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Commentary

Jaks and cytokine receptors—An intimate relationship

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Abbreviations:
EPO, erythropoietin
FERM, four-point-one, ezrin, radixin, moesin
IFN, interferon
IL, interleukin
Jak, Janus kinase
JH, Jak homology
LIF, leukaemia inhibitory factor
OSM, oncostatin M
R, receptor
SCID, severe combined immunodeficiency
SH2, src-homology 2
STAT, signal transducer and activator of transcription
YFP, yellow fluorescent protein

ABSTRACT

Most cytokine receptors lack intrinsic kinase activity and many of them signal via Janus kinases (Jaks). These tyrosine kinases are associated with cytokine receptor subunits, they become activated upon receptor triggering and subsequently activate downstream signalling events, e.g. the phosphorylation of STAT transcription factors.

The successful interplay between cytokines, their receptors and the connected Jaks not only determines signalling competence but is also vital for intracellular traffic, stability, and fate of the cognate receptors. Here, we will discuss underlying mechanisms as well as some structural features with a focus on Jak1 and two of the signal transducing receptor subunits of interleukin (IL)-6 type cytokines, gp130 and OSMR.

Regions that are critically involved in Jak-binding have been identified for many cytokine receptor subunits. In most cases the membrane-proximal parts comprising the box1 and box2 regions within the receptor are involved in this association while, within Jaks, the N-terminal FERM domain, possibly together with the SH2-like domain, are pivotal for binding to the relevant receptors. The exclusive membrane localisation of Jaks depends on their ability to associate with cytokine receptors. For gp130 and Jak1, it was shown that the cytokine receptor/Jak complex can be regarded as a receptor tyrosine kinase since both molecules have the same diffusion dynamics and are virtually undissociable. Furthermore, Jaks take an active role in the regulation of the surface expression of at least some cytokine receptors, including the OSMR and this may provide a quality control mechanism ensuring that only signalling-competent receptors (i.e. those with an associated Jak) would be enriched at the cell surface.

1. Introduction

Cytokines are involved in a variety of biological processes including hematopoiesis and the regulation of the immune system. Upon cytokine-induced receptor aggregation, Janus kinases (Jaks) auto-activate and transphosphorylate themselves. Tyrosine residues within the cytoplasmic tail of the cytokine receptor are subsequently phosphorylated by Jaks,

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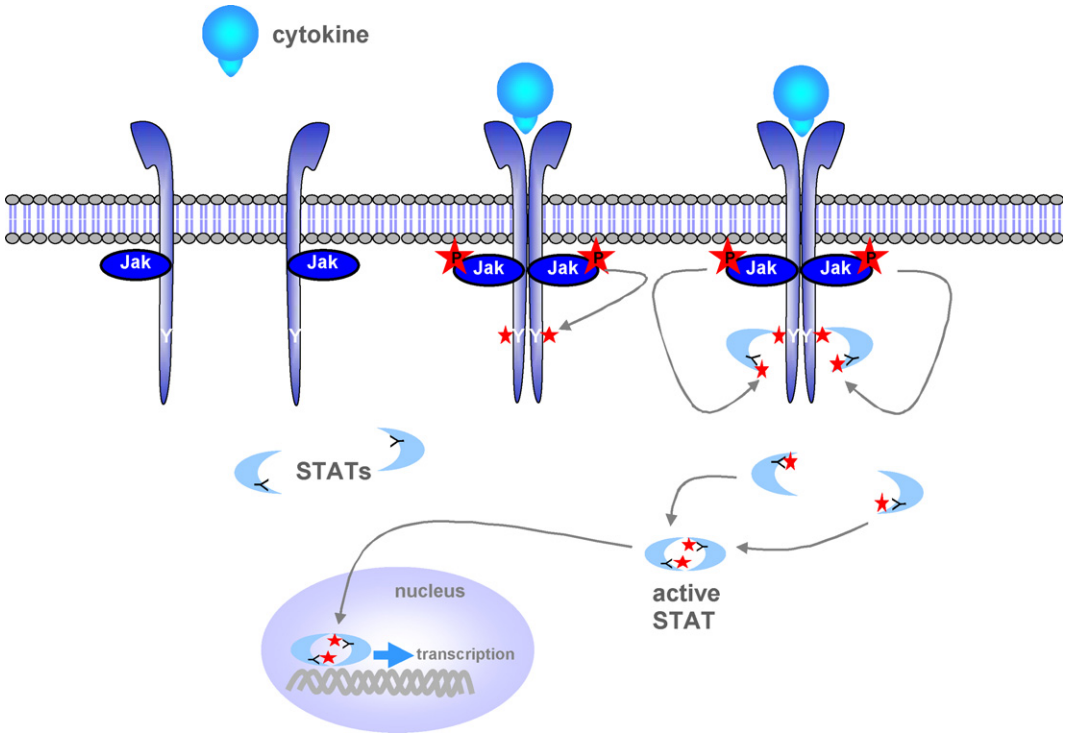

Fig. 1 – Activation of the Jak-STAT pathway by cytokine binding to its receptor. Cytokine-induced receptor aggregation leads to Janus kinase (Jak) phosphorylation and activation. Tyrosine residues within the cytoplasmic tail of the cytokine receptor are subsequently phosphorylated by the Jaks, providing docking sites for SH2 domain-containing signalling proteins including STATs. Tyrosine-phosphorylated STATs translocate to the nucleus where they bind to promoter regions of their respective target genes.

providing docking sites for SH2 domain-containing signalling proteins including signal transducers and activators of transcription (STATs). Tyrosine-phosphorylated STATs dimerise and translocate to the nucleus where they bind to specific DNA sequences in the promoter regions of their respective target genes (Fig. 1) [1–3].

The Jak family of cytoplasmic tyrosine kinases comprises four mammalian members. They are large tyrosine kinases (MW: 120–140 kDa), which are pre-associated with the cytoplasmic regions of signal transducing cytokine receptor subunits [1,2]. Three of the Jaks, Jak1, Jak2 and Tyk2, are expressed in a wide variety of tissues, whereas Jak3 expression is restricted to cells of the hematopoietic system [1,2].

To date, knockout mice of all four Jaks exist and their phenotypic analysis yielded valuable information for the understanding of their physiological role. These mice all show phenotypes that are linked to cytokine signalling deficiencies [4–11]. Evidently, Jaks are involved in a number of inflammatory disorders in which cytokines play crucial roles. Deregulated Jaks have been described to be involved in a number of pathologies, i.e. severe combined immunodeficiency (SCID) [12], some chronic myeloproliferative disorders [13–17] and cancer [18–22].

The focus of the present commentary is the Jak–receptor complex, the first relay station where the information of cytokine binding at the outside of the target cell is transmitted into intracellular signalling cascades ultimately changing nuclear gene expression patterns. An overview about the structural features and functions of Jaks and their receptors is followed by a more detailed presentation of structural aspects governing Jak–receptor interaction, its dynamic behaviour and its implications on receptor trafficking and stability.

2. The Jak/receptor interaction

2.1. *The Jak side of the medal*

Due to the lack of crystallographic data, the structure/function-relationship of the interaction between cytokine receptors and Janus kinases still remains largely elusive. Based on sequence similarities between Jak family members seven Jak homology (JH) domains have been described [23] (Fig. 2), which match the domain structure of Jaks only partially. The JH1 domain at the C-terminus is a classical kinase domain. It is N-terminally preceded by the JH2 domain (pseudokinase domain), which has a kinase domain fold but lacks crucial residues for catalytic activity and for nucleotide binding. The pseudokinase domain has been described to modulate kinase activity [24,25]. The JH3–JH7 regions, which constitute the N-terminal half of the Jaks, are involved in binding to cytokine receptors [26–30]. A part of the N-terminal region of the Jaks (corresponding to amino acids 24–415 in Jak1) shares significant sequence similarity with so called four-point-one, ezrin, radixin and moesin (FERM) domains, and it has been suggested that Jaks might harbour a divergent type of FERM domain [31].

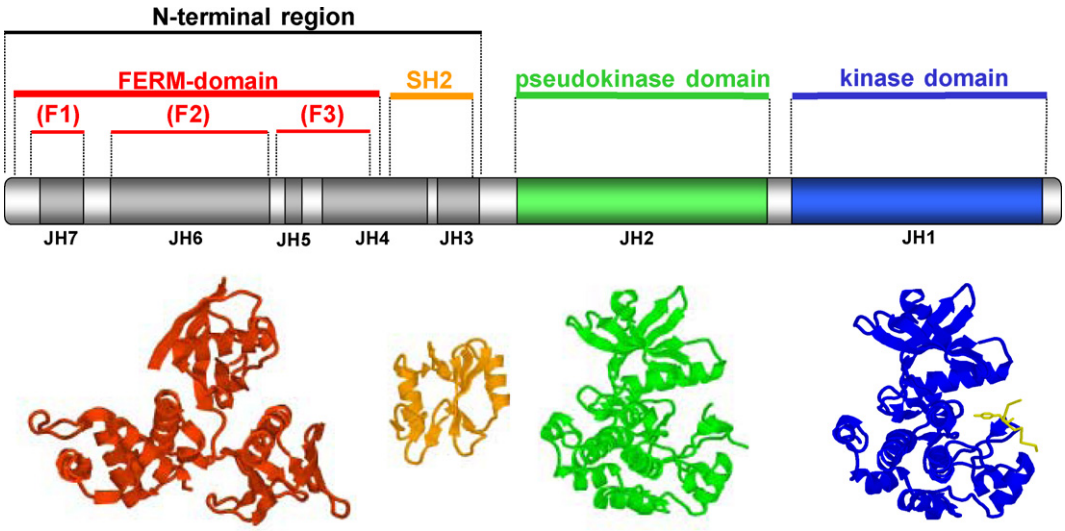

Fig. 2 – The putative structural organisation of Jaks. The ribbon representation of the solved structures of the moesin FERM domain (red), the src-kinase SH2 domain (orange) and the insulin receptor kinase domain (green and blue) give a general idea of the globular organisation of Janus kinases. Note that the kinase domains of Jak2 and Jak3 have been solved recently [73,74]. JH: Jak homology region, SH2: src homology 2 domain, FERM: four-point-one, ezrin, radixin, moesin.

The clover-shaped FERM domains comprise three subdomains: subdomain F1 with a ubiquitin-like β-grasp fold, F2 with an acyl-CoA-binding-protein-like fold, and F3, which shares the fold of the phosphotyrosine binding (PTB) or PH (pleckstrin homology) domains [32]. Structural data of a growing number of solved FERM domains [32–36] illustrates that the JH7 domain corresponds roughly to the F1 subdomain of the FERM domain while JH6 represents the F2 subdomain. JH3–JH5 do not match any defined structural domains, but the region they encompass contains the F3 subdomain of the FERM domain and a putative SH2 domain.

Shortened fragments comprising only the JH6/JH7 domains of Tyk2, Jak2, and Jak3 were able to associate with appropriate receptors upon overexpression [26–29]. Intact cell experiments with different overexpressed Jak1/Jak3 chimeras have shown that substitution of the putative Jak3 FERM domain with that from Jak1 can confer the ability to bind to gp130, the common signal transducing chain of IL-6-type cytokines [37]. Using structural data from FERM domains, a model of the Jak1 F1 subdomain, and site-directed mutagenesis, we have previously shown that mutation of the conserved and structurally important residue Y107 in the F1 subdomain of Jak1 leads to loss of gp130 binding [38] as was expected for a mutant that disrupts the fold of a domain that is involved in binding. The mutated Jak1 residue in question corresponds to Y100 in Jak3, which, when mutated to cysteine, caused SCID in a patient and was no longer able to bind to the IL-2Rγ. Interestingly, Y100 of Jak3 has also been mutated to phenylalanine and this mutant bound the IL-2Rγ chain like wild-type Jak3, which can be explained by the assumption that the phenylalanine residue stabilizes the F1 FERM subdomain by hydrophobic core interactions, similar to the original tyrosine residue. Further mutagenesis data of a region comprising amino acids 98–102 of Jak3 also strongly argue in favour of a FERM domain presence in Jaks. The amino acids L98 and I102, located around the aforementioned Y100, are likewise crucial for Jak3-binding to the common IL-2 receptor γ chain [29]. All these residues are conserved as hydrophobic residues in FERM domains (and also in Jaks) and in the solved FERM structures they, too, are important for hydrophobic core interactions.

To further investigate whether Jak1 contains a FERM-subdomain we used the same approach previously described for the F1-FERM-subdomain, i.e. mutation of conserved residues involved in hydrophobic core stabilisation and applied it to the F2-FERM-subdomain of Jak1. We found that mutation of such residues leads to a loss of Jak1-binding and/or inhibition of Jak1 function (unpublished data). Taken together, these studies provide evidence for the presence of a FERM domain in Jaks and underscore their importance for receptor association.

2.2. *The receptor side of the medal*

Jaks associate with the membrane-proximal region of cytokine receptors. Amongst receptors, there is little sequence homology except for short stretches called the box1 region, a proline-rich motif of eight amino acids, and the box2 region, a cluster of hydrophobic amino acid residues often followed by charged amino acids [39,40]. In a few receptors, areas C-terminal of box2 have been implicated in Jak-binding and activation [41–43], while, in gp130, sequences downstream of box2 do not seem to contribute to binding and activation of Jak [44]. Within the relatively long membrane-proximal stretch, box1 and box2 regions as well as residue W666 were essential for Jak1-binding to gp130 [45]. Interestingly, W652 (a residue in box1) was not involved in binding Jak1 but was crucial for Jak1 phosphorylation and activation [44]. Thus, the role of box1 in cytokine receptor signalling is not restricted to mere Jak-binding. The W652 mutation even behaved dominantly negative, since no signalling occurred when only a single cytoplasmic chain of a gp130 dimer contained the mutation. In fact, mutation of the corresponding residue (W258) in the erythropoietin receptor

(EpoR) leads to impaired Jak activation and is thought to be part of an α-helically organised region, whose precise orientation is necessary to promote signalling [46].

There is growing evidence that rigidity of the transmembrane regions, likely due to α-helical structures, can extend into the intracellular [46,47] as well as to the extracellular region [48]. In this context, addition of the box1–2 region to the C-terminal part of a gp130 mutant, which cannot associate with Jaks, did not restore Jak association [49]. Membrane proximity and/or structural information extending from the extracellular/transmembrane domains again proves to be essential. Signalling through the gp130 homodimer can also be elicited using antibodies. Interestingly, efficient gp130 activation could only be achieved by two distinct agonistic monoclonal antibodies [50,51] supporting the notion that the sterical information of ligand/receptor binding must be transmitted through the transmembrane region into the cell to elicit Jak activation [52,53].

Taken together, it seems to be a general phenomenon that the mere proximity of Jaks in ligand-associated receptor complexes is not sufficient for their activation. Rather, it is likely that the Jak/receptor interaction interface (involving large portions of the receptor box1/2 region and of the N-terminal region of Jaks) is involved in positioning Jaks correctly so that ligand-induced receptor dimerisation and reorientation can lead to their mutual activation.

2.3. *Jaks and receptors: both sides of the medal*

As discussed above, the structural integrity of an α-helical structure, which is more membrane-proximal than box1, and which can be regarded as the continuation of the transmembrane helix is important for the Jak association [46,49]. In many cytokine receptors, the C-terminal part of the box1 motif contains two to three proline residues. It is likely, that at this point the α-helix ends since proline residues are typical α-helix breaking residues and that this proline-rich stretch adopts other secondary structures (i.e. polyproline type II helical structure) or the receptor might have a less ordered conformation from there on. Up to date there are no structural data on the cytoplasmic parts of cytokine receptors. The receptor–Jak interaction might induce a re-structuring of certain receptor residues into defined interaction interfaces. This ''induced fit-like'' scenario seems probable since the length of the non-structured 65 amino acids (23 nm) is about three to four times the dimension of the FERM domain (6–7 nm across) [35]. Alternatively, a non-structured cytoplasmic tail of gp130 may have to adopt a loop structure, which would have to pass several times through the clefts or along the surface of the FERM domain.

It is also conceivable that the activation of Jaks involves structural re-organisation of the Jak/receptor binding interface, and that residues, such as W652 of gp130, are involved in this dynamic process. Moreover, residues like W652 might enforce allosteric interactions essential for Jak1 enzymatic activity. In addition, these possibilities are not necessarily mutually exclusive, rendering the Jak activation mechanism even more complex.

Importantly, the involvement of rather long sequence stretches within the receptor and Jak1 suggests that the Jak1/gp130 binding might be mediated by multiple contact sites, which dictate the Jak position in a defined orientation, and which ultimately become critical for activation. This also harbours the potential for a very tight and long-lasting interaction.

3. The Jak/receptor complex behaves like a receptor tyrosine kinase

Using Jak-deficient cells as controls we demonstrated that endogenous Jak1 (as well as Jak2 and Tyk2) predominantly locates at the plasma membrane and is not found in significant amounts within the nucleus or in the cytoplasm [54] (as was previously reported [55–57]). In immunofluorescence studies none of the tested Jak1, Jak2 or Tyk2 antibodies were suited to detect Jaks specifically (using the Jak-deficient cells as negative controls). Jak localisation was also investigated in Jak1-deficient cells in which endogenous levels of Jak1, Jak1-YFP and the respective non-receptor binding mutants thereof had been reconstituted. In living cells, Jak1-YFP localised to the plasma membrane while the receptor-binding deficient Jak1-L80A/Y81A-YFP was evenly distributed throughout the cytoplasm. Fractionation experiments with cells stably expressing Jak1 and the aforementioned mutant Jak1-L80A/Y81A also revealed a membrane localisation of Jak1 and again a cytoplasmic localisation of Jak1-L80A/Y81A. This was an interesting observation since a membrane-bound protein, like Jak1, without a transmembrane domain might also directly bind to the membrane by lipid modifications (e.g. myristoylation, palmitoylation, farnesylation), by lipid binding domains (e.g. FERM-, PH-, FYFE-domains), through membrane penetrating structures, by electrostatic forces, by binding to other membrane-associated proteins, or by a combination of some of these mechanisms. However, this does not seem to be the case for Jak1. This is also in accordance with the observation that the residues, which mediate phospholipid binding in the FERM domain of radixin [33] are not conserved in Jaks. Moreover, after cytokine stimulation, Jak1 also remained localised at the plasma membrane [54].

Data from the Jak1/receptor interaction study and those from the localisation of endogenous levels of Jak1, Jak1-YFP and non-receptor-binding mutants thereof suggest that Jak1 is recruited to membranes by tight association with cytokine receptors. Interestingly, the half lives of gp130 and Jak1 are also identical [58] and this again argues in favour of a ''common fate'' of the two proteins. Quantitative fluorescence recovery after photo-bleaching (FRAP) at the plasma membrane revealed equal mobilities for overexpressed gp130-YFP and Jak1-YFP. Jak1-YFP diffuses like a transmembrane protein indicating that there is no rapid exchange of bleached Jaks from a transient cytoplasmic pool. By a dual-color FRAP approach it was possible to show that immobilisation of gp130-CFP by a pair of cross-linking monoclonal antibodies also led to the immobilisation of Jak1-YFP [59]. Thus, Jak molecules do not exchange between different receptors at the plasma membrane and the gp130/Jak1 complex can be considered as an inseparable entity resembling a receptor tyrosine kinase.

4. The unconventional Jak SH2 domain

The presence of a Src homology 2 (SH2) domain sequence similarity within Jaks, directly C-terminal to the FERM domain, has been discussed since the first descriptions of these enzymes [23]. Alignments of Jak SH2 domains with SH2 domains of other proteins support this hypothesis, since critical residues involved in the hydrophobic core of the domain (e.g. positions αA9, βB2, βB3, βB4, βC3, βC5, βD7, αB2 and αB5) are strictly conserved in the SH2 domains (hydrophobic amino acid side chains in 100% of the 420 publicly available SH2 sequences) as well as in all Jaks. Furthermore, secondary structure prediction analysis of the Jak family members revealed the typical secondary structure pattern found in SH2 domains. For functionality, SH2 domains depend on the arginine residue at position βB5, which contacts the phosphate group of a binding phosphotyrosine motif. Accordingly, this residue was present in 419 sequences (99.8%) in an alignment of 420 reference SH2 domain sequences, highlighting the strict requirement for this amino acid at this position. Strikingly, this arginine is not equally well conserved in Jaks and is only found in 80% of all Jak SH2 sequences. Therefore, taking all available Jak sequences into account, there is a surprising discrepancy of conservation between structural (conserved in all) and functional residues (conserved in most but not all Jaks).

In an in-depth study of the Jak1 SH2 domain [60] we investigated the functionality of the Jak1 SH2 domain by stably reconstituting Jak1-defective cells with endogenous amounts of Jak1, Jak1-YFP and a mutant of these in which the crucial arginine residue R466 within the SH2 domain had been replaced by lysine (a common loss of function mutation used in SH2 domain studies [61–63]). The subcellular distribution of endogenous amounts of Jak1-R466K was unchanged as assessed by cell fractionation and confocal microscopy of living cells. Mutation of this residue also did not influence receptor-binding as shown for the OSMR and gp130. Likewise, the signalling capacity of Jak1 was not affected by this point mutation as the signal strength and the kinetics of Jak activation and STAT factor activation were indistinguishable. Taken together, the classical Jak1 SH2 domain function was not required for IL-6-type cytokine or interferon induced Jak/STAT signalling [60]. Similarly, mutation of the corresponding arginine in human Jak2 to alanine also had no effect on STAT1 DNA binding upon IFN-γ treatment [30]. However, in truncation mutants and SH2 domain swapping mutants, the SH2 domain of Jak1 was shown to be structurally important for binding to the OSMR and consequently for efficient OSMR surface expression. In contrast, for gp130, EpoR and the interferon-α receptor 1 (IFNαR1), the SH2 domain of Jak1, Jak2 or Tyk2, respectively, were not necessary for receptor-binding, although the SH2 domain was required for the upregulation of receptor surface expression of EpoR and IFNαR1 [37,64,65].

Taken together, the SH2 domain in Jak1 does not seem to fulfil a classical SH2 domain function, adding to the mystery of these kinases that already harbour a kinase domain with a non-classical function. Further support for these unconventional features of the Jak SH2 domain can be deduced from the alignments and models. The absence of the well conserved tryptophan, anchoring the N-terminal tail at the back of the SH2 domain and directing it away from the phosphotyrosine recognition site, indicates that the domain preceding the SH2 domain, namely the FERM domain, could be positioned aside and not behind the SH2 domain.

5. Quality control: Jaks affect stability and trafficking of cytokine receptors

Jaks are not only crucial for signal transduction of cytokines but also for the regulation of the surface expression of at least some cytokine receptors. The surface expression of the human OSMR is substantially enhanced when Jak1, Jak2, or Tyk2 are co-expressed, an effect that can be seen upon overexpression as well as in cells with endogenous protein levels. Studies with mutant proteins revealed that the up-regulation at the cell surface requires the association between the Jak and OSM receptor while kinase activity is dispensable for this effect [66]. We have identified three dileucine-like motifs within the interbox1/2 region of the OSMR that prevent efficient surface expression in the absence of associated Jaks, possibly by lysosomal targeting and destabilisation of the receptor. Although it is conceivable that these dileucine-like motifs are part of a specific sorting signal, it is also possible that they contribute to a general hydrophobicity impeding correct receptor folding, unless they are properly masked by Jaks. In fact, the region between box1 and box2 of the OSMR contains more hydrophobic residues than the corresponding regions of gp130 and LIFR (13 versus 6). Interestingly, LIFR and gp130 chimeric receptor constructs truncated after the box1/box2 region display a strong surface expression that cannot be further increased by co-expressed Jaks [67].

Jaks have also been reported to up-regulate the surface expression of other cytokine receptors, thereby contributing to a quality control mechanism ensuring that signalling-competent receptors (i.e. those with an associated Jak) would be enriched at the cell surface. In this context, Jak2 is crucial for EpoR surface expression and it has been hypothesised that Jak2 supports the folding process of the receptor in the endoplasmic reticulum [65]. Furthermore, Jak2 increases the stability of the mature growth hormone receptor [68], while the surface expression of the thrombopoietin receptor can be enhanced by Jak2 and Tyk2 [69]; inhibitor studies imply the involvement of lysosomal or proteasomal degradation, respectively. Expression of Tyk2 is needed for stable surface expression of the IFNαR1 and the IL-10 R2. In the case of the IFNαR1, Tyk2 has been shown to prevent receptor internalisation from the plasma membrane [64], a mechanism that could be excluded for the OSMR. Interestingly, Jak3-negative cells express slightly higher levels of the common gamma chain (γ_c) than Jak3 containing cells. However, γ_c expression could be further enhanced by overexpression of Jak3 [70]. Preliminary data from our lab indicate that the surface expression of the IL-4Rα and of full-length gp130 can be positively affected by Jak1 and Jak2. In contrast, expression of full length LIFR seems completely unaffected by co-expressed Jaks.

Apparently, the strictness of quality control imposed on the cytokine receptor seems to vary between different cytokine receptor/Jak complexes. There is evidence that competition exists between different receptors for a limiting amount of Jak, as demonstrated for the IFNαR1/IL-12-receptor system [71].

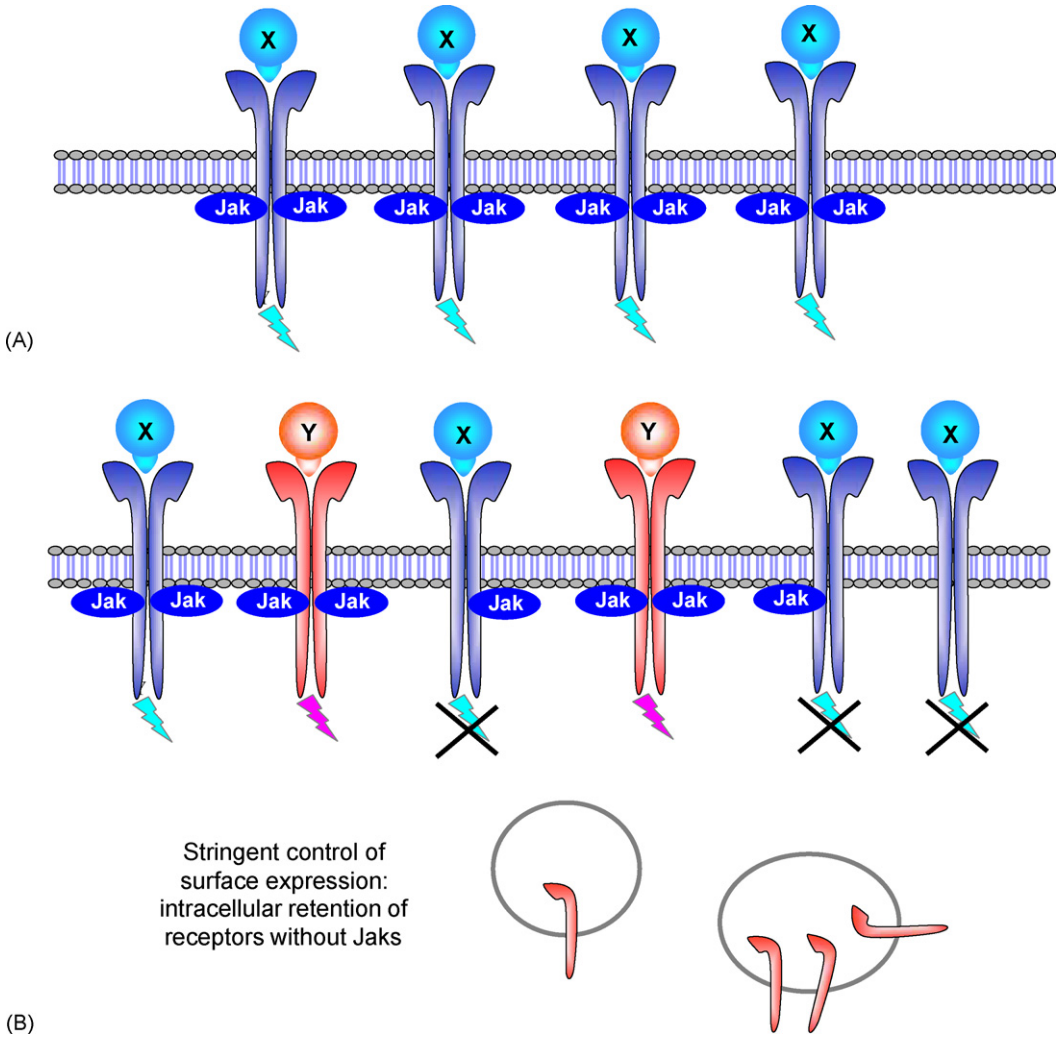

Fig. 3 – Competition for Jaks favours signalling via receptors with stringent Jak-mediated control of their surface expression. In panel A, the cell is fully responsive to cytokine X, all receptors shown are signalling-competent. In B, the cell has expressed the same amount of receptors for cytokine Y as for cytokine X while the amount of the shared Jak remains constant and therefore limiting. Assuming the same affinity of both receptors for the Jak, only half of the receptor subunits for cytokines X and Y are now equipped with an associated kinase. All receptor chains for cytokine X are at the membrane ("no Jak-control of surface expression"). However, only one of the four dimeric receptor complexes shown is signalling competent, the others even act antagonistically by sequestrating the ligand. In contrast, only half of the receptors for cytokine Y are at the cell surface ("stringent Jak-control of surface expression") while the others are retained intracellularly. However, both dimeric receptor complexes at the cell surface are responsive to cytokine Y. Hence, the differential Jak-mediated control of surface expression boosts the relative cellular response to cytokine Y while suppressing response to X.

Thus, one would expect that a more stringent control should ensure a better signalling capacity of the corresponding receptor whereas a loose control should lead to a higher percentage of signalling-incompetent receptors, which nonetheless bind the cytokine and could thus serve as decoy receptors. (It should be noted that a receptor without an associated kinase even acts in a dominant negative fashion because it precludes signal transduction even when present in a dimer with a Jak-carrying receptor subunit [45,72].) A differential degree of quality control may add another layer of complexity by offering a higher degree of flexibility when a cell needs to rapidly and sustainably respond to changes imposed by the environment (or a developmental program), as illustrated in Fig. 3.

6. Concluding remarks

Deregulated Jaks have been described to be involved in a number of pathologies and are therefore considered interesting pharmacological targets. The very recently published structures of the kinase domains of Jak3 and Jak2 represent the first structural information about Jaks [73,74]. The kinase domains of these two Jaks are of interest for the development of small molecule inhibitors. Mutations in Jak3 can lead to severe combined immunodeficiency (SCID) in humans [12] indicating that Jak3 inhibitors could act as immune suppressants and indeed, the first existing specific Jak3 inhibitors (e.g. CP-690550) block allograft rejection or extend allograft survival by reducing NK- and T-cell numbers [75,76]. TEL-Jak2 fusion proteins,

caused by chromosomal translocation, are constitutively active and evoke lymphoid and myeloid leukemia [18,19]. Recently, an acquired gain-of-function mutation of Jak2, V617F, was reported in some chronic myeloproliferative disorders such as polycythemia vera, chronic idiopathic myelofibrosis and essential thrombocythemia [13–17]. Constitutively active Jaks (Jak1 and Jak3) can also be found in malignant transformations engendered by viruses [20–22]. Thus, Jak inhibitors might be useful to suppress uncontrolled growth of (cancer) cells harbouring deregulated Jak molecules. An inhibitor of Tyk2 may become a potential future drug for autoimmune diseases since this kinase has been associated with Systemic Lupus Erythematosus [77].

Here, we have discussed data, which demonstrate that the FERM domain of Jaks is crucial for receptor association and that, at least in some cases, the SH2-like domain may also be involved in this interaction. Nevertheless, the real situation is likely to be still more complex and even the kinase domain may affect receptor-binding [78]. Structural information on the receptor/kinase complex is a crucial prerequisite to understand the binding specificity and the activation process of Jaks, which is the initial event of intracellular cytokine signal transduction. Interestingly, the intracellular regions of different cytokine receptors do not show much similarity concerning the nature and the position of the residues involved in Jak-binding although they bind the same Janus kinase. Hence, interfering with the receptor/Jak interaction by just disturbing one interaction site of a defined cytokine receptor might be a more specific way to inhibit signal transduction via a certain cytokine than using kinase inhibitors for Jaks, because the ubiquitously expressed Janus kinases (Jak1, Jak2 and Tyk2) are often active in different cytokine signalling pathways. In disease treatment this approach might represent an alternative to cytokine antagonists, neutralising antibodies or kinase inhibitors or might be of use for a combination treatment targeting certain cytokines.

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