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Review

Molecular mechanisms of cytokine receptor activation

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Abstract

Cytokine receptors are transmembrane proteins that transmit a signal into the cell upon ligand binding. Commonly, these molecules have one hydrophobic segment of about 20–26 amino acids that is believed to span the membrane as a helix and this divides these receptors into extra- and intracellular components. By utilizing two different epitopes, the cytokines bridge two receptor chains, resulting in a close proximity of the intracellular component and thereby initiating the intracellular signalling cascade. The dimerization event is believed to be the mechanism by which the signal is transmitted across a membrane. In the light of new results obtained for the erythropoietin receptor, James A. Wells questioned whether any dimer would be sufficient. This review will expand upon the above question by discussing the more complex signal-transducing receptor subunits of the Interleukin-6 type family of cytokines. Based on the recently solved quaternary structure of the Insulin receptor, possible analogies will be confronted.

Keywords: Cytokine receptor; gp130; Dimerization; Activation

1. Introduction

Information in an organism is transmitted by the interaction between molecules. Proteins and the formation of protein complexes play a central role in cell communication. The flow of information is organized in signalling cascades which are initiated by the interaction of a ligand with a membrane-spanning receptor. Upon ligand binding, these receptors become activated and thereby transmit the signal from the outside to the inside of a cell where a cascade of events is initiated. This cascade is complex and not fully understood so far. The intrinsic regulation and even the crosstalk between different signal-transducing pathways have been studied extensively during the last years. These cascades mostly involve several phosphorylation/dephosphorylation events that result in protein activation, enzymatic activity or susceptibility for interaction with other proteins. The initial ligand/receptor interaction seems to be the most specific step. The specificity of ligand/receptor interaction has been well studied in the field of cytokines and their receptors [1–3].

The growth hormone (GH) receptor complex was the first four-helical cytokine receptor to be crystallized together with its ligand [4] and has therefore become paradigmatic for the understanding of four-helical cytokine receptor complexes. One GH molecule binds to two receptor molecules via two contact epitopes designated as site I and site II. Remarkably, the two receptors use identical amino acid residues to bind the two different epitopes of the cytokine [4,5]. One paradigmatic conclusion derived from these studies is that generally cytokines are recognized by their cognate receptors at sites equivalent to site I and site II of GH [3,4]. The X-ray structure of the viral Interleukin-6/gp130 complex revealed that this paradigm does not fully explain the situation in IL-6 type cytokines as biochemical studies have demonstrated the existence of a site III on IL-6 and the activation of gp130 requires two distinct binding epitopes on the receptor [6–12]. This structural information not only contributed remarkably to the understanding of protein/protein interactions but also provided the basis for the design of new cytokines and deliberate manipulation of cytokine signalling. The fact that at least two receptors are needed to induce signalling has led to the assumption that receptor dimerization is the crucial step for transmitting the signal through the membrane. For the erythropoietin receptor it has been shown that dimerization is indispensable but not sufficient for activation [13–16]. Besides dimerization, a specific orientation of the involved receptor chains seems to be mandatory [16,17]. In cases where receptors already

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exist as preformed dimers, ligand binding might replace binding sites engaged in the formation of such dimers and thereby changing their orientation. This review will focus on cytokine receptors and their dimerization as one mechanism for the transmission of information across a membrane.

2. Results and discussion

2.1. The cytokines

More than a decade ago, Fernando Bazan presented two seminal papers in which he suggested that known cytokines shared a common protein fold [18,19]. This work was mainly based on theoretical considerations and grouped these proteins into the family of "four-helix bundle" cytokines. These four helices are arranged in an up-up-down-down topology (Fig. 1A), which as yet has not been identified in any other non-cytokine protein and is therefore to be regarded as the typical cytokine fold. This topology demands that the three loops connecting the four helices are long-short-long.

All these cytokines transmit their signal into the cell by multi-subunit receptor complexes. Subfamilies of cytokines have been defined based on the fact that certain signal-transducing receptor subunits are shared between them. One of such receptor-subunits is gp130 which is shared by the Interleukin-6 (IL-6) type cytokines (IL-6, IL-11, ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), cardiotrophin-1 like cytokine (CLC), leukemia inhibitory factor (LIF), oncostatin M (OSM)) as part of the signal-transducing complex. Similarly, the β-subunit of the IL-3 receptor is shared by IL-3, IL-5 and granulocyte macrophage colony stimulating factor (GM-CSF), whilst the IL-2γ receptor is the common subunit for IL-2, IL-4, IL-7 and IL-15 (see Table 1). Another possibility to allocate all these cytokines (IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-11, the p35 subunit of IL-12, IL-13, IL-15, granulocyte colony stimulating factor (G-CSF), GM-CSF, erythropoietin (EPO), GH, prolactin (PRL), CNTF, CT-1, CLC, LIF, OSM, interferon α (IFNα) and IFNγ) [20,21] into subfamilies is based on structural considerations and has been suggested by Bazan. This classification divides the cytokines into short-chain (IL-3, IL-4, IL-5, IL-15 and GM-CSF) or long-chain cytokines (the remainder) [22]. The so far known 3D structures solved by crystallography or NMR of these cytokines have confirmed Bazan's landmark prediction (Table 2). As new cytokines are still being identified there may be other possibilities to define subfamilies. One such possibility is the number of different receptors engaged in the final signal-transducing receptor complex, which is tantamount to the number of interaction epitopes present on the corresponding cytokine. As an example, GH, EPO or IL-4 have two ligand/receptor interaction sites, whereas cytokines like CNTF, IL-11, IL-15 and IL-6 have three [4,23,24]. These sites were designated I, II and III (Fig. 1B).

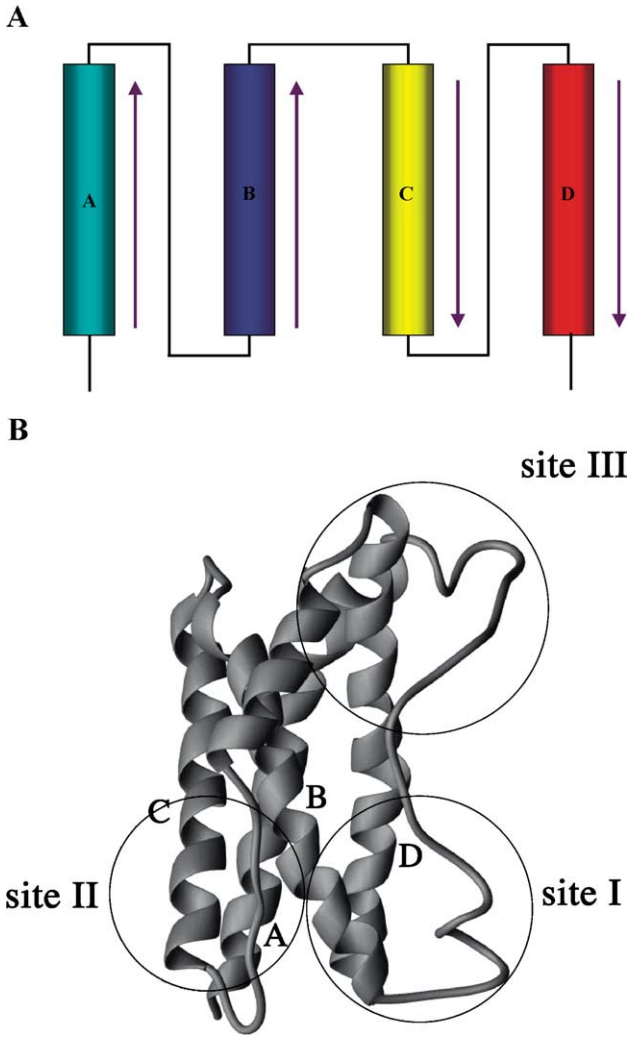

Fig. 1. The 'four helix bundle' cytokines. (A) Schematic representation of the 'four helix bundle' cytokines with an up-up-down-down topology. The four helices are designated as: A, B, C, and D. (B) A ribbon representation of the Interleukin-6 structure solved by NMR spectroscopy [49]. The interaction epitopes with the different receptor subunits (site I: IL-6R, site II: gp130 cytokine receptor homology region (CRH) and site III: gp130 Ig-like domain) are encircled and marked.

The use of a different number of epitopes and their relative orientation to each other raises the questions (1) whether the mechanism by which the signal is transmitted into the cell is also different and (2) to what extent the topology of the receptors, here defined as the relative orientation of domains to each other, plays a role in this process.

2.2. Structural complexity of receptors

Type I cytokine receptors are proteins spanning the cellular membrane with their NH_2 terminus at the extracellular and the C terminus at the intracellular side. The extracellular part is responsible for the interaction with a ligand. The intracellular part exhibits either intrinsic kinase activity or contains the so-called adapter domains, which are

Table 1
Receptor sharing by cytokines

Cytokines	α-Receptor[a]	β1-Receptor[b]	β2-Receptor[b]
IL-6	IL-6R	gp130	gp130
IL-11	IL-11R	gp130	gp130
CNTF	CNTFR	gp130	LIFR
CLC	CNTFR	gp130	LIFR
CT-1	(?)	gp130	LIFR
OSM		gp130	LIFR or OSMR
LIF		gp130	LIFR
IL-3		IL-3R	IL-3β
IL-5		IL-5R	IL-3β
GM-CSF		GM-CSFR	IL-3β
IL-2	IL-2Rα	IL-2Rb	IL-2Rγ
IL-4		IL-4R or IL-13R	IL-2Rγ
IL-7		IL-7R	IL-2Rγ
IL-15	IL-15Rα	IL-2Rβ	IL-2Rγ

[a] α-Receptors are defined as non-signalling ligand binding receptor subunits.
[b] β-Receptors are defined as signalling receptor subunits.

able to interact with kinases. In both instances, binding of a ligand to the extracellular part initiates the intracellular signalling cascade. Almost all molecules involved in these cascades show a modular architecture, i.e. they consist of several domains. These domains are either enzymes, like kinases, phosphatases or proteases, and/or consist of several docking modules responsible for the interaction with other molecules of the signalling cascade. As an example, the insulin receptor (INSR) contains an intrinsic kinase and the signal transducers of cytokines, like gp130, EpoR, GHR and IL-4R, are constitutively associated with tyrosine kinases of the Janus-kinase (Jak) family [25,26]. In contrast, the tumor necrosis factor receptor (TNFR)-1 and -2 show neither intrinsic enzymatic activity nor are they directly associated with enzymes. Although activated by the same ligand, they initiate different signalling cascades depending on their different intracellular modules. The intracellular death domain of the TNFR1 interacts with proteins named TRADD that leads to the activation of a series of proteases belonging to the caspase family. The TNFR2 does not contain a death domain but interacts with the FADD protein (for review see Ref. [27]).

Like the intracellular parts, the extracellular portions of cytokine receptors are built up by modules. Some of these modules are found in several receptor superfamilies. In the following, I will focus on the TNFR superfamily, the insulin/insulin-like receptors and the 4-helical cytokine-receptor superfamily. For example, the extracellular part of the TNFR consists of four cysteine-rich regions [28]. Three of these repeats are also found in the INSR where they are flanked by the so-called L1 and L2 domains (see Fig. 2) [29]. In addition to these three domains, which are responsible for ligand binding, this receptor contains three membrane proximal domains sharing the fibronectin type III (FNIII) fold [30]. This architecture is also present in the signal-transducing receptor subunits of the IL-6 type family of cytokines namely gp130, LIFR and OSMR (see Fig. 2) [19,22]. These belong to the family of cytokine receptors which is defined by the presence of the so-called cytokine-receptor homology region (CRH) consisting of two immunoglobulin domains, whereby the loops connecting the β-sheets establish the specific contact to the ligand [1]. The membrane proximal immunoglobulin domain contains a typical sequence motif tryptophan-serine-X-tryptophan-serine (WSXWS) in which X can be any amino acid residue.

The above described receptors can be divided into two classes with respect to their complexity. For the remainder of this review receptors like GHR, EpoR and IL-4R, which contain only domains in the extracellular region that are involved in ligand binding, are called 'simple' receptors. Receptors like gp130, G-CSFR and INSR, which contain additional domains apparently not involved in ligand binding, are designated as 'complex' receptors.

2.3. 'Simple' receptors

The class of 'simple' receptors can be further subdivided into two groups, which either homo- or heterodimerize upon ligand binding. Examples of ligands for these receptors are GH or IL-4, respectively, and they differ in the mode of interaction with the receptors [5,31]. The GH receptor complex is depicted in Fig. 3. One GH molecule binds two receptor molecules via two contact epitopes designated site I and II. Remarkably, the two receptors use identical amino acid residues to bind the two different epitopes of the cytokine [4,5,32]. However, the two interaction sites differ in their free energy of binding. One paradigmatic conclusion derived from these studies was that cytokines generally are recognized by their cognate receptors at sites equivalent to sites I and II of GH [4,32]. A detailed analysis of the interaction epitopes suggested a common design [4,5]. Using an alanine scan, in which all residues in the interaction area have been mutated to alanine, the contribution of each amino acid residue to the binding energy has been

Table 2
Overview of known cytokine, cytokine-receptor and cytokine/cytokine-receptor structures

Cytokines		Cytokine receptors	Cytokine/cytokine-receptor complexes
Long chain	IL-2 [47]	gp130 (D_2-D_3) [57]	GH/$(GHR)_2$ [4]
	IL-6 [48,49]	gp130 (D_3) [58]	GH/PRLR [62]
	IL-10 [50]	EpoR [13]	IL-4/IL-4-R [31]
	CNTF [51]	G-CSFR (D_3) [59]	EPO/$(EpoR)_2$ [17]
	LIF [52]	IL-3Rβ [60,61]	G-CSF/GCSFR [63]
	G-CSF [53]		IL-12α/IL-12β [64]
	EPO [54]		$(viral\text{-}IL\text{-}6)_2$/
	GH [55]		$(gp130)_2$ [6]
	OSM [56]		
Short chain	IL-3 [65]		
	IL-4 [66,67]		
	IL-5 [68]		
	IL-13 [69]		
	GM-CSF [70]		

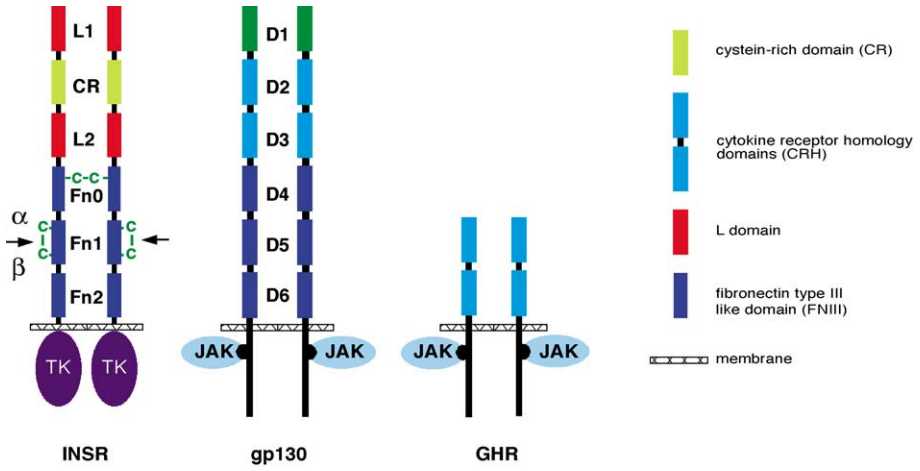

Fig. 2. Receptors sharing common extracellular modules. Schematic representations of the domains present in the extracellular parts of the Insulin receptor (INSR), gp130 and the human growth hormone receptor (GHR) as examples for 'complex' and 'simple' receptors, respectively. The cysteine bridges are depicted in green, arrows mark the cleavage site between the α- and β-sub-units in the insulin pro-receptor. TK: intrinsic tyrosine kinase domain of the insulin receptor; Jak: Janus-kinases; L1, CR, L2, Fn0, Fn1, Fn2: insulin receptor domains; D1, D2, D3, D4, D5, D6: gp130 domains.

studied. Hydrophobic residues, aliphatic parts of polar side chains and parts of the backbone were involved in the most important interactions. This hydrophobic core is surrounded by a region consisting of polar and charged amino acid residues which only slightly contribute to the binding energy, but are involved in the specific recognition of the two molecules [5]. In contrast to this architecture, the IL-4/IL-4R interaction epitopes have been described as 'avocado clusters', where a nucleus is also enveloped by an oily shell [31]. Instead of having a hydrophobic core, the central interaction is mediated by charged side chains or amino acid residues capable of forming hydrogen bonds. This central area is surrounded by a shell of hydrophobic amino acids [31].

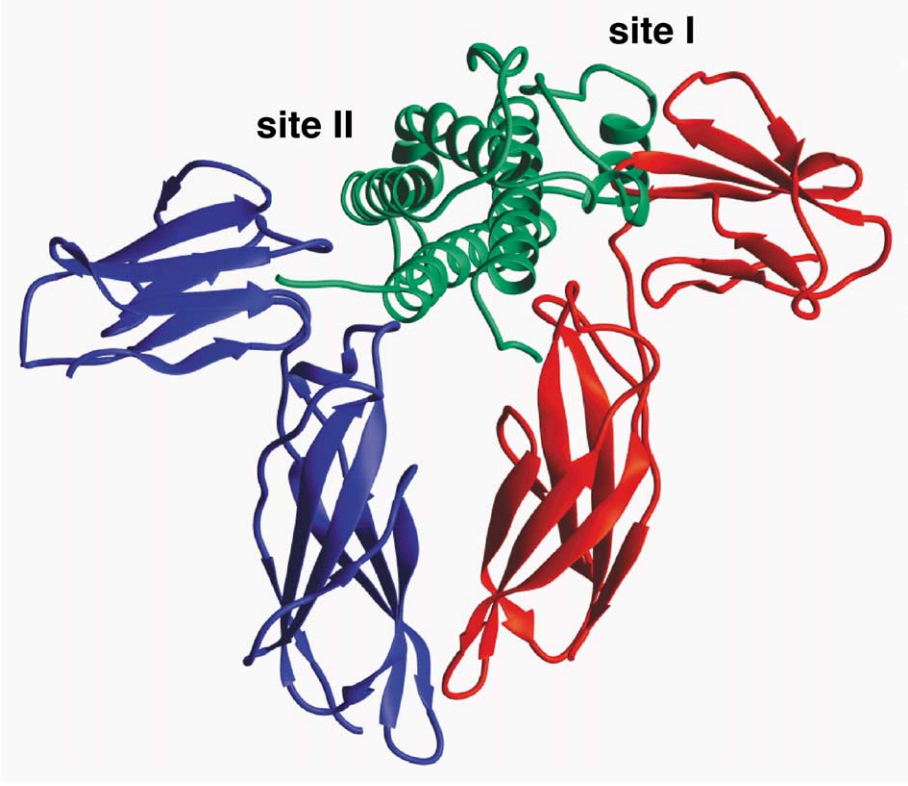

Fig. 3. The growth hormone receptor complex. Ribbon representation of the X-ray structure of the GH (green) and GHR (red and blue) complex [4]. The two receptor chains bind one molecule of GH via sites I and II.

Regardless of the specific mode of interaction, GH and IL-4 were thought to cause dimerization of the receptor chains by bringing the intracellular parts in close proximity and thereby initiating the intracellular signalling cascade. Therefore, it appeared that dimerization is needed and sufficient for the onset of the signal transduction.

Most interestingly, it has been shown that dimerization is needed but not sufficient for some of the homodimeric receptors and that they exist as preformed dimers on the cell surface. Crystallographic and biochemical studies have demonstrated that the EpoR as well as the TNFR-1 are dimers even in the absence of their ligands [13,33].

Although the X-ray structure of the TNF/TNFR-1 complex consists of a trimeric ligand bound to a trimeric receptor, the unliganded receptor forms dimers [33,34]. Further biochemical data support the idea that a dimerization of the TNFR-1 is sufficient to initiate signal transduction and that the trimeric form of the complex seen in the crystal structure might not reflect the situation on the cell surface [35].

In the case of the EpoR, there is crystallographic evidence for preformed dimers of this receptor. Distinct dimeric configurations exist for this receptor dependent on being unliganded, Epo bound or bound to agonistic or antagonistic peptides [13,14,16]. The presence of a ligand and its agonist or antagonist activity resulted in different distances and orientations of the receptor parts close to the membrane [14].

Furthermore, in vivo fragment complementation assays were used to study this allosteric model of receptor activation. Complementary fragments of the enzyme dihydrofolate reductase were fused to the intracellular part of EpoR and bind a fluorescein-conjugated inhibitor methotrexate (fMTX) during reassembling inside the cell. The fMTX is retained in cells by this complex whereas unbound fMTX is actively transported out of the cells [36]. The fMTX–DHFR complexes can then be monitored by fluorescence. These sophisticated experiments revealed that unliganded receptor dimers exist in a configuration that prevents activation of the signalling cascade but they can undergo a ligand-induced change in the orientation of the two receptors that allows activation of the involved intracellular kinases [16].

Beyond the evidence that preformed dimers exist, the structures of the unliganded receptors revealed that the dimerization occurs via epitopes that are also involved in ligand binding [13]. Therefore, ligand binding to these receptors might be regarded as a competition event in which the ligand supersedes the two receptors bound to each other and thereby changes their relative orientation. This concept would suggest that mutations in the ligand-binding epitope of the receptor would not only affect ligand binding but also inhibit the formation of preformed dimers which might be a prerequisite for efficient signal initiation. Such an interesting case has been observed in the class of complex receptors which now will be discussed.

2.4. 'Complex' receptors

The 'complex' receptors consist of more domains than needed for ligand binding and can therefore be divided into two parts, one responsible for ligand binding and the other involved in transmitting the signal into the cell. One paradigmatic conclusion derived from the studies of the GH/GHR complex was that generally, cytokines are recognized by their cognate receptors at sites equivalent to site I and site II of GH [3,4]. This paradigm does not hold true for IL-6 type cytokines and their 'complex' receptors gp130, LIFR and OSMR. The existence of three distinct receptor binding epitopes has been clearly demonstrated for IL-6, IL-11 and CNTF. In analogy to GHR that occupies site I (end of AB-loop, C-terminal D-helix) and site II (A/C-helix) of the GH, the cognate α-receptor is located at site I and the common signal transducer gp130 at site II of these cytokines. A third β-receptor binding epitope (site III) is not present on GH and is occupied by a second gp130 molecule (IL-6, IL-11) or serves as a specific LIFR binding site on CNTF [7,10–12]. Site III consists of the C-terminal A-helix, the N-terminal AB-loop, the BC-loop with adjacent amino acid residues, the C-terminal CD-loop and the N-terminal D-helix [7,10]. Based on mutagenesis studies in combination with molecular modelling studies, it has been shown that the sites I and II of the IL-6 type cytokines exclusively interact with the corresponding CRH of the involved receptors, whereas site III is utilizing the Ig-like domain of the 'complex' receptors. Thus, the 'complex' receptors have two distinct binding regions. These data, mostly derived from biochemical studies, have been confirmed by the recently solved X-ray structure of the viral IL-6 molecule in complex with two gp130 molecules. In this complex, the CRH of one gp130 molecule is indeed bound to the ligand via its CRH to site II and the second gp130 molecule uses its Ig-like domain to bind to site III of the viral IL-6 [6,8,9]. Since one gp130 molecule has two distinct binding sites, two gp130 molecules are able to bind two ligands in a symmetrical arrangement as seen in the X-ray structure [6] depicted schematically in Fig. 4A. But most interestingly, only one of these two symmetrical binding sites per gp130 molecule is needed to induce signal transduction. This has been shown by Pflanz et al. who used two mutants of gp130 that either lack the Ig-like domain or contain a distinct mutation within the CRH. Both mutants were to be unable to induce IL-6 signal transduction [37]. After co-transfection of both inactive mutations IL-6 bioactivity was restored (Fig. 4B). Since the combination of the two gp130-muteins is able to bind only one IL-6/IL-6R complex, the formation of a tetrameric complex, consisting of one IL-6, one IL-6R and two gp130 molecules is sufficient for biological activity. In addition, the same authors showed that the two epitopes sequentially co-operate upon IL-6-induced receptor activation and combining the two mutations restores the high affinity of ligand binding [37]. How can two intact binding epitopes cooperate within two different molecules? One attractive explanation might be the formation of pre-

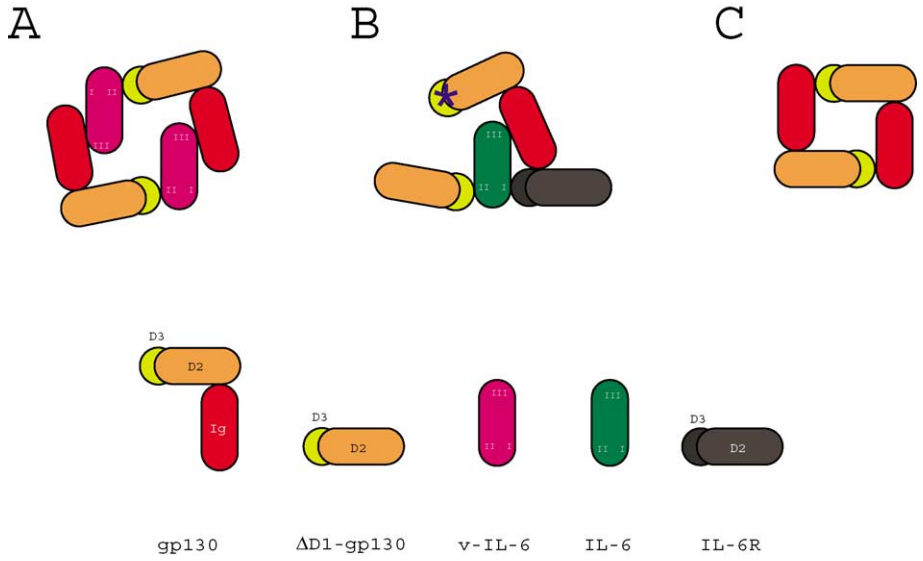

Fig. 4. (A) Schematic representation of the (gp130/viral IL-6)$_2$ complex as solved by X-ray crystallography [6]. (B) Schematic representation of the IL-6/IL-6R/(gp130)$_2$ complex in which both gp130 molecules are mutated, with molecules either lacking the Ig-like domain (ΔD1) or carry mutations in the CRH (marked by *). (C) Pre-formed gp130 dimer in which the Ig-like domain of one gp130 would interact with the CRH (D2,D3) of the second and vice versa.

formed dimers. In analogy to the situation of the EpoR, where same epitopes are responsible for dimerization as well as ligand binding, in the case of gp130 the Ig-like domain of one gp130 would interact with the CRH of the second and vice versa (Fig. 4C). Therefore, mutation in the CRH or removal of the Ig-like domain would affect both interaction sites and thereby prevent dimerization, whereas a combination of the two mutated gp130 molecules would still allow the formation of dimers by the combination of the two intact epitopes present in each molecule. In the case of the heterodimeric receptor complex gp130/OSMR, it has been shown that this complex exists as a preformed dimer on the cell surface by co-immunoprecipitation experiments [38]. Since these cells were not transfected with the corresponding cDNAs, the observed dimers cannot be an experimental artifact due to overexpression. Furthermore, mutations within the CBM of gp130 abrogate signalling by IL-6 but not by LIF or OSM [39,40]. In the case of IL-6, these mutations might be able to interfere with the formation of a symmetric preformed dimeric gp130 but not within the preformed asymmetric gp130/LIFR or gp130/OSMR.

In contrast to the ‘simple’ receptors, the ‘complex’ ones have more than one epitope for the interaction with the ligand. To make the situation even more ‘complex,’ they also have domains that are not involved in ligand binding. The signal transducing subunits of the IL-6 type family of cytokines, namely gp130, LIFR and OSMR, contain three membrane-proximal fibronectin type III domains (see Fig. 2), an architecture that is also seen in the G-CSFR. Mutational studies on soluble and membrane bound gp130 shed light on the role that these domains may play. As two strategies, deletion and substitution mutants have been created to study their contribution in signal transduction [41,42].

Deletion of any of these domains in the soluble protein had no impact on the binding characteristics [42]. In the membrane bound form, the results were contradictory. All these muteins were not able to initiate signal transduction, but more surprisingly, some of them were no longer able to bind the ligand. Deletions of the fourth and sixth domain led to a complete loss of binding, whereas deletion of the fifth revealed some residual binding [42]. Therefore, these domains seem to be necessary to position the upper domains in a specific way that enables these domains to bind the ligand. Another study used chimeras of gp130 and G-CSFR, in which the three membrane-proximal fibronectin type III-like domains were exchanged between the two receptors: (GR-FNIII)gp130, which contains the membrane-proximal FNIII modules of G-CSFR, and its complement the chimera (gp130−FNIII)GR [41]. Both chimeras were able to bind their corresponding ligands, demonstrating that the three membrane-distal domains, which are responsible for recognising the ligands, are presented in an appropriate orientation. This picture completely changed when these chimeras were tested on their ability to induce biologically activity. Whereas cells transfected with the cDNA coding for the chimera (gp130−FNIII)GR were fully responsive to G-CSF, cells transfected with the other chimera did not respond to the IL-6/IL-6R complex, but to an agonistic anti-gp130 monoclonal antibody. Since these cells were also expressing the LIFR, the authors asked the question whether a heterodimer of (GR-FNIII)gp130/LIFR is able to induce LIF dependent signalling. This heterodimeric receptor failed to bind to LIF with high affinity and the cells did not respond to LIF.

A paper of Kurth et al. [42] utilized a similar strategy of substitution. The authors exchanged only one of the FNIII domains, namely D5, in gp130 by the corresponding G-

CSFR domain. Surprisingly, in cells overexpressing this mutein, the activation of this chimera became ligand independent, suggesting this domain may play a role in the activation of the receptor.

Taken together, the above described results show clearly that the three membrane-proximal domains play a pivotal role in the transmission of the signal, but it is unable to be explained by a unique model. If dimerization is needed but not sufficient, and the three membrane-proximal FNIII domains are important for receptor activation, the question that remains is whether we can learn something about their activation mechanism from receptors which are covalently linked dimers.

2.5. The insulin receptor as a template for cytokine receptors?

The INSR can be regarded as a covalently linked homodimer, since division into an α- and β-chain is a proteolytic, posttranslational process. Like gp130, the INSR can be divided into two regions, one for binding the ligand (L1-CR-L2), the other one responsible for signal transmission. Like in gp130, the latter consists of three membrane-proximal FNIII domains (Fig. 2). Interestingly, the quaternary structure of the insulin receptor bound to one insulin molecule has recently been solved by cryo-electron microscopy [43]. Although the reported structure has no atomic resolution, the overall shape of the whole receptor, the location and relative orientation of the different domains could be deduced (for review see Ref. [44]). Surprisingly, this study revealed that the receptor molecule has the shape of a globular protein instead of being clearly divided into a extra- and intracellular part divided by the membrane (Fig. 5A). The L2 domain, which is involved in ligand binding, and the two membrane-proximal FNIII domains seem to be in direct contact with the intracellular kinase domain. This direct contact might suggest that the extracellular and intracellular parts are able to communicate directly which each other and that the information need not be transmitted through a transmembrane region. In fact, the two hydrophobic amino acid segments of about 25 residues that were supposed to span the membrane as helices are far apart from each other and are unable to come into contact. The two membrane-proximal FNIII domains (Fn2 and Fn1), lying parallel to the membrane, are described by the authors as one part of a pontoon. In between the two pontoons, the ligand binding domains are mounted with the help of the third FNIII domain (Fn0). In a recent review, the same authors discuss details about the mechanism by which this topology might be used to transmit the signal to the kinase domains [44]. Because all these receptors can be divided into two regions, one for the specific binding of the ligand, and a second, membrane-proximal region consisting of three FNIII domains. The latter can be regarded as a dance floor on which each dancer performs their own specific choreography. Nevertheless, the mechanism by which the noise of the dancing steps is transmitted through the floor is the same.

Can the above described results on deletion and substitution mutants of gp130 be explained by a model which assumes that the overall topology of gp130 and INSR is the same? The deletion studies can be explained easily. Removal of one of the FNIII domains in the soluble forms of gp130 would have no influence on the orientation of the ligand-binding domains, since they are free to rotate and translate in solution. The situation is different when these constructs are fixed to the membrane. Now, the remaining membrane-proximal domains direct the ligand-binding domains into different orientations and thereby prevent the correct interaction with the ligand. Results obtained with the chimeric receptor constructs fit also into this picture. With both molecules, the dance floor is built up by two times

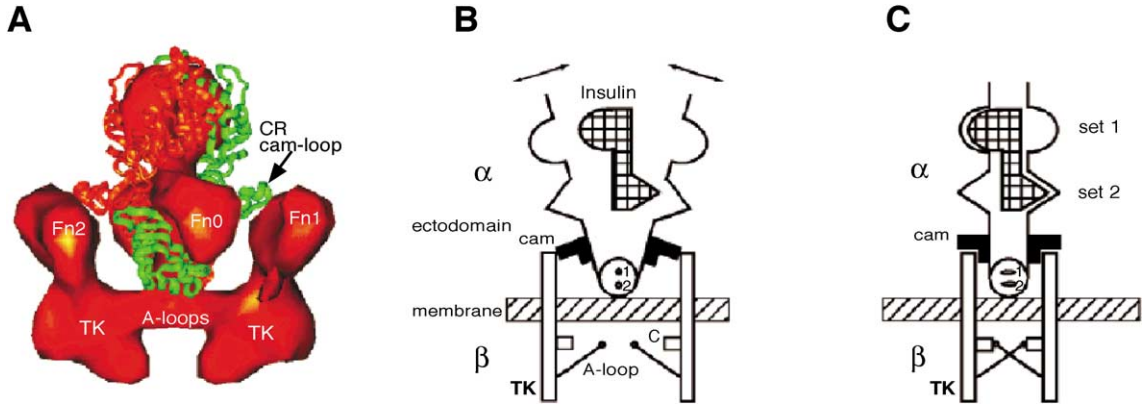

Fig. 5. Quaternary structure of the insulin receptor. (A) Full-mass representation of the insulin receptor structure as determined by cryo-electron microscopy [43]. (B and C) Simplified schematic of the change in domain orientation during activation of the insulin receptor. (B) Inactive configuration: The ectodomains of the α-subunits each with two different insulin binding sites and one blocking cam touching the Fn2 domain, thereby keeping the tyrosine kinases (TK) separated. (C) Active configuration: The blocking cams has been rotated allowing the β-subunits to move closer to the center of the ectodomain and the activation loop (A-loop) of each kinase is positioned for transphosphorylation. (This figure is taken from Ref. [44] with kind permission of the American Chemical Society).

three FNIII domains of the same molecule, either G-CSFR or gp130 and is therefore functional, as observed for G-CSF and the agonistic anti-gp130 antibody. The situation for IL-6 might be different since the activating ligand is not IL-6 alone but the IL-6/IL-6R complex. Due to the dense packing of the domains in an INSR-structure based gp130 model, it must be assumed that the IL-6R has numerous contacts with gp130, even with the membrane-proximal domains. Replacing these by the corresponding domains of G-CSFR, this interaction may prevent complex formation. Since the viral IL-6 is interacting directly with gp130, without the help of a specific α-receptor [45,46], it should be able to induce signal transduction via this chimeric receptor.

In case of LIF as the ligand, the dance floor would be a combination of the GCFSR–FNIII domains and the corresponding domains of the LIFR, a heterodimeric combination that has never been observed and therefore might not be able to dimerize.

The fifth domain seems to play a special role. As described above, the membrane bound deletion mutant is still able to bind the ligand, but is unable to initiate the signalling cascade. Replacement of this domain by the corresponding one of the G-CSFR, at least in cells overexpressing this chimera, leads to constitutive activation. This result again suggests that preformed dimers exist and the exchange of the fifth domain results in a topological change of the domains which corresponds to the activated receptor (Fig. 5B and C). In the INSR, the fifth and sixth domains are in a rod-like orientation, thereby representing one half of the pontoon, suggesting strong interactions between them. Furthermore, the sixth domain is directly involved in the activation mechanism, since the cysteine-rich domain touches it. This contact is thought to transmit the information of ligand binding to the dance floor, thereby permitting the approach of the tyrosine kinases (Fig. 5B and C). Replacing the fifth domain in gp130 by the corresponding one of G-CSFR would weaken the connection between the fifth and sixth domain, thereby relaxing the stiffness between them and may thereby allow the intracellular parts to approach and induce signal transduction.

The contribution of the intracellular associated JAK kinases to the formation of dimeric receptors has not been examined so far, but may have an important impact on the activation mechanism. Since the extracellular part acts in this model as a gate that has to be opened by the ligand, the prediction would be that removal of the whole extracellular part, but not of the transmembrane region, will lead to a constitutively active receptor.

2.6. The tasks ahead

Much of what has been suggested above is of a hypothetical nature. Further experimental evidence for the suggested models is needed. So far, the existence of a pre-formed dimer of gp130 on the cell surface has not been shown unambiguously, although there are many indications for their existence. The quaternary structure of the INSR might be used as a template for the domain organization of the signal-transducing receptor subunits of the IL-6 type cytokines, especially gp130, and may lead to a unique explanation of the so far published experiments about the role of the FNIII domains. The INSR shows a negative co-operativity upon insulin binding and seems to be more than a simple on/off switch. Again, there are hints that gp130 behaves the same in the case of its IL-6/IL-6R induced activation, but detailed studies to prove this point are not yet available. Nevertheless, the suggested analogy has to be proven by more experimental data, but may guide future work to establish an activation mechanism for these receptors.

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