**Regulation of NF-κB signalling cascade by immunophilins**

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**ABSTRACT**

The fine regulation of signalling cascades is a key event required to maintain the appropriate functional properties of a cell when a given stimulus triggers specific biological responses. In this sense, cumulative experimental evidence during the last years has shown that high molecular weight immunophilins possess a fundamental importance in the regulation of many of these processes. It was first discovered that TPR-domain immunophilins such as FKBP51 and FKBP52 play a cardinal role, usually in an antagonistic fashion, in the regulation of several members of the steroid receptor family via its interaction with the heat-shock protein of 90-kDa, Hsp90. These Hsp90-associated cochaperones form a functional unit with the molecular chaperone influencing ligand binding capacity, receptor trafficking, and hormone-dependent transcriptional activity. Recently, it was demonstrated that the same immunophilins are also able to regulate the NF-kB signalling cascade in an Hsp90 independent manner. In this article we analize these properties and discuss the relevance of this novel regulatory pathway in the context of the pleiotropic actions managed by NF-kB in several cell types and tissues.

**INTRODUCTION**

The ability of a cell to identify the moment when it should differentiate, grow, divide or die depends on extracellular signals such as hormones, small molecules of varied nature, proteins attached to neighboring cells, or small peptides, and also how the cell process this information. Receptors are the transmitters of those extracellular signals, most likely to the nucleus, through cascades of reactions and interactions between proteins as well as metabolic reactions. Signalling cascades of the cell can be considered as a steady-state homeostatic system that results of a highly dynamic inflow and outflow of biological information. The concept of treating signalling cascades as highly dynamic steady-state systems was first introduced by Boon Chock and Earl Stadtman to the regulation of metabolic enzymes [1]. Such pioneer concept can also be applied to those biological cascades related to the various processes of the cell since most of them are also related to some type of enzymatic activity catalyzing protein modifications, i.e. phosphorylation, dephosphorylation, acetylation, isomerization, methylation, etc., which ultimately lead to establish a sort of steady-state condition of a whole chain of reactions able to be regulated separately at many steps [2]. These regulatory systems control the cell cycle, cell differentiation, and cell proliferation processes as a response to specific signal inputs.

Molecular chaperones and their cochaperones are responsible for the interaction of multiple key components of those signalling pathways able to regulate growth, differentiation, and development. The molecular relationships between these proteins and various signalling proteins and their partners appear to be decisive for the appropriate biological action of signal transduction cascades, and the relative expression of these proteins is important for the regulation of the response since insufficient or excessive amounts could generate an aberrant control of essential cell processes such as proliferation, division, development and/or growth [3]. Both genetic and molecular interactions between regulatory proteins and the various components of the signalling pathways show us that the cross-talk between these proteins can regulate proliferation and development by preventing or enhancing cell growth and cell death as the levels of these molecular chaperones change in response to various stimuli. In this article we will focus on the recently discovered regulatory action by FKBP51 and FKP52, two Hsp90-binding chaperones belonging to the immunophilin family, on the biological properties of the transcription factor NF-κB.

## IMMUNOPHILINS

Immunophilins are endogenous proteins with peptidyl-prolyl-(*cis/trans*)-isomerase (PPIase) activity, i.e., the reversible *cis/trans* enzymatic interconvertion of Xaa-Pro bonds. These proteins are grouped in a common family of proteins whose signature domain is the PPIase domain. In turn, they are classified into two major subfamilies according to their capacity to bind immunosuppressant ligands [4], whose binding site is the PPIase domain. When immunophilins bind the cyclic undecapeptide cyclosporine A (CsA), they are called *cyclophilins* (or CyPs), and those that are able to bind the cyclic macrolide tacrolimus (or FK506) are named *FK506-binding proteins* (or FKBPs). Many members of the FKBP subfamily also bind the drug sirolimus (or rapamycin). There is a third subfamily of immunophilins referred to as *Parvulins* that show certain homology with the PPIase domain of the other subfamilies and may show enzymatic activity, but they are not able to bind immunosuppressive drugs. The most relevant parvulin in humans is Pin1, which recognizes a specific motif of a phosphorylated serine or threonine residue preceding a proline [5].

Immunophilins were first described as intracellular receptors for immunosuppressive drugs. Even though most of them bind these drugs, only the low molecular weight immunophilins FKBP12 and CyPA, the archetypal members of each subfamily, are related to the immunosuppressive effect when the FK506•FKBP12 or CsA•CyPA complex inhibits the phosphatase activity of calcineurin, a PP2B

class of Ser/Thr protein-phosphatase. The inhibition of such activity prevents the dephosphorylation and subsequent activation of the transcription factor NFAT (*Nuclear Factor of Activated T cells*), which remains cytoplasmic. Therefore, the production of interleukines and interferon-γ is prevented (see [6] for a recent review).

High molecular weight immunophilins have a more complex architecture and are not related to the immunosupression process (Fig.1). The archetype of this subfamily is the 52-kDa FK506-binding protein, FKBP52 [7]. In addition to the active PPIase domain (also called FKBD1 or FK1 domain in FKBP proteins), which resembles the structure of the immunosuppressive factor FKBP12, there are other additional domains only present in the high molecular weight subfamily. The best studied is the TPR domain formed by sequences of 34 amino acids repeated in tandem through which they bind to Hsp90 via the MEEVD C-terminal conserved sequence of this chaperone [8] (Fig.1). TPR-domain immunophilins such as FKBP51 and FKBP52 are abundant and ubiquitous proteins that were first discovered associated to steroid receptors. The four more classical TPR domain immunophilins that have been relatively well characterized due to their association with these receptors are FKBP52 (gene name *FKBP4*), FKBP51 (gene name *FKBP5*), the cyclophilin CyP40 (gene name *PPID*), and the FKBP-like protein phosphatase PP5 (gene name *PPP5C*). All of them have their counter-part in plants [9], are highly ubiquitous, and are also able to form complexes (many of them still to be characterized) with several factors, although their biological functions and many aspects of their molecular mechanism of action are poorly understood.

Another important TPR-domain immunophilin is FKBP37 (gene name *AIP*), also known as XAP2/AIP. It was first discovered associated to AhR (aryl-hydrocarbon receptor, or "dioxane" receptor), where the immunophilin favors the biological actions of the receptor [10, 11]. FKBP37 is also able to interact and repress the biological activity of other member of the nuclear receptor superfamily, PPARα (peroxisome proliferator-activated receptor-α), an Hsp90-binding transcription factor [12] that modulates lipid metabolism, inflammation, and blood pressure [13].

There are two more relevant TPR-domain immunophilins whose biological roles have been elucidated more recently. One is FKBP38, which shows a mitochondrial localization signal and has been related to apoptosis (see [14] for a recent review). In spite of its almost identical three-dimensional structure of the PPIase domain with the immunosuppressive immunophilins FKBP12, FKBP38 lacks enzymatic activity and does not bind immunosuppressive drugs. However it provides a scaffold platform to facilitate protein-protein interactions. This is particularly important for the case of

anti-apoptotic factors [15] such as the proto-oncogene Bcl-2 (*B-cell lymphoma 2*). This contributes to tumorigenesis and chemoresistance [16].

The other relevant TPR-domain immunophilin is FKBP36 because it is crucial to spermatogenesis since it is able to interact with components of the synaptonemal complex [17], and is also a natural inhibitor of GADPH activity [18]. GADPH is involved in the mechanism of vesicle transport from the endoplasmic reticulum to the Golgi and is also recruited by Rab2 to the vesicular-tubular clusters of the reticulum where it helps to form vesicles. Consequently, FKBP36 shows the additional potential to affect vesicle trafficking and the secretory pathways [18]. To date, there are no compounds able to recognize specifically this immunophilin.

In the mid-1990s, a 92 residue member of the parvulins was identified in *Escherichia coli,* forming the prototype of the third family of PPIases [19]. Soon after, human isoenzymes were described and the small subfamily of parvulins was born. It has only three members in humans: Pin1, Par14, and Par17 [20]. Pin1 is the best studied and its name is often used as synonym of parvulin itself. They are also able to accelerate protein folding in vitro, but they show unique specificity for prolines preceded by phosphorylated Ser or Thr residues. Thus, Pin1 possesses the potential to regulate several phosphorylation signalling cascades by modifying the conformation of the target protein around its phosphorylation site, making Ser or Thr residues less or more accessible for dephosphorylation (see a recent reviewed in [21]). In this sense, Pin1 may act as a molecular timer to make the first move or bring to an end signalling cascades at certain time points of the cell cycle [22]. Pin1 is prevalently overexpressed in human cancers and its expression levels correlate with poor clinical outcome [23].

Pin1 inhibitors may simultaneously block multiple oncogenic signalling pathways and thereby overcome cancer-cell resistance to inhibition of specific kinases or phosphatases [21]. The best known Pin1 inhibitors include the natural product juglone, the small molecule PiB and others of peptidic nature (see [24] for a recent review), but their specificity and potency remain a major concern, and further design and optimization of novel small molecule Pin1 inhibitors are required. Even though recent efforts have been made to obtain better compounds with higher cell membrane permeability and better affinity for this immunophilin [25, 26], the specificity of these novel small molecules still remains to be proved.

**NF-κB**

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) constitutes a family of highly related transcription factors able to regulate the expression of a great number of genes related to several processes such as inflammatory responses, cell growth, immune responses, cell development, synaptic plasticity, memory, cancer processes, etc [27]. This family of transcription factors belongs to the rapid-acting set of cell factors able to be activated by a large variety of signals and stressful situations; that includes cytokines, reactive oxygen species, bacterial and viral antigens, cell injuries, ionizing radiation, UV light, beta-adrenergic agonists, cocaine, etc. Since NF-κB was discovered in 1986 as a transcription factor able to bind to the enhancer element of the immunoglobulin κ light-chain of activated B cells [28], it became clear that in addition to having a crucial role in innate immunity, it is also able to regulate many other basic functions of the cell such as inflammatory responses, immune development, chronic inflammation, autoimmunity diseases, cancer promotion, cell development, programmed cell death, proliferation control, tumorigenesis, etc. (see [29-31] for recent comprehensive updates). NF-κB is actually regarded as a family of structurally related homologues that comprise p50 (NF-κB1), p52 (NF-κB2), p65 (Rel A), Rel B, and c-Rel. All of them share a conserved DNA-binding and dimerization domain. Potentially, they may associate in different combinations such as they can form up to fifteen types of dimers. Nonetheless, the physiological existence of all of these potential dimers has not been demonstrated to date, the p50•p65/RelA heterodimer being unquestionably the most abundant in all cell types [32]. On the other hand, the NF-κB family may be divided from the transcriptional perspective into two groups based on the occurrence of the $C_T$-transactivation domains, which are only present in RelA, RelB, and c-Rel [32].

NF-κB proteins bind to members of the inhibitory-κB family (or IκB) that serve as regulators of biological activity. The members of the IκB subfamily are the classical IκB proteins (IκBα, IκBβ, and IkBε), NF-κB precursor proteins (p100 and p105), and the nuclear IκBs (IκBζ, Bcl-3, and IκBNS). IκB proteins show an $N_T$-signal receiving domain (SRD), a central domain (ARD), and a $C_T$-PEST sequence (Pro-, Glu-, Ser-, and Thr-rich domain). IκBα was originally described as disrupting factor of preformed NF-κB•DNA complexes that favors the dissociation of those complexes [33, 34]. Inasmuch as the expression of IκBα is in turn regulated by NF-κB [35]; IκB is able to regulate both NF-κB activation and inactivation.

**NF-κB SIGNALLING CASCADE**

In the canonical activation pathway of NF-κB (Fig. 2-A), excitatory signals activate Toll-like receptors (TLRs), tumor necrosis factor receptor (TNFR) or interleukin-1 receptors (IL-1R). Archetypal stimulating molecules are lipopolysaccharides (LPS), tumor necrosis factor α (TNFα), and interleukin-1 β (IL-1β), respectively [29]. This leads to the activation of the IκB kinase (IKK) complex, which phosphorylates IκBα. This complex is formed by IKKα and IKKβ subunits and at least one non-catalytic accessory protein, the IKKγ subunit, also known as NEMO (NF-κB Essential Modulator) [36] (see Fig. 2-A). In turn, this IκB kinase complex associates to additional factors and interacts with other upstream signalling molecules and kinases. The phosphorylation of IκB favors its release from the p50•RelA/p65 dimer followed by proteasomal degradation of the inhibitor factor. Thus, the free heterodimer is retrotransported to the nucleus.

An alternative NF-κB activation pathway known as the "non-canonical pathway" (Fig.2-B) originates from different types of receptors [37], including CD40, RANK (Receptor Activator for Nuclear Factor kappa B), BAFFR (B-cell Activation Factor), LTβR (Lymphotoxin β-Receptor) or TNFR type II. In this pathway, NF-κB is activated by the kinase NIK, which phosphorylates and activates predominantly IKKα, whose activity phosphorylates p100. This favors p100 ubiquitination and its partial degradation to generate the p52 subunit, that usually associates to RelB [38].

Besides the canonical (Fig. 2-A) and the alternative pathways (Fig. 2-B), a third manner for NF-κB activation also exists and is named the "atypical activation pathway" (Fig. 2-C). Actually, there is more than one, but the most typical is the activation of the IKK complex after genotoxic stress via the ATM kinase (Ataxia-Telangiectasia Mutated protein-kinase) leading to ubiquitination of IKKγ/NEMO [39]. In all the above-described pathways, following the liberation of the NF-κB dimers by activation of IKKs, their steady state localization is normally shifted to the nucleus and the Rel Homology Domains (RHD) (Fig. 1) are free to bind cognate DNA-sequences in the enhancer elements of target gene promoters. Depending on the accessibility of the genome regulated by epigenetic mechanisms and the cell type, hundreds of different target genes can be transcriptionally activated and regulated by additional transcription factors. This may either enhances or reduces the NF-κB biological action. The level of complexity and crosstalks between NF-κB with other signalling pathways and transcription factors are some of the reasons that explain the pleiotropic actions of this factor.

There is general consensus that NF-κB proteins bind as a homo- or heterodimer to a 10-base-pair DNA sequence (which was first identified in the enhancer region of the immunoglobulin κ-light-chain gene of mature B cells [33, 40]). The first structure of a Rel-homology region was discovered from the structure of a p50 homodimer bound to an idealized κB target DNA sequence [41-43]. Various

structures of NF-κB dimers bound to DNA have been reveled to date, showing a common pattern of structures that resemble a butterfly, where the dimer represents the wings around a cylindrical DNA structure [42]. NF-κB seems to encircle the target DNA. The analysis of different types of these structures has shown plasticity for these sequences of NF-κB homo/heterodimers. The canonical p50•RelA/p65 heterodimer recognizes the binding sequence through the p50 subunit bound to a 5-GGPyN half-site and via RelA/p65 binding to another 5-GGPyN site. Importantly, IκB is one of the proteins induced by RelA/p65, which implies the existence of a self-regulated NF-κB feedback that helps to restore the original cytoplasmic localization of NF-κB [35].

## THE FKBP•NF-κB CONNECTION

As it was advanced before, all Rel factors form homodimers or heterodimers with the sole exception of Rel B, which forms only heterodimers. The relative abundance of different NF-κB proteins may vary in different tissues and cell types, whereas the p50•RelA/p65 heterodimer is highly ubiquitous and also the most frequent in most cell types and tissues [44]. In unstimulated cells, even though p50•RelA/p65 heterodimers are retained in the cytosol by IκB and translocate to the nucleus of the NF-κB via the dynein/dynactin motor complex [45] upon cell stimulation, both cytoplasmic and nuclear complexes undergo a dynamic nuclear-cytoplasmic shuttling [40, 46, 47]. This allows a low basal transcriptional activity of NF-κB given the fact that the IκB/NF-κB complex is also subject to dynamic dissociation/reassociation events. This nuclear-cytoplasmic shuttling of NF-κB resembles that observed for steroid receptors, where the inactive cytoplasmic form of these ligand-dependent transcription factors must translocate to the nucleus upon cell stimulation with steroid hormones [48-50].

In previous studies, our laboratory and others have reported that the 51-kDa and 52-kDa FK506-binding proteins FKBP51 and FKBP52 are responsible in a mutually exclusive fashion for the retrotransport mechanism of GR [51, 52] and MR [53, 54]. Both FKBPs are also regulators of the ligand-dependent transcriptional activity for those receptors [54-56] and other members of the family such as PR [57, 58], AR [59, 60] and, to a minor degree, ERα [58, 61]. These Hsp90-binding immunophilins are highly homologous and share 60% homology and 75% similarity [62]. As shown in Fig.1, they are structurally characterized by the presence of two key sequences: the TPR domain, through which they bind to Hsp90, and the peptidyl-prolyl isomerase (PPIase) domain [63], where the macrolide FK506 and also the dynein/dynactin motor complex bind. Both domains are essential for the retrotransport mechanism of steroid receptors [50, 64], the first because of its interaction with the chaperone and the

second due to its capacity to bind motor proteins. Nonetheless, the enzymatic activity of the PPIase domain does not appear to be essential. Upon steroid binding, FKBP51 is released from the receptor•Hsp90 heterocomplex and is replaced by FKBP52, which recruits dynein/dynactin motor proteins favoring the transport of the receptor to the nucleus on microtubule tracks [65] (Fig.3). While FKBP52 favors steroid binding and transcriptional activity, FKBP51 impairs both effects.

Both transcription factors, steroid receptors and NF-κB, show similar requirements for subcellular redistribution upon the onset of a given activating stimulus. A recent investigation showed that FKBP51 and FKBP52 affects the nuclear translocation of RelA/p65 and also influence the transcriptional activity of NF-κB [66]. FKBP51 delays the nuclear translocation of p50•RelA/p65 and also shows inhibitory action on transcriptional activity, an effect related to its incapacity to interact with dynein/dynactin [55], whereas FKBP52 shows a strong stimulatory effect on transcription. In contrast to steroid receptors, these biological actions for NF-κB are Hsp90-independent for NF-κB. This is confirmed by the fact that point mutants in the TPR domains of the FKBPs unable to bind Hsp90 show similar effects as the wild type immunophilins [66]. This Hsp90-independence indicates an innovative mechanism of regulation in divergence with the steroid receptors, where their ligand-dependent activation and transcriptional activity are mostly Hsp90-dependent. However Hsp90 has been associated to NF-κB activation by its role on IKK regulation, as it is described below. Importantly the PPIase activity of FKBP52 appears to be exceptional for this stimulatory action, whereas that enzymatic activity is not required for the FKBP51 inhibitory action. A similar independent effect on the PPIase activity was described for the regulation of the GR by FKBP51 [55, 67], whereas a PPIase-dependent mechanism is implicated for FKBP52 [55, 68].

One interesting extrapolation of these effects is that the biological action of NF-κB may be regulated in different tissues and cell types by the overall expression balance of both immunophilins, which could contribute in part to the pleiotropic actions of NF-κB. Moreover, our assays showed that Hsp70 is also a RelA/p65-interactor, which is in agreement with a very recent report in neurons [69] where the nuclear translocation of both RelA/p65 and Hsp70 was postulated to occur as a protein-protein complex. Interestingly, the up-regulation of Hsp70 was also reported to induce nuclear translocation of RelA/p65 in rat liver cells [70]. Nevertheless, the RelA/p65 and Hsp70 interaction not always leads to positive effects on NF-κB activation. The initial reports showed interactions that involve stress-induced situations where the effects of heat-shock proteins reduce the inflammatory responses [71]. In this scenery, it was proposed that overexpressed Hsp70 is able to interact with NF-κB, suggesting that Hsp70 may substitute IκB by anchoring NF-κB to the cytoplasm. Only increased accumulation of the chaperone could result in an inhibition complex of NF-κB [72]. It was also

reported that Hsp70 shows similar effects on various intracellular immune pathways and signalling in the brain [73], although it cannot be ruled out that the biological actions may be cell- and stimulus-dependent. Also, it has been shown that Hsp70 interacts with the IKK complex to decrease NF-κB signalling [74] as well as with other members of the inflammatory signalling cascade preventing their actions [75, 76]

The IκB/NF-κB cytosolic complex is subject to dynamic dissociation/reassociation events. Experiments with IKKα knockout mice [77] demonstrated defective cell proliferation and differentiation and have also shown that IKKα is dispensable for IκB degradation. Moreover IKKα has been reported to be required for the termination of NF-κB activation [78]. A physical interaction between FKBP51 and the IKK complex has been demonstrated, most likely via the IKKα subunit bound to Hsp90 [79, 80], but the biological function of FKBP51 on IKK signalling is still unclear [80] (Fig.3). While down-modulation of Hsp90α and Hsp90β likewise resulted in reduced kinase activity, it has been shown that FKBP51 is not a constitutively associated component of the IKK complex [80], and its down-modulation interfered with neither TNFα-induced IKK activity nor IκBα degradation and RelA/p65 translocation. Actually, the experimental evidence shows that the prevailing complex for the IKK•Hsp90 complex to generate an activated state is the one that recruits Cdc37 rather than FKBP51 [80] (Fig.3), both factors being transiently associated with NF-κB [80]. Importantly, in the same study it was also reported that TNFα is unable to modify the association of IKK with those interacting factors. In short, the role of FKBP51 on NF-κB signalling cascade remained elusive to date. It is unlikely that both Hsp90-interacting factors, Cdc37 and FKBP51, are part of the same IKK complex since it has been demonstrated that the binding of a TPR protein to Hsp90 precludes the binding of Cdc37 and vice versa, perhaps due to the fact that both proteins bind to adjacent domains of the chaperone [81]. Because FKBP51 is able to associate to IKK, and because Cdc37 is essential for the maturation of *de novo* synthesized IKKs into enzymatically competent kinases, but not for assembly of an IKK holocomplex, it could be possible that the role of FKBP51 is more related to the assembly process with IKK in similar fashion as the Hsp90 co-chaperone Hop (Heat-shock organizing protein) is intermediary for GR•Hsp90 assembly, but it is not present in mature complexes [82].

Fig.3 depicts the proposed novel mechanism for the regulation of NF-κB biological actions by FKBP51 and FKBP52. These immunophilins affect NF-κB activation at different levels, i.e. nuclear transport, nuclear retention, and transcriptional activity. It is important to emphasize that endogenous FKBP51 was found constitutively associated to the promoter regions of an NF-κB target gene, whereas FKBP52 replaces FKBP51 in stimulated cells. Moreover, while FKBP51 represses NF-κB transcription,

FKBP52 greatly enhances this activity in a mechanism that involves its PPIase enzymatic activity (and is therefore impaired by PPIase inhibitory drugs) (Fig.3).

A crucial nuclear mechanism for gene expression is the modification of the chromatin environment of the respective genes. It has been shown that when NF-κB is activated, histone phosphorylation can be mediated by nuclear IKKα that is recruited to the promoter sites of NF-κB-regulated genes [83, 84]. Among a number of chromatin remodelers is the PPIase protein Pin1 [85], an immunophilin-like protein that also targets RelA/p65 [86]. Because PPIase-induced conformational changes have functional effects on target proteins, the action of Pin1 on RelA/p65 is reflected in a more efficient nuclear accumulation of RelA/p65 and also a greater stability by preventing its ubiquitin-mediated proteolysis [86]. In certain types of cancer cells, Pin1 is usually up-regulated [87-89] whereas the E3-ubiquitin-ligase of RelA/p65, SOCS1 is down-regulated [90-92] or mutated [93], all of which may contribute to the constitutive activation of NF-κB in those cancers. A similar mechanism can be proposed here for the expression balance of FKBP51 and FKBP52, in particular for the latter immunophilin that shows an important stimulatory action dependent on its PPIase activity.

## FUTURE PERSPECTIVES

Given the role played in the initiation and progression of cancer, the NF-κB signalling pathway is a potent node of pharmacological interference in the clinic. Because NF-κB is also an essential protein in the immunological response against cancer, there has been a reluctance to use NF-κB-targeting inhibitors for the treatment of such malignancies. Nevertheless, combining classical chemotherapeutics with inhibitors of NF-κB activation seems to result in a promising synergistic strategy [94, 95]. Elevated NF-κB activity and/or higher half-life persistence in the nucleus of cancer cells (like that observed with FKBP52) provide a survival mechanism by up-regulating anti-apoptotic genes, thereby representing a major causative factor for drug resistance [96].

The development of immunophilin ligands appears to have promising perspectives in the coming years [97]. Thus, the ability to regulate the functions of a specific protein using cell-permeable small molecules like those that bind FKBPs is an unquestionably powerful method, not only to study biological systems, but also a desired alternative to be used in therapeutic treatments. In line with this aim, it has recently been reported the synthesis of two novel compounds named SAFit1 and SAFit2, that are highly selective inhibitors of FKBP51 [98]. This new class of ligands achieves selectivity for this immunophilin

by an induced-fit mechanism that is much less favorable for FKBP52. By using these ligands, it was confirmed and original report showing that the selective inhibition of FKBP51 enhances neurite elongation in neuronal cultures [99] and, even more importantly, that these drugs improve neuroendocrine feedback in vivo as well as stress-coping behavior [98].

Ideally, the biological function of certain nuclear factors could be regulated if we can influence the mechanisms by which they reach their sites of action. In this sense, because NF-κB is active in many cancer cells and its persistent localization in the nucleus strengthens or directly leads to tumor development. Therefore, based on the model shown in Fig.3, it is tempting to think that targeting specifically the PPIase activity of FKBP52 (essential for its enhancement action on NF-κB effects) could be a promising novel regulatory approach to prevent NF-κB activity.

## REFERENCES

[1] P.B. Chock, E.R. Stadtman, Covalently interconvertible enzyme cascade systems, Methods Enzymol, 64 (1980) 397-325.

[2] E. Helmreich, The Biochemistry of Cell Signalling, Oxford University Press, Oxford, U.K., 2001.

[3] E.A. Nollen, R.I. Morimoto, Chaperoning signaling pathways: molecular chaperones as stress-sensing 'heat shock' proteins, J Cell Sci, 115 (2002) 2809-2816.

[4] C.B. Kang, Y. Hong, S. Dhe-Paganon, H.S. Yoon, FKBP family proteins: immunophilins with versatile biological functions, Neurosignals, 16 (2008) 318-325.

[5] J.U. Rahfeld, K.P. Rucknagel, B. Schelbert, B. Ludwig, J. Hacker, K. Mann, G. Fischer, Confirmation of the existence of a third family among peptidyl-prolyl cis/trans isomerases. Amino acid sequence and recombinant production of parvulin, FEBS Lett, 352 (1994) 180-184.

[6] H. Li, A. Rao, P.G. Hogan, Interaction of calcineurin with substrates and targeting proteins, Trends Cell Biol, 21 (2011) 91-103.

[7] I. Callebaut, J.M. Renoir, M.C. Lebeau, N. Massol, A. Burny, E.E. Baulieu, J.P. Mornon, An immunophilin that binds M(r) 90,000 heat shock protein: main structural features of a mammalian p59 protein, Proc Natl Acad Sci U S A, 89 (1992) 6270-6274.

[8] D.F. Smith, Tetratricopeptide repeat cochaperones in steroid receptor complexes, Cell Stress Chaperones, 9 (2004) 109-121.

[9] K. Aviezer-Hagai, J. Skovorodnikova, M. Galigniana, O. Farchi-Pisanty, E. Maayan, S. Bocovza, Y. Efrat, P. von Koskull-Doring, N. Ohad, A. Breiman, Arabidopsis immunophilins ROF1 (AtFKBP62) and ROF2 (AtFKBP65) exhibit tissue specificity, are heat-stress induced, and bind HSP90, Plant Mol Biol, 63 (2007) 237-255.

[10] Q. Ma, J.P. Whitlock, Jr., A novel cytoplasmic protein that interacts with the Ah receptor, contains tetratricopeptide repeat motifs, and augments the transcriptional response to 2,3,7,8-tetrachlorodibenzo-p-dioxin, J Biol Chem, 272 (1997) 8878-8884.

[11] L.A. Carver, C.A. Bradfield, Ligand-dependent interaction of the aryl hydrocarbon receptor with a novel immunophilin homolog in vivo, J Biol Chem, 272 (1997) 11452-11456.

[12] W.K. Sumanasekera, E.S. Tien, R. Turpey, J.P. Vanden Heuvel, G.H. Perdew, Evidence that peroxisome proliferator-activated receptor alpha is complexed with the 90-kDa heat shock protein and the hepatitis virus B X-associated protein 2, J Biol Chem, 278 (2003) 4467-4473.

[13] A. Hiukka, M. Maranghi, N. Matikainen, M.R. Taskinen, PPARalpha: an emerging therapeutic target in diabetic microvascular damage, Nat Rev Endocrinol, 6 (2010) 454-463.

[14] F. Edlich, C. Lucke, From cell death to viral replication: the diverse functions of the membrane-associated FKBP38, Curr Opin Pharmacol, 11 (2011) 348-353.

[15] M. Shirane, K.I. Nakayama, Inherent calcineurin inhibitor FKBP38 targets Bcl-2 to mitochondria and inhibits apoptosis, Nat Cell Biol, 5 (2003) 28-37.

[16] B.H. Choi, H.S. Yoon, FKBP38-Bcl-2 interaction: a novel link to chemoresistance, Curr Opin Pharmacol, 11 (2011) 354-359.

[17] M.A. Crackower, N.K. Kolas, J. Noguchi, R. Sarao, K. Kikuchi, H. Kaneko, E. Kobayashi, Y. Kawai, I. Kozieradzki, R. Landers, R. Mo, C.C. Hui, E. Nieves, P.E. Cohen, L.R. Osborne, T. Wada, T. Kunieda, P.B. Moens, J.M. Penninger, Essential role of Fkbp6 in male fertility and homologous chromosome pairing in meiosis, Science, 300 (2003) 1291-1295.

[18] F. Jarczowski, G. Jahreis, F. Erdmann, A. Schierhorn, G. Fischer, F. Edlich, FKBP36 is an inherent multifunctional glyceraldehyde-3-phosphate dehydrogenase inhibitor, J Biol Chem, 284 (2009) 766-773.

[19] J.U. Rahfeld, A. Schierhorn, K. Mann, G. Fischer, A novel peptidyl-prolyl cis/trans isomerase from Escherichia coli, FEBS Lett, 343 (1994) 65-69.

[20] S.F. Gothel, M.A. Marahiel, Peptidyl-prolyl cis-trans isomerases, a superfamily of ubiquitous folding catalysts, Cell Mol Life Sci, 55 (1999) 423-436.

[21] K.P. Lu, X.Z. Zhou, The prolyl isomerase PIN1: a pivotal new twist in phosphorylation signalling and disease, Nat Rev Mol Cell Biol, 8 (2007) 904-916.

[22] C.H. Lin, H.Y. Li, Y.C. Lee, M.J. Calkins, K.H. Lee, C.N. Yang, P.J. Lu, Landscape of Pin1 in the cell cycle, Exp Biol Med (Maywood), (2015).

[23] M. Theuerkorn, G. Fischer, C. Schiene-Fischer, Prolyl cis/trans isomerase signalling pathways in cancer, Curr Opin Pharmacol, 11 (2011) 281-287.

[24] T.H. Lee, L. Pastorino, K.P. Lu, Peptidyl-prolyl cis-trans isomerase Pin1 in ageing, cancer and Alzheimer disease, Expert Rev Mol Med, 13 (2011) e21.

[25] L. Dong, J. Marakovits, X. Hou, C. Guo, S. Greasley, E. Dagostino, R. Ferre, M.C. Johnson, E. Kraynov, J. Thomson, V. Pathak, B.W. Murray, Structure-based design of novel human Pin1 inhibitors (II), Bioorg Med Chem Lett, 20 (2010) 2210-2214.

[26] C. Liu, J. Jin, L. Chen, J. Zhou, X. Chen, D. Fu, H. Song, B. Xu, Synthesis and biological evaluation of novel human Pin1 inhibitors with benzophenone skeleton, Bioorg Med Chem, 20 (2012) 2992-2999.

[27] J. Napetschnig, H. Wu, Molecular basis of NF-kappaB signaling, Annu Rev Biophys, 42 (2013) 443-468.

[28] R. Sen, D. Baltimore, Multiple nuclear factors interact with the immunoglobulin enhancer sequences, Cell, 46 (1986) 705-716.

[29] B. Hoesel, J.A. Schmid, The complexity of NF-kappaB signaling in inflammation and cancer, Mol Cancer, 12 (2013) 86.

[30] G. Diamant, R. Dikstein, Transcriptional control by NF-kappaB: elongation in focus, Biochim Biophys Acta, 1829 (2013) 937-945.

[31] L. Tornatore, A.K. Thotakura, J. Bennett, M. Moretti, G. Franzoso, The nuclear factor kappa B signaling pathway: integrating metabolism with inflammation, Trends Cell Biol, 22 (2012) 557-566.

[32] A. Oeckinghaus, S. Ghosh, The NF-kappaB family of transcription factors and its regulation, Cold Spring Harb Perspect Biol, 1 (2009) a000034.

[33] W.F. Tam, R. Sen, IkappaB family members function by different mechanisms, J Biol Chem, 276 (2001) 7701-7704.

[34] U. Zabel, P.A. Baeuerle, Purified human I kappa B can rapidly dissociate the complex of the NF-kappa B transcription factor with its cognate DNA, Cell, 61 (1990) 255-265.

[35] M.L. Scott, T. Fujita, H.C. Liou, G.P. Nolan, D. Baltimore, The p65 subunit of NF-kappa B regulates I kappa B by two distinct mechanisms, Genes Dev, 7 (1993) 1266-1276.

[36] M.S. Hayden, S. Ghosh, Shared principles in NF-kappaB signaling, Cell, 132 (2008) 344-362.

[37] N.D. Perkins, Integrating cell-signalling pathways with NF-kappaB and IKK function, Nat Rev Mol Cell Biol, 8 (2007) 49-62.

[38] G. Xiao, E.W. Harhaj, S.C. Sun, NF-kappaB-inducing kinase regulates the processing of NF-kappaB2 p100, Mol Cell, 7 (2001) 401-409.

[39] T.T. Huang, S.M. Wuerzberger-Davis, Z.H. Wu, S. Miyamoto, Sequential modification of NEMO/IKKgamma by SUMO-1 and ubiquitin mediates NF-kappaB activation by genotoxic stress, Cell, 115 (2003) 565-576.

[40] T.T. Huang, N. Kudo, M. Yoshida, S. Miyamoto, A nuclear export signal in the N-terminal regulatory domain of IkappaBalpha controls cytoplasmic localization of inactive NF-kappaB/IkappaBalpha complexes, Proc Natl Acad Sci U S A, 97 (2000) 1014-1019.

[41] F. Fusco, A. Pescatore, E. Bal, A. Ghoul, M. Paciolla, M.B. Lioi, M. D'Urso, S.H. Rabia, C. Bodemer, J.P. Bonnefont, A. Munnich, M.G. Miano, A. Smahi, M.V. Ursini, Alterations of the IKBKG locus and diseases: an update and a report of 13 novel mutations, Hum Mutat, 29 (2008) 595-604.

[42] C.W. Muller, F.A. Rey, M. Sodeoka, G.L. Verdine, S.C. Harrison, Structure of the NF-kappa B p50 homodimer bound to DNA, Nature, 373 (1995) 311-317.

[43] C.W. Muller, S.C. Harrison, The structure of the NF-kappa B p50:DNA-complex: a starting point for analyzing the Rel family, FEBS Lett, 369 (1995) 113-117.

[44] A. Hoffmann, G. Natoli, G. Ghosh, Transcriptional regulation via the NF-kappaB signaling module, Oncogene, 25 (2006) 6706-6716.

[45] T.D. Gilmore, The Rel/NF-kappaB signal transduction pathway: introduction, Oncogene, 18 (1999) 6842-6844.

[46] A. Birbach, P. Gold, B.R. Binder, E. Hofer, R. de Martin, J.A. Schmid, Signaling molecules of the NF-kappa B pathway shuttle constitutively between cytoplasm and nucleus, J Biol Chem, 277 (2002) 10842-10851.

[47] I. Mikenberg, D. Widera, A. Kaus, B. Kaltschmidt, C. Kaltschmidt, Transcription factor NF-kappaB is transported to the nucleus via cytoplasmic dynein/dynactin motor complex in hippocampal neurons, PLoS One, 2 (2007) e589.

[48] C. Elbi, D.A. Walker, G. Romero, W.P. Sullivan, D.O. Toft, G.L. Hager, D.B. DeFranco, Molecular chaperones function as steroid receptor nuclear mobility factors, Proc Natl Acad Sci U S A, 101 (2004) 2876-2881.

[49] A.P. Madan, D.B. DeFranco, Bidirectional transport of glucocorticoid receptors across the nuclear envelope, Proc Natl Acad Sci U S A, 90 (1993) 3588-3592.

[50] M.D. Galigniana, P.C. Echeverria, A.G. Erlejman, G. Piwien-Pilipuk, Role of molecular chaperones and TPR-domain proteins in the cytoplasmic transport of steroid receptors and their passage through the nuclear pore, Nucleus, 1 (2010) 299-308.

[51] M.D. Galigniana, C. Radanyi, J.M. Renoir, P.R. Housley, W.B. Pratt, Evidence that the peptidylprolyl isomerase domain of the hsp90-binding immunophilin FKBP52 is involved in both dynein interaction and glucocorticoid receptor movement to the nucleus, J Biol Chem, 276 (2001) 14884-14889.

[52] T.H. Davies, Y.M. Ning, E.R. Sanchez, A new first step in activation of steroid receptors: hormone-induced switching of FKBP51 and FKBP52 immunophilins, J Biol Chem, 277 (2002) 4597-4600.

[53] M.D. Galigniana, A.G. Erlejman, M. Monte, C. Gomez-Sanchez, G. Piwien-Pilipuk, The hsp90-FKBP52 complex links the mineralocorticoid receptor to motor proteins and persists bound to the receptor in early nuclear events, Mol Cell Biol, 30 (2010) 1285-1298.

[54] L.I. Gallo, A.A. Ghini, G. Piwien Pilipuk, M.D. Galigniana, Differential recruitment of tetratricorpeptide repeat domain immunophilins to the mineralocorticoid receptor influences both heat-shock protein 90-dependent retrotransport and hormone-dependent transcriptional activity, Biochemistry, 46 (2007) 14044-14057.

[55] G.M. Wochnik, J. Ruegg, G.A. Abel, U. Schmidt, F. Holsboer, T. Rein, FK506-binding proteins 51 and 52 differentially regulate dynein interaction and nuclear translocation of the glucocorticoid receptor in mammalian cells, J Biol Chem, 280 (2005) 4609-4616.

[56] D.L. Riggs, P.J. Roberts, S.C. Chirillo, J. Cheung-Flynn, V. Prapapanich, T. Ratajczak, R. Gaber, D. Picard, D.F. Smith, The Hsp90-binding peptidylprolyl isomerase FKBP52 potentiates glucocorticoid signaling in vivo, Embo J, 22 (2003) 1158-1167.

[57] S. Tranguch, J. Cheung-Flynn, T. Daikoku, V. Prapapanich, M.B. Cox, H. Xie, H. Wang, S.K. Das, D.F. Smith, S.K. Dey, Cochaperone immunophilin FKBP52 is critical to uterine receptivity for embryo implantation, Proc Natl Acad Sci U S A, 102 (2005) 14326-14331.

[58] Z. Yang, I.M. Wolf, H. Chen, S. Periyasamy, Z. Chen, W. Yong, S. Shi, W. Zhao, J. Xu, A. Srivastava, E.R. Sanchez, W. Shou, FK506-binding protein 52 is essential to uterine reproductive physiology controlled by the progesterone receptor A isoform, Mol Endocrinol, 20 (2006) 2682-2694.

[59] J. Cheung-Flynn, V. Prapapanich, M.B. Cox, D.L. Riggs, C. Suarez-Quian, D.F. Smith, Physiological role for the cochaperone FKBP52 in androgen receptor signaling, Mol Endocrinol, 19 (2005) 1654-1666.

[60] S. Periyasamy, T. Hinds, Jr., L. Shemshedini, W. Shou, E.R. Sanchez, FKBP51 and Cyp40 are positive regulators of androgen-dependent prostate cancer cell growth and the targets of FK506 and cyclosporin A, Oncogene, 29 (2010) 1691-1701.

[61] J.P. Schulke, G.M. Wochnik, I. Lang-Rollin, N.C. Gassen, R.T. Knapp, B. Berning, A. Yassouridis, T. Rein, Differential impact of tetratricopeptide repeat proteins on the steroid hormone receptors, PLoS One, 5 (2010) e11717.

[62] D.L. Cioffi, T.R. Hubler, J.G. Scammell, Organization and function of the FKBP52 and FKBP51 genes, Curr Opin Pharmacol, 11 (2011) 308-313.

[63] J.C. Sivils, C.L. Storer, M.D. Galigniana, M.B. Cox, Regulation of steroid hormone receptor function by the 52-kDa FK506-binding protein (FKBP52), Curr Opin Pharmacol, 11 (2011) 314-319.

[64] C.L. Storer, C.A. Dickey, M.D. Galigniana, T. Rein, M.B. Cox, FKBP51 and FKBP52 in signaling and disease, Trends Endocrinol Metab, 22 (2011) 481-490.

[65] M.D. Galigniana, Steroid receptor coupling becomes nuclear, Chem Biol, 19 (2012) 662-663.

[66] A.G. Erlejman, S.A. De Leo, G.I. Mazaira, A.M. Molinari, M.F. Camisay, V. Fontana, M.B. Cox, G. Piwien-Pilipuk, M.D. Galigniana, NF-kappaB Transcriptional Activity Is Modulated by FK506-binding Proteins FKBP51 and FKBP52: A ROLE FOR PEPTIDYL-PROLYL ISOMERASE ACTIVITY, J Biol Chem, 289 (2014) 26263-26276.

[67] C. Cluning, B.K. Ward, S.L. Rea, A. Arulpragasam, P.J. Fuller, T. Ratajczak, The helix 1-3 loop in the glucocorticoid receptor LBD is a regulatory element for FKBP cochaperones, Mol Endocrinol, 27 (2013) 1020-1035.

[68] D.L. Riggs, M.B. Cox, J. Cheung-Flynn, V. Prapapanich, P.E. Carrigan, D.F. Smith, Functional specificity of co-chaperone interactions with Hsp90 client proteins, Crit Rev Biochem Mol Biol, 39 (2004) 279-295.

[69] C. Klenke, D. Widera, T. Engelen, J. Muller, T. Noll, K. Niehaus, M.L. Schmitz, B. Kaltschmidt, C. Kaltschmidt, Hsc70 is a novel interactor of NF-kappaB p65 in living hippocampal neurons, PLoS One, 8 (2013) e65280.

[70] K. Dokladny, R. Lobb, W. Wharton, T.Y. Ma, P.L. Moseley, LPS-induced cytokine levels are repressed by elevated expression of HSP70 in rats: possible role of NF-kappaB, Cell Stress Chaperones, 15 (2010) 153-163.

[71] D.L. Feinstein, E. Galea, D.A. Aquino, G.C. Li, H. Xu, D.J. Reis, Heat shock protein 70 suppresses astroglial-inducible nitric-oxide synthase expression by decreasing NFkappaB activation, J Biol Chem, 271 (1996) 17724-17732.

[72] I.V. Guzhova, Z.A. Darieva, A.R. Melo, B.A. Margulis, Major stress protein Hsp70 interacts with NF-kB regulatory complex in human T-lymphoma cells, Cell Stress Chaperones, 2 (1997) 132-139.

[73] F.R. Sharp, X. Zhan, D.Z. Liu, Heat shock proteins in the brain: role of Hsp70, Hsp 27, and HO-1 (Hsp32) and their therapeutic potential, Transl Stroke Res, 4 (2013) 685-692.

[74] R. Ran, A. Lu, L. Zhang, Y. Tang, H. Zhu, H. Xu, Y. Feng, C. Han, G. Zhou, A.C. Rigby, F.R. Sharp, Hsp70 promotes TNF-mediated apoptosis by binding IKK gamma and impairing NF-kappa B survival signaling, Genes Dev, 18 (2004) 1466-1481.

[75] R.G. Giffard, R.Q. Han, J.F. Emery, M. Duan, J.F. Pittet, Regulation of apoptotic and inflammatory cell signaling in cerebral ischemia: the complex roles of heat shock protein 70, Anesthesiology, 109 (2008) 339-348.

[76] J.Y. Kim, M.A. Yenari, The immune modulating properties of the heat shock proteins after brain injury, Anat Cell Biol, 46 (2013) 1-7.

[77] A.K. Sil, S. Maeda, Y. Sano, D.R. Roop, M. Karin, IkappaB kinase-alpha acts in the epidermis to control skeletal and craniofacial morphogenesis, Nature, 428 (2004) 660-664.

[78] T. Lawrence, M. Bebien, G.Y. Liu, V. Nizet, M. Karin, IKKalpha limits macrophage NF-kappaB activation and contributes to the resolution of inflammation, Nature, 434 (2005) 1138-1143.

[79] T. Bouwmeester, A. Bauch, H. Ruffner, P.O. Angrand, G. Bergamini, K. Croughton, C. Cruciat, D. Eberhard, J. Gagneur, S. Ghidelli, C. Hopf, B. Huhse, R. Mangano, A.M. Michon, M. Schirle, J. Schlegl, M. Schwab, M.A. Stein, A. Bauer, G. Casari, G. Drewes, A.C. Gavin, D.B. Jackson, G. Joberty, G. Neubauer, J. Rick, B. Kuster, G. Superti-Furga, A physical and functional map of the human TNF-alpha/NF-kappa B signal transduction pathway, Nat Cell Biol, 6 (2004) 97-105.

[80] M. Hinz, M. Broemer, S.C. Arslan, A. Otto, E.C. Mueller, R. Dettmer, C. Scheidereit, Signal responsiveness of IkappaB kinases is determined by Cdc37-assisted transient interaction with Hsp90, J Biol Chem, 282 (2007) 32311-32319.

[81] A.M. Silverstein, N. Grammatikakis, B.H. Cochran, M. Chinkers, W.B. Pratt, p50(cdc37) binds directly to the catalytic domain of Raf as well as to a site on hsp90 that is topologically adjacent to the tetratricopeptide repeat binding site, J Biol Chem, 273 (1998) 20090-20095.

[82] W.B. Pratt, M.D. Galigniana, Y. Morishima, P.J. Murphy, Role of molecular chaperones in steroid receptor action, Essays Biochem, 40 (2004) 41-58.

[83] K.J. Park, V. Krishnan, B.W. O'Malley, Y. Yamamoto, R.B. Gaynor, Formation of an IKKalpha-dependent transcription complex is required for estrogen receptor-mediated gene activation, Mol Cell, 18 (2005) 71-82.

[84] G.Y. Park, X. Wang, N. Hu, T.V. Pedchenko, T.S. Blackwell, J.W. Christman, NIK is involved in nucleosomal regulation by enhancing histone H3 phosphorylation by IKKalpha, J Biol Chem, 281 (2006) 18684-18690.

[85] N. Raghuram, H. Strickfaden, D. McDonald, K. Williams, H. Fang, C. Mizzen, J.J. Hayes, J. Th'ng, M.J. Hendzel, Pin1 promotes histone H1 dephosphorylation and stabilizes its binding to chromatin, J Cell Biol, 203 (2013) 57-71.

[86] A. Ryo, F. Suizu, Y. Yoshida, K. Perrem, Y.C. Liou, G. Wulf, R. Rottapel, S. Yamaoka, K.P. Lu, Regulation of NF-kappaB signaling by Pin1-dependent prolyl isomerization and ubiquitin-mediated proteolysis of p65/RelA, Mol Cell, 12 (2003) 1413-1426.

[87] R.W. Pang, T.K. Lee, K. Man, R.T. Poon, S.T. Fan, Y.L. Kwong, E. Tse, PIN1 expression contributes to hepatic carcinogenesis, J Pathol, 210 (2006) 19-25.

[88] K.P. Lu, Y.C. Liou, X.Z. Zhou, Pinning down proline-directed phosphorylation signaling, Trends Cell Biol, 12 (2002) 164-172.

[89] G.M. Wulf, A. Ryo, G.G. Wulf, S.W. Lee, T. Niu, V. Petkova, K.P. Lu, Pin1 is overexpressed in breast cancer and cooperates with Ras signaling in increasing the transcriptional activity of c-Jun towards cyclin D1, EMBO J, 20 (2001) 3459-3472.

[90] Y. Oshimo, K. Kuraoka, H. Nakayama, Y. Kitadai, K. Yoshida, K. Chayama, W. Yasui, Epigenetic inactivation of SOCS-1 by CpG island hypermethylation in human gastric carcinoma, Int J Cancer, 112 (2004) 1003-1009.

[91] J. Oh, S.H. Kim, S. Ahn, C.E. Lee, Suppressors of cytokine signaling promote Fas-induced apoptosis through downregulation of NF-kappaB and mitochondrial Bfl-1 in leukemic T cells, J Immunol, 189 (2012) 5561-5571.

[92] X.D. Zhao, W. Zhang, H.J. Liang, W.Y. Ji, Overexpression of miR -155 promotes proliferation and invasion of human laryngeal squamous cell carcinoma via targeting SOCS1 and STAT3, PLoS One, 8 (2013) e56395.

[93] B. Schif, J.K. Lennerz, C.W. Kohler, S. Bentink, M. Kreuz, I. Melzner, O. Ritz, L. Trumper, M. Loeffler, R. Spang, P. Moller, SOCS1 mutation subtypes predict divergent outcomes in diffuse large B-Cell lymphoma (DLBCL) patients, Oncotarget, 4 (2013) 35-47.

[94] O. Fuchs, Transcription factor NF-kappaB inhibitors as single therapeutic agents or in combination with classical chemotherapeutic agents for the treatment of hematologic malignancies, Curr Mol Pharmacol, 3 (2010) 98-122.

[95] C. Fabre, N. Mimura, K. Bobb, S.Y. Kong, G. Gorgun, D. Cirstea, Y. Hu, J. Minami, H. Ohguchi, J. Zhang, J. Meshulam, R.D. Carrasco, Y.T. Tai, P.G. Richardson, T. Hideshima, K.C. Anderson, Dual inhibition of canonical and noncanonical NF-kappaB pathways demonstrates significant antitumor activities in multiple myeloma, Clin Cancer Res, 18 (2012) 4669-4681.

[96] K. Salem, C.O. Brown, J. Schibler, A. Goel, Combination chemotherapy increases cytotoxicity of multiple myeloma cells by modification of nuclear factor (NF)-kappaB activity, Exp Hematol, 41 (2013) 209-218.

[97] S. Gaali, R. Gopalakrishnan, Y. Wang, C. Kozany, F. Hausch, The chemical biology of immunophilin ligands, Curr Med Chem, 18 (2011) 5355-5379.

[98] S. Gaali, A. Kirschner, S. Cuboni, J. Hartmann, C. Kozany, G. Balsevich, C. Namendorf, P. Fernandez-Vizarra, C. Sippel, A.S. Zannas, R. Draenert, E.B. Binder, O.F. Almeida, G. Ruhter, M. Uhr, M.V. Schmidt, C. Touma, A. Bracher, F. Hausch, Selective inhibitors of the FK506-binding protein 51 by induced fit, Nat Chem Biol, 11 (2015) 33-37.

[99] H.R. Quintá, D. Maschi, C. Gomez-Sanchez, G. Piwien-Pilipuk, M.D. Galigniana, Subcellular rearrangement of hsp90-binding immunophilins accompanies neuronal differentiation and neurite outgrowth, J Neurochem, 115 (2010) 716-734.

## LEGENDS TO FIGURES

**FIGURE 1. Structures of FKBPs, Hsp90, IκB and NF-κB**.

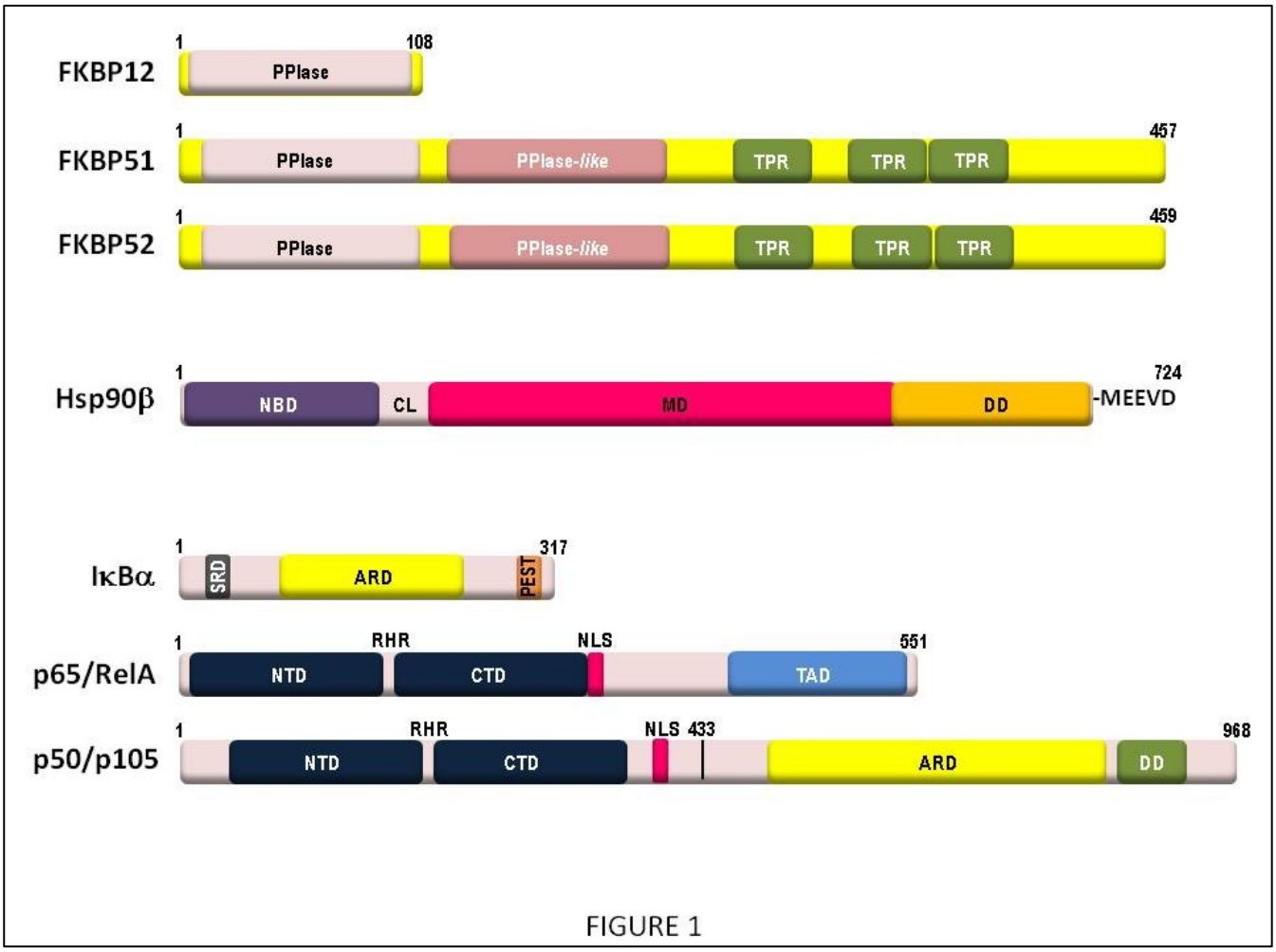
The upper part of the figure depicts the structural domains of FKBP51 and FKBP52 compared to FKBP12, the archetypical immunophilin responsible for immunosuppression that was the first protein well characterized of this family. The PPIase domain (also named FK1 domain) is responsible for the rotamase enzymatic activity and is also the binding site for the immunosuppressive macrolide FK506, which inhibits that activity. The PPIase-like domain (also named FK2) is the nucleotide-binding domain. The TPR domains (absents in FKBP12) are the responsible for interactions with Hsp90. This chaperone (shown in the middle of the figure) has two isoforms, α and β, the later being the active form for most signalling cascade factors. It shows four domains —NBD, nucleotide binding domain; CL, charged linker; MD, middle domain; DD, dimerization domain. The lower part of the figure depicts the structures of the two most frequent subunits of NF-κB and the domains of its inhibitor, IκB. Abbreviations are as follows: NTD, N-terminal domain; CTD, C-terminal domain; NLS, nuclear localization signal; RHR, Rel-homology region; TAD, C-terminal transactivation domain; ARD, ankyrin repeat–containing domain; DD, dimerization domain; PEST, signal–receiving domain C-terminal Pro-, Glu-, Ser-, and Thr-rich sequence; SRD, N-terminal signal–receiving domain. All these structures correspond to human proteins.

**FIGURE 2. Canonical, non-canonical, and atypical NF-κB signalling pathways**.

***A)*** In the canonical NF-κB signalling pathway, lipopolysaccharides (LPS), tumor necrosis factor α (TNFα) or interleukin-1 (IL-1) activate Toll-like receptors (TLRs), tumor necrosis factor receptor (TNFR), and interleukin-1 receptor (IL-1R), respectively, leading to the activation of the IKK complex. This kinase phosphorylates IκBα, a prerequisite for its subsequent polyubiquitination followed by proteasomal degradation. NF-κB homo- or heterodimers are then translocated to the nucleus and activate gene transcription. ***B)*** In the non-canonical NF-κB signalling pathway, activation of CD40, receptor activator for nuclear factor κB (RANK), B-cell activation factor (BAFFR), or lymphtoxin β-receptor (LTβR), leads to activation of IKKα by the NF-κB-inducing kinase (NIK). IKKα phosphorylates the p100 subunit leading to its polyubiquitination and subsequent partial proteosomal processing to yield the p52 subunit. p52•RelB heterodimers can then activate transcription of target genes. ***C)*** In the atypical NF-κB signalling pathway, genotoxic stress leads to a translocation of IKKγ (also called NEMO) to the nucleus, where it is sumoylated and subsequently ubiquitinated. This

process is mediated by the ataxia telangiectasia mutated (ATM) checkpoint kinase. IKKγ and ATM return to the cytosol where they are able to activate IKKβ.

**FIGURE 3. Role of FKBP51 and FKBP52 on glucocorticoid receptor and NF-κB action.** Unliganded glucocorticoid receptor (GR) forms cytosolic heterocomplexes with Hsp90 dimers (90), Hsp70 (70), the cochaperone p23, and FKBP51 (51). Hormone (H) binding favors the exchange of FKBP51 by FKBP52 (52), which in turn recruits the dynein /dynactin motor complex (Dyn) able to power the GR retrotransport on cytoskeletal tracks. In the nucleus, the Hsp90-based heterocomplex dissociates and GR dimers induce the expression of target genes. NF-κB dimers also exchange FKBP52 by FKBP51 upon cell stimulation, although this mechanism is Hsp90-independent. The activation by IKK•Cdc37 complex phosphorylates IκB (which is targeted to proteosomal degradation), allowing the dynein/dynactin retrotransport of NF-κB dimers. FKBP51 shows inhibitory action on the transcriptional activity of both, GR and NF-κB, whereas FKBP52 shows stimulatory effects. FK506 prevents such stimulatory action of NF-κB indicating the relevance of the PPIase activity of FKBP52 for this effect. Note that NF-κB and GR can neutralize one another by a typical trans-repression mechanism. FKBP51 can also form complexes with the IKK•Hsp90 complex, but its role is still poorly understood, and is perhaps related to complex assembly, Cdc37 being the major factor in mature IKK complexes.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108
PPIase
FKBP51
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457
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FKBP52
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PPIase
PPIase-like
TPR
TPR
TPR
Hsp90β
1
724
NBD
CL
MD
DD
-MEEVD
IκBα
1
317
SRD
ARD
PEST
p65/RelA
1
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FIGURE 1

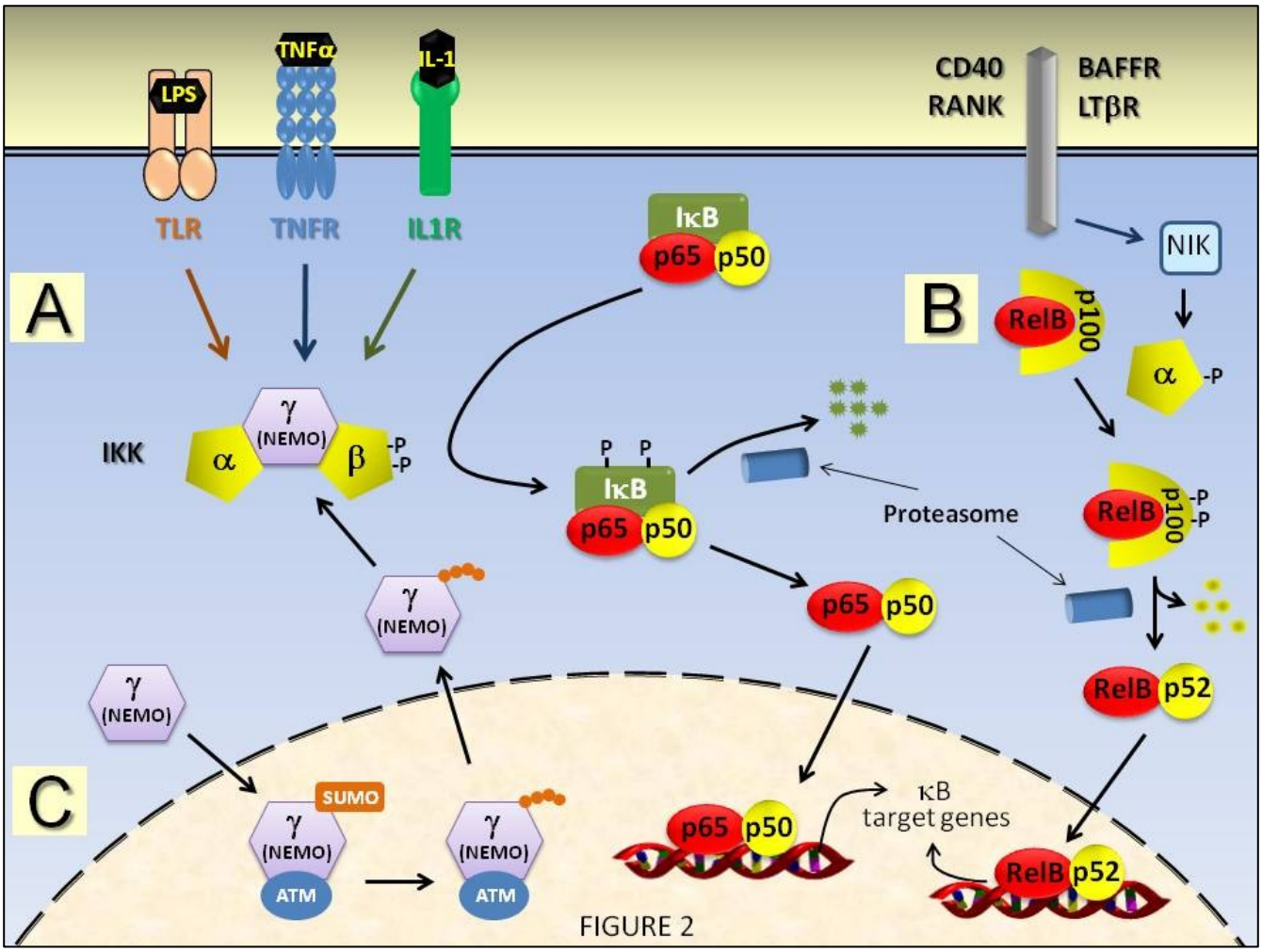
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FIGURE 2

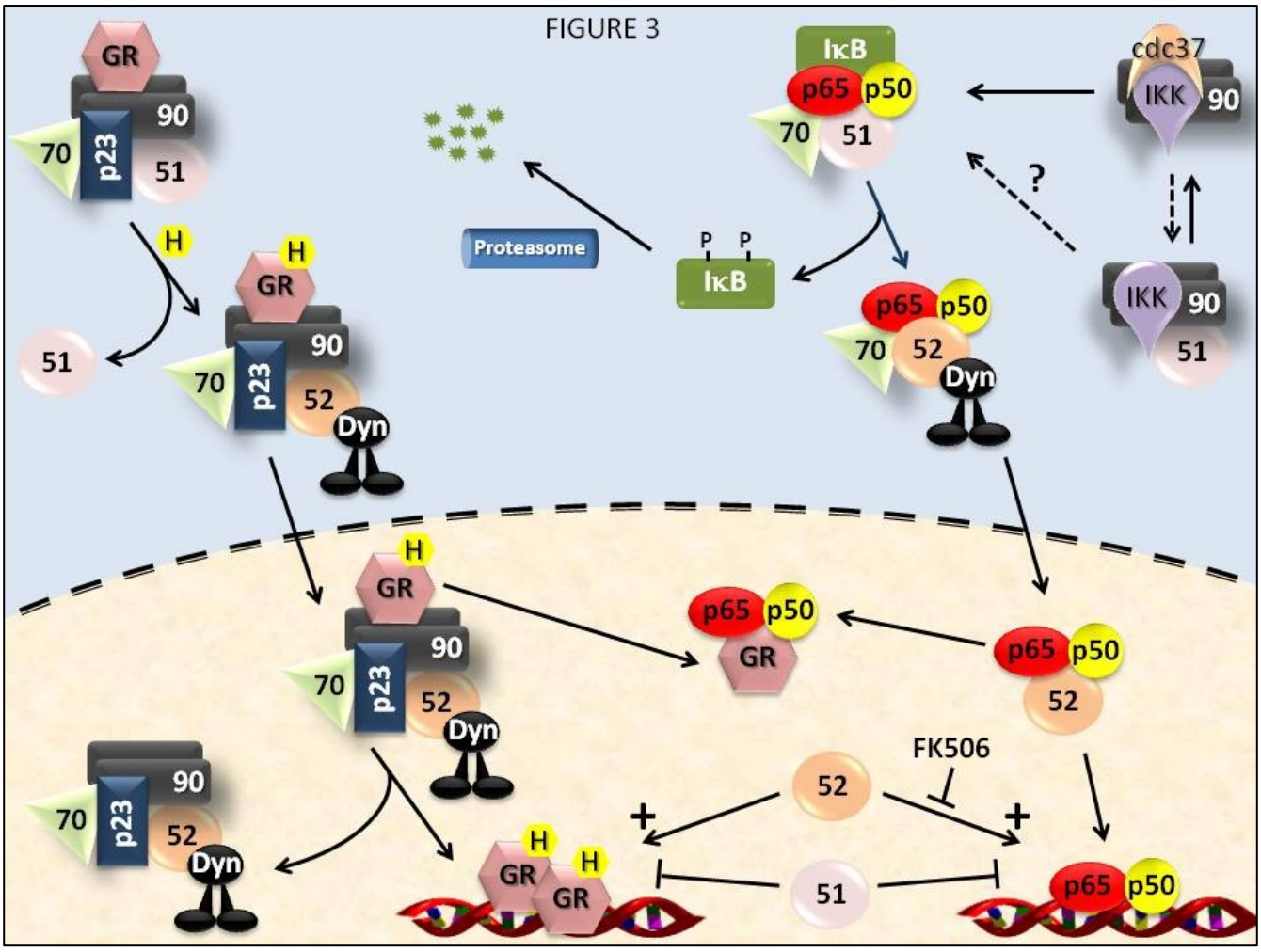
FIGURE 3
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