tyrosine phosphatase. Mol. Cell. Biol. **22**, 5662–5668

175 Yamamoto, T., Sekine, Y., Kashima, K., Kubota, A., Sato, N., Aoki, N. and Matsuda, T. (2002) The nuclear isoform of protein-tyrosine phosphatase TC-PTP regulates interleukin-6-mediated signaling pathway through STAT3 dephosphorylation. Biochem. Biophys. Res. Commun. **297**, 811–817

176 Zhu, W., Mustelin, T. and David, M. (2002) Arginine methylation of STAT1 regulates its dephosphorylation by T cell protein tyrosine phosphatase. J. Biol. Chem. **277**, 35787–35790

177 Valdez, B. C., Henning, D., Perlaky, L., Busch, R. K. and Busch, H. (1997) Cloning and characterization of Gu/RH-II binding protein. Biochem. Biophys. Res. Commun. **234**, 335–340.

178 Chung, C. D., Liao, J., Liu, B., Rao, X., Jay, P., Berta, P. and Shuai, K. (1997) Specific inhibition of Stat3 signal transduction by PIAS3. Science (Washington, D.C.) **278**, 1803–1805

179 Liu, B., Liao, J., Rao, X., Kushner, S. A., Chung, C. D., Chang, D. D. and Shuai, K. (1998) Inhibition of Stat1-mediated gene activation by PIAS1. Proc. Natl. Acad. Sci. U.S.A. **95**, 10626–10631

180 Gross, M., Liu, B., Tan, J., French, F. S., Carey, M. and Shuai, K. (2001) Distinct effects of PIAS proteins on androgen-mediated gene activation in prostate cancer cells. Oncogene **20**, 3880–3887

181 Liu, B., Gross, M., ten Hoeve, J. and Shuai, K. (2001) A transcriptional corepressor of Stat1 with an essential LXXLL signature motif. Proc. Natl. Acad. Sci. U.S.A. **98**, 3203–3207

182 Liu, B. and Shuai, K. (2001) Induction of apoptosis by protein inhibitor of activated Stat1 through c-Jun NH_2-terminal kinase activation. J. Biol. Chem. **276**, 36624–36631

183 Rödel, B., Tavassoli, K., Karsunky, H., Schmidt, T., Bachmann, M., Schaper, F., Heinrich, P., Shuai, K., Elsässer, H. P. and Möröy, T. (2000) The zinc finger protein Gfi-1 can enhance STAT3 signaling by interacting with the STAT3 inhibitor PIAS3. EMBO J. **19**, 5845–5855

184 Tan, J. A., Hall, S. H., Hamil, K. G., Grossman, G., Petrusz, P. and French, F. S. (2002) Protein inhibitors of activated STAT resemble scaffold attachment factors and function as interacting nuclear receptor coregulators. J. Biol. Chem. **277**, 16993–17001

185 Kotaja, N., Karvonen, U., Janne, O. A. and Palvimo, J. J. (2002) PIAS proteins modulate transcription factors by functioning as SUMO-1 ligases. Mol. Cell. Biol. **22**, 5222–5234

186 Sachdev, S., Bruhn, L., Sieber, H., Pichler, A., Melchior, F. and Grosschedl, R. (2001) PIASy, a nuclear matrix-associated SUMO E3 ligase, represses LEF1 activity by sequestration into nuclear bodies. Genes Dev. **15**, 3088–3103

187 Jackson, P. K. (2001) A new RING for SUMO: wrestling transcriptional responses into nuclear bodies with PIAS family E3 SUMO ligases. Genes Dev. **15**, 3053–3058

188 Yoshimura, A., Ohkubo, T., Kiguchi, T., Jenkins, N. A., Gilbert, D. J., Copeland, N. G., Hara, T. and Miyajima, A. (1995) A novel cytokine-inducible gene CIS encodes an SH2-containing protein that binds to tyrosine-phosphorylated interleukin 3 and erythropoietin receptors. EMBO J. **14**, 2816–2826

189 Matsumoto, A., Masuhara, M., Mitsui, K., Yokouchi, M., Ohtsubo, M., Misawa, H., Miyajima, A. and Yoshimura, A. (1997) CIS, a cytokine inducible SH2 protein, is a target of the JAK–STAT5 pathway and modulates STAT5 activation. Blood **89**, 3148–3154

190 Starr, R., Willson, T. A., Viney, E. M., Murray, L. J., Rayner, J. R., Jenkins, B. J., Gonda, T. J., Alexander, W. S., Metcalf, D., Nicola, N. A. and Hilton, D. J. (1997) A family of cytokine-inducible inhibitors of signalling. Nature (London) **387**, 917–921

191 Endo, T. A., Masuhara, M., Yokouchi, M., Suzuki, R., Sakamoto, H., Mitsui, K., Matsumoto, A., Tanimura, S., Ohtsubo, M., Misawa, H. et al. (1997) A new protein containing an SH2 domain that inhibits JAK kinases. Nature (London) **387**, 921–924

192 Naka, T., Narazaki, M., Hirata, M., Matsumoto, T., Minamoto, S., Aono, A., Nishimoto, N., Kajita, T., Taga, T., Yoshizaki, K. et al. (1997) Structure and function of a new STAT-induced STAT inhibitor. Nature (London) **387**, 924–929

193 Magrangeas, F., Boisteau, O., Denis, S., Jacques, Y. and Minvielle, S. (2001) Negative cross-talk between interleukin-3 and interleukin-11 is mediated by suppressor of cytokine signalling-3 (SOCS-3). Biochem. J. **353**, 223–230

194 Verdier, F., Chretien, S., Muller, O., Varlet, P., Yoshimura, A., Gisselbrecht, S., Lacombe, C. and Mayeux, P. (1998) Proteasomes regulate erythropoietin receptor and signal transducer and activator of transcription 5 (STAT5) activation. Possible involvement of the ubiquitinated CIS protein. J. Biol. Chem. **273**, 28185–28190

195 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) The JAK-binding protein JAB inhibits Janus tyrosine kinase activity through binding in the activation loop. EMBO J. **18**, 1309–1320

196 Sasaki, A., Yasukawa, H., Suzuki, A., Kamizono, S., Syoda, T., Kinjyo, I., Sasaki, M., Johnston, J. A. and Yoshimura, A. (1999) Cytokine-inducible SH2 protein-3 (CIS3/SOCS3) inhibits Janus tyrosine kinase by binding through the N-terminal kinase inhibitory region as well as SH2 domain. Genes Cells **4**, 339–351

197 Schmitz, J., Weissenbach, M., Haan, S., Heinrich, P. C. and Schaper, F. (2000) SOCS3 exerts its inhibitory function on interleukin-6 signal transduction through the SHP2 recruitment site of gp130. J. Biol. Chem. **275**, 12848–12856

198 Nicholson, S. E., De Souza, D., Fabri, L. J., Corbin, J., Willson, T. A., Zhang, J. G., Silva, A., Asimakis, M., Farley, A., Nash, A. D. et al. (2000) Suppressor of cytokine signaling-3 preferentially binds to the SHP-2-binding site on the shared cytokine receptor subunit gp130. Proc. Natl. Acad. Sci. U.S.A. **97**, 6493–6498

199 Sasaki, A., Yasukawa, H., Shouda, T., Kitamura, T., Dikic, I. and Yoshimura, A. (2000) CIS3/SOCS-3 suppresses erythropoietin (EPO) signaling by binding the EPO receptor and JAK2. J. Biol. Chem. **275**, 29338–29347

200 Eyckerman, S., Broekaert, D., Verhee, A., Vandekerckhove, J. and Tavernier, J. (2000) Identification of the Y985 and Y1077 motifs as SOCS3 recruitment sites in the murine leptin receptor. FEBS Lett. **486**, 33–37

201 Bjørbæk, C., Lavery, H. J., Bates, S. H., Olson, R. K., Davis, S. M., Flier, J. S. and Myers, Jr, M. G. (2000) SOCS3 mediates feedback inhibition of the leptin receptor via Tyr 985. J. Biol. Chem. **275**, 40649–40657

202 Hörtner, M., Nielsch, U., Mayr, L. M., Johnston, J. A., Heinrich, P. C. and Haan, S. (2002) Suppressor of cytokine signaling-3 is recruited to the activated granulocyte-colony stimulating factor receptor and modulates its signal transduction. J. Immunol. **169**, 1219–1227

203 Hörtner, M., Nielsch, U., Mayr, L. M., Heinrich, P. C. and Haan, S. (2002) A new high affinity binding site for suppressor of cytokine signaling-3 on the erythropoietin receptor. Eur. J. Biochem. **269**, 2516–2526

204 De Souza, D., Fabri, L. J., Nash, A., Hilton, D. J., Nicola, N. A. and Baca, M. (2002) SH2 domains from suppressor of cytokine signaling-3 and protein tyrosine phosphatase SHP-2 have similar binding specificities. Biochemistry **41**, 9229–9236

205 Nicholson, S. E., Willson, T. A., Farley, A., Starr, R., Zhang, J. G., Baca, M., Alexander, W. S., Metcalf, D., Hilton, D. J. and Nicola, N. A. (1999) Mutational analyses of the SOCS proteins suggest a dual domain requirement but distinct mechanisms for inhibition of LIF and IL-6 signal transduction. EMBO J. **18**, 375–385

206 Zhang, J. G., Farley, A., Nicholson, S. E., Willson, T. A., Zugaro, L. M., Simpson, R. J., Moritz, R. L., Cary, D., Richardson, R., Hausmann, G. et al. (1999) The conserved SOCS box motif in suppressors of cytokine signaling binds to elongins B and C and may couple bound proteins to proteasomal degradation. Proc. Natl. Acad. Sci. U.S.A. **96**, 2071–2076

207 Kamura, T., Sato, S., Haque, D., Liu, L., Kaelin, W. G. J., Conaway, R. C. and Conaway, J. W. (1998) The Elongin BC complex interacts with the conserved SOCS-box motif present in members of the SOCS, ras, WD-40 repeat, and ankyrin repeat families. Genes Dev. **12**, 3872–3881

208 Frantsve, J., Schwaller, J., Sternberg, D. W., Kutok, J. and Gilliland, D. G. (2001) Socs-1 inhibits TEL-JAK2-mediated transformation of hematopoietic cells through inhibition of JAK2 kinase activity and induction of proteasome-mediated degradation. Mol. Cell. Biol. **21**, 3547–3557

209 Kamizono, S., Hanada, T., Yasukawa, H., Minoguchi, S., Kato, R., Minoguchi, M., Hattori, K., Hatakeyama, S., Yada, M., Morita, S. et al. (2001) The SOCS box of SOCS-1 accelerates ubiquitin-dependent proteolysis of TEL-JAK2. J. Biol. Chem. **276**, 12530–12538

210 Ungureanu, D., Saharinen, P., Junttila, I., Hilton, D. J. and Silvennoinen, O. (2002) Regulation of Jak2 through the ubiquitin-proteasome pathway involves phosphorylation of Jak2 on Y1007 and interaction with SOCS-1. Mol. Cell. Biol. **22**, 3316–3326

211 Rui, L., Yuan, M., Frantz, D., Shoelson, S. and White, M. F. (2002) SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. J. Biol. Chem. **277**, 42394–42398

212 De Sepulveda, P., Ilangumaran, S. and Rottapel, R. (2000) Suppressor of cytokine signaling-1 inhibits VAV function through protein degradation. J. Biol. Chem. **275**, 14005–14008

213 Kamura, T., Burian, D., Yan, Q., Schmidt, S. L., Lane, W. S., Querido, E., Branton, P. E., Shilatifard, A., Conaway, R. C. and Conaway, J. W. (2001) Muf1, a novel Elongin BC-interacting leucine-rich repeat protein that can assemble with Cul5 and Rbx1 to reconstitute a ubiquitin ligase. J. Biol. Chem. **276**, 29748–29753

214 Chen, X. P., Losman, J. A., Cowan, S., Donahue, E., Fay, S., Vuong, B. Q., Nawijn, M. C., Capece, D., Cohan, V. L. and Rothman, P. (2002) Pim serine/threonine kinases regulate the stability of Socs-1 protein. Proc. Natl. Acad. Sci. U.S.A. **99**, 2175–2180

215 Hanada, T., Yoshida, T., Kinjyo, I., Minoguchi, S., Yasukawa, H., Kato, S., Mimata, H., Nomura, Y., Seki, Y., Kubo, M. and Yoshimura, A. (2001) A mutant form of JAB/SOCS1 augments the cytokine-induced JAK/STAT pathway by accelerating degradation of wild-type JAB/CIS family proteins through the SOCS-box. J. Biol. Chem. **276**, 40746–40754

216 Cohney, S. J., Sanden, D., Cacalano, N. A., Yoshimura, A., Mui, A., Migone, T. S. and Johnston, J. A. (1999) SOCS-3 is tyrosine phosphorylated in response to interleukin-2 and suppresses STAT5 phosphorylation and lymphocyte proliferation. Mol. Cell. Biol. **19**, 4980–4988

217 Winzen, R., Kracht, M., Ritter, B., Wilhelm, A., Chen, C. Y., Shyu, A. B., Muller, M., Gaestel, M., Resch, K. and Holtmann, H. (1999) The p38 MAP kinase pathway signals for cytokine-induced mRNA stabilization via MAP kinase-activated protein kinase 2 and an AU-rich region-targeted mechanism. EMBO J. **18**, 4969–4980

218 Neininger, A., Kontoyiannis, D., Kotlyarov, A., Winzen, R., Eckert, R., Volk, H. D., Holtmann, H., Kollias, G. and Gaestel, M. (2002) MK2 targets AU-rich elements and regulates biosynthesis of tumor necrosis factor and interleukin-6 independently at different post-transcriptional levels. J. Biol. Chem. **277**, 3065–3068

219 Chen, C. Y., Del Gatto-Konczak, F., Wu, Z. and Karin, M. (1998) Stabilization of interleukin-2 mRNA by the c-Jun NH2-terminal kinase pathway. Science (Washington, D.C.) **280**, 1945–1949

220 Ming, X. F., Stoecklin, G., Lu, M., Looser, R. and Moroni, C. (2001) Parallel and independent regulation of interleukin-3 mRNA turnover by phosphatidylinositol 3-kinase and p38 mitogen-activated protein kinase. Mol. Cell. Biol. **21**, 5778–5789

221 Bank, U., Kupper, B., Reinhold, D., Hoffmann, T. and Ansorge, S. (1999) Evidence for a crucial role of neutrophil-derived serine proteases in the inactivation of interleukin-6 at sites of inflammation. FEBS Lett. **461**, 235–240

222 Siewert, E., Müller-Esterl, W., Starr, R., Heinrich, P. C. and Schaper, F. (1999) Different protein turnover of interleukin-6-type cytokine signalling components. Eur. J. Biochem. **265**, 251–257

223 Gerhartz, C., Dittrich, E., Stoyan, T., Rose-John, S., Yasukawa, K., Heinrich, P. C. and Graeve, L. (1994) Biosynthesis and half-life of the interleukin-6 receptor and its signal transducer gp130. Eur. J. Biochem. **223**, 265–274

224 Schaefer, T. S., Sanders, L. K. and Nathans, D. (1995) Cooperative transcriptional activity of Jun and Stat3 β, a short form of Stat3. Proc. Natl. Acad. Sci. U.S.A. **92**, 9097–9101

225 Chakraborty, A., White, S. M., Schaefer, T. S., Ball, E. D., Dyer, K. F. and Tweardy, D. J. (1996) Granulocyte colony-stimulating factor activation of Stat3α and Stat3β in immature normal and leukemic human myeloid cells. Blood **88**, 2442–2449

226 Caldenhoven, E., van Dijk, T. B., Solari, R., Armstrong, J., Raaijmakers, J. A., Lammers, J. W., Koenderman, L. and de Groot, R. P. (1996) STAT3β, a splice variant of transcription factor STAT3, is a dominant negative regulator of transcription. J. Biol. Chem. **271**, 13221–13227

227 Schaefer, T. S., Sanders, L. K., Park, O. K. and Nathans, D. (1997) Functional differences between Stat3α and Stat3β. Mol. Cell. Biol. **17**, 5307–5316

228 Yoo, J. Y., Huso, D. L., Nathans, D. and Desiderio, S. (2002) Specific ablation of Stat3β distorts the pattern of Stat3-responsive gene expression and impairs recovery from endotoxic shock. Cell (Cambridge, Mass.) **108**, 331–344

229 Bode, J. G., Nimmesgern, A., Schmitz, J., Schaper, F., Schmitt, M., Frisch, W., Häussinger, D., Heinrich, P. C. and Graeve, L. (1999) LPS and TNFα induce SOCS3 mRNA and inhibit IL-6-induced activation of STAT3 in macrophages. FEBS Lett. **463**, 365–370

230 Stoiber, D., Kovarik, P., Cohney, S., Johnston, J. A., Steinlein, P. and Decker, T. (1999) Lipopolysaccharide induces in macrophages the synthesis of the suppressor of cytokine signaling 3 and suppresses signal transduction in response to the activating factor IFN-γ. J. Immunol. **163**, 2640–2647

231 Ahmed, S. T. and Ivashkiv, L. B. (2000) Inhibition of IL-6 and IL-10 signaling and Stat activation by inflammatory and stress pathways. J. Immunol. **165**, 5227–5237

232 Niemand, C., Nimmesgern, A., Haan, S., Fischer, P., Schaper, F., Rossaint, R., Heinrich, P. C. and Müller-Newen, G. (2003) Different sensitivities of IL-10- and IL-6-induced STAT3 activation towards SOCS3 induction and other inhibitory mechanisms in primary human macrophages. J. Immunol. **170**, 3263–3272

233 Stoiber, D., Stockinger, S., Steinlein, P., Kovarik, J. and Decker, T. (2001) Listeria monocytogenes modulates macrophage cytokine responses through STAT serine phosphorylation and the induction of suppressor of cytokine signaling 3. J. Immunol. **166**, 466–472

234 Sengupta, T. K., Talbot, E. S., Scherle, P. A. and Ivashkiv, L. B. (1998) Rapid inhibition of interleukin-6 signaling and Stat3 activation mediated by mitogen-activated protein kinases. Proc. Natl. Acad. Sci. U.S.A. **95**, 11107–11112

235 Andus, T., Geiger, T., Hirano, T., Kishimoto, T. and Heinrich, P. C. (1988) Action of recombinant human interleukin 6, interleukin 1 β and tumor necrosis factor α on the mRNA induction of acute-phase proteins. Eur. J. Immunol. **18**, 739–746

236 Bode, J. G., Fischer, R., Häussinger, D., Graeve, L., Heinrich, P. C. and Schaper, F. (2001) The inhibitory effect of IL-1β on IL-6 induced α_2-macroglobulin expression is due to activation of NF-κB. J. Immunol. **167**, 1469–1481

237 Kordula, T. and Travis, J. (1996) The role of Stat and C/EBP transcription factors in the synergistic activation of rat serine protease inhibitor-3 gene by interleukin-6 and dexamethasone. Biochem. J. **313**, 1019–1027

238 Brown, R. T., Ades, I. Z. and Nordan, R. P. (1995) An acute phase response factor/NF-κB site downstream of the junB gene that mediates responsiveness to interleukin-6 in a murine plasmacytoma. J. Biol. Chem. **270**, 31129–31135

239 Horvai, A. E., Xu, L., Korzus, E., Brard, G., Kalafus, D., Mullen, T. M., Rose, D. W., Rosenfeld, M. G. and Glass, C. K. (1997) Nuclear integration of JAK/STAT and Ras/AP-1 signaling by CBP and p300. Proc. Natl. Acad. Sci. U.S.A. **94**, 1074–1079

240 Symes, A., Gearan, T., Eby, J. and Fink, J. S. (1997) Integration of Jak–Stat and AP-1 signaling pathways at the vasoactive intestinal peptide cytokine response element regulates ciliary neurotrophic factor-dependent transcription. J. Biol. Chem. **272**, 9648–9654

241 Zhang, X., Wrzeszczynska, M. H., Horvath, C. M. and Darnell, Jr, J. E. (1999) Interacting regions in Stat3 and c-Jun that participate in cooperative transcriptional activation. Mol. Cell. Biol. **19**, 7138–7146

242 Zhang, Z., Jones, S., Hagood, J. S., Fuentes, N. L. and Fuller, G. M. (1997) STAT3 acts as a co-activator of glucocorticoid receptor signaling. J. Biol. Chem. **272**, 30607–30610

243 Kunz, D., Zimmermann, R., Heisig, M. and Heinrich, P. C. (1989) Identification of the promoter sequences involved in the interleukin-6 dependent expression of the rat α_2-macroglobulin gene. Nucleic Acids Res. **17**, 1121–1138

244 Wegenka, U. M., Buschmann, J., Lütticken, C., Heinrich, P. C. and Horn, F. (1993) Acute-phase response factor, a nuclear factor binding to acute-phase response elements, is rapidly activated by interleukin-6 at the posttranslational level. Mol. Cell. Biol. **13**, 276–288
245 Zhang, X. and Darnell, Jr, J. E. (2001) Functional importance of Stat3 tetramerization in activation of the α_2-macroglobulin gene. J. Biol. Chem. **276**, 33576–33581
246 Zhang, Z. and Fuller, G. M. (1997) The competitive binding of STAT3 and NF-κB on an overlapping DNA binding site. Biochem. Biophys. Res. Commun. **237**, 90–94
247 Zhang, Z. and Fuller, G. M. (2000) Interleukin 1β inhibits interleukin 6-mediated rat γ fibrinogen gene expression. Blood **96**, 3466–3472
248 Hiroi, M. and Ohmori, Y. (2003) The transcriptional coactivator CREB-binding protein cooperates with STAT1 and NF-κ B for synergistic transcriptional activation of the CXC ligand 9/monokine induced by interferon-γ gene. J. Biol. Chem. **278**, 651–660
249 Korzus, E., Torchia, J., Rose, D. W., Xu, L., Kurokawa, R., McInerney, E. M., Mullen, T. M., Glass, C. K. and Rosenfeld, M. G. (1998) Transcription factor-specific requirements for coactivators and their acetyltransferase functions. Science (Washington, D.C.) **279**, 703–707
250 Zhang, J. J., Vinkemeier, U., Gu, W., Chakravarti, D., Horvath, C. M. and Darnell, Jr, J. E. (1996) Two contact regions between Stat1 and CBP/p300 in interferon γ signaling. Proc. Natl. Acad. Sci. U.S.A. **93**, 15092–15096
251 Paulson, M., Pisharody, S., Pan, L., Guadagno, S., Mui, A. L. and Levy, D. E. (1999) Stat protein transactivation domains recruit p300/CBP through widely divergent sequences. J. Biol. Chem. **274**, 25343–25349
252 Pfitzner, E., Jahne, R., Wissler, M., Stoecklin, E. and Groner, B. (1998) p300/CREB-binding protein enhances the prolactin-mediated transcriptional induction through direct interaction with the transactivation domain of Stat5, but does not participate in the Stat5- mediated suppression of the glucocorticoid response. Mol. Endocrinol. **12**, 1582–1593
253 Zhu, M., John, S., Berg, M. and Leonard, W. J. (1999) Functional association of Nmi with Stat5 and Stat1 in IL-2- and IFNγ-mediated signaling. Cell (Cambridge, Mass.) **96**, 121–130
254 Luo, G. and Yu-Lee, L. (2000) Stat5b inhibits NFκB-mediated signaling. Mol. Endocrinol. **14**, 114–123
255 Nakajima, H., Brindle, P. K., Handa, M. and Ihle, J. N. (2001) Functional interaction of STAT5 and nuclear receptor co-repressor SMRT: implications in negative regulation of STAT5-dependent transcription. EMBO J. **20**, 6836–6844
256 Campos, S. P. and Baumann, H. (1992) Insulin is a prominent modulator of the cytokine-stimulated expression of acute-phase plasma protein genes. Mol. Cell. Biol. **12**, 1789–1797.
257 Wang, Y., Ripperger, J., Fey, G. H., Samols, D., Kordula, T., Wetzler, M., Van Etten, R. A. and Baumann, H. (1999) Modulation of hepatic acute phase gene expression by epidermal growth factor and Src protein tyrosine kinases in murine and human hepatic cells. Hepatology **30**, 682–697
258 Nakayama, K., Kim, K. W. and Miyajima, A. (2002) A novel nuclear zinc finger protein EZI enhances nuclear retention and transactivation of STAT3. EMBO J. **21**, 6174–6184

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