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REVIEW ARTICLE

Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway[1]

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The family of cytokines signalling through the common receptor subunit gp130 comprises interleukin (IL)-6, IL-11, leukaemia inhibitory factor, oncostatin M, ciliary neurotrophic factor and cardiotrophin-1. These so-called IL-6-type cytokines play an important role in the regulation of complex cellular processes such as gene activation, proliferation and differentiation. The current knowledge on the signal-transduction mechanisms of these cytokines from the plasma membrane to the nucleus is reviewed. In particular, we focus on the assembly of receptor complexes after ligand binding, the activation of receptor-associated kinases of the Janus family, and the recruitment and phosphorylation of transcription factors of the STAT family, which dimerize, translocate to the nucleus, and bind to enhancer elements of respective target genes leading to transcriptional activation. The important players in the signalling pathway, namely the cytokines and the receptor components, the Janus kinases Jak1, Jak2 and Tyk2, the signal transducers and activators of transcription STAT1 and STAT3 and the tyrosine phosphatase SHP2 [SH2 (Src homology 2) domain-containing tyrosine phosphatase] are introduced and their structural/functional properties are discussed. Furthermore, we review various mechanisms involved in the termination of the IL-6-type cytokine signalling, namely the action of tyrosine phosphatases, proteasome, Jak kinase inhibitors SOCS (suppressor of cytokine signalling), protein inhibitors of activated STATs (PIAS), and internalization of the cytokine receptors via gp130. Although all IL-6-type cytokines signal through the gp130/Jak/STAT pathway, the comparison of their physiological properties shows that they elicit not only similar, but also distinct, biological responses. This is reflected in the different phenotypes of IL-6-type-cytokine knock-out animals.

CYTOKINES

Cytokines play an important role in the communication between cells of multicellular organisms. As intercellular mediators acting in nanomolar-to-picomolar concentrations they regulate survival, growth, differentiation and effector functions of cells. They are key players in the regulation of the immune response, particularly during infections, inflammatory joint, kidney, vessel and bowel diseases, or neurological and endocrinological autoimmune diseases. Unlike hormones, cytokines are not stored in glands as preformed molecules, but are rapidly synthesized and secreted by different cells mostly after stimulation. Most cytokines are difficult to detect in serum because their producer cells are often adjacent to the target cells and usually only small amounts of cytokines are released. Cytokines act on many different target cells (pleiotropism) and frequently affect the action of other cytokines in an additive, synergistic or antagonistic manner. Besides their pleiotropic effects, cytokine actions are often redundant, i.e. similar biological responses can be achieved by several different cytokines. They exert their actions, which can be auto-, para- or endo-crine, via specific cell-surface receptors on their target cells. Cytokines are polypeptides of low molecular mass; their structures are often stabilized by N- and/or O-glycosylation and by intramolecular disulphide bridges.

Cytokines have been classified (1) on the basis of their biological responses into pro- or anti-inflammatory cytokines, (2) according to the receptors used or (3) according to their three-dimensional structures [1].

Despite the lack of amino-acid-sequence similarities, Bazan [2] first proposed a family of cytokines characterized by a four-α-helix bundle structure. These were later subclassified into short-chain and long-chain α-helix-bundle cytokines [1]. The latter subfamily comprises interleukin (IL)-6, IL-11, leukaemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin (CT)-1, erythropoietin (Epo), granulocyte colony-stimulating factor (G-CSF), IL-12, growth hormone, prolactin, IL-10, interferon (IFN) α/β, and leptin. Among these cytokines IL-6, IL-11, LIF, OSM, CNTF and CT-1 use the common receptor subunit gp130 for signal transduction and as a consequence elicit similar and overlapping physiological responses. Therefore we refer to this group of mediators as 'IL-6-type cytokines'. The major steps in IL-6-type cytokine signalling have recently been elucidated. It was found that these cytokines signal via the activation of Janus kinases (Jaks) and transcription factors of the STAT family.

In the present review we discuss the structural properties of IL-6-type cytokines and their receptors, their physiological responses and the molecular mechanisms involved in their signal transduction via the Jak/STAT pathway.

Abbreviations used: AP-1, activator protein 1; APP, acute-phase protein; C/EBP, CCAAT enhancer binding protein; CBM, cytokine-binding module; CREB, cAMP response element binding factor; CBP, CREB-binding protein; CNTF, ciliary neurotrophic factor; CT, cardiotrophin; EGF, epidermal growth factor; Epo, erythropoietin; FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; gp, glycoprotein; GR, glucocorticoid receptor; Grb, growth-factor-receptor-bound protein; hsp, heat-shock protein; IFN, interferon; IL, interleukin; IRF, interferon regulatory factor; IRS, insulin receptor substrate; Jak, Janus kinase; JAB, Jak-binding protein; JH, Jak homology; LIF, leukaemia inhibitory factor; MAPK, mitogen-activated protein kinase; NF, nuclear factor; NLS, nuclear localization signal; OSM, oncostatin M; PDGF, platelet-derived growth factor; PIAS, protein inhibitor of activated STAT; R, receptor; s, soluble; SP-1, stimulating protein 1; SH2, Src homology 2; SH3, Src homology 3; SHP, SH2-domain-containing tyrosine phosphatase; SOCS, suppressor of cytokine signalling; STAT, signal transducer and activator of transcription; SSI, STAT signalling inhibitor; TIMP, tissue inhibitor of metalloproteinases; Trk, tyrosine receptor kinase.

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IL-6-TYPE CYTOKINES AND THEIR RECEPTORS

IL-6-type cytokines

All IL-6-type cytokines are polypeptides with molecular masses of about 20 kDa (Table 1). With the exception of CNTF and CT-1, IL-6-type cytokines are classical secretory proteins synthesized with N-terminal signal peptides; IL-6, LIF and OSM are also N-glycosylated. CNTF and CT-1 are released upon injury of their producer cells. It remains to be elucidated whether there is an alternative release mechanism under physiological conditions.

While no structural data are available for any of the IL-6-type cytokine receptors, the tertiary structures of LIF [3], CNTF [4] and IL-6 [5,6] have been solved by X-ray crystallography or NMR spectroscopy (Figure 1). All structures confirm the predicted four-α-helix-bundle topology of these cytokines. Helix A is connected by a long loop with helix B in such a way that helix B lies parallel with helix A. Helix B is separated from helix C by a very short loop, allowing only an antiparallel packaging. Helix C is again joined by a long loop with helix D, resulting in parallel packaging of the C-terminal helices. As a consequence the

Table 1 Biochemical properties of human IL-6-type cytokines

Owing to space limitations we have omitted references for the information given here. However, a complete set of references is available from P. C. H. on request.

Property	IL-6	IL-11	LIF	CNTF	CT-1	OSM
Number of amino acids						
Precursor	212	199	202	200*	201*	252
Mature protein	184	178	180	200	201	196†
Molecular mass (kDa)						
Predicted	20.8	19.1	20	22.9	21.2	22.1
Observed	21–28	23	45	21–28	21.5	28
Glycosylation						
Potential N-glycosylation sites	2	0	6	0	0	2
N-glycosylation demonstrated	Yes, 1 of 2	0	Yes	0	0	Yes
Number of cysteine residues	4	0	6	1	2	5
Number of S–S bridges	2	0	3	0	?	2
mRNA size (kb)	1.3	1.5, 2.5	1.8, 4‡	1.0	1.7	2
Number of exons	5	5	3	2	3	3
Chromosomal localization	7p21–p14	19q13.3–13.4	22q14	11q12	16p11.1–11.2	22q12

* No signal sequence.
† Processed N- and C-terminally.
‡ Major form.

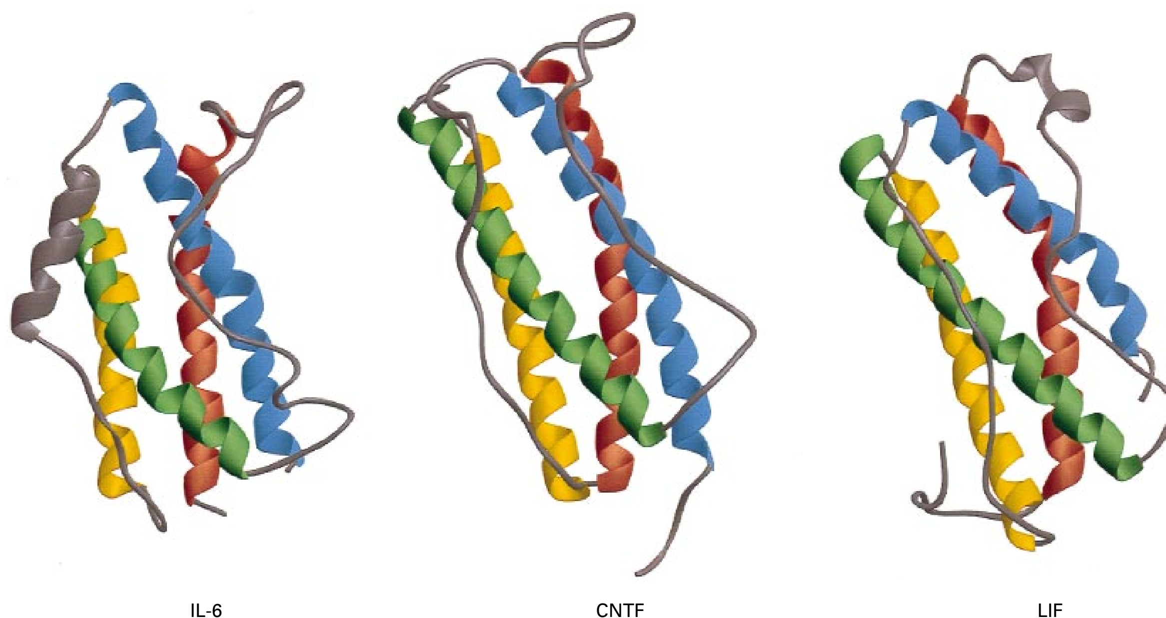


Figure 1 Structures of IL-6, CNTF and LIF (ribbon representation)

The four long α-helices, A (red), B (green), C (yellow) and D (blue), and the connecting loops (grey), as far as they have been defined, are shown. The Brookhaven Databank accession numbers are 1IL6, 1CNT and 1LKI for IL-6, CNTF and LIF respectively.

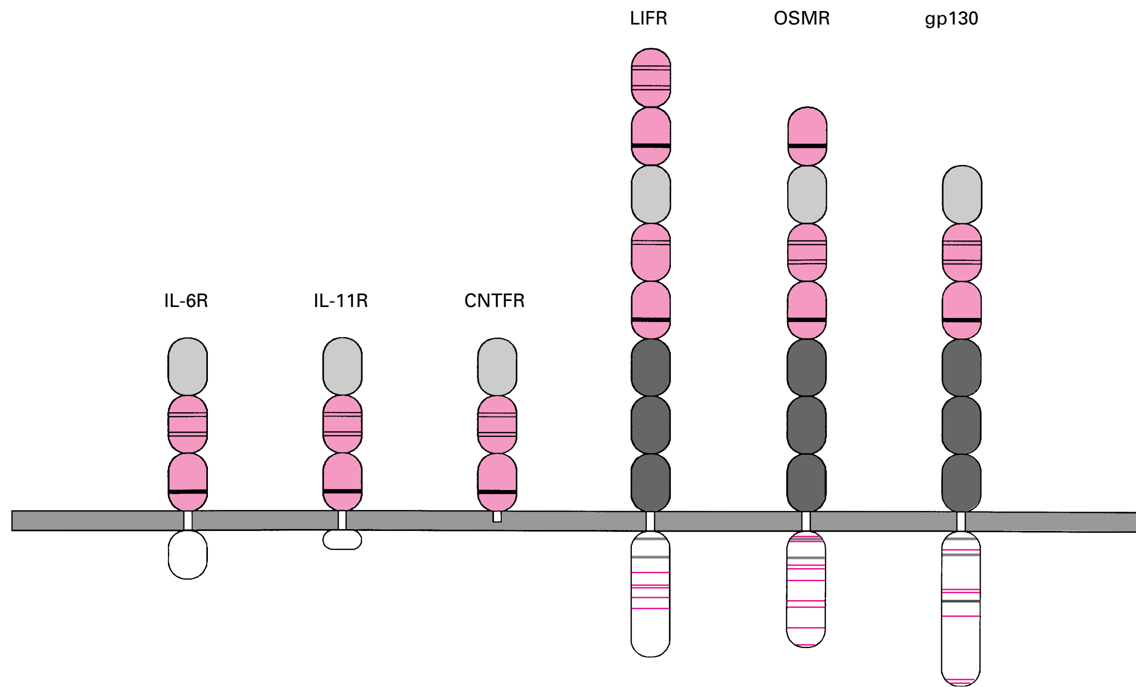


Figure 2 Domain composition of receptors involved in IL-6-type-cytokine signalling

Predicted IgG-like domains are shown light grey, fibronectin-type-III-like domains dark grey and CBMs dark pink. The horizontal bars in the CBMs define the conserved cysteine residues (thin black lines) or the WSXWS motif (broad black bars). The lengths of the cytoplasmic parts (white) correspond to the respective numbers of amino acids. Tyrosine residues in the cytoplasmic domains are represented as red lines, box 1 and 2 as grey bars and the dileucine motif in gp130 as a dark-grey bar.

Table 2 Biochemical properties of human IL-6-type cytokine receptors

Owing to space limitations we have omitted references for the information given here. However, a complete set of references is available from P. C. H. on request. Abbreviation: n.d., not determined.

Property	IL-6R	IL-11R	CNTFR	LIFR	OSMR	gp130
Number of amino acids						
Precursor	468	422	372	1097	979	918
Mature protein	449	400 (368*)	352‡	1053	952	896
Extracellular domain	339	343	352	789	712	597
Transmembrane domain	28	26		26	22	22
Intracellular domain	82	31		238	218	277
Molecular mass (kDa)						
Predicted	49.9	43.1	38.9	111	107	101
Observed	80	n.d.	72	190	180	130–150
Glycosylation						
Potential N-glycosylation sites	5	2	4	19	15	10
N-glycosylation demonstrated	Yes	n.d.	n.d.	Yes	n.d.	Yes
mRNA size (kb)	5	1.9	2	4.5, 5§, 6	> 5	7
Number of exons	n.d.	12	n.d.	20	n.d.	n.d.
Chromosomal localization	n.d.	9p13	9p13	5p12–13	n.d.	5, 17
Soluble forms	Yes*†	n.d.	Yes	Yes*	n.d.	Yes*

* Generated by alternative splicing.
† Generated by shedding.
‡ Glycosylphosphatidylinositol anchor.
§ Minor form.

overall fold shows an 'up–up–down–down' topology of the four long helices. The four-α-helix-bundle cytokines are one of the rare examples of proteins where pulling at the N- and C-termini would result in the formation of a knot.

IL-6-type cytokine receptors

The receptors involved in IL-6-type cytokine signalling identified so far are type I membrane proteins (extracellular N-terminus,

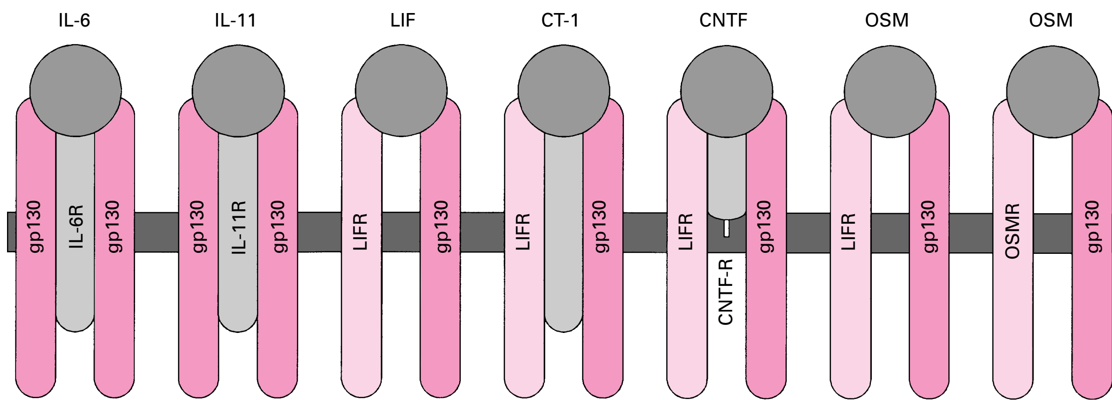


Figure 3 IL-6-type cytokine receptor complexes

The cytokines are schematically depicted as grey circles. Signal-transducing-receptor subunits are dark pink (gp130) or light pink (LIFR and OSMR). α-Receptor subunits are shown in light grey.

one transmembrane domain), with the exception of the CNTFR (CNTF receptor), which is linked to the plasma membrane by a glycosylphosphatidylinositol anchor [7]. They all belong to the cytokine receptor class I family. This receptor family is defined by the presence of at least one cytokine-binding module (CBM) consisting of two fibronectin-type-III-like domains of which the N-terminal domain contains a set of four conserved cysteine residues and the C-terminal domain a WSXWS motif [2]. Figure 2 shows the domain composition of the receptors involved in IL-6-type cytokine signalling deduced from sequence comparison and secondary structure predictions, and Table 2 lists their biochemical properties. All receptors contain an IgG-like domain located either at the N-terminus or, in the case of the LIFR and OSMR, between the two CBMs (the N-terminus of which is incomplete in the OSMR). The signal-transducing receptor chains have three additional membrane-proximal fibronectin-type-III-like domains. Compared with the α-receptors they are furthermore characterized by a considerably longer cytoplasmic part, reflecting their functional involvement in signal transduction.

IL-6-type cytokine receptor complexes

The first event in activation of the Jak/STAT signalling pathway is the ligand-induced homo- or hetero-dimerization of signal-transducing receptor subunits. All IL-6-type cytokines recruit gp130 to their receptor complexes. They either signal via gp130 alone [8] or in combination with LIFR [9] or the recently cloned OSMR [10], which are all able to activate Jaks and to recruit STAT proteins. IL-6 induces gp130-homodimerization [11], whereas CNTF [12], LIF [13], and CT-1 [14] signal via hetero-dimerization of gp130 and LIFR (Figure 3). OSM was first described to signal via gp130–LIFR heterodimers [13], but at least in mice the physiological signalling receptor consists of gp130 and OSMR [15]. For IL-11 it is not clear whether, similarly to IL-6, it induces gp130 homodimer formation [16] or, alternatively, recruits an additional, as-yet-unidentified, receptor subunit [17].

IL-6, IL-11 and CNTF first bind to specific α-receptor subunits (IL-6R [18], IL-11R [19], CNTFR [7]) which are not involved in the intracellular signal-transduction cascade (Figure 3). After ligand binding the complexes of cytokine and α-receptor are able to efficiently recruit the corresponding signal-transducing receptor components. The membrane-bound α-receptors can be functionally replaced by their soluble forms, which lack the transmembrane and cytoplasmic parts [20–22]. The responsiveness of a given cell type to these cytokines is therefore mainly determined by expression of the respective α-receptor subunits or the presence of the corresponding soluble receptors. LIF and OSM directly bind to the signal-transducing receptor components without the requirement for an additional α-receptor subunit. For CT-1 an α-receptor subunit has been proposed [23], but thus far not verified by molecular cloning of the corresponding cDNA.

Soluble IL-6R and gp130

Soluble cytokine receptors are found in body fluids of different mammalian species (for reviews, see [24–26]). For example, soluble (s) IL-6R is found in human plasma at a concentration of 30–70 ng/ml [27,28]. In several conditions, such as HIV infection [27], multiple myeloma [29] and juvenile chronic arthritis [30], elevated levels of soluble IL-6R have been observed. Although *in vitro* experiments have shown that the soluble IL-6R can be released by shedding (i.e. limited proteolysis of the membrane-bound form [31]) the physiological mode of generation seems to be secretion of the soluble receptor after translation of an alternatively spliced mRNA [28,32]. It is not clear whether, under physiological conditions, the soluble IL-6R acts as an agonist, since relatively high concentrations of soluble gp130 (300 ng/ml) have also been found in human blood [33]. Soluble gp130 is probably translated from an alternatively spliced mRNA [34] and can neutralize IL-6–sIL-6R complexes, thereby acting as an antagonist [33]. The plasma pool of sIL-6R and sgp130 may act in combination as a kind of buffer to modulate systemic effects of IL-6 (G. Müller-Newen and P. C. Heinrich, unpublished work).

IL-6-type-cytokine–receptor interactions

The cytokine–α-receptor interaction has been studied in detail for the binding of IL-6 to IL-6R. Since the IgG-like domain of the IL-6R is dispensable for biological activity [35], the residues crucial for ligand binding are located in the CBM. Extensive mutagenesis studies revealed that mainly residues in the loops near the hinge region between the two domains of the CBM are involved in recognition of the ligand [35–38]. Thus the binding

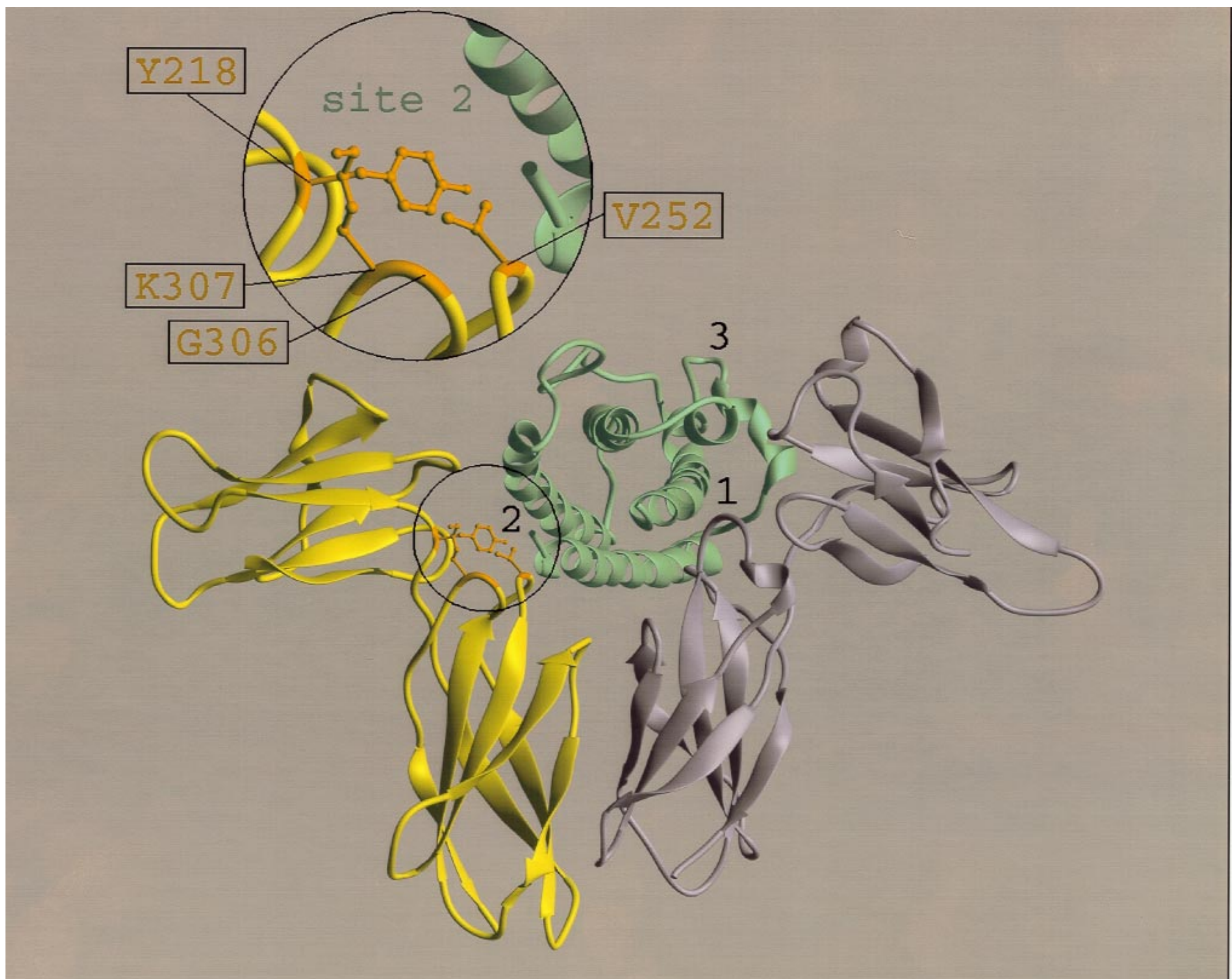


Figure 4 Model of the IL-6–IL-6R–gp130 ternary complex

IL-6 is coloured green and the CBMs of IL-6R and gp130 are violet and yellow respectively. The numbers 1, 2 and 3 designate the different interaction sites of IL-6 with IL-6R (site 1) and gp130 (sites 2 and 3). Amino acid side chains analysed by mutagenesis are depicted in the inset and labelled in orange: Y218, tyrosine-218; K307, lysine-307; G306, glycine-306; V252, valine-252; numbering of the residues is according to that given in [8]. Reproduced with permission from [42].

mode is similar to the one observed in the structure of the related growth hormone–growth hormone receptor complex [39].

In a ternary-complex-formation assay using deletion mutants of soluble gp130 it has been shown that the three membrane-proximal fibronectin-type-III-like domains are dispensible for IL-6–IL-6R [40] or IL-11–IL-11R [41] binding and therefore ligand binding is confined to the CBM and IgG-like domains. Recently, valine-252 in the BC-loop of the C-terminal domain of the CBM has been shown to be important for the interaction of gp130 with IL-6–IL-6R (Figure 4) [42] as well as IL-11–IL-11R complexes [41]. This residue is located at a corresponding position to that of a tryptophan residue in the human-growth-hormone receptor that is crucial for ligand binding [43]. This suggests a conserved mode of ligand binding in the cytokine receptor family. A functional role for the IgG-like domain in ligand binding has been demonstrated for the LIFR [44] and may be expected to be similar in gp130 and OSMR.

Mutagenesis studies on IL-6, CNTF and LIF (reviewed in [38,45]) revealed that the IL-6-type cytokines have three distinct receptor-binding sites referred to as sites 1, 2 and 3. Site 1 is formed by the C-terminal residues of helix D and the C-terminal part of the AB-loop and determines the specificity of α-receptor binding. Site 2 consists of residues located near the middle of helices A and C and seems to be the universal gp130-binding site of all IL-6-type cytokines. Depending on the cytokine, site 3 is most probably used for recruitment of LIFR, OSMR or a second gp130 molecule. Here, mutagenesis data suggest that amino acid residues at the end of the CD-loop, the N-terminus of the D-helix and the N-terminal part of the AB-loop are involved in receptor binding. Possibly, LIFR and OSMR use their two CBMs (the first of which is incomplete in the OSMR) to simultaneously occupy site 1 and site 3 located on the same face of the respective cytokines.

Ternary-complex-formation assays using IL-6 [46], mutants thereof [47] and the soluble forms of the receptor components revealed that the soluble receptor complex consists of two

molecules of IL-6, IL-6R and gp130. Alternatively, a tetrameric complex consisting of one molecule each of IL-6 and IL-6R and two molecules of gp130 has been proposed [38]. A hexameric complex was also described for CNTF and consists of two molecules each of CNTF and CNTFR and one molecule each of gp130 and LIFR [48]. Whether these findings mirror the physiological situation of membrane-bound receptor molecules on the cell surface has to await experimental confirmation.

SIGNALLING OF IL-6-TYPE CYTOKINES

In 1994 it was discovered that IL-6-type cytokines utilize tyrosine kinases of the Jak family and transcription factors of the STAT family as major mediators of signal transduction (shown in Scheme 1), a feature they share with the IFNs and many other cytokines and growth factors [49–51]. gp130-associated kinases Jak1, Jak2, and Tyk2 become activated upon stimulation, and the cytoplasmic tail of gp130 is phosphorylated. Several phosphotyrosine residues of gp130 are docking sites for STAT factors with matching SH2 domains, mainly STAT3 and STAT1 [52,53]. Subsequently, STATs also become phosphorylated, form dimers and translocate to the nucleus, where they regulate transcription of target genes. The tyrosine phosphatase SHP2 binds to phosphorylated gp130 as well [52], thereby possibly forming a link to the mitogen-activated protein kinase (MAPK) pathway, which is also activated upon IL-6-type cytokine stimulation.

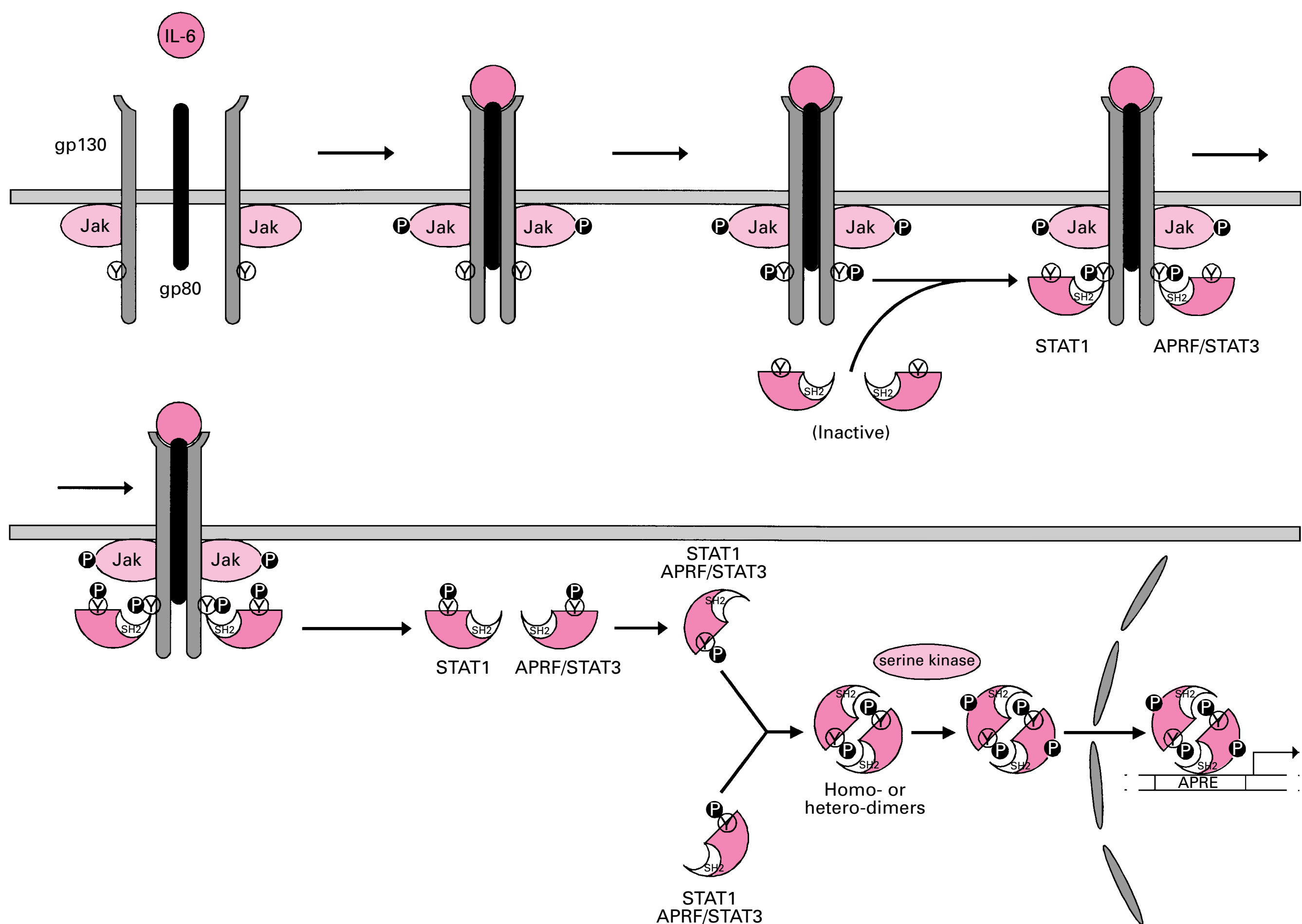
In the following sections we introduce in greater detail the key players of the scenario depicted in Scheme 1 and discuss their relevance to signal transduction of IL-6-type cytokines.

JANUS KINASES

Structure–function relationship

Jaks are intracellular tyrosine kinases with molecular masses of 120–140 kDa. Four members are known in mammalian cells: Jak1, Jak2 and Tyk2, which are widely expressed, and Jak3, which is mainly found in cells of haematopoietic origin. The structural organization of Jaks is shown in Figure 5. A typical kinase domain, also called the Jak homology (JH)-1 domain, is located at the C-terminus. It is preceded by a kinase-like domain (JH2). The N-terminal half of the Jaks contains five additional regions with high sequence similarity between the different Jaks (JH3–JH7) (see [54–56] for recent reviews).

Within the *kinase domain*, Jaks show considerable similarity to other kinases with respect to an activation loop implicated in



Scheme 1 IL-6 signalling via the gp130/Jak/STAT pathway

IL-6 leads to dimerization of gp130 molecules. Associated Jaks become activated and phosphorylate the cytoplasmic part of gp130, thereby creating docking sites for STAT factors STAT1 and STAT3. STATs also become phosphorylated, form homo- or hetero-dimers and translocate into the nucleus, where they regulate gene transcription. In addition, STAT factors are also substrates of (a) serine kinase(s). Further abbreviations used: APRF, acute-phase response factor; APRE, acute-phase response element; encircled Y, tyrosine; white P in black circle, phosphate.

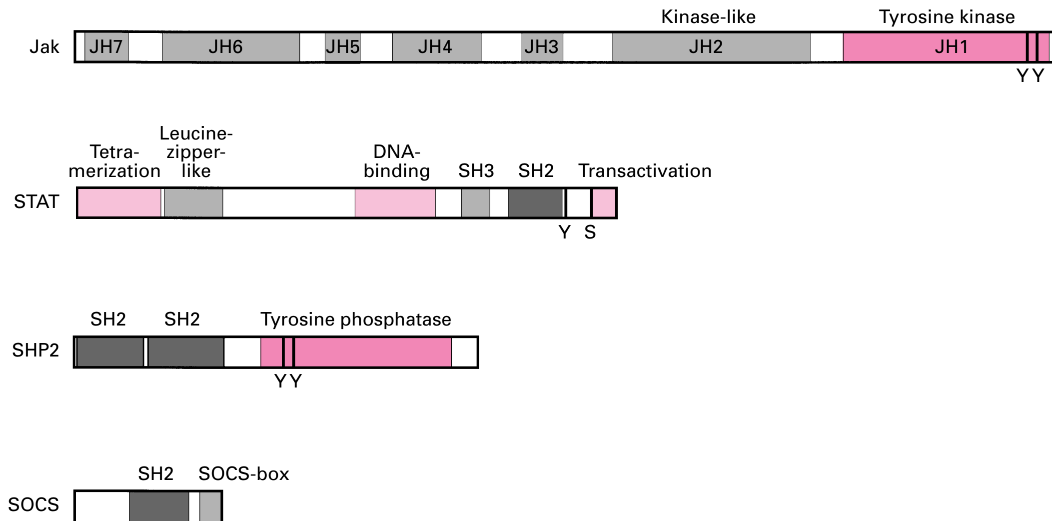


Figure 5 Structural organization of Jaks, STAT factors, tyrosine phosphatase SHP2 and SOCS proteins

The important tyrosine and serine residues which are phosphorylated are shown as black bars.

regulation of kinase activity (see [57] for a review). Indeed, substitution of two adjacent tyrosine residues in the predicted activation loop of Tyk2 (tyrosine-1054 and tyrosine-1055) by phenylalanine leads to an inactive kinase [58]. Two tyrosine residues at the corresponding positions of Jak2 (tyrosine-1007 and tyrosine-1008) were found to be phosphorylated, and a single mutation of tyrosine-1007 eliminated essentially all tyrosine kinase activity [59].

Ligand-induced receptor dimerization is thought to bring the associated Jaks into close proximity, leading to their activation via inter- or intra-molecular phosphorylation at sites necessary for catalytic activity [55,56,60]. Also, dimerization of different receptor ectodomains covalently fused to Jak2 or the Jak2 JH1 domain induced kinase activity [61,62]. Moreover, when Jaks are highly overexpressed in insect or mammalian cells, they become tyrosine-phosphorylated and display kinase activity even in the absence of an exogenous stimulus ([63–65]; H. M. Hermanns, C. Haan and I. Behrmann, unpublished work). This indicates that close contact between these enzymes may be sufficient to trigger their activation. However, the recent description of the influence of the redox state on kinase activity points to a further level of regulation imposed on the Jaks [56].

The significance of the *kinase-like domain* is not clear. It has limited sequence similarity to the canonical kinase domain, but lacks conserved sequences necessary for catalytic activity. Accordingly, Jaks inactivated by point mutations in the kinase domain or by a deletion thereof have no enzymic activity, in spite of the presence of an intact kinase-like domain [58,59,66]. However, the kinase-like domain can have an influence on the kinase activity, although no clear picture emerges from the literature as to whether this is a positive or a negative one. For example, deletion of the Tyk2 kinase-like domain leads to a protein no longer able to phosphorylate an exogenous substrate [67]. Deletion of a large portion of the kinase-like domain of Jak2, however, did not result in a catalytically inactive protein [68]. A negative regulatory function of this domain has been suggested by the observation that a mutation in the kinase-like domain of the *Drosophila* Jak homologue *hopscotch* leads to a hyperactive enzyme. Mutation of the corresponding position in Jak2 also resulted in a molecule with increased catalytic activity [69].

The *N-terminal half of the Jaks* (regions JH7–JH3, Figure 5) is involved in receptor association. Whereas the whole N-terminal half of Jak1 within a Jak1–Jak2 chimera is necessary to functionally reconstitute Jak1-negative cells, specific binding of Jak2 to the IFNγR2 is determined by its JH7 and JH6 domains at the very N-terminus [70]. The JH7–6 domains of Jak2 even interact in far-Western-blot analysis with the cytoplasmic part of the granulocyte–macrophage colony-stimulating factor (GM-CSF) receptor β chain [68]. Also for Jak3, the JH7–6 domains were necessary and sufficient for association with the IL-2R γ chain [71]. The binding of Tyk2 to IFNαR1, however, requires the JH7–JH3 domains, and within this region the JH3 and JH6 domains appear to be of particular importance [72]. Thus, receptor interaction is complex, possibly involving multiple and discontinuous regions, and the structural requirements for interaction may vary between the different Jaks. Moreover, the way a given Jak associates with cytoplasmic parts of cytokine receptors may depend on the respective receptor complex. This is reflected in the finding that Jak1 with a point mutation within its JH5 domain lost its ability to transmit signals via gp130, but still was capable of sustaining IFN responses (I. M. Kerr, personal communication).

It should be noted that Jaks, apart from being receptor-associated enzymes, may fulfill additional functions. For Tyk2, a structural role has been demonstrated: it is necessary for surface expression of the IFNαRI receptor as well as for high-affinity binding of IFNα [58,67,73]. Moreover, Jaks may have adaptor functions, since several proteins (such as Tec, Btk, Raf, SHP1, SHP2, STAM, SH2-Bβ) were reported to associate with Jaks [74–79].

Jaks involved in signalling via receptors of IL-6-type cytokines

IL-6 leads to the activation of Jak1, Jak2 and Tyk2 [49,50,80]. This holds true also for the other IL-6-type cytokines IL-11, LIF,

OSM, CT-1 and CNTF. Which kinases are involved and to what extent a certain kinase is activated varies among cells (e.g. [50,81]) and possibly reflects different expression levels of Jaks. For certain cell types gp130-mediated activation of other tyrosine kinases such as Hck, Fes, Btk, and Tec has been reported [82–84]. Their significance in IL-6-type cytokine signalling remains to be determined.

To address the question whether the three Jaks are interchangeable or whether there is a hierarchy, advantage was taken of fibrosarcoma-derived cell lines lacking either Jak1, Jak2 or Tyk2. It was demonstrated that signalling of IL-6 in the presence of the soluble IL-6 receptor (i.e. tyrosine phosphorylation of gp130, STAT3, and SHP2) crucially depends on the presence of Jak1. Jak2 and Tyk2, although phosphorylated, could not substitute for Jak1 in Jak1-negative cells [66]; F. Schaper, C. Gendo and P. C. Heinrich, unpublished work). Jak1 was equally essential when gp130 signalling of these fibrosarcoma cells was induced by IL-11–soluble IL-11R complexes [41]. LIF signalling in embryonic stem cells requires Jak1 as well: upon reduction of the Jak1 level by antisense technology, larger amounts of the cytokine were needed to retain pluripotentiality [85]. These findings are corroborated by the phenotypes of Jak1- and Jak2-deficient mice: several types of cells derived from Jak1-deficient mice failed to manifest biological responses to IL-6-type cytokines [86], whereas Jak2-deficient ES cells, as well as fibroblasts derived from Jak2-deficient mice, responded to LIF and IL-6 respectively [87,88]. It is noteworthy, however, that overexpression of dominant negative forms of Jak2 represses signalling via gp130 [66,89]. This indicates that Jak2 and Jak1 may compete for common binding sites on the receptor.

Receptor sequences important for Jak association

Receptor association of Jaks is thought to be mediated by the membrane-proximal box1/box2 regions that are conserved among many cytokine receptors [55,90]. Box1 is a proline-rich motif of eight amino acid residues essential for Jak association, whereas box2, a cluster of hydrophobic amino acid residues followed by positively charged amino acids, is necessary for Jak association only with some receptors. Also, the region between the two boxes is variably important, depending on the receptor investigated. Some receptors, however, e.g. the IFNRs, do not fulfill the classical box1/box2 criteria described above, but are still able to bind Jaks.

The *signal transducer gp*130 also contains box1 and box2 sequences within the membrane-proximal part of the cytoplasmic region. Mutations within the *box1* region reduced the ability of gp130 to associate with Jaks [91], abolished ligand-induced activation of Jak1 and Jak2 [80] and further receptor functions [65,90]. Also *box*2 contributes to Jak-binding: studies with progressive gp130 truncations revealed a reduction of Jak2 binding [65] and abrogation of certain biological effects upon deletion of box2 [65,90]. However, Jaks are able to associate with gp130 devoid of box2 when they are overexpressed ([65,91,92]; C. Haan, H. M. Hermanns and I. Behrmann, unpublished work).

The *LIF receptor* and the *OSM receptor* also contain membrane-proximal box1/box2-like regions [9,10]. It was shown that the first 65 amino acid residues in the cytoplasmic domain of the LIF receptor, in combination with gp130, could mediate signalling [93]. We could demonstrate co-precipitation of Jak1, Jak2 and Tyk2 with receptors containing cytoplasmic parts of the LIFR when we co-expressed the respective Jaks in COS-7 cells (H. M. Hermanns and I. Behrmann, unpublished work). Here again just the membrane-proximal 72 residues were sufficient to mediate this interaction.

THE STAT FAMILY OF TRANSCRIPTION FACTORS

Up until now, seven mammalian STAT genes have been cloned and localized in three chromosomal clusters, suggesting that this family of proteins has evolved by gene duplication. The mammalian STAT factors are designated as STAT1, 2, 3, 4, 5a, 5b and 6 (reviewed in [55]). Except for STAT2, alternatively spliced forms have been described [94–97]. However, in the case of STAT4 and STAT6 the corresponding proteins could not be identified. Homologous proteins were found in *Drosophila* (D-STAT/*marelle*) [98,99] and recently also in *Dictyostelium* [100]. Since no STAT-like genes have been identified in the genomes of unicellular systems, including yeast, STATs might have evolved with the development of multicellular organisms.

Regulation of STAT activity

With the exception of STAT4, STAT factors are ubiquitously expressed. STAT4 expression is more restricted to myeloid cells and testis [101]. The regulation of STAT synthesis does not seem to play a major role in cytokine signalling. STAT activity is predominantly regulated by post-translational modifications, i.e. tyrosine and serine phosphorylation. STATs are mainly activated after stimulation of cytokine receptors. However, there is a growing number of reports demonstrating STAT activation also via receptor tyrosine kinases {epidermal-growth-factor (EGF) receptor, fibroblast-growth-factor (FGF) receptor, platelet-derived-growth-factor (PDGF) receptor, CSF-1R, c-kit, insulin receptor, c-met [102–111]} and G-protein-coupled receptors (angiotensin II receptor [112]). Ligands signalling through the same class of receptor complexes usually activate the same set of STAT factors [102], e.g. all IL-6-type cytokines activate STAT3 and STAT1.

Structure–function relationship of STATs

STATs are proteins with a conserved structural organization (Figure 5). They consist of 750–850 amino acids (STAT1, 750 amino acids; STAT3, 770 amino acids) and various domains within the STAT molecules have been defined: a tetramerization domain and a leucine-zipper-like domain at the N-terminus, a DNA-binding domain in the middle, an Src homology 3 (SH3)-like domain, an SH2 domain and a transactivation domain at the C-terminal end. In all STATs a tyrosine residue near the C-terminus is phosphorylated upon receptor activation.

The function of the highly conserved *SH2 domain* is well established. This domain is responsible for binding of the STATs to the tyrosine-phosphorylated receptor motifs [52,113,114] and also for homo- and hetero-dimerization with other tyrosine-phosphorylated STAT molecules [115]. Whereas STAT3 binds to pYXXQ motifs [52,53], STAT1 is recruited to a more restricted sequence (pYXPQ) in gp130 [53]. In order to demonstrate the pivotal role of the SH2 domains in STAT activation, a chimaeric STAT3 molecule containing the SH2 domain of STAT1 instead of its own was constructed. This chimaera, STAT3/1(SH2), showed the same pattern of activation through gp130 phosphotyrosine motifs as wild-type STAT1, i.e. it was activated via YXPQ motifs but not via motifs lacking the proline in position $Y+2$ [116]. Thus an exchange of solely the SH2 domain converted STAT3 from a factor that is able to bind all gp130 YXXQ motifs into a factor that selectively associates with YXPQ motifs. Also the isolated recombinant STAT3 SH2 domain was shown to specifically interact with the pYXXQ motifs of gp130 (S. Haan and P. C. Heinrich, unpublished work). STAT1 binding to the IFNγR and its phosphorylation-dependent dissociation and

subsequent dimerization has been described as an ordered, reversible, and affinity-driven process [117].

STAT1 and STAT3 – both activated via the common signal transducer gp130 – are able to form homo- and heterodimers [101]. Charged amino acids C-terminal to the SH2 domains have been proposed to contribute to dimer formation (U. Hemmann, F. Horn and A. Grötzinger in [118]). The tyrosine phosphorylation site which is essential for dimerization of STATs is located around amino acid position 700 (STAT1, tyrosine-701; STAT3, tyrosine-705) [119,120]. Interestingly the STAT phosphotyrosine motifs (STAT1, pYIKT; STAT3, pYLKT) do not agree with the consensus sequences deduced from the gp130 recruitment sites [53]. A weak association of STAT1 with STAT2 or STAT3 prior to cytokine stimulation has been described [121]. Since no data on their phosphorylation status were presented, the mechanism responsible for this interaction still needs to be elucidated.

In some cell lines STAT5 activation has been observed in response to IL-6-type cytokines. Activation does not require receptor tyrosine phosphorylation [122]. Interestingly there is evidence for a direct interaction of STAT5 with Jak kinases [123]. In spite of the sequence similarity to functional SH3 domains, the proposed SH3 domain in STATs does not seem to be involved in binding proline-rich sequences, since crucial amino acids are lacking [101].

STAT dimerization is a prerequisite for *DNA binding* [115]. The DNA-binding domain of STAT proteins is located almost in the middle of the molecule (amino acids 300–480), and its primary structure is not related to any other known protein sequence [124]. Binding of the various STAT factors to similar DNA elements (TTN_5AA) [125,126] is probably due to the highly conserved amino acid sequences within the DNA-binding domains. The sequences of the preferred DNA elements depend on the composition of the STAT dimer complex, e.g. STAT1 and STAT3 preferentially bind to $TTCN_3GAA$ present in the promoter regions of acute-phase-protein (APP) genes [126,127]. Recently, the structure of the N-terminal part of STAT4 (123 amino acids) has been solved by X-ray analysis and has been suggested to be important for co-operative DNA binding of multiple STAT dimers [128].

The activity of the C-terminal *transactivation domain* of STATs is at least partially regulated by a serine phosphorylation (serine-727 in STAT1 and STAT3) [129–132]. The kinase responsible for this serine phosphorylation depends on the signalling pathway and the cellular context. After growth-hormone stimulation, STAT3 is MAPK-dependently serine-phosphorylated; in contrast, after stimulation with IL-6, STAT3 is phosphorylated at the same amino acid independently from MAPK activation. This phosphorylation is sensitive to the serine/threonine protein kinase inhibitor H7. The kinase still has to be identified [133,134].

The STAT1 splice variant STAT1β lacking 38 C-terminal amino acids is phosphorylated on tyrosine-701 and translocates to the nucleus after cytokine stimulation, but does not activate transcription of IFNγ-responsive genes [94,135]. Owing to complex formation with STAT2 it is able to transactivate IFNα/β-inducible genes [135]. In STAT3β, the C-terminal 55 amino acids of STAT3α are replaced by an unrelated sequence of seven amino acids [95,96]. This product of alternative splicing leads to constitutively tyrosine-phosphorylated STAT3β dimers. Only in certain cell lines are these STAT3β dimers able to transactivate STAT3-responsive genes [136]. Other reports show STAT3β to inhibit transcriptional responses to IL-5 [95] and thereby act as a dominant negative form of STAT3α. In contrast, Schaefer et al. showed a STAT3β-specific synergistic activity with c-Jun [96]. These contradictory data require further clarifying experiments. Using STAT3α- and -3β-specific peptide antibodies we could hardly detect STAT3β in more than 20 commonly used cell lines (E. Siewert, W. Müller-Esterl and P. C. Heinrich, unpublished work) and therefore the physiological significance of this splice variant needs to be proven.

Nuclear translocation of STATs

STATs are activated in the cytoplasm, but they exert their function in the nucleus (Scheme 1). Thus, after tyrosine phosphorylation, they have to be transported into the nuclear compartment. This translocation can be followed by indirect immunofluorescence. In untreated cells both STAT1 and STAT3 are distributed diffusely in the cytoplasm and the nucleus and within minutes after IL-6 treatment both transcription factors are found preferentially in the nucleus. This translocation is transient, since after 2 h the staining pattern is comparable with the one obtained for untreated cells [130]. A similar transient nuclear translocation was observed when the localization of a STAT3 molecule which was tagged C-terminally with the green fluorescent protein was monitored (L. Terstegen, P. Gatsios and L. Graeve, unpublished work).

The mechanism by which STATs enter the nucleus is unknown. Transport of macromolecules in and out of the nucleus is a signal-mediated and energy-dependent process [137]. Proteins to be transported to the nucleus exhibit a so-called nuclear localization sequence (NLS), which usually is a short motif characterized by a cluster of basic amino acids. This NLS is recognized by the NLS receptor importin-α, which, together with importin-β, mediates the docking of the transported protein to the cytoplasmic face of the nuclear-pore complex. The NLS-containing protein, together with the importins, is then translocated across the pore, a step which requires the GTPase Ran and GTP hydrolysis [137]. A close inspection of the amino acid sequences of all cloned STATs does not reveal a conventional NLS. Thus, nuclear translocation of STATs is achieved either via an untypical NLS or via a shuttle protein that binds to activated STATs and carries an NLS. In this respect the finding that activated STAT5b [138], as well as STAT3 [139], form a complex with the glucocorticoid receptor (GR) is very interesting, since GR contains two NLSs [140]. However, for none of the other STATs has such an interaction been demonstrated to date, so this might not be a general mechanism. For STAT1 it was shown that tyrosine phosphorylation is essential for nuclear import and that the GTPase activity of Ran is crucial for this process [141]. The importin-α family member NPI-1 was identified as the NLS receptor for STAT1 [142]. Thus at least STAT1 enters the nucleus via the conventional import pathway.

STATs and gene regulation

After dimerization and nuclear translocation STATs bind to specific enhancer sequences and stimulate, and in certain cases possibly also repress, transcription of respective target genes. During the past years many target genes for IL-6-type cytokines have been identified and, in a number of cases, functional STAT binding sites were found in the promoter regions of these genes.

Among IL-6-type cytokine target genes that are activated via STATs are (1) APP genes such as C-reactive protein [143], α_1-antichymotrypsin [144], α_2-macroglobulin (rat) [127], lipopolysaccharide-binding protein [145] and tissue inhibitor of metalloproteinases (TIMP)-1 [146], (2) transcription factors such as Jun B [147], c-Fos [148], interferon regulatory factor (IRF)-1 [149] and CCAAT enhancer binding protein (C/EBP)δ [150] and (3) a variety of other genes, such as interstitial collagenase [151],

vasoactive intestinal peptide [152], pro-opiomelanocortin [153], heat-shock protein hsp90 [154], bcl-x [155] and the IL-6 signal transducer gp130 [156].

From the promoter studies of such IL-6-type-cytokine-responsive genes and other STAT-regulated genes, at least two concepts emerge. (1) STAT-binding sites are often in close proximity to binding sites for other transcription factors, suggesting a co-operativity of these factors in gene regulation. For STAT3, possible co-operativity with C/EBPβ/nuclear factor (NF)–IL-6 [145,154], NF-κB [157], activator protein (AP)-1 [145,146,151,152] and GR [139] has been reported. However, a direct protein–protein interaction between STAT3 and other factors has only been demonstrated in a few cases [96,139]. STAT1 also associates with members of the IRF-1 family [158] and SP1 [159]. (2) STAT-binding sites are often arranged as a tandem, e.g. in the rat α_2-macroglobulin or the human α_1-antichymotrypsin promoters. This suggests that STAT dimers might form multimers on clustered binding sites. Such multimerization was recently demonstrated for STAT1, and its N-terminus was found to be essential for this process [160,161]. These two modes of co-operative action support the notion that gene regulation of IL-6-type-cytokine-responsive genes, and probably gene regulation in general, is an integrative process in which several transcription factors together modulate the rate of transcription of a certain gene.

In spite of the fact that detailed information concerning the STAT–enhancer interaction has become available, it is still not clear how STAT factors and co-operating transcription factors transfer their activation signal on to the basal transcription machinery. In this respect the observation that STAT1 interacts with CREB-binding protein (CBP) and p300 [162,163] is very interesting, since CBP and p300 are believed to serve as bridges between transcription factors and RNA polymerase II [164]. CBP enhances the STAT3-induced transcription of the human α_1-antichymotrypsin gene, suggesting that STAT3 also interacts with CBP (U. Schniertshauer, F. Horn and P. C. Heinrich, unpublished work). Thus, although some information on gene regulation by STAT factors has accumulated to date, the picture emerging is far from clear, and it is expected that, within the next few years, many more details about this crucial process will be worked out.

TYROSINE PHOSPHATASE SHP2

After IL-6-type-cytokine stimulation, not only Jaks and STATs, but also the tyrosine phosphatase SHP2, is recruited to gp130 and subsequently phosphorylated. Whereas the role of the activated STATs in signalling is well established, that of SHP2 is less clear. This enzyme is involved in tyrosine kinase receptor [PDGFR, EGFR, FGFR, c-kit, insulin receptor, insulin-like-growth-factor receptor, tyrosine receptor kinase (Trk)B], G-protein-coupled-receptor (angiotensin receptor, PAR2) as well as cytokine receptor (IL-2R, IL-3R, IL-5R, IL-6R, IL-11R, GM-CSFR, IFNα/βR, EpoR) signal-transduction pathways. In addition to receptor binding, SHP2 interacts with adaptor proteins such as insulin receptor substrate (IRS)-1, growth-factor-receptor-bound protein (Grb)2 and Grb7 [165–167].

Structure and function of SHP2

SHP2 is a ubiquitiously expressed tyrosine phosphatase of 585 amino acids and a molecular mass of about 65 kDa. An alternative splice product containing four additional amino acids (ALLQ) within the phosphatase domain has been detected [168]. Detailed biochemical analysis demonstrated a decreased enzymic activity of this splice form [169]. SHP2, like its homologue SHP1, contains two SH2 domains at its N-terminus (Figure 5). The phosphatase domain extends from position 273 to 510. An SHP2 homologue in *Drosophila*, *corkscrew*, contains an insert of 150 amino acids within its phosphatase domain [170,171].

Both *SH2 domains* are required for recruitment of SHP2 to the phophotyrosine motifs of the activated receptors, but they also play a role in the regulation of the phosphatase activity. Phosphotyrosine peptides of IRS-1 specifically interacting with the SH2 domains of SHP2 synergistically enhance SHP2 enzymic activity [172]. Similarly, incubation of SHP2 with a phosphotyrosine peptide corresponding to its recruitment site in the PDGFR increased phosphatase activity [173]. An autoinhibition of SHP2 by its SH2 domains, which bind in a phosphorylation-independent manner to the catalytic domain, was proposed to explain this phenomenon [174], and this has recently been confirmed by the crystal structure of SHP2 [175].

The function of SHP2 *phosphatase activity* in signalling is still obscure. Protein-tyrosine phosphatase activity, but not tyrosine phosphorylation of SHP2, is required for immediate-early responses (e.g. MAPK activation) to EGF, but not to PDGF [176]. Src and c-kit have been shown to be dephosphorylated by SHP2 [174,177,178]. Controversial data have been reported for PDGFR [174,177,179,180]. It is likely that other substrates for this enzyme will be identified. There is evidence that an auto-dephosphorylation of SHP2 [181] might regulate the interaction of SHP2 with SH2-domain-containing proteins.

SHP2 can be phosphorylated by many tyrosine kinases. *In vitro* phosphorylation by purified PDGFR kinase correlated with enhanced phosphatase activity [177]. However, it is more likely that the tyrosine phosphorylation of SHP2 is necessary for signalling in an alternative manner. The phosphotyrosine motifs in SHP2 are involved in the interaction with other SH2-domain-containing proteins. Grb2 binds SHP2 through its SH2 domain after stimulation with PDGF [182,183]. Interestingly, in the case of the EGF signalling pathway, the interaction of SHP2 and Grb2 is not phosphotyrosine-dependent, but mediated by the Grb2-SH3 domain [166]. Gp130–SHP2–Grb2 complex formation was also demonstrated [184,185]. The recruitment of Grb2 to gp130/LIFR via SHP2 thus links the MAPK pathway to the Jak/STAT pathway [89].

SHP2 and IL-6-type-cytokine signalling

SHP2 becomes phosphorylated by tyrosine receptor kinases as well as by cytoplasmic kinases like Src [186], bcr-abl [187] and Jaks. A Jak–SHP2 interaction and the phosphorylation of SHP2 by Jaks has been demonstrated [77]. Jak1 and Jak2, but not Jak3, associate with SHP2 after overexpression in co-transfected COS cells. The N-terminal region of Jak2, but not its kinase activity, is essential for the interaction with SHP2. The interaction does not depend on SHP2 phosphatase activity, phosphotyrosines in SHP2 or the SHP2-SH2 domains [77]. Tyrosine-304 and -327 within the phosphatase domain are phosphorylated by Jak1 and Jak2. Subsequently Grb2 can bind to phosphotyrosine-304 of SHP2. In contrast, after PDGF stimulation, phosphotyrosine-546 or -580 of SHP2 provides the binding site for Grb2 [77,182,183]. In spite of the SHP2–Jak1 or SHP2–Jak2 complexes formed, no dephosphorylation of Jaks could be demonstrated [77]. Recently, an enhanced and prolonged phosphorylation of gp130, Jak and STAT was observed when SHP2 activation was prevented ([188]; F. Schaper, M. Eck and P. C. Heinrich, unpublished work). Substrates for the enzyme SHP2 in the signalling of IL-6-type cytokines still have to be identified.

SHP2 binds to phosphotyrosine-759 of gp130 [52,189]. The motif Y^{759}STV agrees well with the consensus sequence

YXXL/V/I deduced from SHP2 binding to the PDGFR, EGFR and IRS-1 [190]. Activation of SHP2 through gp130 requires Jak1 (F. Schaper, C. Gendo and P. C. Heinrich, unpublished work). Only a few reports on the role of SHP2 in IL-6-type-cytokine signalling have been published, and further clarification is needed. IL-6 and other IL-6-type cytokines mediate the proliferation of gp130-transfected Ba/F3-cells. Fukada et al. [184] demonstrated that proliferation depends on STAT3 and SHP2 activation. Whereas STAT3 elicits an anti-apoptotic signal, SHP2 mediates the mitotic signal via the MAPK pathway. Furthermore, SHP2 might be involved in the induction of serine phosphorylation of STAT1 and STAT3 via activation of the MAPK pathway. A modulatory effect of SHP2 on the induction of IL-6-responsive genes has recently been demonstrated ([188,191]; F. Schaper, J. Schmitz and P. C. Heinrich, unpublished work).

NEGATIVE REGULATION OF THE gp130/Jak/STAT PATHWAY

In most systems the activation of STATs is transient. This suggests that efficient mechanisms for STAT inactivation must exist. Theoretically, two mechanisms can be envisioned, namely dephosphorylation or proteolytic degradation. In the first case, dephosphorylated STATs would most likely re-enter the cytoplasm in order to undergo additional rounds of activation. In the latter case, newly synthesized STATs would have to replenish the degraded pool. Most evidence obtained so far favours the first scenario. As mentioned above, the transient shift of STAT1 and STAT3 from the cytoplasm to the nucleus and back has been followed by immunofluorescence, and no evidence for a major loss of protein was obtained. Furthermore, metabolic labelling studies have indicated that at least STAT3 has a long half-life ($>$ 8 h) and that this half-life is not dramatically altered upon stimulation (E. Siewert and F. Schaper, unpublished work). Nevertheless, a few studies have directly addressed this question for STAT3 and STAT1 activation. We have used the proteasome inhibitor lactacystin to study the possible involvement of the proteasome in STAT3 turnover. There was no measurable effect of this inhibitor on the activation kinetics of STAT3 (A. Küster and P. C. Heinrich, unpublished work). Kim and Maniatis found that the proteasome inhibitor MG132 stabilizes activated STAT1 in the nucleus and that STAT1 becomes ubiquitinated [192]. From these studies they concluded that STAT1 is inactivated, at least partially, via the proteasome pathway. However, they could not rule out the possibility that the ubiquitin–proteasome pathway functions at other steps in the signal-transduction pathway, e.g. at the level of the receptor or the Jaks. Haspel et al. [193] also found that proteasome inhibitors prolong the half-life of activated STAT1; however, this was not due to an inhibition of STAT degradation, but to a prolonged signalling from the receptor. When the fate of ^{35}S-labelled STAT1 was monitored, they found that activated molecules promptly enter the nucleus and later return quantitatively to the cytoplasm as non-phosphorylated molecules [193]. This strongly argues for dephosphorylation as being the major means of inactivating STATs.

Recently, a protein inhibitor of activated STAT3, named PIAS3, has been discovered in various human tissues [194]. The 68 kDa protein co-immunoprecipitates with tyrosine-phosphorylated STAT3, but not STAT1. It also blocks DNA binding of activated STAT3 as well as STAT3-mediated gene activation (Scheme 2). A similar protein has been identified for STAT1. The authors speculate that there may exist a specific PIAS for every STAT factor, and, moreover, that the ratio of STAT and PIAS expression may determine the strength of the STAT signal. How the activity of PIAS3 is regulated is as yet unknown.

Feedback inhibition of Jak/STAT signalling

Another mechanism to down-regulate the Jak/STAT pathway has recently been discovered with the cloning and identification of suppressor-of-cytokine-signalling (SOCS) proteins, also referred to as Jak-binding protein (JAB) or STAT-induced STAT inhibitors (SSIs) [195–197]. These proteins comprise a family of negative-feedback inhibitors, since their transcription is induced by IL-6 and LIF and since they inhibit tyrosine phosphorylation of gp130, STAT1 and STAT3. SOCS-1/JAB/SSI-1 contains an SH2 domain and was shown to directly interact with the kinase domain (JH1) of Jak2 [196]. Association with the other members of the Jak family was also demonstrated and shown to result in a reduced tyrosine kinase activity. Thus SOCS-1/JAB/SSI-1 is a Jak inhibitor that is induced via the Jak/STAT pathway and eventually leads to feedback regulation of this pathway (Scheme 2).

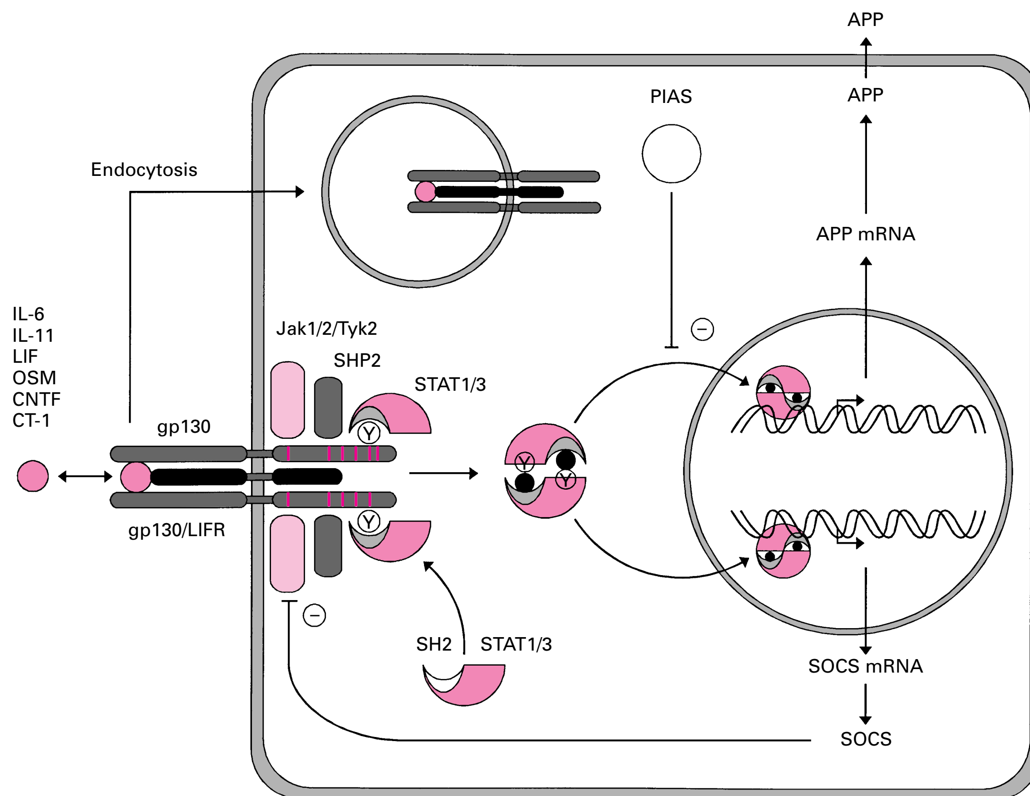
Endocytosis of the IL-6–receptor complex

Cytokine receptors are transmembrane proteins and therefore follow the typical membrane-protein-transport pathways throughout the cell. They are synthesized on membrane-bound polysomes in the endoplasmic reticulum, packed into vesicles and transported to the Golgi apparatus, where N-glycan processing occurs [198], before they are finally translocated to the plasma membrane. However, cytokine receptors are not permanently located at the cell surface, but also undergo endocytosis. Information on cytokine-receptor trafficking has been obtained only recently.

A rapid removal of inflammatory cytokines from the circulation is essential for re-establishment of homoeostasis. When ^{125}I-labelled IL-6 was injected into rats, it was cleared from the circulation with a plasma half-life of about 2 min [199]. The major organ in which IL-6 accumulated was the liver, where it was degraded [200,201]. Thus the hepatocyte is not only a major target for IL-6 in respect of APP gene regulation, but is also the major site within the organism to eliminate IL-6.

The molecular mechanism of IL-6 internalization has been studied in some detail. Binding studies with rat hepatocytes and the hepatoma cell line HepG2 revealed that about 4500 and 2000 receptors respectively were expressed at the cell surface. All these binding sites were of high affinity, with dissociation constants of 39 and 60 pM respectively [201,202]. Interestingly, attempts by several groups to reconstitute this high-affinity binding in transfected cells have so far failed. When gp80 and gp130 were co-expressed in COS-7 cells, dissociation constants of about 0.3–3 nM were observed [13,203] This suggests that a high-affinity converting subunit is still missing in the current models of the IL-6–receptor complex.

After binding IL-6 is efficiently internalized, which results in a complete depletion of IL-6 surface binding sites within 30–60 min [202,204]. Thus, IL-6 down-regulates its surface receptor. *De novo* protein synthesis is necessary to replenish IL-6 binding sites, suggesting that, after internalization, receptor and ligand are degraded, probably in the lysosomal compartment. This could actually be demonstrated using radiolabelled soluble IL-6 receptor [205]. There is strong evidence that the IL-6 receptor is endocytosed via clathrin-coated vesicles, although this has not been demonstrated directly. Expression studies using wild-type and truncated forms of gp80 and gp130 revealed that the cytoplasmic domain of gp130 is crucial for efficient endocytosis and receptor down-regulation [206]. The IL-6 receptor, when



Scheme 2 Negative regulation of the IL-6-type cytokine signal-transduction pathway

Several mechanisms possibly lead to down-regulation of IL-6-type cytokine-mediated signals, including receptor endocytosis, induction of feedback inhibitors (SOCS) or further inhibitory proteins like PIAS. Also for the phosphatase SHP2 a negative regulatory role is being discussed.

expressed alone, was internalized only inefficiently (E. Dittrich and L. Graeve, unpublished work).

Internalization of receptors via the clathrin-coated pit pathway is mediated through two sorts of signal sequences localized within the cytoplasmic part: tyrosine-based motifs which form a tight β-turn and dileucine motifs [207]. A detailed mutational analysis of the cytoplasmic domain of gp130 revealed that it contains a dileucine internalization motif [206]. When fused C-terminally to the Tac-antigen intracellular domain, the gp130 dileucine motif was also capable of mediating the efficient internalization of this protein, which usually is not endocytosed. The fate of internalized gp130 is still unclear. Complexed with IL-6 and gp80 it could be targeted to lysosomes, as is often observed for proteins containing dileucine motifs [207]. Alternatively, gp130 could be separated from the IL-6–gp80 complex and recycled back to the cell surface. Some indirect evidence favours the first scenario [208], but future studies have to address this question more directly.

In order to study the relationship between endocytosis of gp130 and Jak/STAT signalling, chimaeric receptors were used that consisted of the extracellular parts of the α- and β-chains of the IL-5 receptor and the transmembrane and cytoplasmic parts of gp130. These chimaeras endocytosed IL-5 with kinetics similar to those found for IL-6 and the IL-6–receptor complex [209,210]. In the presence of IL-5 they also activated the Jak/STAT pathway. A mutant form of these chimaeric molecules with the dileucine motif replaced by two alanine residues did not endocytose IL-5, but Jak/STAT signalling was still intact. Furthermore, a mutant defective in signalling was endocytosed as efficiently as the wild-type [210]. Thus, it appears that endocytosis is uncoupled from the Jak/STAT pathway. In addition, agonistic and antagonistic monoclonal antibodies against the signal transducer gp130 were internalized with comparable kinetics. These findings suggest that endocytosis of gp130 is not ligand-mediated (the ligand being IL-6/gp80), but constitutive. However, it is also possible that it is not the activation of the Jak/STAT pathway itself, but the dimerization of gp130, that triggers endocytosis. Studies in which internalization rates of monomeric and dimeric gp130 are measured will solve this issue.

Recent evidence suggests that tyrosine- as well as dileucine-based internalization motifs are recognized by the adaptor complexes AP-1 and/or AP-2 [211,212]. If gp130 is internalized constitutively via clathrin-coated pits, it should interact with the plasma-membrane adaptor AP-2. Preliminary data support this notion (S. Thiel and L. Graeve, unpublished work).

Internalization was also reported for LIF and CNTF [213,214]; however, the mechanisms of this endocytosis have not been analysed in detail. One can easily envision that, in all possible receptor complexes, gp130 is the crucial component mediating the interaction with the endocytotic machinery. However, the LIFR and the OSMR might also contain independent internalization motifs. Future studies have to address this question.

BIOLOGICAL RESPONSES TO IL-6-TYPE CYTOKINES

The result of IL-6-type cytokine signalling is the regulation of a variety of complex cellular processes such as proliferation, differentiation and gene activation. The many biological re-

Table 3 Physiological properties of human IL-6-type cytokines

Owing to space limitations we have omitted references for the information given here. However, a complete set of references is available from P. C. H. on request. Further abbreviations used: A, astrocytes; ACTH, adrenocorticotropic hormone; CH, chondrocytes; EC, endothelial cells; ECM, extracellular matrix; ES, embryonic stem cells; F, fibroblasts; GI, gastrointestinal; K, keratinocytes; KU, Kupffer cells; LDL, low-density lipoprotein; LPS, lipopolysaccharide; M, monocytes/macrophages; MA, mast cells; MC, melanoma cells; MMP, matrix metalloproteinase; N, neurons; NGFR, nerve-growth-factor receptor; O, osteoblasts; PGE, prostaglandin E; PTH, parathyroid hormone; S, synoviocytes; SCH, Schwann cells, SM smooth-muscle cells; T, T-cells; TEC, thymic epithelial cells; TGFβ, transforming growth factor β; TNFα, tumour necrosis factor α.

	IL-6	IL-11	LIF
Sites of synthesis			
Tissues	Many tissues including blood, cartilage, bone marrow, skin, lung and central nervous system	Haematopoietic tissues, lung, gastrointestinal tract, bone, central nervous system, thymus, connective tissues, skin, uterus and testis	Many tissues, including heart, liver, endometrium, pituitary, central nervous system, gut, kidney, lung and thymus
Cells	M, EC, F, T, CH, S, O, KU, SM	F, EC, O, CH, S, K, N	M, F, T, K, TEC, S, CH, O, A
Stimulators	IL-1, TNFα, TGFβ, OSM, LPS	IL-1, TGFβ	IL-1, TNFα, TGFβ, IL-8, EGF, IL-3, OSM, LPS, PDGF, PTH-related proteins
Inhibitors	Glucocorticoids, oestrogens, IL-4, IL-10	?	Glucocorticoids, oestrogens, IFNγ
Responses	Haematopoiesis	Haematopoiesis (megakaryopoiesis, thrombopoiesis, erythropoiesis)	Blastocyst implantation
	Differentiation and proliferation of B- and T-cells	Growth control of GI epithelial cells	Haematopoiesis
	Stimulation of proliferation of mesangial cells and keratinocytes	Osteoclast development	Differentiation factor for pituitary corticotrophic cells
	Regulation of APP synthesis	Neurogenesis	Regulation of APP synthesis
	Up-regulation of TIMP-1	(Weak) stimulator of APP synthesis	Up-regulation of TIMP-1
	Stimulation of ACTH production	Up-regulation of TIMP-1	Inhibition of differentiation of ES cells
	Osteoclast development	Inhibition of adipogenesis	Switch to cholinergic function of sympathetic neurons
	Fever		Survival factor for neurons
			Proliferation of myoblasts

	CNTF	CT-1	OSM
Sites of synthesis			
Tissues	Nervous system	Heart, skeletal muscle, ovary, colon, prostate, testis, fetal kidney and lung	Testis, blood
Cells	SCH, A		M, T
Stimulators	Release after injury of peripheral nerve cells Increased synthesis in astrocytes after injury, IFNγ	?	T-cell activators, PMA, IL-2, IL-3, Epo
Inhibitors	?	?	?
Responses	Anti-apoptotic effect after nerve injury	Induction of hypertrophy of neonatal cardiac myocytes	Growth inhibitor of EC, F and MC
	Inhibition of developmentally determined apoptosis	Inhibition of cardiac myocyte apoptosis	Growth stimulator of SM, ES, O
	Promotes the cholinergic phenotype in sympathetic nerves	Survival factor for spinal motor neurons	Inhibition of differentiation of ES
	Activation of choline acetyltransferase in motoneurons	Stimulation of cholinergic differentiation of sympathetic neurons	Survival of Sertoli cells and gonocytes
	Stimulation of outgrowth of neurites *in vitro*	Increase in platelet and red-blood-cell counts	Up-regulation of LDL-R
	Down-regulation of pro-inflammatory cytokines (IL-1, IL-8) and PGE_2	Inhibition of LPS-stimulated TNFα production	Regulation of APP synthesis
	Regulation of APP synthesis	Stimulation of APP synthesis	Induction of cytokines (IL-6, G-CSF, GM-CSF, LIF, bFGF)
	Up-regulation of CNTFR and NGFR		Effect on ECM (TIMP-1 $\uparrow$, TIMP-3 $\downarrow$, MMP1 $\uparrow$, MMP3 $\uparrow$, MMP9 $\uparrow$, proteoglycans $\uparrow$, collagen type I and III $\uparrow$)
			Up-regulation of adhesion molecules in EC

sponses of the IL-6-type cytokines are listed in Table 3. It should be noted that most of the information presented has come from *in vitro* experiments where single recombinant cytokines and many different cell lines have been used. It is currently difficult to assess whether the observed *in vitro* effects reflect the *in vivo* situation. Interesting insights into the possible roles of IL-6-type cytokines *in vivo* have come from transgenic and knock-out animal studies (Table 4). On the one hand these studies have confirmed some of the *in vitro* observations; on the other hand new and unexpected functions have become evident. Thus the phenotype of the IL-6 knock-out mice confirmed IL-6 to be the major mediator of the acute-phase response at least during sterile experimental inflammation [215]. In contrast, IL-11R knock-out mice did not show abnormal haematopoiesis, as expected from *in vitro* studies [216], but surprisingly female infertility was observed [217]. It is interesting that gene knock-outs of the central players in IL-6-type cytokine signalling (STAT3 [218], gp130 [219], Jak1 [86]) lead to mice with lethal phenotypes (Table 4), whereas mice deficient in one of the cytokines display only relatively mild defects. One surprising outcome of CNTF and CNTFR knock-out mice was a marked difference in phenotype: whereas CNTF-deficient mice showed a loss of motoneurons only in adult animals, the CNTFR knock-outs died between 12 and 24 h after birth, suggesting the existence of a second CNTF-like cytokine ('CNTF-2') [220]. Thus, it can be expected that further IL-6-type cytokines, and possibly receptor chains, will be discovered in the near future.

Since all IL-6-type cytokines signal through the gp130/Jak/

Table 4 Phenotypes of transgenic (TG) and knock-out (k.o.) mice

Owing to space limitations we have omitted references for the information given here. However, a complete set of references is available from P. C. H. on request.

Cytokine	TG or k.o.	Effects
IL-6	TG	IgG1 plasmacytosis but no plasmacytomas in C57BL/6 mice
		Transplantable monoclonal plasmacytoma, chromosomal translocation t (12,15) in BALB/c mice
	k.o.	Normal embryogenesis and development
		Lymphoid development not seriously affected
		Bone metabolism disturbed, higher rate of bone turnover in female mice
		Impaired anti-viral response
		Reduced bactericidal activity
		No mucosal IgA
		Acute-phase response to turpentine, but not to LPS, severely compromised
		Defect in liver regeneration after partial hepatectomy
		Failure to evoke fever response to endogenous IL-1β and exogenous pyrogens
IL-6R (gp80)	TG	Stimulation of osteoblast development in the presence of IL-6 in co-cultures with normal spleen cells
	k.o.	Not examined
IL-11	TG	Inflammatory and fibrotic responses
	k.o.	Not examined
IL-11R	TG	Not examined
	k.o.	Female infertility due to defective decidualization
		Normal haematopoiesis
CNTF	TG	Prevention of motoneuron degeneration
		Loss of body weight
		Protection of neurons from apoptosis during embryonal development
	k.o.	Loss of motoneurons in adult mice and subsequent muscle atrophy
CNTFR	TG	Not examined
	k.o.	Mice die between 12 and 24 h after birth
		Defect in motoneuron development
LIF	TG	Overexpression in ES-cells:
		M-LIF (matrix-associated form): no differentiation of embryonic chimaeras
		D-LIF (soluble form): normal gastrulation of embryonic chimaeras
		Intrapituitary LIF overexpression:
		Arrested pituitary maturation with formation of pituitary Rathke's cleft cysts
		Growth-hormone deficiency
		Diminished body weight
		Overexpression in T-cells:
		B-cell hyperplasia, polyclonal hypergammaglobulinaemia and mesangial proliferative glomerulonephritis
		Interconversion of thymic and lymph node phenotypes due to disruption of stroma–lymphocyte interactions
		Overexpression in pancreatic islands:
		Neurotransmitter switching of sympathetic neurons (to cholinergic phenotype)
	k.o.	Female infertility due to an implantation defect
		Smaller size
		Partial loss of stem-cell pool
		Reduced thymic T-cell responses
		Reduced ability of sympathetic neurons to survive injury
LIFR	TG	Not examined
	k.o.	Perinatal lethality
		Defects in placental architecture
		Dramatic decrease in bone volume
		Reduction of astrocyte numbers in spinal cord and brain stem
		Significant loss in motoneurons of the facial nucleus and lumbar spinal cord
		Overall 50% reduction in neurons of the nucleus ambigous
		Elevated stores of glycogen in late-gestation fetal liver
OSM	TG	Overexpression in neurons and keratinocytes is lethal
		Abnormalities in bone growth and spermatogenesis
		Overexpression in pancreatic cells leads to fibrosis
		Overexpression in thymocytes results in abnormal lymphoid tissue development
		Stimulation of extrathymic T-cell development
	k.o.	Not examined
OSMR	TG	Not examined
	k.o.	Not examined
CT-1	TG	Not examined
	k.o.	Not examined
CT-1R	TG	Not examined
	k.o.	Not examined
gp130	TG	Not examined
	k.o.	Embryos die between day 12.5 and term
		Heart abnormality
		Haematopoietic abnormality

Table 4 (*cont.*)

Cytokine	TG or k.o.	Effects
Jak1	TG	Not examined
	k.o.	Perinatal lethality
		Failure to nurse
		Decrease in birth rate
		Smaller thymus
		Abnormal lymphocyte development
		Impaired response to cytokine signalling via gp130, γ_c and class II cytokine receptors
Jak2	TG	Not examined
	k.o.	Embryonic lethality
		Absence of definitive erythropoiesis
		Impaired response to Epo, thrombopoietin, IL-3, GM-CSF and IFNγ
Tyk2	TG	Not examined
	k.o.	Not examined
SHP2	TG	Not examined
	k.o.	Embryos die between day 10 and term
STAT1	TG	Not examined
	k.o.	No overt developmental abnormalities but lack of response to IFN-α, -β and -γ
		Highly susceptible to microbial and viral pathogens
		Normal response to other cytokines that activate STAT1
STAT3	TG	Not examined
	k.o.	Embryos die before day 8.5
		Development is normal until day 6, thereafter rapid degeneration and failure in mesoderm formation
STAT5A	TG	Not examined
	k.o.	Females fail to lactate
		Bone-marrow-derived macrophages show decreased proliferation upon GM-CSF treatment
		Reduced STAT5B levels, reduced levels of STAT5B phosphorylation
		Decreased whey-acidic-protein expression
STAT5B	TG	Not examined
	k.o.	Minor effect on lactation
		Reduced fertility
		20% weight reduction in males
		Increased expression of cytochrome *P*-450 in the liver
STAT5A/B	Double k.o.	Reduced viability
		Females are sterile, males are fertile
		20–40% reduction in weight
		Liver phenotype
		T-cell hyporesponsiveness upon IL-2 treatment

STAT pathway, their redundancy is understandable. But how is specificity *in vivo* achieved, particularly in light of the fact that gp130, Jak kinases and STATs are ubiquitously expressed? Several mechanisms seem to provide specificity to the action of the different IL-6-type cytokines. First, cytokine synthesis and release are spatially and temporally restricted. Secondly, responsiveness is regulated by differential cell-surface expression of ligand-binding receptor subunits. Furthermore, gp130/LIFR or gp130/OSMRβ heterodimers may signal in a way different from gp130 homodimers [221,222]. Thus specific characteristics emerge for each of the IL-6-type cytokines. Whereas IL-6, and to some extent OSM, represent typical defence hormones involved in the activation of the immune and acute-phase response, IL-11 and LIF play a crucial role in fertility [223]. Although exhibiting overlapping biological functions with the other IL-6-type cytokines, CNTF and CT-1 are more restricted to local actions on injured nerve cells and cardiac myocytes respectively (Table 3).

PERSPECTIVES

Although exciting contributions to the elucidation of the molecular mechanism of the gp130/Jak/STAT pathway have been made during the last 5 years, many open questions still remain to be answered. The molecular basis for the promiscuity of gp130 that is able to recognize at least six different cytokines and to form complexes with six different receptors needs to be understood. Whereas the function of the CBMs in ligand binding is well established, the role of the IgG-like and the membrane-proximal fibronectin-type-III-like domains of the signal-transducing-receptor chains remains obscure. An important issue is the elucidation of the stoichiometry of the native receptor complexes on the plasma membrane. Furthermore, there is presently no information on the structure of the cytoplasmic domains of gp130, LIFR and the OSMR β chain and not even a molecular model has been proposed.

While Jak1 plays a major role in IL-6-type cytokine signalling, the contribution of Jak2 and Tyk2 needs to be clarified. Also, the question of whether Jak kinases are not only necessary, but also sufficient, for downstream phosphorylation events has to be answered. The significance of other kinases activated upon stimulation with IL-6-type cytokines needs to be clarified. The dimerization and nuclear-translocation mechanisms of STATs are not understood. Further unanswered questions exist concerning details of STAT–DNA binding, co-operation with other transcription factors as well as the mechanism of *trans*-activation. It is not known how different responses such as proliferation, growth inhibition or the regulation of APP synthesis and secretion are elicited by the action of a single cytokine on different cells. Cross-talks between gp130/Jak/STAT signal transduction and other signalling pathways may be crucial for specific cellular responses. Finally, the *in vivo* function of the different IL-6-type cytokines, in particular their local versus systemic actions as well as their modulation by soluble receptors, is far from being understood, in spite of the fact that transgenic and knock-out animal models have greatly advanced our knowledge.

Note added in proof (received 21 July 1998)

During typesetting of this review the three-dimensional structures of both STAT1 and STAT3β bound to DNA have been published [224,225]. The structures confirm most of the STAT features summarized in this review and beyond that they provide considerable additional insight into the function of these transcription factors. It should be noted that the STAT SH3 domains proposed do not exist.

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