REVIEW

The molecular details of cytokine signaling via the JAK/STAT pathway

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Abstract: **More than 50 cytokines signal via the JAK/STAT pathway to orchestrate hematopoiesis, induce inflammation and control the immune response. Cytokines are secreted glycoproteins that act as intercellular messengers, inducing proliferation, differentiation, growth, or apoptosis of their target cells. They act by binding to specific receptors on the surface of target cells and switching on a phosphotyrosine-based intracellular signaling cascade initiated by kinases then propagated and effected by SH2 domain-containing transcription factors. As cytokine signaling is proliferative and often inflammatory, it is tightly regulated in terms of both amplitude and duration. Here we review molecular details of the cytokine-induced signaling cascade and describe the architectures of the proteins involved, including the receptors, kinases, and transcription factors that initiate and propagate signaling and the regulatory proteins that control it.**

Keywords: **cytokine; Cytokine Signaling; JAK/STAT; SOCS; cytokine receptor; hematopoiesis**

Introduction

Cytokines are secreted glycoproteins that act as intercellular messengers to control the hematopoietic and immune systems and the inflammatory response. The term "cytokine" arises from the Greek κύτος and κίνησις (cell and movement) consistent with their ability to mobilize cells to sites of infection and inflammation. Historically, the plethora of different cytokines have been divided into five groups: (A) TNF-alpha and related molecules, (B) IL-1 family members, (C) TGF-Betas, (D) factors that signal through receptor tyrosine kinases such as M-CSF, (D) chemokines, and (E) cytokines that signal through the JAK/STAT pathway (Fig. 1). The latter group is perhaps the largest and comprises both hematopoietic growth factors such as EPO as well as immunomodulatory cytokines such as IL-2 and inflammatory cytokines such as interferon gamma (Table I). This review will focus entirely on this class and henceforth we use the term cytokine to describe only those that signal via the JAK/STAT cascade.

JAK/STAT signaling: from the cell-surface to the nucleus

The molecular details of the JAK/STAT pathway were largely uncovered in a series of ground breaking studies from the laboratories of James Darnell, George Stark and Ian Kerr more than two decades ago.[1] It is an elegantly simple signaling cascade in which the cytokine requires only three components (receptor, kinase, and transcription factor) to elicit a response (Fig. 2). Each cytokine binds to a specific receptor on the surface of its target cell. These receptors contain intracellular domains which are constitutively associated with members of the JAK (Janus Kinase) family of tyrosine kinases.[2–6] JAKs are inactive prior to cytokine exposure however binding of cytokine to its receptor induces their auto-activation by *transphosphorylation*.[7] Once activated, JAKs phosphorylate the intracellular tails of the receptors on specific tyrosines which in turn act as docking sites for members of the Signal Transducers and Activators of Transcription (STAT) family of transcription factors (Fig. 2).[8] Receptor-localized STATs are then phosphorylated by JAK[9,10] which leads to their disassociation from the receptor and translocation to the nucleus, where they drive the expression of cytokine-responsive genes,[11] often leading to proliferation and/or differentiation. To ensure that signaling is switched off appropriately, a number of proteins act to attenuate cytokine signaling at multiple levels of the pathway. Notably, the suppressors of cytokine signaling (SOCS) family are negative feedback inhibitors of the signaling cascade.[12,13] Although there are exceptions, a general rule of cytokine signaling is that each cytokine binds to a specific receptor, this induces activation of specific JAK(s) and STAT(s) and signaling is switched off by a particular SOCS protein (Fig. 3).

Evolutionarily, the JAK/STAT pathway first arose in Bilateria; Drosophila for example contains the complete set of pathway components (cytokine, receptor, JAK, STAT). Although the simplicity of the system's architecture has been maintained, there has
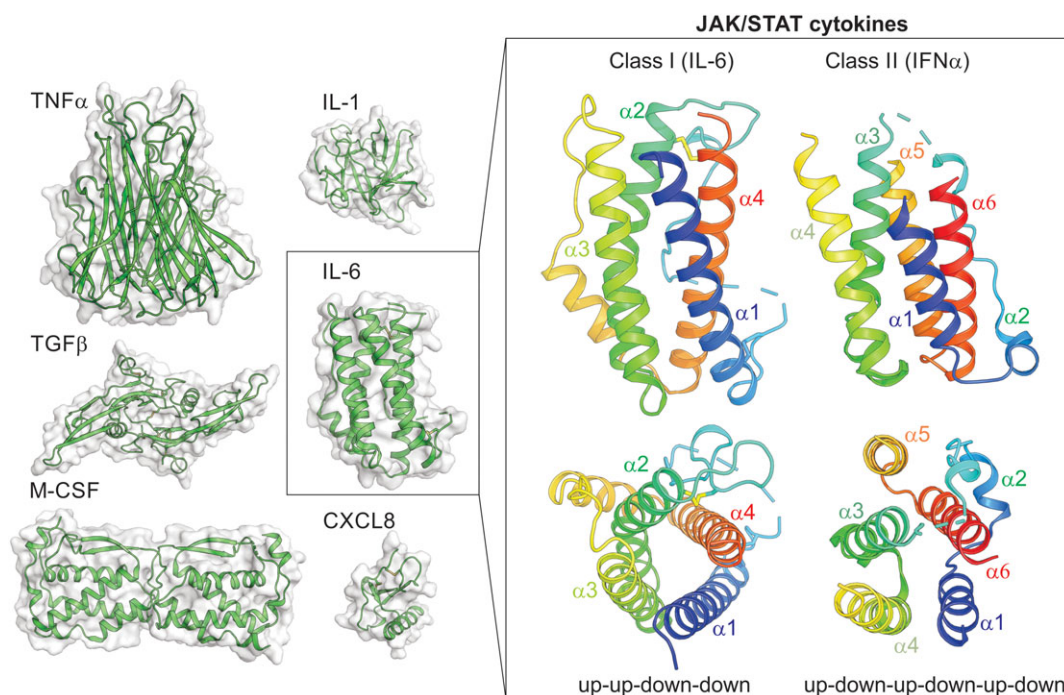

Figure 1. *Cytokines*. Structures of members of the TNFα-family, TGFβ-family, IL-1-like cytokines, chemokines (CXCL8), cytokines that signal through receptor tyrosine-kinases (M-CSF) or the JAK/STAT pathway (IL-6) are shown on the left. JAK/STAT cytokines are helical bundle cytokines and can be divided into two classes. Examples of these two classes are shown on the right.

Table I. *List of Cytokines that Signal through the JAK/STAT Pathway*

Abbreviation	Name	Major Functions
Class I cytokines		
IL-2 family		
IL-2	Interleukin-2	Immune response, T-cell differentiation
IL-4	Interleukin-4	T_H2 differentiation
IL-7	Interleukin-7	T-, B-cell growth factor
IL-9	Interleukin-9	Pleiotropic, Stimulates, T-, B- and NK cells
IL-15	Interleukin-15	Stimulates T- and NK-cells
IL-21	Interleukin-21	Stimulates, T-, B- and NK cells
IL-3 family		
IL-3	Interleukin-3	Multi-lineage haematopoietic growth factor
IL-5	Interleukin-5	B-cell development, eosinophils
GM-CSF	Granulocyte/Macrophage Colony Stimulating Factor	Multi-lineage haematopoietic growth factor, especially monocytes, neutrophils, eosinophils and basophils
IL-6 family		
IL-6	Interleukin-6	Pleiotropic, haematopoiesis, acute phase response, lymphoid differentiation
LIF	Leukemia Inhibitory Factor	Pleiotropic, blastocyst implantation, bone remodeling, CNS
CNTF	Ciliary NeuroTrophic growth Factor	Neuronal growth factor
CT1	Cardiotrophin 1	Cardiac myocytes growth factor
CLC	Cardiotrophin-like cytokine	Neurological growth factor
OSM	Oncostatin M	Pleiotropic, bone formation
IL-31	Interleukin-31	Inflammatory, cell-mediated immunty
NP	Neuropoietin	Neural growth factor
Homodimeric		
G-CSF	Granulocyte Colony Stimulating Factor	Stimulates granulocyte production, mobilises stem cells
EPO	Erythropoietin	Stimulates formation of erthrocytes
TPO	Thrombopoietin	Stimulates formation of megakaryocytes/platelets
GH	Growth Hormone	Growth
PRL	Prolactin	Milk production
LEP	Leptin	Regulates appetite
Others		
IL-12	Interleukin-12	Stimulates T- and NK-cells
IL-13	Interleukin-13	Pleiotropic, airway epithelia, allergic response
IL-23	Interleukin-23	Inflammation
TSLP	Thymic Stromal LymphoPoietin	Inflammatory, stimulates T- and B-cells
Class II cytokines		
Type I interferon		
IFNα	Interferon alpha (23 subtypes)	Anti-viral, secreted by lymphocytes, fibroblasts and monocytes
IFNβ	Interferon beta	Anti-viral, ubiquitously expressed
IFNε	Interferon epsilon	Anti-viral, expressed in female reproductive tract
IFNκ	Interferon kappa	Anti-viral, expressed by keratinocytes
IFNω	Interferon omega	Anti-viral, secreted by leukocytes
Type II interferon		
IFNγ	Interferon gamma	Pro-inflammatory, secreted by T- and NK-cells, activates macrophages/monocytes
Type III interferon		
IFNλ1	Interferon lambda1	Anti-viral, similar to type I but acts on fewer cell-types
IFNλ2	Interferon lambda2	Anti-viral, similar to type I but acts on fewer cell-types
IFNλ3	Interferon lambda3	Anti-viral, similar to type I but acts on fewer cell-types
IL-10 family		
IL-10	Interleukin-10	Anti-inflammatory, inhibits macrophage activation
IL-19	Interleukin-19	Inflammatory, acts on dermal cells
IL-20	Interleukin-20	Inflammatory, acts on dermal cells
IL-22	Interleukin-22	Inflammatory, secreted by Th1 cells, acts on dermal cells
IL-24	Interleukin-24	Inflammatory, acts on dermal cells
IL-26	Interleukin-26	Antimicrobial, T_H17 cytokine

been a large expansion in the numbers of each of the components throughout the course of evolution. For example, *Drosophila* has three cytokine-like molecules (*unpaired, unpaired-2, unpaired-3*), a single receptor (*domeless*), one JAK (*hopscotch*), one STAT (*marelle*/STAT92E), and three SOCS. In contrast, the human genome encodes >50 cytokines, a roughly equivalent number of receptor chains, four JAKs,
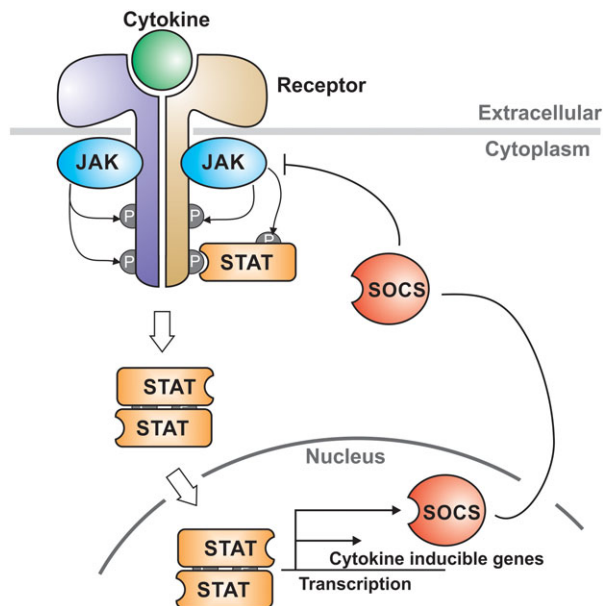
Figure 2. The JAK/STAT pathway. Schematic of the signaling cascade induced by cytokines that signal via the JAK/STAT pathway. Cytokine binds to a specific receptor and allows transactivation of the associated Janus Kinases (JAKs). Activated JAKs then phosphorylate tyrosines on the intracellular domains of the receptor which recruit the Signal Transducers and Activators of Transcription (STAT) transcription factors. STATs are translocated into the nucleus and upregulate the transcription of cytokine-responsive genes. SOCS proteins are direct targets of STAT and act as negative-feedback inhibitors to switch off the signaling cascade.

seven STATs and eight SOCS (Table II) and these numbers are not dramatically different in any vertebrates so far examined.[45]

Cytokines and their receptors

Most cytokines are small helical-bundle proteins usually ca. 150–200 amino acids in length. They are divided into two classes based on motifs found in their receptors (see below). Class I cytokines consist of four α-helices in a characteristic up-up-down-down configuration. Some of these, such as IL-5 exist as dimers but the topology is conserved. The unusual up-up-down-down configuration necessitates two long loops to connect the up-up and down-down pairs. In class II cytokines, one or both of these loops is replaced by an extra α-helix resulting in 5–6 helices in total arranged in an anti-parallel fashion. Again, some (such as IFNγ and IL-10) function as dimers, where the 2 C-terminal helices of one molecule are domain-swapped into a second.

Cytokine receptors consist of multiple (usually two) protein chains. These receptor chains are type I single-pass transmembrane proteins with conserved intracellular and extracellular features. The extracellular domains contain a region termed the hemopoietin domain or cytokine receptor homology region (CHR),[46] formed by a pair of Fibronectin type III (FnIII) domains

oriented at nearly right angles to one-another (Fig. 4). FnIII domains are small ca. 100 residue domains that form a β sandwich comprised of a three- and a four-stranded β-sheet. The principal binding site for cytokines is at the junction between the two FnIII domains within the CHR, and flexible, variable loops from each domain determine specificity at this binding site.[47,48]

The CHRs of the receptors for Class I cytokines contain four conserved cysteines arranged in a $CX_{(9-10)}$-$CXWX_{(26-32)}$-$CX_{(10-15)}$-C sequence within the first FnIII domain (forming two intra-domain disulfide bonds) and a "WSXWS motif" in the second FnIII domain.[49] The CHRs of Class II receptors do not have the WSXWS motif and although they too share conserved cysteines, they are arranged differently. Intracellularly, both Class I and Class II receptors share sequences that allow for recruitment of JAKs and STATs.

Class I cytokine receptors

Class I receptors represent the largest group, with 34 Class I receptor chains encoded in the human genome.[50] All Class I receptors contain a CHR but many also contain additional extracellular domains such as Ig domains, extra FnIII domains or even a second CHR. Class I receptors bind to a broad array of interleukins, hematopoietins, and growth factors whilst Class II receptors are more restricted, recognizing only interferons and IL-10 family cytokines (Fig. 3).

As shown in Figure 3, there are three major shared chains utilized by Class I cytokines. These are gp130, beta common and gamma common, utilized by IL-6, IL-3, and IL-2 family cytokines, respectively. In addition to these, there are two other shared chains used by cytokines in the IL4/13 and IL-12/23 subgroups. Finally, there are the homodimeric receptors, consisting of two identical chains such as those used by EPO, TPO, GH, PRL, Leptin, and G-CSF. Within each of these classes, the cytokine IL-12Rβ2 receptor stoichiometry and organization can differ. A common theme within the non-homodimeric receptors is that there will be a cytokine-specific chain (nominally the "alpha" chain) that recognizes cytokine with high affinity, and the resulting dimer will then recruit a "shared" chain in order to initiate signaling. The alpha-chain may or may not contain the intracellular motifs required to recruit a JAK kinase. Although there are differences in the number of individual chains that comprise a Class I receptor, receptors usually contain precisely two signaling chains (those whose cytoplasmic domain binds to a JAK family member to initiate signaling).

Homodimeric receptors. The homodimeric cytokine receptors are a family of structurally diverse receptors that are categorized by their use of two identical receptor chains. Some, like EPOR,[51] GHR[47] and PRLR[52] are the most simple of all receptors in terms of architecture, the ectodomain of each receptor chain consisting
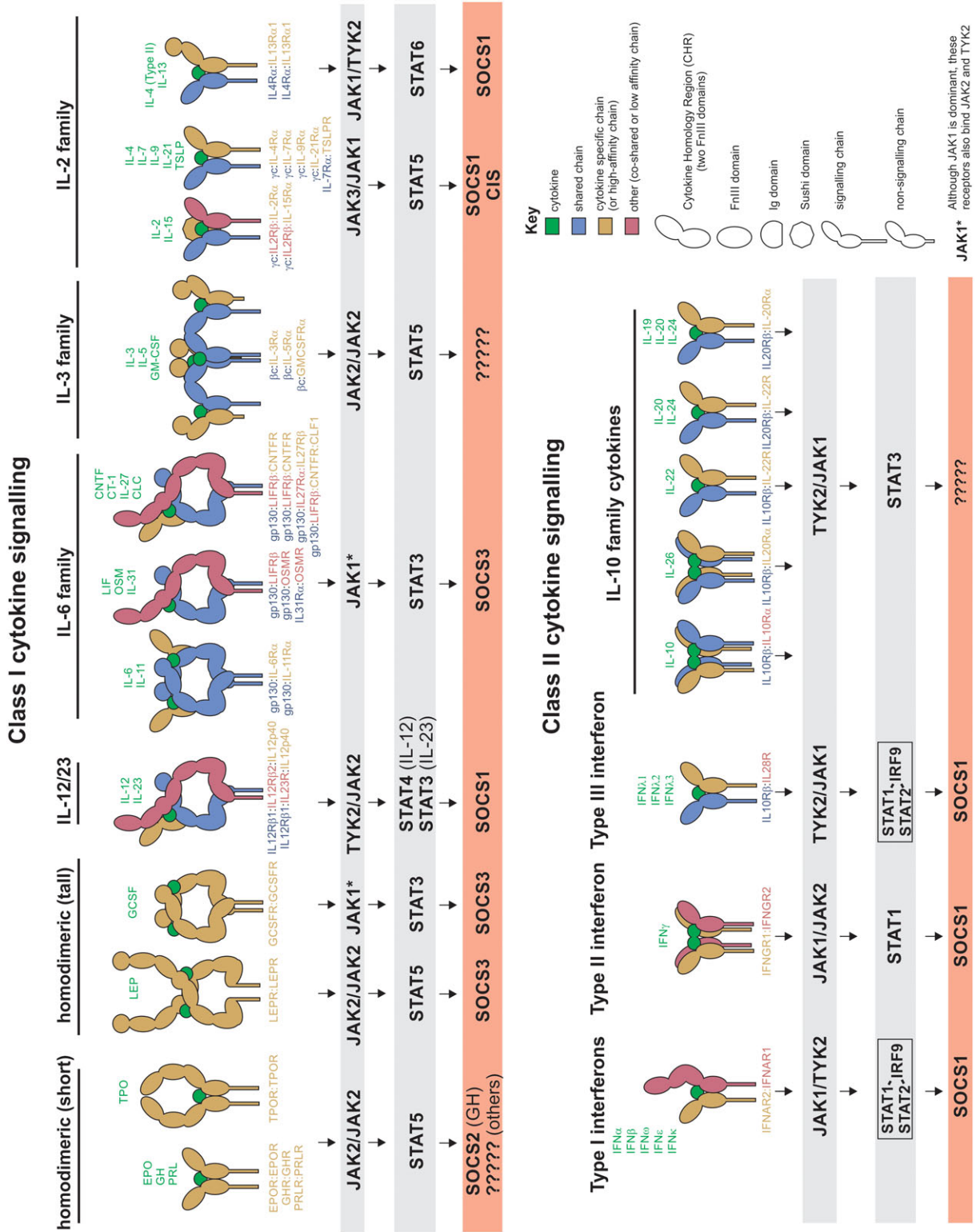
Figure 3. Class I and Class II cytokines. Families of cytokines and the receptors they bind to are shown above the JAK-, STAT-, and SOCS-family members they signal through.

Cytokine Signaling via the JAK/STAT Pathway

Table II. *JAK/STAT/SOCS Family Members*

| Gene	Knockout mouse phenotype	Cytokines dysregulated	Reference
Jak1	Early post-natal lethality, neurological defects; SCID	IL-2, IL-6 family cytokines, IFNs	14
Jak2	Embryonic lethal, failure of definitive erythropoiesis.	EPO, TPO, GH, PRL, IL-3, IL-5, GM-CSF and IFNγ	15–18
Jak3	SCID	IL-2 family cytokines	19–21
Tyk2	Resistance to LPS, reduced IL-12 response	IL-12	22–24
Stat1	Impaired antiviral response	Type I/II/III interferons	25
Stat2	Impaired antiviral response	Type I/III interferon	26
Stat3	Embryonic lethal	IL-6, IL-10 family cytokines, G-CSF	27
Stat4	Impaired Th1 development	IL-12	28
Stat5a/b	Impaired mammary gland development, infertility, no NK cells	GH, PRL, IL-3 family cytokines	29,30
Stat6	Impaired Th2 development	IL-4/13	31
Cish	Increased susceptibility to allergic asthma. Increased tumor immunity	IL-2, IL-4, IL-15	32,33
Socs1	neonatal lethality	IFNγ/α/β IL-12/23, IL4/13 IL-2 family cytokines	34–41
Socs2	gigantism,	GH	42
Socs3	embryonic lethality; placental defects.	IL-6 family cytokines, G-CSF, Leptin	43,44

only of a single CHR unit. Studies on EPOR and GHR in particular have formed the basis of the general paradigm for cytokine signaling. These three receptors, alongside the larger TPOR (which contains two CHRs per ectodomain chain), bind a single molecule of cytokine to form a signaling-competent ternary complex.

Although these complexes are by nature asymmetric, it is the hinge regions between the two FnIII domains of the CHRs on both chains that participate in cytokine binding. One chain binds to the cytokine with high-affinity, and the other chain binds to a lower-affinity site on the cytokine. This phenomenon is observed for all classes of cytokine receptor.

The Leptin and G-CSF receptors are also homodimeric but structurally have more in common with the IL-6 receptor family (see below and Fig. 3). They are

referred to as "tall receptors" as they contain "legs," composed of two or three FnIII domains that link their cytokine-binding region to the membrane. This places the cytokine binding region of these receptors as far as 120 Å from the cell-surface. The leptin receptor is the largest of all cytokine receptors, consisting of (from N- to C-terminus): Ig-CHR-Ig-CHR-FnIII-FnIII. Both the leptin and G-CSF receptors bind two molecules of cytokine, forming signaling competent *tetramers* with a similar architecture to that seen in many IL-6 family receptors[53] (Figs. 3 and 4).

IL-6 family cytokine receptors. The IL-6 family of cytokines includes IL-6, IL-11, IL-27, LIF, OSM, CNTF, CT-1, and CLC. The majority of these cytokines act via receptors that include the common
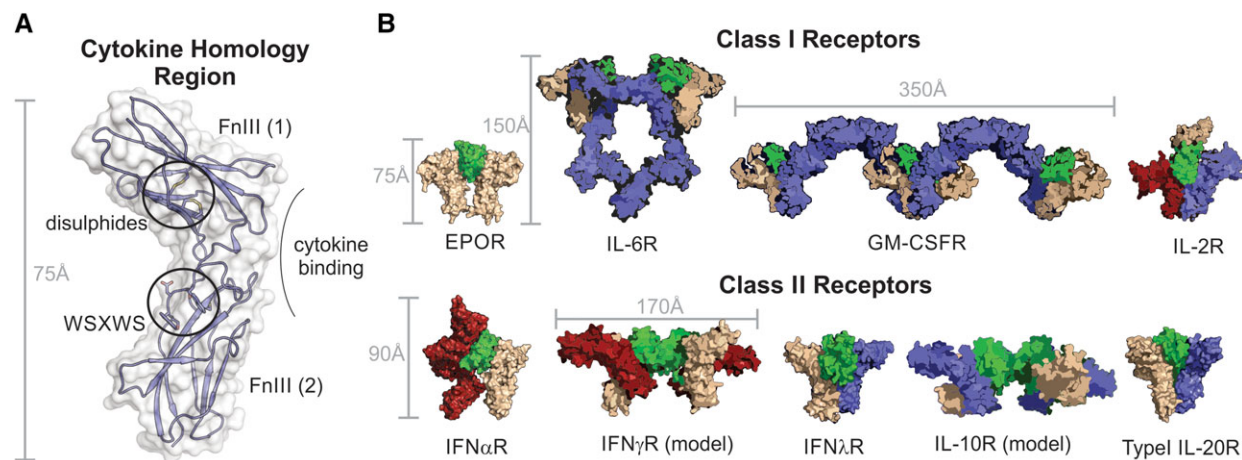
Figure 4. The cytokine homology region (CHR) forms the basis of all cytokine receptors. (A) The CHR from a Class I receptor (Growth Hormone Receptor) is shown with the two FnIII domains, disulfide bonds and WSXWS motif highlighted. All receptors contain a CHR however many receptors, especially those that recognize Class I cytokines, have additional FnIII and Ig domains and this results in a large variety of receptor architectures and stoichiometries. (B) Structures and models of a diverse range of cytokine:receptor complexes.

gp130 chain. Like the G-CSF and Leptin receptors, gp130 is also a tall receptor chain. Its extracellular domain consists of an Ig domain, a CHR, followed by three FnIII domains that form the legs. Within the IL-6 family are two other tall receptor chains which are shared between cytokines on a more limited basis, these are LIFRβ and OSMR.

The signaling competent complex between IL-6 or IL-11 and their receptors are hexamers. These consist of two molecules each of cytokine, gp130, and a cytokine-specific alpha chain (IL-6Rα or IL-11Rα).[54–56] Two copies of gp130 are required because the intracellular domain of the cytokine-specific alpha chains of these receptors do not bind JAK. Signal transduction is, therefore, achieved by transactivation of two JAK molecules bound to the two gp130 receptor chains.

Whilst the IL-6 and IL-11 signaling complexes are similar in structure and composition, other gp130 cytokines signal via different topological assemblies. For example, the complexes between CNTF, CLC, or CT-1 and their receptors are tetramers. These cytokine/receptor complexes are not built around two gp130 chains but instead are built around a heterodimer between gp130 and another JAK-binding tall receptor called LIFRβ.[57,58] As is the case for IL-6, these cytokines first bind to cytokine-specific non-signaling alpha receptor chains and the resulting complexes then recruit gp130 or LIFRβ. Finally, LIF, OSM, and IL-31 signal via trimeric complexes with the receptor being a heterodimer of two tall chains without the requirement for an alpha chain.

IL-12/23 receptors.

IL-12 and IL-23 also signal via tall receptors. Although there is no structure of the full receptors for these cytokines they are likely to be of similar architecture to the LIF receptor[59,60] (Fig. 4). IL-12 and IL-23 are unusual cytokines as they are both composed of two disulfide-linked subunits that arise from different genes.[61] The first of these subunits (IL-12p35 and IL-23p19) are classic four-helix bundle cytokines. The second subunit (IL-12p40, which is shared by both IL-12 and IL-23) is more like a receptor in structure, consisting of a CHR, replete with WSXWS motif, and an N-terminal Ig domain, but lacking a transmembrane domain. Indeed, the p40 receptor-like subunit associates with the 4-helix bundle subunit in a classic receptor-like manner, using the hinge region between the two FnIII domains of the CHR.[62,63] The receptors for IL-12 and IL-23 are also heterodimers which share a common chain, the interleukin-12 receptor subunit β1 (IL-12Rβ1). The other monomer in each receptor is a cytokine-specific alpha chain (IL-12Rβ2 or IL-23R). As one would expect, the common receptor subunit recognizes the p40 subunit whilst the specific alpha chains recognize the classic 4-helix bundle moieties within each cytokine heterodimer.[64]

IL-3 family cytokines.

The IL-3 family (IL-3,-5, GMCSF) all signal via receptors that contain the common beta chain (beta-common, βc).[65] The extracellular region of βc is comprised of two CHRs (i.e. four FnIII domains). βc has no measurable affinity for any of the three cytokines alone, instead each of the three cytokines within this family has a specific alpha-chain that facilitates a high affinity interaction with βc.[66] The primary site of interaction with the alpha-chain is the usual hinge region between the two FnIII domains of the CHR however these alpha-chains also contain a N-terminal Ig domain which wedges over the top of the cytokine, providing extra affinity. It is this binary complex which associates with βc with high affinity.

βc forms a closely associated dimer with the individual FnIII domains of each monomer drawn out like beads on a string, allowing the dimer to associate in an almost domain-swapped fashion.[67] The two cytokine binding sites on the βc dimer are thus formed at the spatial junction between an FnIII domain from both monomers. The structure of the signaling competent GM-CSF/receptor complex shows the most surprising stoichiometry of all, forming dodecamers consisting of four cytokines, four βcs, and four alpha receptors.[67] This dodecameric structure is the minimum structure that can allow juxtaposition of the intracellular JAKs, leading to transactivation and downstream signaling.

IL-2 family cytokines.

Finally, the IL-2 family cytokines (IL-2, -4, -7, -9, -15, -21) all signal via receptors that contain the common gamma chain (gamma common, γc), a JAK3-associated receptor subunit. The extracellular region of γc is much smaller than that of gp130 or βc, consisting of just a single CHR. Most members of this family of cytokines signal via heterodimeric receptors (γc plus a specific alpha receptor), thus forming ternary signaling complexes upon the addition of cytokine. IL-4, IL-7, IL-9, and IL-21 all signal in this manner. The general paradigm for signaling by these cytokines is that γc (like gp130 or βc) has little affinity for the cytokines alone but binds strongly to a complex between the cytokine and the alpha receptor chain.

Despite being the defining family member of this group of cytokines, IL-2 itself, along with IL-15, are unusual in that they also require a third receptor subunit called IL2Rβ. IL-2Rβ is a JAK1-associated receptor subunit and is essential for signaling. The alpha receptors for IL-2 and IL-15 are non-signaling chains distinct from other cytokine receptors as they consist of sushi domains (another beta-sandwich fold) rather than a CHR. The full IL-2 receptor is found on activated T- and NK-cells, and binds IL-2 with extremely high affinity (10pM). IL-15 signals similarly to IL-2 but IL-15Rα usually presents IL-15 from a different cell (i.e. *in trans*) thus IL-15 requires cell–cell contact in order for signaling to occur.[68]

Just as is seen for the gp130 family, the common gamma receptor family also contains chains that are shared on a more limited basis. For example, IL-7Rα can either heterodimerize with γc to form the IL-7 receptor or with TSLPR to form the receptor for TSLP. Likewise, IL-4Rα can heterodimerize with γc to form the Type I IL4R (found on hematopoietic cells); however, it can also heterodimerize with IL-13Rα1 to form the Type II IL4R (found on non-hematopoietic cells). Both IL-4 and IL-13 can signal via this complex and this is one of only a few examples of different Class I cytokines signaling via the same receptor, a phenomenon that is common for Class II cytokines as we shall now discuss.

Class II cytokine receptors

The Type II family cytokines encompass the interferons (IFN α,β,γ,λ,ε,κ,ω) and IL-10 family cytokines.[69] Signaling via Class II cytokine receptors (unlike Class I) adheres to a more common set of rules regarding stoichiometry and receptor assembly. Each Class II receptor is a heterodimer and each of these receptors associate with one molecule of cytokine to initiate signaling. The only exceptions to this rule are IL-10 (and possibly IL-26) and IFNγ which are dimeric cytokines and the stoichiometry of the entire signaling complex is, therefore, doubled (Figs. 3 and 4). All Type II cytokine receptor chains bind to JAK, unlike many Type I receptor alpha chains. Finally, the ectodomain architecture of all Class II receptors consist of just a single CHR (with the sole exception of IFNαR1 which has two) and are not decorated by extra Ig or FnIII domains.

Similar to Class I, the Class II receptor family consists of both shared chains and cytokine-specific chains. However, a characteristic of class II cytokine signaling is the plasticity seen within the system, in many cases a single receptor can bind multiple cytokines and a single cytokine can in some cases bind multiple receptors.

The Type I interferon receptor (IFNα/β receptor).

The Type I interferon receptor is a heterodimer consisting of IFNαR1 and IFNαR2. IFNαR1 has a large extracellular domain that consists of two CHRs while IFNαR2, similar to all other Class II cytokine receptors has only a single CHR. IFNαR2 is the high affinity chain, interacting with ligand with sub-nanomolar affinity whilst IFNαR1 binds with an affinity approximately two orders of magnitude lower.[70]

There is a vast array of type I IFNs, including IFN-α (which itself has at least 13 different subtypes), IFN-β, IFN-ε, IFN-κ, and IFN-ω. Other interferons are found in different mammalian species. Remarkably, these all bind to the same receptor, even though the downstream consequences elicited by each Type I interferon differs. Structural studies have shown that each ligand binds the receptor at the same position; however, the stability of the resultant complexes differ.[71] Thus, the half-life of the cytokine/receptor complex appears to determine the downstream signaling outcomes.

The Type II interferon receptor (IFNγ receptor).

The interferon gamma receptor does not consist of any shared receptor chains and interacts only with a single ligand, interferon gamma. Likewise, IFNγ does not bind any other receptors. IFNγ acts as a homodimer and binds two copies of IFNγR1 with sub-nanomolar affinity. The active signaling complex is then formed by recruiting two copies of IFNγR2,[72] making the signaling complex hexameric.

The Type III interferon receptor (IFNλ receptor).

The Type III cytokines IFN-λ1, -λ2, and -λ3 (also known as IL-29, IL-28A, and IL-28B, respectively) signal via a single receptor. This Type III receptor consists of two chains, IL-28R and IL-10Rβ, the latter of which is shared with receptors for the IL-10 family. The structure of IFNλ bound to its receptor is reminiscent of the GH:GHR complex structure with the ligand occupying a specially similar position albeit with a very different angle of occupation.[73] The IL-28R chain binds cytokine with high affinity and this binary complex then recruits IL10Rβ.

IL-10 family receptors.

IL-10 family cytokines (IL-10, IL-19, IL-20, IL-22, IL-24, and IL-26) can be subdivided into two classes. Those that use the shared IL10Rβ chain (IL-10, 22, 26) and those that use the shared IL20Rβ chain (IL-19, 20, 24).

Like IFNγ, IL-10 (and probably IL-26) are homodimeric cytokines and their receptors likewise contain two copies of each of the two individual chains. The shared chain for these two receptors is IL10Rβ and the cytokine-specific chains are IL-10Rα and IL-20Rα, respectively. In each case, the cytokine-specific chains bind with high affinity to ligand (sub-nanomolar), while IL-10Rβ is the lower affinity (high micromolar – mM) receptor.[74,75] IL10Rβ also forms half of the receptor for IL-22, a monomeric cytokine.[75] The cytokine specific chain can be IL22Rα1, or alternatively it can recruit a soluble receptor (IL22BP) that can mediate its biological effects.

The remaining IL-10 family cytokines (IL-19, 20, 24) bind to two distinct receptors. The so-called Type II IL-20 receptor consists of a shared IL20Rβ and a cytokine-specific subunit IL22R. The type I receptor contains the same shared IL20Rβ chain along with the IL20Rα subunit. IL-20 and IL-24 can signal via both receptors whereas IL-19 binds to the type I receptor only. In the Type I receptor, IL20Rβ is the high affinity subunit.[76] Overall the structure of the cytokine:receptor complex is similar to that of IFNλ. Modeling of the type II receptor suggests a similar overall architecture.[77]

Cytokine receptor cytoplasmic domains

Both Class I and II cytokine receptors are complexes of single pass transmembrane-domain containing proteins. Many cytokine-specific alpha receptor chains contain a short cytoplasmic region with no known function, but each functional receptor complex always consists of at least two (most often precisely two) individual receptor chains with long intracellular regions (several hundred amino acids in length) that are the scaffolds upon which signaling is initiated. These unstructured[59,60] cytoplasmic domains exist to provide sequence-specific docking sites for JAKs and STATs. The JAK-binding regions are known historically as the Box 1 and Box 2 motifs and are membrane proximal whilst the STAT binding motifs are located towards the C-terminus, distal to the membrane. In some cases, in between these two motifs are additional binding sites for negative-regulators such as the SOCS proteins.

The JAK binding motif: Box 1/2.   Mutagenesis studies first identified two regions on the cytoplasmic tail of receptors, termed Box 1 and Box 2, critical for the association of JAKs with receptor.[78] Box 1 is proline rich and is located approximately 10 residues from the C-terminus of the transmembrane region of the receptor, whilst Box 2 is about 10–50 residues further downstream and is rich in hydrophobic residues. Apart from these features, these regions share low sequence homology between different receptors. Moving the Box 1/2 motif further from the membrane abolishes the ability of JAK to associate[79] suggesting that membrane proximity is important for cytokine inducible activation. Specific receptors bind to specific JAKs, although some receptors (most notably the G-CSF and IL-6 family of receptors) can bind multiple JAKs.[80] It is sequence differences within the Box 1 and Box 2 motifs of different receptors that determine which JAK (JAK1, JAK2, TYK2, or JAK3) is bound. For example, structural studies identified a PxxLxF sequence in JAK1-binding Class II receptors as the key motif required for JAK1 interaction.[81] No obvious motifs have yet been defined for other receptors. Very recently, it has been shown for two homodimeric receptors (EPOR and LeptinR) that the small region between the membrane and the Box 1 motif coordinates JAK homodimerization and is necessary for efficient signaling.[82] It may be assumed (but has not yet been shown) that the same region of other receptors may mediate JAK dimerization as well.

STAT-binding motifs.   Once JAKs are activated (see below) they phosphorylate distal tyrosines on the receptor intracellular domains and these sites act as docking sites for the signal transducer and activator of transcription (STAT) transcription factors. Thus, all receptors contain conserved tyrosines that fulfill this function. Just as different receptors bind different JAKs, so they also bind different STATs.[83] The ability of a certain cytokine to induce activation of a particular STAT is driven purely by the STAT-binding sites contained within its receptor. STAT-binding sites from one receptor can be replaced with binding sites for different STATs from other receptors and thereby activate non-physiological STATs.[84,85] In many cases, a single pTyr can recruit multiple members of the STAT family, albeit with differing affinity.[86] It is likely that the affinity for one STAT over another is purely a function of the sequence immediately surrounding the phosphotyrosine, for example pYxxP,[87] pYxxQ,[85] pYxxL,[88] and pYxxF[89] sequences are associated with recruitment of STAT1, STAT3, STAT5, and STAT6, respectively.

Some receptors contain multiple STAT binding sites, for example the IL-6 receptor signaling chain (gp130) contains four STAT3 binding motifs[90] and EPOR contains four STAT5 binding motifs on each chain.[88] In other cases, such as in IFNγR, only a single STAT binding site is found.[87]

Other stimulatory sites.   In addition to stimulating signaling by STATs, many cytokines also induce additional signaling pathways via the same receptors,[91,92] such as the MAPK and PI(3)K pathways. For example, IL-6 family cytokines stimulate both these pathways. Although the mechanism of PI(3)K stimulation is unclear, the MAPK pathway is activated via the phosphatase SHP2:[93] SHP2 binds to phosphotyrosine 759 on the gp130 subunit of the IL-6 receptor; cytokine exposure activates SHP2; and this leads to Ras/Raf signaling which stimulates the MAPK cascade and ultimately transcriptional activators such as Elk.

Negative-regulatory sites.   In addition to stimulatory sites on the intracellular domains of cytokine receptors, there are often sites for regulatory proteins that inhibit signaling. In general, these inhibitory proteins interact with phosphotyrosine motifs on the receptors via SH2 (Src-homology 2) domains (as do the STATs) and hence they are only recruited once the receptors are phosphorylated. The SOCS proteins are a family of negative-regulatory proteins that all contain SH2 domains and many bind to specific receptor sites to inhibit signaling.[94] Particularly well-characterized sites are found on the IL-6 and G-CSF receptors (for SOCS3) and the GHR (for SOCS2). In all cases these SOCS binding sites are located C-terminal to the JAK-binding region of the receptor.

Janus Kinases (JAKs)

There are four members of the JAK family found in all vertebrates: JAK1, JAK2, JAK3, and TYK2[3–5,95] (see Table II). Each JAK is ca. 1000 residues in length and consists of four distinct domains: An N-terminal FERM (band 4.1, Ezrin, Radixin, Moesin) domain followed by an SH2 domain and two kinase domains (Fig. 5). The
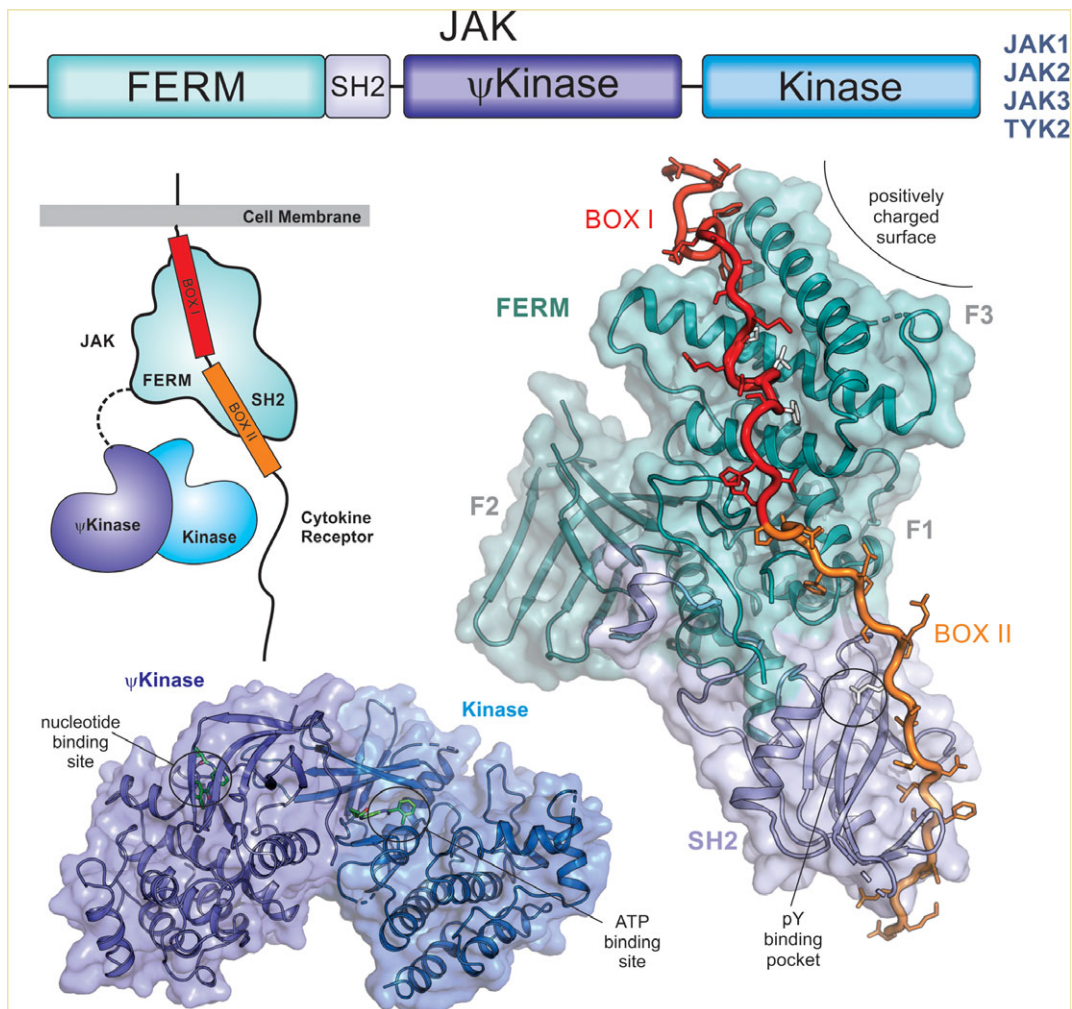
Figure 5. Janus kinases (JAKs). There are four members of the JAK family (JAK1, JAK2, JAK3, and TYK2) and all share similar domain architecture (top). The FERM and SH2 domains tether JAK to the receptor, binding Box I and Box II respectively (structure shown on the right, PDB ID: 5L04)). The pseudokinase (ψkinase) regulates the activity of the catalytically active kinase domain (bottom, PDB ID: 4OLI) via a mechanism that is unclear. There is no structure of a full-length JAK protein and hence the relative orientation of the N- and C-terminal halves of the protein is unknown (indicated schematically on the left).

first of these kinase domains is catalytically-inactive and is therefore a pseudokinase domain (also termed the JAK Homology 2 or JH2 domain). The C-terminal kinase domain is the catalytic domain in each JAK, historically termed the JH1 domain.

FERM/SH2 domains. The FERM and SH2 domains together are responsible for binding to the receptor. This provides the specificity required to target a particular JAK to a particular receptor chain. With a few exceptions, a receptor chain only binds to a single, specific member of the JAK family. The FERM and SH2 domains are closely associated and form a single structural unit.[81,96,97] Together they provide the binding site for the Box 1 and Box 2 motifs found in all cytokine receptors. The FERM domain is a three-lobed structure consisting of an F1 lobe (ubiquitin-like), an F2 lobe (acyl-CoA-binding protein-like), and an F3 lobe (pleckstrin homology domain). The F2 lobe is primarily responsible for binding the membrane-

proximal Box 1 motif on the receptor and in addition contains a large surface with significant positive charge that probably interacts with the cell membrane.[81,96,97] The SH2 domain interacts with the Box 2 motif. Interestingly, it coordinates a glutamate in the same way that other SH2 domains coordinate phosphotyrosine. Together, the Box 1 and Box 2 motifs form a long (85 Å), largely extended epitope that buries over 3000 Å^2 of JAK.[97] The relevant contributions of Boxes I and II toward overall affinity differ between receptors. For example, in the IFNλ receptor, Box 1 provides most of the affinity whilst the addition of Box 2 adds a further 10-fold increase.[81] In contrast, TYK2 binding to the IFNα receptor appears to be dominated by Box 2.[96]

Pseudokinase domain. The pseudokinase domain, critical for modulating the activity of the C-terminal tyrosine kinase domain,[98–100] is the most enigmatic of the JAK domains. Although it adopts a typical kinase

fold,[100–106] it is catalytically defective. The pseudokinase domain of JAK2 shows some residual activity that may be responsible for autophosphorylation *in cis* on two auto-inhibitory phosphorylation sites, Ser523 and Tyr570[100]; however, it is likely to be completely inactive in other members of the JAK family,[103] despite maintaining the ability to bind ATP.[107] An emerging idea is that ATP binding by pseudokinase domains functions as a "molecular switch"[107]; however, this remains to be established for the JAK family.

The ability of the pseudokinase domain to regulate the activity of the kinase domain has been recognized for some time, since the observation that a mutation in the pseudokinase domain of the *Drosophila* JAK homolog, hop, resulted in hyperactive kinase activity and a leukemia-like disease.[108] Importantly, deletion of the pseudokinase domain increases the basal level of kinase activity but prevents a further increase in activity in response to cytokine.[98,99] The importance of the pseudokinase domain was further highlighted in 2006 when it was discovered that a V617F point mutation in human JAK2 was causative of a range of myeloproliferative neoplasms.[109–111] This group of diseases, which includes Polycythemia Vera, Essential Thrombocythemia and the more severe Primary Myelofibrosis display aberrantly high levels of myeloid cells such as erythrocytes and platelets. The V617F mutation leads to an increased basal activity of JAK2 and cytokine-independent signaling through the EPO and TPO receptors. The analogous mutation in other JAK family members has also been shown to cause aberrant signaling.[103] Further mutations in the linker between the SH2 and pseudokinase domains, the so-called exon 12 mutations, have since been discovered to lead to the same diseases.[112,113] Given that the pseudokinase domain plays a role in both switching on the kinase domain and controlling its maximal activity and that mutations within this domain cause disease there have been many efforts to understand this at a molecular level. To date, there is only a single experimentally-determined structure of the tandem pseudokinase-kinase domains (from Tyk2).[102] This structure showed that the two domains adopt a back-to-back orientation, and activating mutations cluster together at the interface between these domains indicating that a physical interaction between the pseudokinase and kinase domains is required for correct regulation of JAK activation. Although equivalent structures of other JAKs have not been forthcoming, sophisticated modeling of the analogous construct from JAK2 gave a near identical prediction of the overall structure[114] even prior to the TYK2 structure being solved.

Kinase domain. The kinase domain of JAK is the domain required for phosphorylation of receptor and subsequently the STAT transcription factors. All JAKs are tyrosine kinases and their kinase domains display a typical fold.[115–119] Tyrosine kinases catalyze the transfer of a phosphate from ATP to a tyrosine-containing protein. The general organization of a kinase includes two lobes: an N-terminal lobe consisting mostly of β-strands and a larger, mostly α-helical C-terminal lobe. The ATP-binding site (and thus the active site) exists between these lobes. The conserved residues for ATP-binding lie mainly on the N-terminal lobe whilst the motifs for tyrosine binding, phosphotransfer and Mg^{2+}-binding are found mostly on the C-terminal lobe. Magnesium is an essential factor for kinases, aiding the co-ordination of ATP. A number of important motifs are characteristic of all kinases and these include (i) the VAIK motif of the β3 strand in the N-lobe, in which the lysine anchors and orients the α- and β-phosphates of ATP; (ii) an aspartic acid in a HRD motif of the catalytic loop (β6–β7) which provides the catalytic base; (iii) an aspartic acid in the DFG motif within the activation loop (β8–β9) that binds the Mg^{2+} coordinating the β- and γ-phosphates of ATP[120]; and (iv) a glycine-rich loop (G-loop; GXGXXG) between the β1 and β2 strands of the N-lobe. The latter is a key factor in binding ATP through hydrogen-bond formation between the backbone of the loop and the ATP γ-phosphate.[121] In addition to these, two hydrophobic regulatory "spines" determine whether a kinase is capable of enzymatic activity, the Regulatory-spine and the Catalytic-spine.[122,123]

Another important motif is the activation loop. This loop contains two consecutive tyrosines and the phosphorylation-state of the first of these determines whether the enzyme is active or inactive. JAKs phosphorylate one-another at this position *in trans* upon cytokine stimulation.[7] Although many structures have been solved of JAK kinases domains with their activation loop in the phosphorylated (active) conformation,[115–119] the inactive conformation has not been observed structurally but can be inferred from studies on other tyrosine kinases, in particular the insulin and IGF receptor kinases, in work led by Stevan Hubbard.[124–127] In the inactive conformation, the activation loop forces the N- and C-lobes apart and blocks the ATP binding site. In addition, the substrate-binding site (which is partly formed by residues within the loop) is not present and the orientation of the DFG motif does not allow magnesium binding.[124] Phosphorylation results in reorientation of the activation loop such that it swings out of the ATP-binding site and lies flat against the solvent exposed surface of the C-lobe. This allows ATP and substrate to bind and catalysis to occur.[127] Structurally trapping a tyrosine kinase in the process of auto-activation (in trans) has only been successfully performed for the IGF1 receptor and the activation loop in this conformation is highly extended, allowing the first tyrosine to access the active site of a second kinase molecule and become phosphorylated.[126] The

second tyrosine within the activation loop has been found fully or partially phosphorylated in a number of JAK structures[116–119]; however, its importance in terms of catalytic activity is unclear. In our studies on JAK2, we observe no difference in the activity of the kinase domain when this residue is mutated to phenylalanine (unpublished data).

The final motif of interest in the JAK kinase domains is the JAK insertion loop that is peculiar to this family.[117] This loop links the αH and αI helices in the C-lobe of the kinases and in JAK1, JAK2, and TYK2 is capped by a "GQM" motif that allows them to bind to SOCS1 and SOCS3, two regulatory proteins that can inhibit the catalytic activity of these kinases. JAK3 does not contain a GQM motif in its JAK insertion loop and is, therefore, immune to SOCS-mediated inhibition.[128]

Signal Transducers and Activators of Transcription (STAT) proteins

The STATs are a family of proteins named for their dual roles of (1) transducing signals from cytokines and (2) promoting transcription of specific genes. The STATs predominantly reside in the cytoplasm as inactive dimers but are rapidly activated upon initiation of cytokine signaling and translocate into the nucleus.[129–131] There are seven mammalian STATs (STAT1-4, STAT5a, STAT5b, and STAT6)[132–134] and each contains several conserved features; an N-terminal region followed by a coiled-coil domain, a DNA binding domain, a linker region, an SH2 domain, and a C-terminal transactivation domain (Fig. 6). Located between the SH2 domain and the transactivation domain is a single conserved tyrosine residue which is the site at which the STAT proteins are phosphorylated by the JAKs and is essential for their activation.[137] STATs exist as dimers both in their active and inactive forms, but the structural arrangement of the two dimeric species is very different. Most STATs function primarily as homodimers; however, heterodimeric complexes do occur and are particularly important for STAT2, which only acts as a heterodimer. STAT2 acts downstream of Type I and III interferons but it does so as part of a complex called ISGF3 (IFN-stimulated gene Factor 3). ISGF3 is a three-protein complex that contains STAT2,
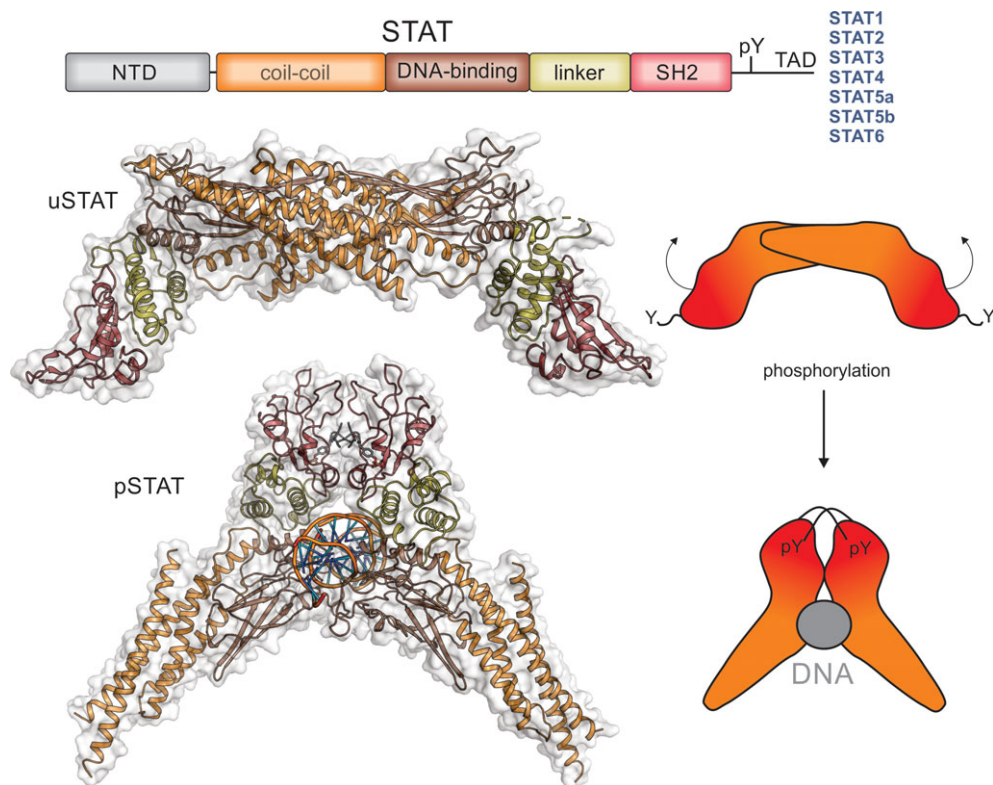
Figure 6. STATs. The Signal Tranducers and Activators of Transcription (STATs) are a family of latent transcription factors that are activated by phosphorylation following cytokine exposure. The same domain architecture is shared by all STAT proteins and is shown schematically above. Unphosphorylated STAT (uSTAT) exists as an antiparallel dimer in the cytoplasm (upper). The SH2 domain (red) of uSTAT binds to phosphotyrosines in cytokine receptors which allows JAK to phosphorylate a specific tyrosine located between the SH2 and transactivation domain (TAD). This phosphotyrosine is then targeted by the SH2 domain of the other monomer inducing a large rotation between the two subunits of the dimer and allowing phosphorylated STAT (pSTAT) to occupy its DNA-binding competent dimeric structure (lower). The structures shown here are of STAT1 (PDB ID: 1YVL,[135] 1BF5[136]) with the colors matching the schematic representation above. The N-terminal domain of STAT does not appear to form a stable interaction with the rest of the molecule and is not shown here. The transactivation domain (TAD) is unstructured but allows binding of accessory factors.

STAT1 and a third transcription factor called IRF9 which binds the STAT2 coiled-coil domain.[138] Figure 3 highlights the dominant STAT family members activated in response to individual cytokines; however, it should be noted that there is often low-level activation of additional STATs.

N-terminal region. The N-terminal domain of the STATs is largely conserved between all seven proteins and forms a bundle of alpha-helices oriented roughly at right angles to one-another.[139,140] Generation of chimeric STAT molecules where the N-terminal domain of STAT1 was replaced with that of STAT2 or STAT5 revealed a role for the N-terminal region in nuclear translocation and deactivation.[141] The N-terminal domain of the STAT proteins also plays a role in cooperative DNA binding[142] between STAT dimers in regions of DNA where there are clusters of STAT binding sites, perhaps accounting for some of the specificity of cellular response to different cytokines.[139]

Coiled-coil domain. The coiled-coil domain of the STAT proteins is a region of approximately 180 amino acids immediately following the N-terminal domain. It comprises four antiparallel alpha-helices which form a bundle in a down-up-down-up topology that is a major site of dimerization in the inactive form but then projects outwards from the core of the protein after activation. Allowing DNA binding and providing a surface for other proteins such as transcription factors to bind.[136]

DNA binding domain. The DNA binding domain allows STATs to function as transcription factors and targets specific DNA sequences.[143] All STATs recognize palindromic DNA sequences with a $TTCN_{2-4}GAA$ motif.[144] While all STATs bind this motif, their sequence preferences differ. STAT1 and STAT5 show preference for sites with a three-base pair spacer between the C and G (N3), with STAT1 also displaying preference for binding sites with a C at the -7 position, relative to the palindrome centre.[145] STAT6, unlike the other STAT proteins, binds to sites with a four-base pair spacer (N4)[145,146]; however, STAT5[147,148] has also been shown to bind weakly to N4 sequences.[145] STAT4 prefers the palindromic sequence (T/A)TTCC(C/G)GGAA(T/A) where the first and last T/A sites outside of the usual motif are also necessary for binding.[149]

Linker and SH2 domain. Immediately downstream of the DNA-binding domain are the linker and SH2 domains. SH2 domains are modules that bind phosphotyrosine (pTyr) when it is embedded in a particular amino acid sequence motif. Each SH2 domain will have its own preferred sequence surrounding the pTyr, usually dictated by residues in the +1 and + 3 positions (relative to the pTyr). Once JAK is activated

it immediately phosphorylates tyrosine residues in the receptor to which it is bound and the presence of an SH2 domain allows STATs to bind to those phosphorylated cytokine receptors. Thus which STATs will be activated by a particular cytokine depends on which receptor(s) their SH2 domain will bind.[150,151] Different STATs can bind to the same sequence with different affinities which accounts for some of the pleiotropy of the STAT family and redundancy of biological outcomes.[152]

STAT activation and transcription. Prior to activation, the STATs are found as inactive dimers in the cytoplasm. The DNA-binding and coiled-coil domains of two STAT monomers interact to form a reciprocal dimer (Fig. 6).[135,153,154] STATs are activated by JAK-catalyzed phosphorylation of a specific tyrosine between the SH2 and transactivation domain. Instead of binding the phosphorylated receptor, the SH2 domain of each STAT monomer now binds the newly created pTyr in the other monomer resulting in dissociation from the receptor and re-orientation to form the active conformation with an exposed DNA-binding domain. These dimers then translocate into the nucleus and induce transcription of genes whose promoters contain the appropriate STAT binding sites. The STATs form a scissor-like structure around the DNA[136] (stabilized by the reciprocal interactions between the SH2 domain of one monomer and the pTyr of the other) and transcription is facilitated by the recruitment of transcriptional co-activators such as CBP/P300. Determining the complete set of genes upregulated by each of the STAT proteins has been difficult due to activation of other transcription factors by pathway crosstalk; however, several hundred to several thousand genes appear to be activated by each activated STAT[155–158] as well as a number of genes being downregulated.[159]

The dephosphorylation of STAT by phosphatases in the nucleus allows them to shuttle back into the cytoplasm for further rounds of activation.[160–162] While STATs are known to be active transcription factors in their phosphorylated state, there are some cases where unphosphorylated STATs also appear to play a role in gene transcription.[163,164] It has also been suggested that unphosphorylated STATs are important for maintaining heterochromatin stability by associating with HP1, a heterochromatin protein necessary for heterochromatin formation.[165,166] Unphosphorylated STAT5 has an important role as a transcriptional repressor in megakaryocytes where it blocks differentiation.[167]

Negative regulation of JAK–STAT signaling

SOCS proteins

Signaling via the JAK–STAT signaling pathway is a dynamic process that involves the rapid transmission

of signal from the cell membrane to the nucleus followed by a highly organized response and subsequent controlled downregulation and attenuation of the initial signal.[137,168] SOCS proteins are the primary drivers of signal attenuation, they are induced by cytokine exposure (via STAT) and then act as negative-feedback inhibitors to switch off the signaling cacade.[13,94,169,170] There are eight SOCS proteins encoded in the human genome, SOCS1-7 and CIS.[13,169–171] All eight contain N-terminal domains of various length and often unknown function but are defined by the presence of a central SH2 domain and a short, C-terminal domain called the SOCS box.[171] CIS and SOCS1/2/3 are the members of the family associated with inhibiting signaling by JAK/STAT-inducing cytokines while other SOCS proteins appear to regulate signaling by factors such as EGF and insulin.

Although induced by many cytokines,[170] and potent inhibitors of many cytokine signaling pathways when artificially overexpressed, knockout studies have shown that each SOCS protein is specific for only a subset of cytokines.[172,173] For example SOCS3 is induced by IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-10, IL-11, IL-12, IL-13, IL-21, IL-22, G-CSF, GM-CSF, LIF, PRL, IFNα, IFNγ, GH, EPO, TPO, OSM, CT1, CNTF, and leptin[170,174–185]; however knockout studies have shown that only IL-6 family cytokines,[172] G-CSF,[173] and Leptin[186] are aberrantly regulated in its absence. This specificity is provided by the SH2 domains of SOCS proteins which in general bind to phosphotyrosine-containing motifs in specific cytokine receptors and therefore only inhibit signaling by cytokines that act via those receptors. For example the SH2 domains of CIS, SOCS2, and SOCS3 bind to motifs in the IL-2,[32] GH,[187] and IL-6[92,188,189] receptors, respectively.

Once bound to cytokine receptors, SOCS proteins induce their ubiquitination and degradation through the activity of their SOCS box domain. The SOCS box is structurally similar to a domain first described in VHL[190] and has two distinct binding properties. The first is a short motif that allows it to bind to elonginBC,[191,192] an adapter complex that is also recruited by VHL. Once the ternary SOCS/elonginBC complex has formed the second motif can then recruit Cullin5, a 100 kDa E3 ligase scaffold.[193] This differs from VHL which uses its SOCS box-like domain to recruit Cullin2.[194] Cullin5 is constitutively associated with a RING domain protein, Rbx2,[194,195] and this provides the E3 ubiquitin ligase activity by recruiting E2 ubiquitin conjugating enzymes to catalyze the ubiquitination of protein substrates. Therefore, SOCS proteins act as the substrate recruitment modules for Cullin5-based E3 ligases and proteins bound by the SOCS SH2 domains (usually receptors) are ubiquitinated and subsequently degraded by the proteasome.

SOCS1 and SOCS3. SOCS1 and SOCS3 are unique amongst the SOCS family in containing a short motif called the Kinase Inhibitory Region (KIR).[196,197] This allows these two SOCS proteins to bind directly to the JAK kinase domain and inhibit its catalytic activity. The KIR is unstructured in the absence of JAK[198,199] but upon binding adopts an extended conformation that sits in the substrate binding groove of the kinase.[200] This occludes the substrate-binding site and prevents JAK from phosphorylating downstream substrates. SOCS1 and SOCS3 inhibit JAK1, JAK2, and TYK2 but not JAK3 due to the absence of a "GQM" sequence in the latter kinase.[128] SOCS1 is approximately 10-fold more potent than SOCS3 at inhibiting JAK, mainly due to sequence differences in the KIR.[128,201]

In vivo, SOCS3 is a specific inhibitor of IL-6 family cytokines,[43,202–204] G-CSF,[172,173] and leptin[186] as these cytokines all signal via receptors that contain binding sites for the SOCS3 SH2 domain.[205] SOCS3 engages the receptor with its SH2 and simultaneously inhibits the JAK1, JAK2 or TYK2 associated with that receptor.[200] SOCS1, on the other hand, is a potent inhibitor of interferon, IL-12/23, IL-4/13 and IL-2 family cytokine signaling[34–41,206–208] however, does not target the receptors for those cytokines with its SH2 domain. The target of the SOCS1 SH2 domain is unclear, it binds tightly to the activation loop sequence of all four JAKs when they are synthesized as synthetic peptides *in vitro*, however, appears to be sterically hindered from doing so when the activation loop is part of an intact kinase domain.[201] Further work is needed to clarify to this issue.

Phosphatases

Association of cytokine with the appropriate receptor triggers a cascade of intracellular tyrosine phosphorylation: JAKs are auto-phosphorylated *in trans*, receptors are then phosphorylated by these activated JAKs and finally the STATs are phosphorylated allowing them to adopt their active conformation. Therefore it is no surprise that phosphotyrosine phosphatases (PTPs) play a crucial role in regulating these signaling pathways.[209,210] Six phosphotyrosine phosphatases in particular have been shown to regulate JAK/STAT signaling: the receptor tyrosine phosphatases CD45 and PTP-RT, two related cytoplasmic phosphatases PTP1b and TC-PTP, and the SH2 domain containing phosphatases SHP1 and SHP2.[211] These phosphatases are constitutively expressed and are, therefore, not feedback-inhibitors. As such, they tend to restrain the amplitude of the signaling cascade rather than controlling its duration. It is the balance between the action of these phosphatases and the activity of the JAKs that determines the flux through the pathway. Determining the true targets of these phosphatases (JAKs, STATs, or receptors) has been difficult and at times contentious.

The receptor phosphatases CD45 and PTPRT. CD45 and PTPRT are both receptor phosphatases comprising an extracellular receptor-like region, a transmembrane domain and two intracellular tandem phosphatase domains. For both CD45 and PTPRT the first phosphatase domain is the catalytically active domain, whereas the second is a catalytically dead pseudophosphatase domain that is thought to have regulatory roles in both proteins. Full length CD45 is ~140 kDa; however, alternative splicing gives rise to several different sized isoforms. The extracellular region of CD45 is comprised of three FnIII domains (Fig. 7). CD45 is highly expressed in hematopoietic cells and thought to dephosphorylate all four JAK proteins.[13,212] Cells deficient in CD45 display extended signaling in response to IL-7, EPO, and interferon stimulation. PTPRT is a large protein containing an N-terminal MAM domain followed by an Ig domain, four FnIII domains, a transmembrane domain and two tandem PTP domains. PTPRT directly interacts with and dephosphorylates the critical tyrosine residue in STAT3, pY705.[213]

The SH2 domain containing phosphatases SHP1 and SHP2. SHP1 and SHP2 are cytoplasmic SH2 domain containing phosphatases. They are approximately 70 kDa, and composed of two SH2 domains and a single PTP domain that is negatively regulated by interactions with the SH2 domains.

The expression of SHP1 is limited to the hematopoietic lineage, where it regulates IL-3, EPO, IFNα, and potentially other cytokine-induced signaling by dephosphorylating JAK1, JAK2, and TYK2.[214–218] SHP2 is ubiquitously expressed, but also plays an essential role in the regulation of hematopoiesis. Knockout of *shp2* leads to increased JAK1 autophosphorylation and upregulation of interferon signaling[217,219] implying a role as a negative regulator. However, SHP2 is better characterized as a *positive* regulator of cytokine signaling. For example, it binds to pY759 on gp130 and activates the MAPK signaling cascade in response to IL-6 and LIF. In fact, SHP2 was the first tyrosine phosphatase to be identified as a proto-oncogene and somatic activating mutations of SHP2 have been identified in acute myeloid leukemia (AML) and B cell acute lymphoblastic leukemia (BALL).

The cytoplasmic phosphatases PTP1B and TC-PTP. PTP1B and TC-PTP are two highly related,
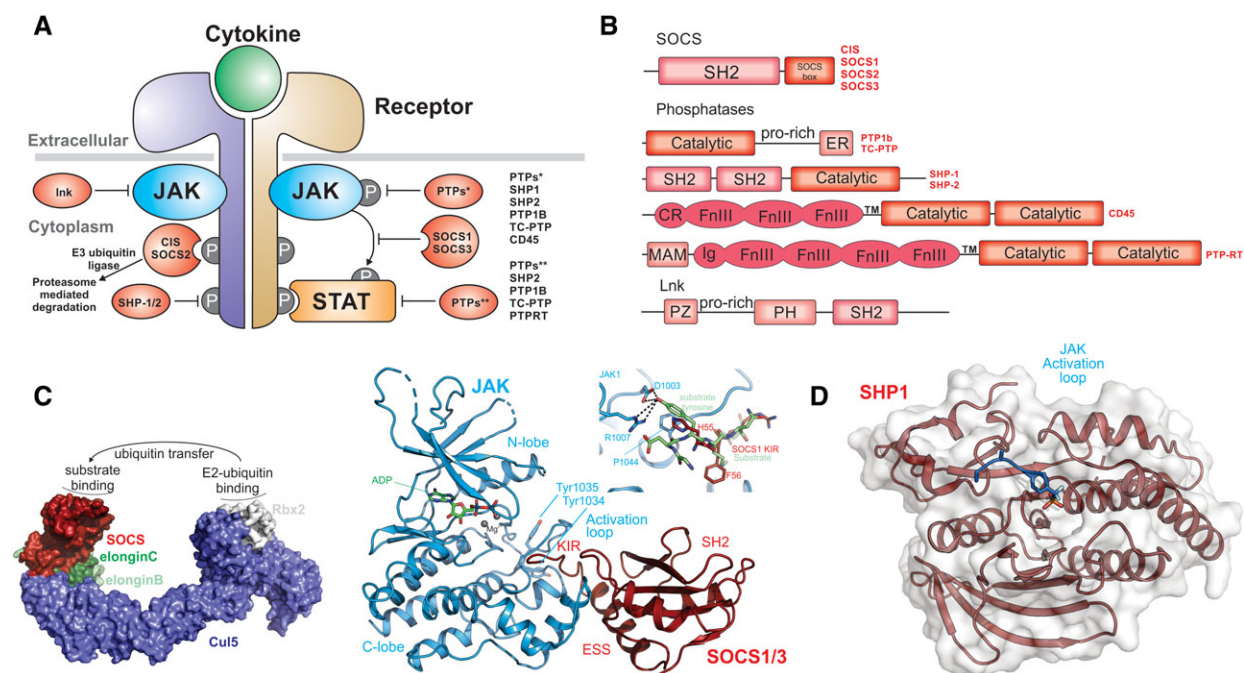
Figure 7. Regulation of cytokine signaling. (A) Schematic diagram showing regulators of cytokine signaling and where they act. (B) Domain architecture of the proteins indicated in A. (C) The primary negative feedback regulators of cytokine signaling are a subset of the SOCS (Suppressors of Cytokine Signaling) family, CIS, SOCS1, SOCS2, and SOCS3. These proteins function as the substrate recruitment modules of an E3 ubiquitin ligase (model structure shown in surface representation) and promote the ubiquitination and degradation of cytokine receptors and potentially other substrates. Substrates bind to the SH2 domain of SOCS proteins (red) and ubiquitin is transferred via an E2 ubiquitin-conjugating enzyme that docks onto the RING-domain protein Rbx2 (white). SOCS1 and SOCS3 (right) can also directly inhibit the JAK kinase domain by using their kinase inhibitory region (KIR) to block the substrate binding site of the kinase (PDB ID: 6C7Y) (model of a substrate overlay shown inset). (D) Six tyrosine phosphatases have been shown to be important regulators of cytokine-pathway activity, acting by dephosphorylating JAKs, STATs, or receptors. The structure of one of these, SHP1, has been solved in complex with the JAK activation loop of JAK2 (PDB ID: 4GSO).

~40 kDa phosphatases that are tethered to the cytoplasmic face of the endoplasmic reticulum (ER).[220–222] Substrate-trapping mutants of PTP1B have been shown to interact directly with the activation loop of JAK2 and TYK2 suggesting these as the targets for its catalytic activity however it may also directly dephosphorylate STAT3.[223–226] PTP1B is a powerful regulator of leptin signaling and knockout mice show increased JAK2 phosphorylation in response to that cytokine.[225] TC-PTP is also tethered to the ER, however, a different isoform which lacks the ER-targeting motif is found in the nucleus and can dephosphorylate STAT3. Both JAK1 and JAK3 are dephosphorylated by TC-PTP and its knockout leads to increased IL-2, IFNα and IFNγ signaling.[227,228]

The adaptor protein, LNK

The lymphocyte adaptor protein, LNK, also known as SH2B3, is a member of the SH2 domain containing adaptor protein family which also comprises APS (SH2B1) and SH2B (SH2B2). This family of proteins contains three distinct domains: a dimerization domain (phenylalanine zipper) which allows homo-dimerization, a Pleckstrin Homology (PH) domain and an SH2 domain.[229] While APS and SH2B appear to activate cytokine signaling, LNK is a negative regulator of cytokines that signal via JAK2, particularly EPO and TPO.[230,231] LNK knockout mice have enhanced numbers of hematopoietic stem cells and are hyperresponsive to EPO and TPO and over-expression of LNK inhibits megakaryocyte development. Consistent with its suppressive role, inactivation mutations in LNK are found in ca. 5% of MPNs and also in rare cases of idiopathic erythrocytosis. The SH2 domain of LNK binds directly to JAK2 (at pTyr813, located between the kinase and pseudokinase domains)[232]; however, it is unclear how this regulates signaling.

Case study: IL-6 signaling

IL-6 represents perhaps the archetypal cytokine, it being the closest homologue to cytokines present in extant insect species. IL-6 (and related family members, Fig. 3) are all highly pleiotropic with roles in hematopoiesis, the acute phase response, development and both pro and anti-inflammatory processes.[233–235] Here we provide a summary of the molecular events involved in IL-6 signaling.

IL-6 production can be induced by a variety of stimuli and by many different cell types. The receptor for IL-6, as discussed above, consists of the gp130 shared signaling chain and the non-signaling cytokine-specific IL-6Rα chain. Although gp130 is found ubiquitously expressed, IL-6Rα is found only on hepatocytes and some leukocytes.[236] However, soluble IL-6Rα is released from the liver and this allows IL-6 to signal to many different cell-types in a process called trans-signaling.[237] Although IL-6 on its own has little affinity for gp130, it binds tightly to IL-6Rα

(either membrane bound or soluble). This dimeric complex can then bind with high affinity to gp130, using regions on both the cytokine and the alpha chain. The resulting ternary complex then dimerizes to form the signaling hexamer.[54,238] Thus, the formation of the signaling competent IL-6 receptor complex is an ordered process. The molecular details of IL-6 binding to its receptor were first described by Boulanger et al., who solved the structure of IL-6 bound to the CHR of IL6Rα and the first three domains of gp130.[54] Combined with the full structure of gp130[239] these studies allow a full model of the IL-6/IL-6 receptor complex to be constructed (Fig. 8).[239]

Once IL-6 has bound to its receptor, bringing together the two JAK-associated gp130 chains, this allows transactivation of the associated JAKs. JAK1, JAK2, and TYK2 can all be found associated with gp130 although knockout studies suggest JAK1 may be dominant.[14] The ability of gp130 to associate with different members of the JAK family is unusual (Fig. 3). Upon activation, these JAKs then phosphorylate five tyrosine residues in the gp130 cytoplasmic domain.[90] The four distal sites (Y767, Y814, Y905, and Y915) are motifs targeted by the STAT3 (and to a lesser extent, STAT1) SH2 domain whilst the proximal phosphotyrosine (Y759) binds the negative regulatory protein SOCS3 and the phosphatase SHP2. STAT3 exists as a pre-formed dimer; however, once it is docked onto the receptor it is phosphorylated by JAK on Y705 and pY705 is then bound by the SH2 domain of the opposing monomers. This results in a re-orientation of STAT3 from an anti-parallel to a parallel dimer (Fig. 6) and subsequent translocation to the nucleus where it induces the transcription of target genes.[11] Activated STAT3 is observed within 15 min of IL-6 exposure and STAT3 target genes are observed almost immediately after[240] highlighting a rapid transcriptional response. Importantly, STAT1 is also activated alongside STAT3.

A well characterized activity of IL-6 is its ability to induce the differentiation of monocytes into macrophages. An important component of this activity is the ability of IL-6 to induce the master transcription factor PU.1 alongside hundreds of other genes (RM, unpublished data). One of the most important of the early genes upregulated by IL-6 is SOCS3.[171] SOCS3 mRNA and protein are observed within 30 min of IL-6 exposure and it is one of the most highly expressed early response genes (RM, unpublished data). SOCS3 protein binds to pY759 on gp130 via its SH2 domain and then uses its kinase inhibitory region to inhibit the associated JAK1 (or JAK2, TYK2). This switches JAK off and prevents any further STAT3 phosphorylation. STAT3 is dephosphorylated in the nucleus by TC45, the nuclear isoform of the T-cell protein tyrosine phosphatase (TC-PTP)[162] and is then shuttled into the cytoplasm by exportin-1 to allow for subsequent activation cycles.[241] SOCS3 inhibits the
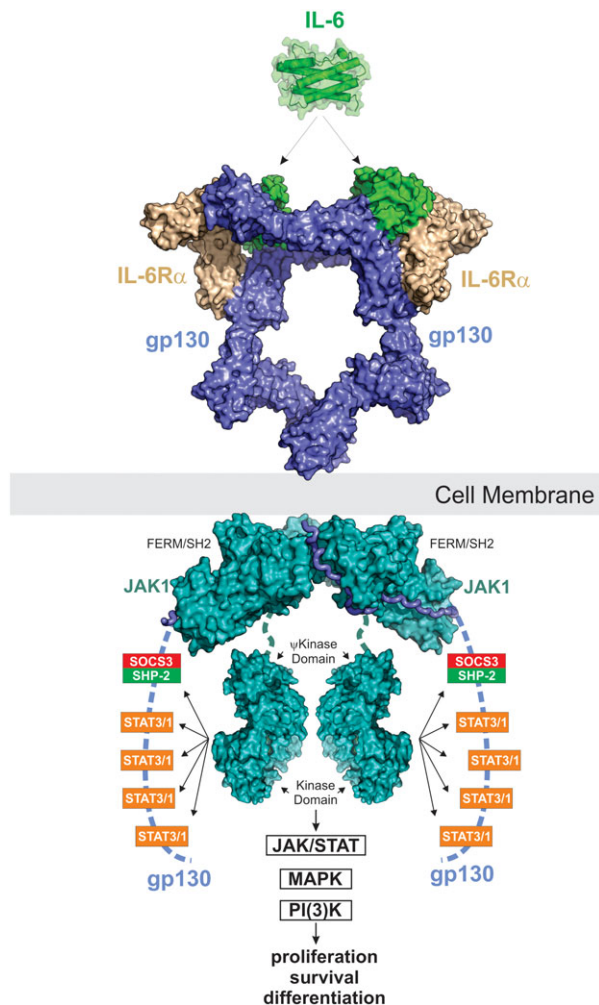
Figure 8. IL-6 signaling. IL-6 signals via a 2:2:2 complex between itself, gp130 and either membrane-bound IL-6Rα (classic signaling) or soluble IL-6Rα (trans-signaling). JAK1, JAK2 and TYK2 can all bind the intracellular domain of gp130; however, JAK1 appears to be the dominant kinase. The structure of JAK1 bound to the gp130 cytoplasmic domain is a model based on the structures of JAK1/IFNλR (PDB ID: 5L04) and the JAK2/EPOR dimeric structure (coordinates kindly provided by R. Ferrao and P. Lupardus). JAK is activated by trans-phosphorylation and then phosphorylates five tyrosine residues on the receptor intracellular domain. The four distal tyrosines are docking sites for STAT3 and to a lesser degree STAT1. Activated STAT3 is then phosphorylated by JAK and translocates into the nucleus to drive the biological response. The MAPK pathway is also stimulated by IL-6 via SHP2 which binds to pY759 using its SH2 domain. The PI(3)K pathway is also activated in response to IL-6. SOCS3 is a direct STAT3 target gene and binds to the SHP2-binding site on the receptor via its SH2 domain. This inhibits MAPK signaling via displacement of SHP2 and also inhibits further STAT3 activation by direct inhibition of JAK catalytic activity.

signaling cascade as described above but may also play a role in shaping the cellular response. Genetic deletion of SOCS3 leads to a wider transcriptional response after IL-6 exposure, in particular there is increased expression of a number of genes associated

with IFNγ (STAT1) signaling. Under normal conditions, therefore, SOCS3 appears responsible for dampening STAT1 transcriptional programs and allowing STAT3 to dominate,[242] while eventually inhibiting both pathways.

Alongside activation of STAT3 (and STAT1), IL-6 stimulates two other signaling cascades: the MAPK and PI(3)K pathways. The phosphatase, SHP2 binds to pY759 on gp130 and promotes activation of the MAPK cascade via a mechanism that is not completely understood but may involve Grb2.[243] SOCS3 also binds to this site and can thereby inhibit both STAT3 and MAPK induced transcriptional responses. How IL-6 induces the PI(3)-kinase pathway is less clear but the end result is activation of the serine/threonine kinase AKT (protein kinase B) at the cell membrane and stimulation of downstream signaling including mTOR.

Unanswered questions

The most important unanswered question in the field is how the activation of JAK (by trans-phosphorylation) is induced by cytokine binding and how this process goes awry in the presence of the activating mutations seen in the pseudokinase domain in human myeloproliferative diseases. The classical explanation given for the process of JAK activation was that simple dimerization of the receptor chains (by cytokine) brought the JAKs into close-enough proximity for their kinase domains to phosphorylate one-another. However it is now clear that many receptors exist as pre-formed dimers even in the absence of cytokine[244] and that it is rather a re-orientation of these chains that allows JAK auto-phosphorylation. In fact, in 2014, Brooks et al. performed a series of FRET-based analyses to show that Growth Hormone induced a *separation* of the intracellular receptor domains and this led to a geometry where the kinase domains of the two JAK molecules were juxtaposed.[245] Such a model supported their earlier analyses which showed that the GHR could be activated by tuning the relative orientation of the TM and juxtamembrane regions even in the absence of cytokine.[246]

This model suggests that prior to cytokine stimulation the pseudokinase domain from one JAK interacts with (and inhibits) the kinase domain from the other. After cytokine stimulation this inhibition is released. The importance of the pseudokinase domain in regulating the kinase domain is of course well-established as described above and by the existence of activating mutations within this domain. The important structure of the TYK2 pseudokinase-kinase domain pair highlighted that activating mutations tend to cluster near the interacting surface between the two domains however did not provide a molecular mechanism for what the pseudokinase domain was actually doing. The only structural information available for transphosphorylation of a tyrosine kinase was provided by crystallographic studies

from the Hubbard laboratory of the IGF1 receptor kinase[126]; however, as far as JAK kinases are concerned, we have no picture of what allows their kinase domains to adopt this position and what prevents them from doing so in the first place. It seems clear that only structures of complete JAK proteins (with and without bound receptor) will provide a full molecular description of how JAK is activated and why this process goes awry upon mutation in human disease.

Disclosure statement

The authors declare no conflicts of interest.

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