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The one thousand and one chaperones of the NF- κ B pathway.

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Abstract

The NF- κ B pathway represents a crucial signaling mechanism in sensing and integrating a multitude of environmental and intracellular stimuli and directing a coordinated response that from the cellular level may impact on the entire organism. A plethora of chaperone proteins works at multiple steps of the pathway, from membrane receptor activation to transcription factor binding to DNA. Indeed, chaperones are required to assist protein conformational changes, to assemble supramolecular complexes and to regulate protein ubiquitination, required for pathway activation. Some chaperones acquired a role as integral components of the signaling complexes, needed for signal progression. Here we describe the chaperones involved in the NF- κ B pathway and their specific roles in the different contexts.

Introduction

NF- κ B is one of the most studied transcription factor playing crucial roles in regulating immune response and inflammation. However, its activity impacts on a wide range of biological processes including cell proliferation, survival and metabolism. Indeed, it is not surprising that NF- κ B aberrant activation has been described in many human diseases, among them autoimmune diseases, neurodegenerative diseases, cardiovascular diseases and cancer [1].

Molecular chaperones are a class of proteins that assist protein folding, promoting the achievement of their functional conformations and orchestrating protein refolding or degradation after stressful events. A subgroup of chaperones is induced by stressful stimuli, like heat shock, hence the name of heat shock proteins (HSPs). However, during evolution, chaperones developed a number of additional roles in different cellular processes, ranging from DNA replication and cell signaling to cell senescence and apoptosis [2]. Interestingly, already 20 years ago, it has been described that heat shock, one of the prototypical HSP-inducing stimuli, suppresses NF- κ B activity [3-9]. Subsequent reports defined the involvement of specific chaperone proteins induced by heat shock in blunting the NF- κ B pathway. Evidence, accumulating over time, describes chaperones both in inhibiting and activating the pathway and even as essential components of its signaling machinery. However, the precise mechanisms of these articulated cross-talks are still far to be completely understood. This review intends to report and discuss the current knowledge on the fundamental role of chaperone proteins in regulating the NF- κ B pathway or in tuning its intensity and its final effects on gene transcription.

The NF- κ B pathway

The family of NF- κ B includes five master transcription factors, NF- κ B1 (also named p50), NF- κ B2 (also named p52), RelA (also named p65), RelB and c-Rel, which regulate the transcription of target genes by binding to specific DNA elements (κ B sites), as various hetero- or homo-dimers. A wide range of stimuli triggers the assembly of multiprotein complexes starting a cascade of protein phosphorylation, non-degradative ubiquitination and higher order oligomerization to activate the I κ B kinases (IKKs) that, consequently, mediates NF- κ B activation. There are two

independent signaling cascades able to mediate NF- κ B nuclear translocation, known as the canonical and the non-canonical (or alternative) NF- κ B pathways [1].

The canonical NF- κ B pathway is activated in response to diverse stimuli transduced by TNF receptor (TNFR) superfamily members, Toll-like receptors (TLRs), cytokine receptors, as well as T-cell (TCRs) and B-cell receptors (BCRs), all converging on the activation of the IKK complex [1]. Two of the most studied pathways generate from the TNFR and TLRs. TNF α binding to the TNFR results in the recruitment of the adaptor proteins tumor necrosis factor receptor type 1-associated DEATH domain protein (TRADD) and the receptor-interacting serine/threonine-protein kinase 1 (RIP1), the E3 ubiquitin ligases TNF-receptor-associated factor 2 (TRAF2), cellular inhibitor of apoptosis protein-1 and 2 (cIAP1 and cIAP2). This protein complex activates the transforming growth factor β activated kinase-1 (TAK1) and the mitogen-activated protein kinase kinase kinase 3 (MEKK3), two kinases required for IKK α and IKK β phosphorylation (Fig. 1) [10]. Instead, TLRs, similarly to the family of IL-1Rs, are characterized by the presence of a Toll/IL-1 receptor (TIR)-domain in the receptor intracellular region, required for the recruitment of the adaptor proteins Myeloid differentiation primary response 88 (MyD88) and MyD88-adaptor-like (Mal) or TIR-domain-containing adapter-inducing interferon- β (TRIF) and TRIF-related adaptor molecule (TRAM). The engagement of different adapters to distinct receptors is probably determined by the folding pattern and the surface properties of the different TIR domains. These adapters, in turn, associate with interleukin-1-receptor-associated kinase (IRAK) proteins or RIP1 that function in a similar manner. Activation of IRAK results in the phosphorylation of TNF-receptor-associated factor 6 (TRAF6), which recruits TAK1 and the IKK complex leading to IKK activation (Fig. 1) [1]. Of note, in both TNFR and TLR signaling, non-degradative ubiquitination plays an important role in regulating the formation of receptor-bound multiprotein complexes. In fact, TRAF protein self-ubiquitination and RIP1 ubiquitination are required to recruit TAK1 and the IKK complex and to activate the downstream signaling [11,12]. Although the precise dynamics of these signaling events is not completely defined, they invariably lead to the activation of the IKK complex [13]. The IKK complex is composed by two kinases (IKK α and IKK β) and an essential regulatory subunit (IKK γ /NEMO), where an IKK γ tetramer binds to two IKK α and IKK β dimers [14]. Although IKK α and β share high degree of sequence homology (approximately 50% identity), it is the phosphorylation of two serine residues (Ser 177 and Ser 181) on IKK β that is required for the IKK complex activation. The IKK γ subunit does not possess a catalytic activity, but its presence in the complex is absolutely required for NF- κ B activation [15,11,13,12,16]. The activated IKK complex phosphorylates I κ B α inducing its ubiquitination and degradation by the proteasome and thus promoting the release of NF- κ B (Fig. 2) that translocates into the nucleus and activates gene transcription (Fig. 3) [17].

While the canonical NF- κ B pathway is involved in almost all aspects of immune responses, the non-canonical pathway likely evolved as a supplementary signaling axis, cooperating with the canonical one in the regulation of specific functions of the adaptive immune system [18]. Indeed, the non-canonical pathway responds to a more specific group of stimuli, including ligands of a subset of TNFR superfamily members such as LT β R, BAFFR, CD40 and RANK [19]. These receptors possess an intracellular TRAF binding domain, essential for the recruitment of TRAF proteins. In absence of stimuli, TRAF2 and TRAF3 associate with the NF- κ B inducing kinase (NIK) facilitating its ubiquitination and proteasomal degradation. Upon stimulation, the recruitment of TRAF proteins to the receptor results in their degradation and in NIK stabilization and activation. Of note, in the non-canonical pathway, IKK α is the only component of the IKK complex required for NF- κ B activation [19,20] and NIK is the kinase responsible for IKK α phosphorylation. Once IKK α is activated, it phosphorylates p100 triggering its cleavage and removal of the I κ B-like ankyrin repeats to generate p52 that together with RelB, enters the nucleus to regulate gene transcription.

Chaperone proteins

Chaperone proteins play an essential role in mediating protein folding in normal and stress conditions. Our cells are equipped with different families of chaperones with specific activities and functions, often working in cooperation to fold native proteins, re-fold denatured polypeptides, inhibit unfolded protein aggregation, direct protein degradation via proteasomal and autophagic pathways, assist the formation of protein complexes, keep proteins in activation-competent conformations and stabilize them during conformational changes required for their activities [21,22]. Chaperones are essential in physiological situations, but they are even more crucial during stress conditions, as in presence of hyperthermia, anoxia, toxins, pathogens, mechanical stress or other stressors. Indeed, chaperone proteins are often transcriptionally induced by different stress conditions to cope with massive protein denaturation and protect cells from death [23,24]. Besides these stress-related functions, chaperone proteins have been described to regulate a number of cellular processes such as cell differentiation, DNA replication, programmed cell death, cellular senescence, cell signaling, inflammation and tumorigenesis [2,25]. In cancer, genomic alterations lead to the expression of mutant and often unstable proteins that require chaperone support to exert their functions. Moreover, tumorigenesis induces a radical breakdown in cellular homeostasis, leading to the hyperactivation of a number of pathways in which chaperones are essential in assisting conformational changes of signaling proteins [26,27]. Accordingly, chaperones have been described to be overexpressed in human cancers, where they sustain proliferative signaling, induce resistance to apoptosis and senescence, promote angiogenesis and mediate cancer cell metabolic reprogramming and evasion from immune surveillance [28]. For these reasons cancer cells develop an addiction to chaperones and therapeutic agents able to interfere with chaperone functions are showing promising results as anti-cancer drugs [29,30]. Chaperones have been classified in different families on the basis of sequence homology, presence of enzymatic activities and molecular weight [31,32]. The most famous are the Heat shock protein 90 (HSP90), Heat shock protein 70 (HSP70) and Heat shock protein 60 (HSP60) families that will be discussed in more depth below. They are ATP-dependent chaperones highly conserved during evolution and involved in folding, stability and functionality of hundred of proteins. The family of the mammalian small heat shock proteins (sHSPs) is perhaps less known and consists of at least eleven low molecular weight chaperones characterized by a highly conserved C-terminal α -crystallin domain. sHSP monomers can associate into dimers and form homo- and hetero-oligomers of different size and composition. sHSPs have been involved in protein quality control by preventing the aggregation of unfolded proteins, regulating their degradation through both the proteasomal and the autophagic pathways and by transferring them to ATP-dependent chaperones to be refolded. sHSPs have been described to interact, stabilize and regulate the activity of many signaling proteins, like kinases and phosphatases, impacting on numerous cellular processes. In turn, sHSPs post-translational modifications, like phosphorylation, have been described to impact on their ability to bind to unfolded proteins and to exert chaperone activity [33,34]. Of note, mutations in some sHSP coding genes are directly associated with motor neuron disorders, cataracts and cardioskeletal myopathies and several sHSPs have been described to exert protective functions in different neurogenerative and neuromuscular diseases [35,33]. Instead, sHSP overexpression has been described in human tumors, where they promote proliferation, progression and chemoresistance [36,30]. It is worth to note that a number of proteins, although not belonging to these well defined chaperone families, have been described to possess chaperone functions and to play crucial roles in specific contexts.

The role of chaperones in the NF- κ B pathway

Different chaperone proteins have been involved in the regulation of many different steps of this signaling cascade. In the sections below, we will describe the current knowledge on the role of chaperones in regulating NF- κ B activation.

HSP27

HSP27 (or HSPB1) is a widely expressed chaperone protein belonging to the small HSP family. It has been described to be involved in the response to different stimuli, including cytokine treatment, heat shock, oxidative stress. Several reports in the literature describe a role for HSP27 in regulating different steps of the NF- κ B pathway, in some cases with opposite effects.

In a first report, HSP27 overexpression has been shown to increase NF- κ B nuclear localization, DNA binding and transcriptional activity in response to etoposide, TNF- α and IL-1 treatment in U937 human leukemic cells. The authors demonstrated that HSP27 does not influence I κ B α phosphorylation, but it enhances its degradation by the proteasome [37]. In a subsequent study, overexpression of HSP27 in HeLa cells has been found to enhance IKK phosphorylation in response to IL-1 β but not TNF α stimulation. Moreover, HSP27 has been reported to co-immunoprecipitate with TRAF6 and to increase its ubiquitination, required for IKK activation. A negative feed-back loop, mediated by the IL-1-dependent activation of kinase mitogen-activated protein kinase-activated protein kinase 2 (MAPKPK2), a p38 downstream kinase, have been also described. MAPKPK2, by phosphorylating HSP27, induces its dissociation from TRAF6 and blunts IKK activation [38,39]. HSP27 has also been described to co-immunoprecipitate with IKK α , IKK β and I κ B α and to promote IKK activity, NF- κ B nuclear localization and target gene expression in hepatocellular carcinoma cells [40]. A subsequent study reported that TNF- α stimulation robustly induces HSP27 phosphorylation and binding to IKK β , promoting the recruitment of I κ B α and ultimately NF- κ B activation in a hepatocellular carcinoma cell model [41]. The same group also demonstrated that HSP27 binds to TAK1 and that the expression of a HSP27 phosphomimetic mutant increases TAK1 ubiquitination and leads to ERK and NF- κ B pro-survival signaling in HeLa cells [42].

HSP60

Heat shock protein 60 (HSP60) belongs to the highly evolutionary conserved family of chaperonins. It assembles in a tetradecameric double ring structure that associates with the co-chaperonin HSP10 to form an isolated chamber dedicated to protein folding. In mammals, HSP60 is mainly located in the mitochondria, where it assists imported polypeptides to fold into their native conformation in an ATP-dependent manner. However, HSP60 also localizes in the cytoplasm where it can associate with different partners and exert functions unrelated with protein folding, like protein trafficking, hormone signaling and taking part to pro-apoptotic and pro-survival pathways [43].

The cytosolic Hsp60 was described to directly interact with IKK α and β in HeLa cells, inducing IKK phosphorylation and NF- κ B transcriptional activity, independently of its chaperone function [44]. The molecular mechanism by which HSP60 promotes IKK phosphorylation has not been investigated, however the authors hypothesize a role for HSP60 in favoring the association of IKK with an upstream kinase or to induce oligomerization-dependent auto-phosphorylation [44]. Of note, the HSP60-mediated activation of the pathway selectively induces a subgroup of NF- κ B target genes, such as Bfl-1/A-1 and MnSOD. These genes code for proteins involved in the regulation of mitochondrial-derived reactive oxygen species (ROS) [44], suggesting that HSP60, in response to the deregulation of mitochondria homeostasis, could translocate into the cytoplasm and activate the NF- κ B pathway, somehow directing its action towards the transcription of genes involved in mitochondria protection and cell survival. The authors hypothesize an involvement of IKK α in determining the target gene specificity, since IKK α may affect chromatin structure by phosphorylating histone H3 on NF- κ B target gene promoters [45,46]. In addition, a possible role for HSP60 in the non-canonical NF- κ B pathway is supported by some experimental evidence, but further studies are awaited to strengthen the results [44].

HSP70

Heat shock protein 70 (HSP70) is an ATP-dependent chaperone highly conserved during evolution [47]. Different homologs exist in different cellular compartments, like the endoplasmic reticulum (Bip, also known as GRP78) and the mitochondria (mortalin). HSP70 interacts with a high variety of proteins in their misfolded state but not with their native counterparts and mediates protein folding, translocation into organelles and solubilization of aggregates [48]. Moreover, HSP70 associates with a limited number of folded and functional proteins, regulating their activity, stability and their assembly in supramolecular complexes [49]. In addition, HSP70 cooperates with HSP90, taking part to the HSP90 chaperone machinery, assisted by its co-chaperone HSP40. When HSP70 binds to a client protein, it can be recruited to the HSP90 dimers by an additional co-chaperone, called HOP (Hsp70/Hsp90 organizing protein), that promotes the transfer of the substrate to HSP90 [21,22].

HSP70 has been described to play mainly an inhibitory role in the NF- κ B pathway. It is well known that high levels of HSP70 protect cells from a number of apoptotic stimuli. Nevertheless, HSP70 has been reported to facilitate cell death induced by specific factors, like Fas ligand and TNF- α [50-52]. Besides activating pro-apoptotic pathways inside the cells, these stimuli also trigger the activation of NF- κ B that dampens cell death by inducing the expression of anti-apoptotic genes [53-55]. Ran and colleagues demonstrated that Hsp70 directly binds to the coiled-coil region of the IKK γ subunit preventing its self-association that is a required event in the activation of the IKK complex [52]. The authors hypothesized that HSP70 chaperone activity may induce a conformational change in IKK γ , impairing the formation of IKK γ oligomers and the assembly of the high molecular weight IKK complex. As a consequence, HSP70 inhibits NF- κ B activation and the expression of anti-apoptotic NF- κ B target genes in response to TNF- α stimulation, promoting cell death in different cell lines (COS-1, HeLa and 293 cells) [52]. Besides binding to IKK γ , HSP70 associates with TRAF6 in RAW cells and blocks its ubiquitination, interfering with LPS-induced NF- κ B activation and the subsequent production of inflammatory mediators [56]. HSP70 has also been described to bind to and sequester TRAF2 in response to TNF- α treatment, interfering with the recruitment of RIP1, TAK1 and the IKK complex to the TNFR, again inhibiting NF- κ B activation [57]. In response to LPS, HSP70 has been reported to translocate into the nucleus and associate with the p65 ubiquitin ligase PDZ and LIM Domain 2 (PDLIM2) and with the proteasome-associated protein BAG-1 (BCL2-associated athanogene 1) in bone marrow derived cells and dendritic cells. HSP70 does not affect p65 ubiquitination, but it facilitates its proteasome degradation attenuating the inflammatory response [58].

Although different studies reported an inhibitory role of HSP70 in the NF- κ B pathway, Lee and colleagues demonstrated that, in human bronchial epithelial cells (BEAS-2B) subjected to heat shock, HSP70 acts preventing the denaturation of IKK, likely through its chaperone activity, potentiating the pathway instead of inhibiting it [59].

BAG3

BAG3 (BCL2-associated athanogene 3), a member of the BAG family of anti-apoptotic proteins, acts as a HSP70 co-chaperone and it is involved in the regulation of the balance between autophagy and proteasome degradation. BAG3 is often overexpressed in different types of tumors conferring resistance to therapies and favoring metastasis formation [60]. BAG3 co-immunoprecipitates with IKK γ and HSP70 in SAOS-2 sarcoma cells and in M14 melanoma cells. In this context, BAG3 overexpression antagonizes the inhibitory binding between HSP70 and IKK γ (see previous paragraph), promoting IKK activation. In addition, BAG3 protects IKK γ from proteasome degradation induced by HSP70, enhancing NF- κ B activity. Indeed, in vivo experiments indicate that human melanoma tumors in which BAG3 has been down-regulated grow less in nude mice and show an increased number of apoptotic cancer cells [61]. The presence of BAG3 also promotes the emergence of castration-resistance in a mouse model of prostate cancer. In

androgen-dependent prostate cancer, the emergence of clones resistant to androgen deprivation is a major concern. Castration per se triggers an important inflammatory reaction that induces IKK α nuclear translocation in residual cancer cells and drives tumor growth via an NF- κ B-independent mechanism. BAG3 is required for the nuclear translocation of IKK α in prostate cancer cells in response to inflammatory cytokines, through an unknown mechanism [62]. Colvin and colleagues define a role for BAG3 in the regulation of NF- κ B by HSP70. In particular, the silencing of HSP70 in MCF7 breast cancer cells induces an increase in NF- κ B activity and phosphorylation and a reduction of I κ B levels. While, the depletion of BAG3 per se does not impact significantly on the activation of the NF- κ B pathway. Instead, BAG3 is required for the activation of NF- κ B induced by the depletion of HSP70 [63].

HSP90

HSP90 is a chaperone protein conserved from bacteria to humans. In mammals, two different highly homologous genes code for two cytoplasmic forms of HSP90: the inducible HSP90 α and the constitutive HSP90 β . Additional homologs are present in the mitochondria (Trap1) and in the endoplasmic reticulum (Grp94). Of note, HSP90 represents the 2% of the total cellular protein content in physiological conditions and its expression is induced to 4-6% by stress. HSP90 binds as a dimer to substrate proteins, called HSP90 clients, and by hydrolyzing ATP through its N-terminal ATPase domain, it induces structural changes in its substrates. HSP90 functions by assembling a dynamic molecular machinery, in which more than twenty co-factors, called co-chaperones, by sequentially and reversibly binding to HSP90, regulate its conformational changes, its enzymatic activity and provide specificity to client protein interactions [64,65,21]. HSP90 interacts with a wide group of client proteins unrelated for structures and functions, among them 60% are kinases, 30% are E3 ligases and 7% are transcription factors [66].

HSP90 plays a pivotal role in the NF- κ B signaling cascade, by assisting different pathway components and participating to multiprotein complexes required for signal progression. A number of proteins involved in the NF- κ B pathway are HSP90 clients, including IRAK1, TAK1, RIP1, MEKK3, NIK, TBK1, IKK α and IKK β [67-73].

IRAK1 and 4 are kinases required to trigger intracellular signaling from Toll-like and IL-1 receptors. IRAK1, but not IRAK4, has been found to bind to HSP90, HSP70 and HOP in a co-immunoprecipitation experiment followed by mass spectrometry. Further analyses indicated the existence of two different complexes, a first one including IRAK1, HSP90, HSP70 and HOP and a second one containing IRAK1 associated to HSP90 and its co-chaperone Cdc37. Pharmacological inhibition of HSP90 induces IRAK1 degradation, supporting the notion that IRAK1 is a bona fide HSP90 client protein. In macrophage, HSP90 inhibition impairs TLR dependent MAPK and NF- κ B signaling and the subsequent cytokine production. Of note, Cdc37 overexpression increases the recruitment of IRAK1 in the Cdc37/HSP90 complex and promotes IRAK1 activation [68].

TAK1 is an important signaling molecule involved in MAPK and NF- κ B activation in response to TNF- α , IL-1 and Toll-like receptor signaling. TAK1 binds to HSP90 and its co-chaperone Cdc37, behaving as a HSP90 client [71,72]. HSP90 is not required for TAK1 activation, but likely it plays a role in the folding and maturation of the nascent TAK1 or in maintaining TAK1 stability when it is not involved in signal transduction [71].

RIP1 is a crucial component of the TNF receptor 1 complex, required for NF- κ B activation in response to TNF signaling [67]. RIP1 has also been characterized as an HSP90 client protein. Indeed, HSP90 inhibition induces RIP1 degradation and sensitizes cells to apoptosis induced by TNF- α . Treatment with TNF- α induces the detachment of RIP1 from HSP90 and its recruitment to the TNFR complex to contribute to signal progression [67].

MEKK3 is activated in response to different inflammatory stimuli, as TNF- α and IL-1, and it has been reported to interact with RIP1 and TAK1 [74-77] and to be involved in the activation of different downstream pathways, like ERK,

p38, JNK and NF- κ B. MEKK3 overexpression has been frequently detected in human cancers, where it activates NF- κ B and improves cell survival [78]. HSP90 interacts with MEKK3 and both pharmacological inhibition and downregulation of HSP90 result in MEKK3 downregulation, likely impacting on NF- κ B signaling [79].

NIK is an IKK kinase involved in both the canonical and the non-canonical NF- κ B pathway [80]. Citri and coworkers, studying the features of the common surface recognized by HSP90 in client kinases, identified NIK as a new HSP90 client protein. Indeed, NIK binds to HSP90 and its co-chaperone Cdc37 and is degraded in response to HSP90 inhibition [70].

HSP90 and Cdc37 have also been described as integral components of the high molecular weight IKK complex independently from TNF- α stimulation, however different studies did not always reach the same conclusions, even when performed on the same cellular models [81,82,73,83,69,84]. Chen and colleagues found that HSP90 and Cdc37 bind stoichiometrically to the IKK α and IKK β kinase domains and described an essential role for HSP90 in the assembly of the IKK complex, rather than in promoting IKK kinase activity or stability. They showed that treatment with the HSP90 inhibitor geldanamycin induces IKK γ dissociation from the IKK complex and abolishes the recruitment of the IKK complex to the TNFR upon TNF- α stimulation [81]. In partial contrast, two studies from Scheidereit's laboratory established that HSP90 is dispensable for the IKK complex formation and that a transient association of HSP90 and Cdc37 with mature and phosphorylated IKKs is required for their complete activation. In particular, they demonstrated the requirement of HSP90 and Cdc37 for NF- κ B activation in response to treatment with TNF- α , IL-1 β and phorbol ester (PMA) [73,84]. In addition, they and others identified a role for HSP90 in the stabilization of the newly synthesized IKK α and β that, in presence of geldanamycin, are polyubiquitinated and degraded by the proteasome [73,84,83]. Instead, Qing and colleagues demonstrated that the degradation of IKKs induced by HSP90 inhibition is a proteasome-independent event and relies on the autophagic pathway [69]. The HSP90-binding immunophilin FK506-binding protein 51 (FKBP51) has also been identified as an IKK α interactor with an essential role in IKK signaling [83].

Morgana

Morgana, also known as CHP-1, is a small chaperone ubiquitously expressed coded by the CHORDC1 gene [85-88]. The absence of Morgana causes centrosome overduplication and genomic instability, impairing embryonic development in mouse and causing larval lethality in *Drosophila* [89]. Morgana is an HSP90 co-chaperone [90-94], endowed with chaperone activity [92] that has been involved in the regulation of different signaling pathways. On the one hand Morgana binds to and inhibits the Rho kinases I and II (ROCK I and II) [89,95], two multifunctional proteins involved in the regulation of cytoskeleton dynamics and of multiple cellular processes, like cell survival, division and migration. On the other hand, Morgana activates the NF- κ B pathway, possibly interconnecting this signal with ROCK activation status. In non-tumorigenic cell lines and in primary cells Morgana overexpression is sufficient to activate NF- κ B and to induce the expression of its target genes, in absence of specific stimuli [96]. In cancer cells in which the NF- κ B pathway is hyperactive, Morgana downregulation hinders NF- κ B activation and impairs the production of extracellular mediators required for the formation of a cancer-permissive microenvironment and for the generation of the pre-metastatic niche in distant organs [96]. Of note, Morgana is overexpressed in a subset of human cancers and, among them, in 36% of human triple negative breast cancers, often characterized by an aberrant activation of NF- κ B [97,95,98]. Morgana takes part in the mature IKK complex but it is dispensable for the recruitment of HSP90 in the complex or for protecting the IKK complex components from degradation. Instead, Morgana is essential for the recruitment of I κ B α to the IKK complex and for enabling its phosphorylation [98].

hTid-1/DNAJ3

hTid-1/DNAJ3 is a DNAJ protein, homologue of the *Drosophila* tumor suppressor Tid56 encoded by the *lethal(2)tumorous imaginal discs* gene [99]. hTid-1/DNAJ3 is a HSP70 co-factor described in different studies to have a role in the modulation of the NF- κ B pathway. Cheng and co-authors demonstrated that hTid-1/DNAJ3 associates both with IKK β and with the I κ B α -NF- κ B complex and inhibits the phosphorylation of I κ B α by IKK β in HEK293, Jurkat T and SAOS-2 cells. This effect is likely achieved through a twofold mechanism: the repression of IKK enzymatic activity and the improvement of I κ B stability. hTid-1/DNAJ3 binds to I κ B through its J domain that is also required for the interaction with different chaperones belonging to the HSP70 family [100,101]. This suggests the possibility that hTid-1 availability may be modulated by stress. Of note, the viral oncoprotein Tax, a strong activator of the NF- κ B pathway, enhances I κ B phosphorylation by displacing hTid-1/DNAJ3 from I κ B [100,101]. hTid-1/DNAJ3 ability to act as a repressor of the NF- κ B pathway contributes to the induction of cell senescence [102] and in reducing the motility and the ability to form metastasis of breast cancer cells [103]. hTid-1/DNAJ3 also regulates macroautophagy induced by nutrient deprivation, independently of HSP70, by mediating the connection between IKKs and beclin, that is part of the autophagy protein complex [104].

hTid-1/DNAJ3 has also been described as an activator of the NF- κ B pathway. In *Drosophila* cells, it is required for the induction of anti-microbial genes in response to gram-negative bacteria infection, while in human HEK293 cells, is essential for TLR5 and TLR4-dependent I κ B α phosphorylation and NF- κ B activation [105].

Nucleophosmin

Nucleophosmin (NPM), also known as B23, NO38 or Numatrin, is an abundant phosphoprotein mainly located in the nucleolus, but actively shuttling between nucleolus, nucleoplasm and cytoplasm. NPM coding gene has been found overexpressed, rearranged, mutated and deleted in several human cancers, where NPM behaves both as an oncogene and as a tumor suppressor [106,107]. NPM is a multifunctional protein involved in several processes such as centrosome duplication, ribosome biogenesis, DNA repair and response to stress [108]. It is also endowed with histone chaperone activity and it mediates the binding of histones to DNA during chromatin assembly [109]. NPM has been first identified, by affinity chromatography coupled to proteomic analysis, as a co-factor for p65 and p50. The interaction between NPM and NF- κ B, already detectable in basal conditions, is enhanced by treatment with cytokines and PMA. Indeed, NPM is required for an efficient induction of the NF- κ B target gene SOD2, in response to PMA and cytokine stimulation in a hepatocarcinoma cell line. In this context, NPM determines some degree of target gene specificity, since IL-8 expression was not affected by NPM downregulation [110]. NPM was subsequently described to associate directly with the p65 subunit in response to TNF- α , mainly in the nucleoplasm. The results suggested that NPM associates with the DNA binding domain of p65 and, by inducing a p65 conformational change, promotes NF- κ B binding to DNA and the assembly of a transcription activation complex, regulating most, but not all, TNF- α induced genes [111]. NPM seems also to play a role as an effector of particular branches of the NF- κ B signaling. In particular, stress conditions, for instance DNA damage, p65 has been shown to translocate from the cytoplasm to the nucleolus. By forcing p65 nucleolar localization, NPM translocates from the nucleolus to the cytoplasm, where it induces apoptosis by promoting BAX accumulation in the mitochondria [112]. A NPM C-terminal deleted mutant fused to a penetrating peptide inhibits NF- κ B target gene expression in primary fibroblasts and delays the onset of the pathology in a NF- κ B-dependent leukemia mouse model. The mutant NPM exerts these functions forming complexes with the endogenous NPM and p65 on the promoters of genes involved in survival and inflammation, blocking p65 transcriptional activity [113]. A role in deregulating NF- κ B signaling has also been identified for oncogenic NPM fusion proteins. The

translocation t(5;17)(q35;q21), occurring in acute promyelocytic leukemia, generates the aberrant protein NPM-RAR α , a fusion of NPM with the retinoic acid receptor α (RAR α). It has been shown that NPM-RAR α binds to TRADD and inhibits caspase activation, while preserving NF- κ B and JNK signaling [114]. The translocation t(2;5)(p23;q35) associated with anaplastic large cell lymphoma generates a fusion protein from NPM and anaplastic lymphoma kinase (NPM-ALK). It has been shown that NPM-ALK disrupts the association of TRAF2 with the cytoplasmic tail of CD30, a member of the TNFR superfamily, blunting NF- κ B constitutive signaling induced by CD30 overexpression in lymphoma cells. Both the N-terminal NPM domain and the ALK kinase activity of NPM-ALK are required for interfering with CD30 signaling and are involved in the phosphorylation of wild type NPM [115].

UXT

Ubiquitously Expressed Transcript (UXT, also known as STAP1 and ART-27) is a small chaperone-like protein belonging to the α -class prefoldin family. UXT has been found associated with the androgen receptor [116,117] and γ -tubulin at centrosome and described as essential for cell viability [118]. UXT comes in two splicing isoforms (UXT-V1 and UXT-V2) displaying different localization inside the cells, being the variant 1 cytoplasmic and the variant 2 mainly localized in the nucleus [119]. UXT-V1 has been described as a component of the TNFR signaling complex, able to associate with TRAF2, preventing the TRAF2-RIP1-TRADD complex from recruiting and activating caspase 8. Hence, UXT-V1 degradation may represent a specific signal to sensitize cells to TNF- α induced apoptosis [120]. UXT-V1 also functions as a regulator of anti-viral pathway in the cytoplasm. In response to retinoic acid-inducible gene I (RIG-I) and melanoma differentiation associated factor 5 (MDA5) viral sensing, a protein complex including TRAF3, TRADD and the mitochondrial antiviral signaling protein (MAVS), assembles on the mitochondrial outer membrane, leading to interferon regulatory factor 3 (IRF3) and NF- κ B activation and the production of type I interferons. UXT-V1 has been shown to be an integral component of this mitochondrial complex, required to stabilize the interaction between TRAF3 and MAVS and trigger anti-viral signal transduction [119].

The nuclear isoform of UXT has been shown to bind to p65 and function as its transcription co-activator [121]. Indeed, upon Epstein-Barr virus (EBV) infection, the EBV kinase BGLF4 phosphorylates UXT on Threonine 3, hampering UXT-NF- κ B binding and NF- κ B enhanceosome activity [122]. UXT also interacts with EZH1 and SUZ12, two subunits of the polycomb repressive complex 2 (PRC2). PRC2 functions as an epigenetic repressor through its histone methyltransferase activity. Instead, the alternative complex consisting of UXT-EZH1-SUZ12 does not regulate histone methylation, but it is required for p65 and Polymerase II recruitment to NF- κ B target genes, functioning as positive regulator of NF- κ B-dependent gene transcription [123].

CCT

Chaperone Containing TCP1 (CCT, also known as TCP-1 ring complex, TRiC) is a chaperonin composed by a cylindrical, multimeric complex with a central cavity consisting of two stacked rings, each composed by eight subunits (CCT α - θ). As other chaperonins, CCT plays a role in ATP-dependent protein folding and assembly of protein complexes and was initially discovered for its crucial role in folding cytoskeletal proteins, like actin and tubulin [124,125]. Thanks to its ability to interact with different partners, CCT has been involved in other essential cellular functions as chromatin modification, signal transduction and cell cycle control [126-128]. CCT has been also involved in the regulation of NF- κ B target gene expression in a promoter-specific manner in HeLa cells. In particular, the CCT η subunit, likely regulating CBP/p300 acetylase activity, modulates the acetylation of p65, modifying its ability to bind to its consensus DNA-binding sites [129].

Conclusion

The ability of cells to sense extracellular signals and environmental modifications is crucial for organisms to adapt and survive. Equally important is the capacity of cells to interpret external signals based on their specific internal conditions. These abilities are even more critical in case of messages activating systemic self-perpetuating signals that may heavily impact on the all organism, like the NF- κ B signaling. Indeed, the NF- κ B pathway is a paradigmatic example of the complexity and specificity achieved through evolution in the integration of an impressive number of extracellular and intracellular signals. It is well accepted that the activation of the NF- κ B pathway in different contexts and in response to different stimuli shows an important degree of specificity in term of final outcomes. Despite the enormous progress in understanding the dynamic of the pathway, the mechanism at the basis of this specificity remains an unsolved issue [130]. The experimental evidence discussed in this review points to a massive involvement of chaperone proteins in tuning different steps of the pathway (Fig.1-3), modulating its activation and conferring specificity in target gene expression. In agreement, different studies underlined a tight interdependence between the NF- κ B pathway and the stress response. Indeed, heat shock or others stressors administered before a proinflammatory stimulus can inhibit the inflammatory responses both in cultured cells and in animal models [9]. The precise molecular mechanisms involved are still under investigation, however, the ability of the stress response to induce and stabilize the expression of I κ B α and to inhibit its phosphorylation seems to be considerable. An additional mechanism depends on the stress-dependent induction of Hsp70, a potent inhibitor of the NF- κ B pathway [9]. Therefore, chaperones, controlling the NF- κ B pathway, regulate the transduction of cytokine-mediated signals, modulating systemic inflammatory responses. On the other hand, accumulating evidence points to a role for cytokines in coordinating stress responses systemically in the entire organism, with different cytokines inducing the expression of definite sets of chaperones in a cell-type specific manner [131,132]. This mutual control generates an interdependent network between immunity and stress signals, interconnecting cellular molecular events with the physiology of the whole organism.

The ability of chaperone proteins in shaping the pathway and its final effects is ensured by their many different properties and capabilities, like their attitude to mediate the assembly of supramolecular complexes, to assist signaling protein conformational changes, to regulate protein degradation and non-degradative ubiquitination or to act as signaling molecules or as co-activators for transcription factors. The sequential conformational changes needed to allow the different components of a complex protein structure to fit in an organized frame highly depend on the action of chaperone proteins. Small chaperones have the ability to oligomerize, representing a potential scaffold for high-order oligomerization reactions. However, in some cases, chaperone proteins have been recognized as integral components of these signaling platforms, required for functional operation of the machine and, ultimately, for signal transduction. This may depend on the ability of chaperones to facilitate conformational changes during enzyme activation, substrate recognition and disabling. The ubiquitin system is another fundamental player in the activation of the NF- κ B pathway, indeed, several signal transduction molecules like IRAK1, RIP1, the ubiquitin ligase TRAF6 and NEMO, are subjected to non-degradative ubiquitination in response to receptor engagement. Ubiquitin chains grown on these proteins may induce conformational changes or act as binding sites for other components, allowing the formation of a functional transduction unit [133]. Downstream in the pathway, the ubiquitination of I κ B α induces its degradation, releasing NF- κ B inhibition and thus promoting its nuclear localization and transcriptional activity. Indeed, it is known that chaperone proteins cooperate with ubiquitin ligases to direct protein degradation [134,66] and to modulate non-degradative ubiquitination [135].

The comprehension of the molecular bases of chaperone-mediated control of the NF- κ B pathway is of paramount importance for the identification of new targets to manipulate inflammation and to increase cell tolerance to stress.

Aberrant NF- κ B overactivation occurs in different pathological conditions, among them, septic shock, stroke, myocardial infarction, infection, autoimmune diseases, neurodegenerative diseases and cancer. In spite of the intense effort in the identification and characterization of NF- κ B inhibitors, none of them has been approved for clinical use, due to the toxicity associated with systemic NF- κ B inhibition [136]. Knowledge on the detailed functions of the different chaperone proteins in finely tuning the NF- κ B pathway may be exploited to shape the signaling outcomes, avoiding a total inhibition of the pathway. The multiple level regulatory framework woven by chaperones might offer dozens of opportunities of intervention that ideally could turn into pharmacological treatments. An attracting perspective is represented by the identification of drugs interfering with the binding between chaperones and different signaling molecules to alter the pathway through different modalities that could result in specific benefits in different pathological contexts. Intensive work is still needed to evaluate the feasibility, effectiveness and safety of this approach.

Conflict of interest

The authors declare that they have no conflict of interest

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Figures

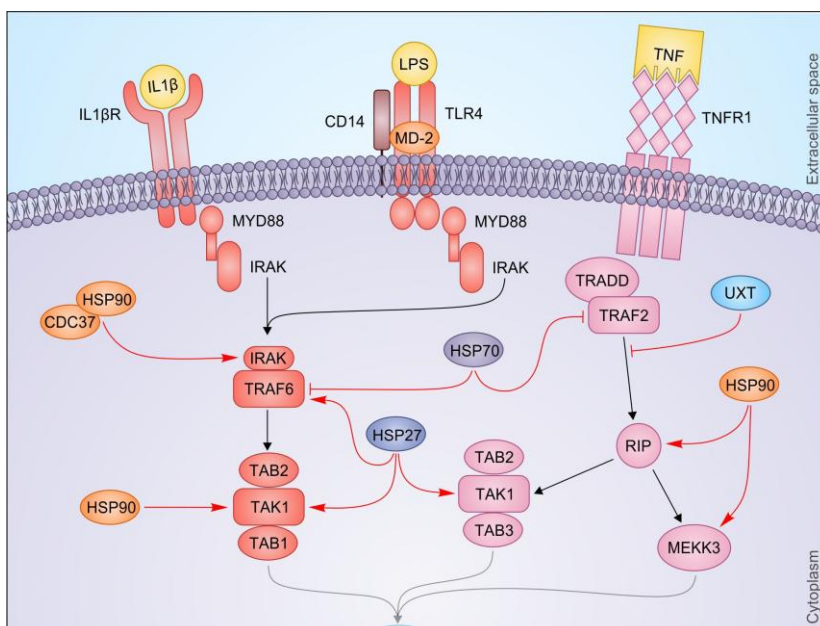


Fig. 1. Schematic model showing the upstream activation of NF- κ B pathway, in response to different stimuli. Chaperone proteins, through positive (red arrows) and negative (red T-shaped lines) regulations, control the activation of the signal.

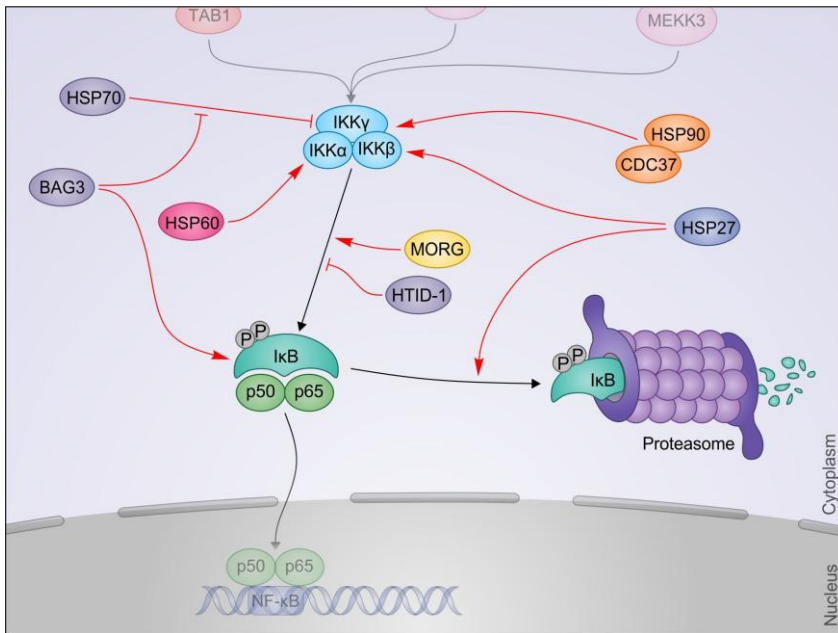


Fig. 2. The figure shows chaperone-mediated control on the IKK complex formation and activation and on IκB phosphorylation. These events lead to p50 and p65 translocation into the nucleus. Chaperone proteins may regulate this process both in a positive and in a negative manner (red arrows and T-shaped lines, respectively).

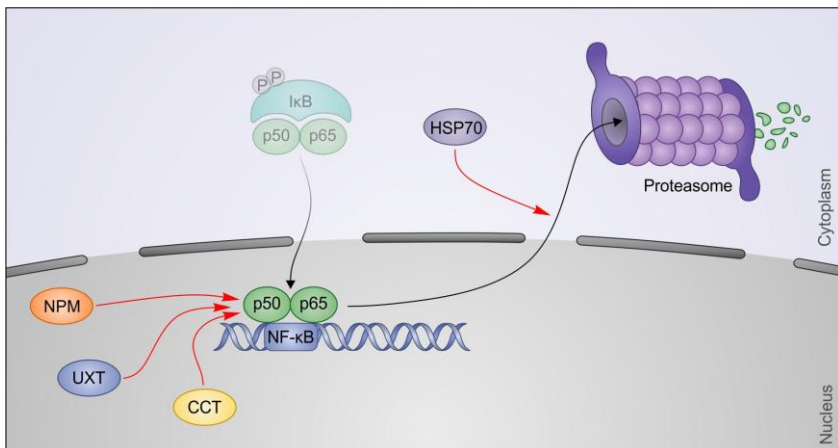


Fig. 3. NPM, UXT and CCT, acting as co-factors of p50 and p65, promote NF-κB transcriptional activity on its target genes. On the contrary, HSP70 facilitates p65 proteasome degradation exerting its inhibitory role on NF-κB pathway.

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