Diverse Roles of the Ubiquitin System in NF-κB Activation

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Abstract:

NF-κB is a transcription factor known to be involved in pleomorphic biological phenomena such as inflammation and immune responses. Abnormal activation of NF-κB has been reported in many pathological conditions, including allergic and auto-inflammatory diseases and malignancies. Therefore, the NF-κB activation pathway has been extensively studied and involvement of the ubiquitin conjugation system in the NF-κB activation pathways has been shown. Also non-degradable roles of the ubiquitin system have been revealed, recently. Several types of polyubiquitin chains exist and the type of chain seems to determine how ubiquitinated proteins are regulated. Roles of non-degradable types of polyubiquitin chains such as K63, linear and K11 chains in NF-κB activation is one of the big issues in NF-κB research. Thus, this short article discusses the differential roles of those polyubiquitin chains in NF-κB activation.

1. The ubiquitin conjugation system

Ubiquitin is a highly conserved globular protein of 76 amino acids in eukaryotic kingdoms, is a protein-based post-translational modifier. The ubiquitin system was identified as part of an energy-dependent degradation system, and the significant roles played by the ubiquitin-proteolytic pathway in biology and medicine are well-documented [1, 2]. The importance of the ubiquitin-proteolytic pathway is realized through its timely and selective recognition of specific substrates, which are then destined for degradation [3]. Timely and selective protein modification is desirable not only for degradation but also a crucial feature of other modes of protein regulation such as signal-induced protein activation [4]. As expected, these non-degradation roles of the ubiquitin system were subsequently recognized and are now widely accepted [5, 6]. The ubiquitin conjugation system regulates functions of proteins by conjugating polyubiquitin chains in most cases. There are several types of polyubiquitin chains, and the type of polyubiquitin chain is thought to determine the mode of regulation of the conjugated proteins [5, 6]. Although it has been believed that polyubiquitin chains are generated via one of seven Lys residues within ubiquitin, we have identified a new type

of polyubiquitin chain, the linear polyubiquitin chain, in which ubiquitin is linked via an N-terminal Met residue rather than a Lys residue of another ubiquitin molecules [7]. Further analysis revealed that linear polyubiquitination is involved in NF-κB activation [8, 9]. It has been well-established that the ubiquitin conjugation system is deeply involved in the NF-κB activation pathway. This review will discuss recent advances in research on the involvement of ubiquitination in the NF-κB activation pathways.

2. The canonical and alternative NF-κB activation pathways and involvement of the ubiquitin proteolytic sysytem in both pathways.

NF-κB is a transcription factor that plays a central role in inflammatory responses by inducing the expression of pro-inflammatory molecules. Besides inflammation, NF-κB is also involved in a broad array of biological phenomena including cell survival and innate and acquired immune responses [10, 11]. NF-κB is inactive in resting cells as it resides in the cytoplasm bound to inhibitor proteins called inhibitors of NF-κBs (IκBs), and is induced by various agents including inflammatory cytokines. Since abnormal activation of NF-κB occurs in many pathological conditions such as allergic and

auto-inflammatory diseases and neoplasms, the NF-κB activation pathway has been extensively studied [11-13]. NF-κB activation pathways are basically subdivided into two distinct pathways, known as the canonical and alternative pathways, and the ubiquitin mediated protein degradation system is involved in both [14, 15].

In the canonical activation pathway, the IKK (IκB kinase) complex, which comprises IKK1, IKK2 and NF-κB essential modulator (NEMO), is activated upon stimulation with various agents such as inflammatory cytokines or Toll-like receptor (TLR) ligands. The activated IKK phosphorylates specific Ser residues within IκBs [16] and phosphorylated IκBs are specifically recognized by ubiquitin ligase complex $SCF^{\beta\text{-}TrCPs}$ to generate polyubiquitin chains and degraded by the proteasome. NF-κB is released from IκBs and translocates to the nucleus to induce the transcription of target genes (Fig. 1) [10, 11]. The alternative pathway is activated by some TNF-receptor family proteins including CD40 and BAFF. Upon stimulation, NIK, which is degraded via ubiquitination by cIAP ubiquitin ligases in resting cells, is stabilized and phosphorylates IKKα. Phosphorylated IKKα induces phosphorylation of NF-κB2/p100, which forms a complex with RelB. The RelB/p100 complex resides in cytoplasm because C-terminal

half of p100 possesses IκB-like function. Phosphorylated NF-κB2/p100 is ubiquitianted by $SCF^{\beta TrCPs}$ E3s followed by the processing (partial degradation) to p52 by the proteasome, which allows translocation of the resultant RelB/p52 heterodimer to the nucleus (Fig. 2) [17]. Recently, it has been shown that NF-κB2/p100 is also ubiquitinated by the SCF^{Fbxw7} ubiquitin ligase in the nucleus [18]. However, the SCF^{Fbxw7}-mediated degradation of NF-κB2/p100 is suggested to be involved in the down regulation of NF-κB activation, but not in the alternative NF-κB activation.

The ubiquitin-proteolytic pathway plays a crucial role in both canonical and alternative NF-κB activation pathways. However, the involvement of different ubiquitin modifications, most of which exert a non-degradable function, has been revealed in the canonical but not in the alternative pathway [19]. Here I will focus on the role of the ubiquitin system in the canonical NF-κB activation to highlight non-degradable function of the ubiquitin conjugation system in signalling.

3. Involvement of different types of polyubiquitin chains in the canonical NF-κB activation pathway

3.1. Importance of non-degradable function of the ubiquitin system in NF-κB activation.

As mentioned before, there exist several types of polyubiquitin chains in cells [5]. Although polyubiquitin chains linked via Lys 48 of ubiquitin (K48 chains) was shown to function as a degradation signal [20], presence and function of other polyubiquitin chains has not been convincingly shown except the involvement of Lys63-linked (K63) chains in DNA repair in yeast [21]. Functions of other chains than K48 chains in signaling was first reported in the canonical NF-κB activation pathway, in which K63 chains was suggested to be involved. The report by the James Chen's group was really breakthrough of the ubiquitin field [22]. Extensive studies have been performed to dissect the role of K63 chains in NF-κB signaling since then, which have convincingly revealed the presence of the non-degradable function of the ubiquitin system [19, 22, 23]. Since excellent reviews on the role of K63 chains in NF-κB signaling have been published [24], the current hypothesis of the roles of K63 chains in IL-1β- and TNF-α-induced canonical NF-κB activation pathway will only be summarized here. Upon binding IL-1β, in addition to adaptor molecules, the TRAF6 ubiquitin ligase is

recruited to the IL-1 receptor complex and K63 chains conjugated mainly onto TRAF6 itself and on IRAK1 recruit the TAK1-TAB1-TAB2/3 complex and the IKK complex via K63-selective binding of TAB2/3 or NEMO, respectively [19]. TAK1 then phosphorylates specific Ser residues (Ser 177 and Ser 181) of IKK2, which leads to the phosphorylation and degradation of IκBs (Fig 3) [25]. K63 chains are shown to be generated upon stimulation with various agents including TNF-α besides IL-1β. When stimulated with TNF-α in addition to adaptor molecules, ubiquitin ligases including TRAF2 and cIAPs are recruited to the TNF receptor 1 and K63 chains conjugated mainly onto RIP1, which are suggested to be generated by cIAPs [19]. The K63 chains are suggested to recruit the IKK and TAK1 complexes and activate the former. Recently, unanchored K63 chains catalyzed by TRAF6 were proposed to be crucial for IKK activation [26] highlighted the important role of K63-linked chains in NF-κB activation. In these settings, K63 chains recruit signaling molecules to the activated receptor complexes. However, the involvement of K63-linked chains in NF-κB activation has been challenged. In cells isolated from KO mice of Ubc13, a crucial component of an Ubc13-Uev1a E2 complex to generate K63 chains specifically [22], NF-κB nor MAPKs

activation mediated by TNF-α is not overtly affected [27]. Moreover, K63 chains are dispensable for TNF-α-, but not IL-1β-, induced canonical NF-κB activation [28]. Thus, although K63 chains play crucial roles in signaling, they might be dispensable for the canonical NF-κB activation, at lease in some occasions including TNF-α stimulation.

3.2. **Newly discovered linear chains and their roles in NF-κB activation**

The ubiquitin chains have been believed to be generated by conjugating C-terminal carboxyl groups of ubiquitin to ε-amino groups of one of seven Lys residue of another ubiquitin [29]. However, generation of a new, different type of ubiquitin chain called the linear ubiquitin chain, in which the C-terminal carboxyl groups of ubiquitin is conjugated to the α-amino group of N-terminus of another ubiquitin has been reported [7]. Here, how the linear chains were discovered is discussed. Although polyubiquitin chains are believed to be generated by the repetitive functions of three enzymes E1, E2 and E3, molecular mechanisms underlying polyubiquitin chain generation has not been completely solved [30]. We have identified the ubiquitin ligase (E3) complex composed of two RING-IBR-RING proteins HOIL-1L and HOIP. HOIL-1L and HOIP possess

multiple putative ubiquitin binding sites (NZFs in HOIL-1L and HOIP) in addition to E2 binding sites (Fig. 4). It was thus hypothesized that the ubiquitin ligase complex recognizes ubiquitin as a substrate and conjugates ubiquitin onto it. Further analyses revealed that it was the case and mass spectrometric analyses showed that HOIL-1L-HOIP complex exclusively generates linear polyubiquitin chains [7]. Although it has been suggested that E2 enzymes determine the type of polyubiquitin chain in the several Lys linked chains [31] (E2 complexes containing Ubc13 (Ubc13-Uev1a and Ubc13-MMS2) only generate K63-linked chains for example [32]), the specificity of linear chains is determined by E3 [7].

During the course of seeking the physiological function, we found that siRNA mediated suppression of HOIP, the catalytic subunit of LUBAC and introduction of the catalytic inactive HOIP mutant suppressed TNF-α-mediated of NF-κB activation, which confirmed LUBAC seems involved in NF-κB activation [33]. Mice lacking HOIL-1L, a regulatory component of LUBAC further provided solid evidences of involvement of LUBAC-mediated linear polyubiquitination in NF-κB signaling. TNF-α-induced nuclear localization of p65, a subunit of NF-κB, is heavily impaired in primary

hepatocytes isolated from HOIL-1L knockout (KO) mice [33]. Moreover, TNF-α-induced IKK activation and expression of target genes of NF-κB are severely attenuated in embryonic fibroblasts (MEFs) from the HOIL-1L KO mice [33]. However, TNF-α-induced NF-κB activation is not completely abolished in HOIL-1L KO mice [33]. Knocking-out of molecules essential for NF-κB activation, such as NEMO or IKK2, is embryonic lethal in mice [34-36], but HOIL-1L KO is not [33]. The expression of HOIP, which is a catalytic subunit of the complex, was heavily decreased but not completely absent in HOIL-1L KO cells [37], which prompt us to hypothesize that HOIP may have another binding partner besides HOIL-1L. SHARPIN, which C-terminus exhibits significant homology with the N-terminal half of HOIL-1L that is essential for binding to HOIP, has been found (Fig. 4) [37-39]. SHARPIN has also been identified as a causative gene in chronic proliferative dermatitis in mice (cpdm) in 2007 and SHARPIN protein was not detected in cpdm cells [40, 41]. Cpdm mice are spontaneous mutant mice exhibiting chronic inflammation including chronic dermatitis and immunodeficiency. However, the precise mechanism why loss of SHARPIN provokes these phenotypes in cpdm mice had not been identified. Further analysis

showed that SHARPIN forms a complex with not only HOIP but also HOIL-1L, namely SHARPIN formed the tertiary complex with HOIL-1L and HOIP. The complex composed of HOIL-1L, HOIP and SHARPIN conjugates to linear polyubiquitin chains and we designated the complex composed of HOIL-1L, HOIP and SHARPIN as LUBAC (linear ubiquitin chain assembly complex) [37-39]. So far, LUBAC is the only reported E3 known to specifically generate linear chains [9]. In cells isolated from cpdm mice, the lack of SHARPIN was found to reduce drastically the amount of the other components of LUBAC, HOIL-1L and HOIP, by destabilizing them, thereby attenuating canonical NF-κB activation induced by TNF-α, CD40 or LT-βR [37-39]. Recently, loss-of-function mutation of HOIL-1L provokes a fatal human inherited disorder characterized by chronic autoinflammation, invasive bacterial infections and muscular amylopectinosis [42]. Loss of HOIL-1L destabilizes the other two components of LUBAC, thereby suppresses signal-induced NF-κB activation in cells isolated from patients as observed in cells from HOIL-1L KO mice [43]. However, no overt phenotypes have been reported in HOIL-1L KO mice although signal-induced canonical NF-κB activation is impaired [33]. Currently, the mechanism underlying

phenotypical difference between HOIL-1L null mice and HOIL-1L null patients or cpdm mice is unknown. Further precise analyses for HOIL-1L KO mice will be needed to clarify it. Thus, loss of regulatory subunits of LUBAC suppresses signal-induced canonical NF-κB activation and provokes diseases characterized by auto-inflammmatory and immunodeficiency phenotypes. The current hypothesis for molecular mechanism underlying LUBAC-mediated NF-κB activation is as follows: upon stimulation with agents such as TNF-α, IL-1β, or TLR ligands, LUBAC recognizes and linearly polyubiquitinates NEMO, which induces IKK activation and subsequent degradation of IκBα (Fig. 5). Although linear polyubiquitination of NEMO is suggested to be involved in IKK activation, further precise analyses will ne definitely necessary to clarify the mechanism underlying IKK activation by LUBAC-mediated linear polyubiquitination.

Although loss of SHARPIN or HOIL-1L attenuates the canonical NF-κB activation, it is of note that canonical NF-κB activation is not completely abolished in cells lacking HOIL-1L or SHARPIN, possibly because residual LUBAC, composed of SHARPIN and HOIP or HOIL-1L and HOIP, respectively, possesses linear polyubiquitination and

NF-κB activation activity. This issue will be discussed later in the article.

3.3. Possible involvement of K11 chains in canonical NF-κB activation

K11 chains, which have been shown to be conjugated to the substrates of the anaphase promoting complex E3, destined the conjugated proteins for degradation by the proteasome [44]. However, it has been shown that K11 chains are also generated by the cIAP ubiquitin ligases and conjugated to RIP1 and involved in the canonical NF-κB activation upon TNF-α stimulation [45, 46]. In the case of the NF-κB pathway, K11 chains do not function as a degradation signal. Thus, currently at least three different ubiquitin chains, K11, K63 and linear chains, are known to be involved in signaling process leading to IKK activation in a non-degradable manner.

4. Functional interplay of distinct non-degradable ubiquitin chains in canonical NF-κB activation

Although involvement of K63, linear and K11 chains in canonical NF-κB activation has been suggested, distinct roles each chain plays in the canonical NF-κB activation has

not been conclusively addressed. Here I'd like to discuss roles of different chains in the canonical IKK activation based on currently available knowledge. NEMO, an indispensable subunit of the canonical IKK complex plays a key role in ubiquitin-mediated activation of the canonical NF-κB pathway [47]. NEMO exhibits the ubiquitin-binding activity that is prerequisite for IKK activation [48, 49]. NEMO possesses two ubiquitin binding domains: one is located in the middle part of the protein, referred to as the UBAN domain [50], and the other is the C-terminal zinc finger (ZF) domain [51]. Mutations of amino acids in the UBAN domain or deletion of ZF cannot restore signal-dependent activation of NF-κB in NEMO-deficient cells [51, 52] confirming the crucial roles of the ubiquitin-binding activity of NEMO in IKK activation. However, the binding specificity of NEMO to ubiquitin chains remains controversial. The UBAN domain is shown to bind preferably to linear di-ubiquitin (1.4 μM) rather than K63 linked di-ubiquitin (131 μM) [53]. Structural analyses confirm high-affinity binding of the UBAN domain to linear di-ubiquitin [52, 53]. However, in the case of longer ubiquitin chains, NEMO containing both the UBAN and ZF domains also bound to other types of ubiquitin chains, including K11 and K63 linkages, albeit

with lower affinity than to linear linkages [45, 51].

In addition to ubiquitin binding activity, NEMO itself is also ubiquitinated. NEMO is shown to be conjugated with K63-linked chains [54-56]. NEMO is also identified as the major target for linear polyubiquitination by LUBAC, which is involved in IKK activation as described above [33, 37-39]. Thus, both ubiquitin conjugation to and recognition by NEMO seems to play crucial roles in the canonical NF-κB activation. However, critical ubiquitin chains leading to the canonical IKK activation cannot be identified only from biochemical analyses.

Then, genetic evidences regarding signal-induced activation of canonical NF-κB activation are discussed here. Although roles of K63 chains in the canonical NF-κB activation have been well-documented, ablation of Ubc13, the critical E2 enzymes for generating K63 chains does not overtly affects TNF-α-mediated NF-κB nor MAP kinase activation. Although NF-κB activation is not overtly affected, IL-1β-induced MAP kinase activation was impaired in Ubc13 null cells [27]. Moreover, as mentioned before, K63 chains are dispensable for TNF-α-, but not IL-1β-induced NF-κB activation [28]. In addition to genetic evidence using HOIL-1L or SHARPIN-lacking

cpdm MEFs, which showed that the linear chain is involved in canonical NF-κB activation, it has been shown that siRNA-mediated knockdown of HOIL-1L in SHARPIN-deficient cpdm MEFs attenuates IL-1β and TNF-α-mediated IκBα degradation almost completely [37]. These results suggested that LUBAC-mediated linear polyubiquitination appears to play a central role in IKK activation upon stimulation with TNF-α or IL-1β [33, 37-39]. K11 chains are shown to be generated by cIAP ubiquitin ligases, which are critical for not only canonical NF-κB but also MAP kinases such as JNK and p38 activation [57]. However, since cIAPs is reported to generate K48, K63 or other type of ubiquitin chains besides K11 chains [45], precise roles of K11 chains in NF-κB activation cannot be clarified using the genetic analyses. Collectively, current evidence suggested that K63 chains are suggested to be involved in the activation of MAPKs as well as NF-κB in IL-1β but not TNF-α stimulation. Linear chains are involved in NF-κB in both TNF-α and IL-1β stimulation. The involvement of linear chains in MAPK activation is still under investigation because some report suggests that linear chains are not involved in MAPK activation [37], but some other reports suggest marginal suppression on JNK activation by TNF-α and IL-1β [38, 39].

K11 chains might be involved in both MAPKs and NF-κB activation in TNF-α but not IL-1β signaling since cIAPs are dispensable for IL-1β signaling.

Therefore, both K63 and linear chains are necessary for IL-1β-mediated NF-κB activation [28, 37-39] and K11 and linear chains seem necessary for TNF-α-mediated NF-κB activation [37-39, 45]. Since profound suppression of linear chains generation by LUBAC selectively suppresses NF-κB activation induced by both IL-1β and TNF-α almost completely [37], linear chains appear to play central roles in IKK activation.

Next, mechanism underlying linear chain mediated activation of IKK is discussed. The IKK complex is activated by phosphorylation of specific Ser residues of IKK2 in the complex. Phosphoryation of kinases are mediated either by trans-autophosphorylation or by upstream kinases [58]. As mentioned before, linear chains are recognized by and conjugated to NEMO. Thus, linear chains conjugated to NEMO can be recognized by NEMO in another IKK complex, which induces multimerization of the IKK complex and IKK2 phosphorylation by trans-autophosphorylation (Fig. 6a) [8]. However, since binding of ubiquitin to the UBAN domain induces conformational changes in NEMO [52], the positions of IKK1 and IKK2 will be changed by linear chain recognition by

NEMO, which leads to phosphorylation of IKK2 possibly by IKK1 (Fig. 6b) [8, 52]. The recently solved crystal structure of IKK2 shows that IKK2 possesses a dimerization domain and dimerization-defective IKK2 mutants fail to be activated [59], which favors that IKK is activated by linear chain-induced dimerization.

Possible roles of K63 and K11 chains in NF-κB activation is also discussed. The ligase activity of cIAPs, which generate K11 chains, is necessary for recruitment of LUBAC to the TNF receptor complex [60]. Also, LUBAC possesses ubiquitin binding activity including K63 chains [60, 61]. Therefore, K63 and K11 chains might function to recruit LUBAC to the IL-1 receptor and the TNF receptor complex, respectively (Fig. 7). Since Ubc13 and cIAPs are involved in IL-1β- and TNF-α-induced activation of MAPKs in addition to canonical NF-κB activation, respectively, these chains may function in the upstream of the signaling cascades and play a role to recruit LUBAC to the activated receptor complexes for IKK activation (Fig. 7). In addition to NEMO, RIP1, which is shown to be conjugated with K11, K48 and K63 chains, is also linearly ubiquitinated by LUBAC upon TNF-α stimulation [39]. Since the ligase activity of LUBAC is necessary for stabilizing the TNF receptor signaling complex [60], linear

polyubiquitination of RIP1 might be involved in stabilization of the TNF signaling complex.

Although it might be plausible that LUBAC-mediated linear polyubiquitination plays a central role in IKK activation, some other mechanism(s) may collaterally exist in the canonical pathway for NF-κB activation. K63 and K11 chains are conjugated to components of the activated receptor complex upon stimulation with various agents. Since NEMO exhibits substantial affinity to the long K11 and K63 polyubiquitin chains, multiple NEMO proteins can recognize one long polyubiquitin chain, which induces multimerization of the IKK complex leading to phosphorylation of IKK2 (Fig 6C, D) [45, 51]. It is also possible that upstream kinases such as TAK1 are recruited to the activated receptor complex together with the IKK complex and phosphorylated IKK2 (Fig. 3) [8]. TAK1, which forms a complex with TAB1 and TAB2/TAB3 [62], is proposed as an upstream kinase for IKK2 phosphorylation [15]. TAK1 is recruited to the stimulated receptor complex by K63 chains because of the K63-specific binding activity of TAB2 and TAB3.

5. Perspectives

Several mechanisms involved in the ubiquitin conjugation system in the canonical and alternative NF-κB activation pathway were introduced. I especially focused on the recent advance regarding involvement of non-degradable ubiquitin chains in the canonical NF-κB activation pathway, which activate the IKK complex by phosphorylation of IKK2 [11, 16]. As discussed here, it might be the case that linear chains play a major role in direct IKK activation upon TNF-α or IL-1β stimulation. However, other ubiquitin chains may play a major role upon stimulation with other molecules, such as the TCR [63]. Also, even after stimulation with TNF-α or IL-1β, K11 or K63 ubiquitin chains may function to activate IKK in some settings. A specific ubiquitin chain exerts its distinct functions by being recognized by ubiquitin binding domains that preferential binds to the specific chain over other chains [49, 64]. Linear chains expressed in yeast *Saccharomyces cerevisiae*, in which linear chains do not exist, function as a degradation signal, albeit very weakly [65]. Because of the lack of linear chains in the yeast, specific binding domains for linear chains may not exist. Thus, linear chains might function as a weak degradation signal by being recognized by

ubiquitin-binding domains on the proteasome that recognize K48 chains with low affinity [66]. Thus, TNF-α- or IL-1β-mediated IKK activation might not be completely abolished in cells lacking synthesis of linear chains because NEMO exhibits substantial affinity to long K63 or K11 chains, which leads to IKK activation. The ubiquitin conjugation system is a regulatory system to regulate protein function [29]. Although the structures of polyubiquitin chains differ from each other, there are many similarities, and the ubiquitin binding domains that preferentially bind to one chain may recognize other chains, albeit with lower affinity. Therefore, in the canonical NF-κB activation pathway, several chains might be involved in direct activation of IKK and the signaling cascades leading from stimulation to IKK activation.

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Figure Legends

Fig. 1. The canonical NF-κB activation pathway.

NF-κB (p65-p50 heterodimer in this Fig) resides within the cytoplasm of resting cells in a form bound to the inhibitor protein, IκBα. Upon binding to TNF-α, TRADD and RIP1 are recruited to the TNF-receptor 1 (TNFR1). TRADD then recruits TRAF2 and cIAPs. In the case of IL-1β signaling, MyD88 is recruited to the IL-1 receptor (IL-1R), which recruits IRAK1 and IRAK4 to the receptor. IRAK1 binds to TRAF6. The ubiquitin ligase activities of TRAF2, TRAF6 and cIAPs are involved in the signaling cascade leading to canonical IKK activation, namely IKK2 phosphorylation. Phosphorylated IκBα is then conjugated with K48-linked polyubiquitin chains by the $SCF^{\beta TrCP}$ ubiquitin ligase and degraded by the proteasome. Liberated NF-κB translocates to the nucleus and induces the expression of target genes.

Fig. 2. The alternativel NF-κB activation pathway.

Upon stimulation with BAFF, NIK, which is degraded via cIAP-mediated ubiquitination in resting cells, is stabilized and phosphorylates IKK1. Phosphorylated

IKK1 induces phosphorylation and ubiquitin-dependent processing (partial degradation) of NF-κB2/p100 to p52, which allows translocation of the RelB/p52 heterodimer to the nucleus.

Fig 3. The proposed role of K63 chains in canonical NF-κB activation.

K63 chains generated upon stimulation by IL-1β recruit the TAK1-TAB1-TAB2/3 complex and the IKK complex via the selective binding of TAB2/3 or NEMO to K63 chains, respectively. TAK1 then phosphorylates IKK2, which leads to the phosphorylation and degradation of IκBs.

Fig. 4. Schematic representation of LUBAC that comprises HOIL-1L, HOIP and SHARPIN.

LUBAC is composed of HOIL-1L, HOIP and SHARPIN. The zinc finger and RING-IBR-RING domains of HOIP are the substrate-binding site and E3 active site, respectively. UBL; ubiquitin-like, NZF; NPl4-type zinc finger, RING; really interesting new gene, IBR; in-between RING, ZF; zinc finger, UBA; ubiquitin-associated domains.

Interaction between HOIL-1L UBL and HOIP UBA is involved in LUBAC formation. Interaction between HOIP NZF2 and SHARPIN UBL has suggested to be involved in LUBAC formation but HOIP lacking NZF2, but not lacking UBA, does bind to SHARPIN in cells.

Fig. 5. The proposed role of linear chains in canonical NF-κB activation.

Upon stimulation with TNF-α and IL-1β, LUBAC, which is composed of HOIL-1L, HOIP and SHARPIN, recognizes and linearly polyubiquitinates NEMO, which leads to IKK activation and subsequent degradation of IκBα.

Fig. 6. The possible roles of the different polyubiquitin chains in IKK activation.

(A) The linear chain conjugated onto NEMO can be recognized by NEMO of another IKK complex, which induces multimerization of the IKK complex. Upon multimerization, IKK2 dimerizes and is phosphorylated by trans-autophosphorylation.

(B) The linear chain conjugated onto NEMO are recognized by the UBAN motif of

another NEMO molecule in the same IKK complex (it has been suggested that two NEMO molecules exist in one IKK complex). Binding of the linear chain to the UBAN domain of NEMO induces conformational changes in NEMO, which affect the position of IKK1 and IKK2, leading to the phosphorylation of IKK2.

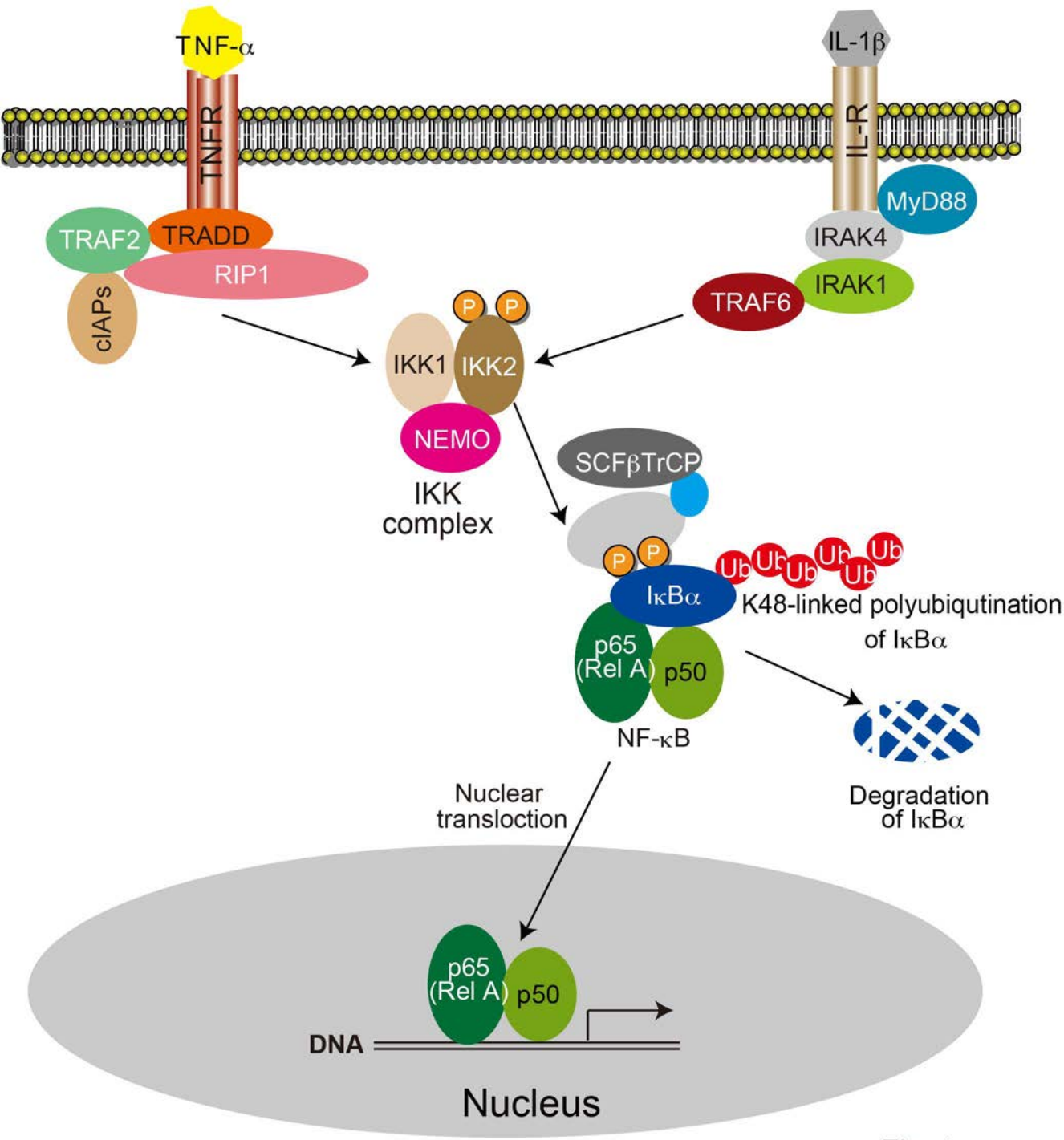
(C) A long K63 chain that is conjugated to components of the IL-1 receptor complex upon stimulation can be recognized by multiple NEMO proteins, which provokes multimerization of the IKK complex leading to phosphorylation of IKK2.

(D) A long K11 chain conjugated to RIP1 upon stimulation with TNF-α by the cIAP ubiquitin ligase can be recognized by multiple NEMO proteins, which provokes multimerization of the IKK complex leading to phosphorylation of IKK2.

Fig. 7. The possible role of K63 and K11 chains in canonical NF-κB activation.

K63 or K11 chains that are conjugated to components of the IL-1 receptor complex or RIP1 in the TNF receptor complex, upon stimulation with IL-1β or TNF-α, respectively may recruit LUBAC to the IL-1 receptor or the TNF receptor, respectively, and induce linear polyubiquitination of NEMO. Linearly polyubiquitinated NEMO induces

IKK2 phosphorylation.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IL-R
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TRADD
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P
IKK1
IKK2
NEMO
IKK
complex
SCFβTrCP
P
P
Ub
IκBα
K48-linked polyubiqutination
of IκBα
p65
(Rel A)
p50
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Degradation
of IκBα
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p50
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Fig. 1

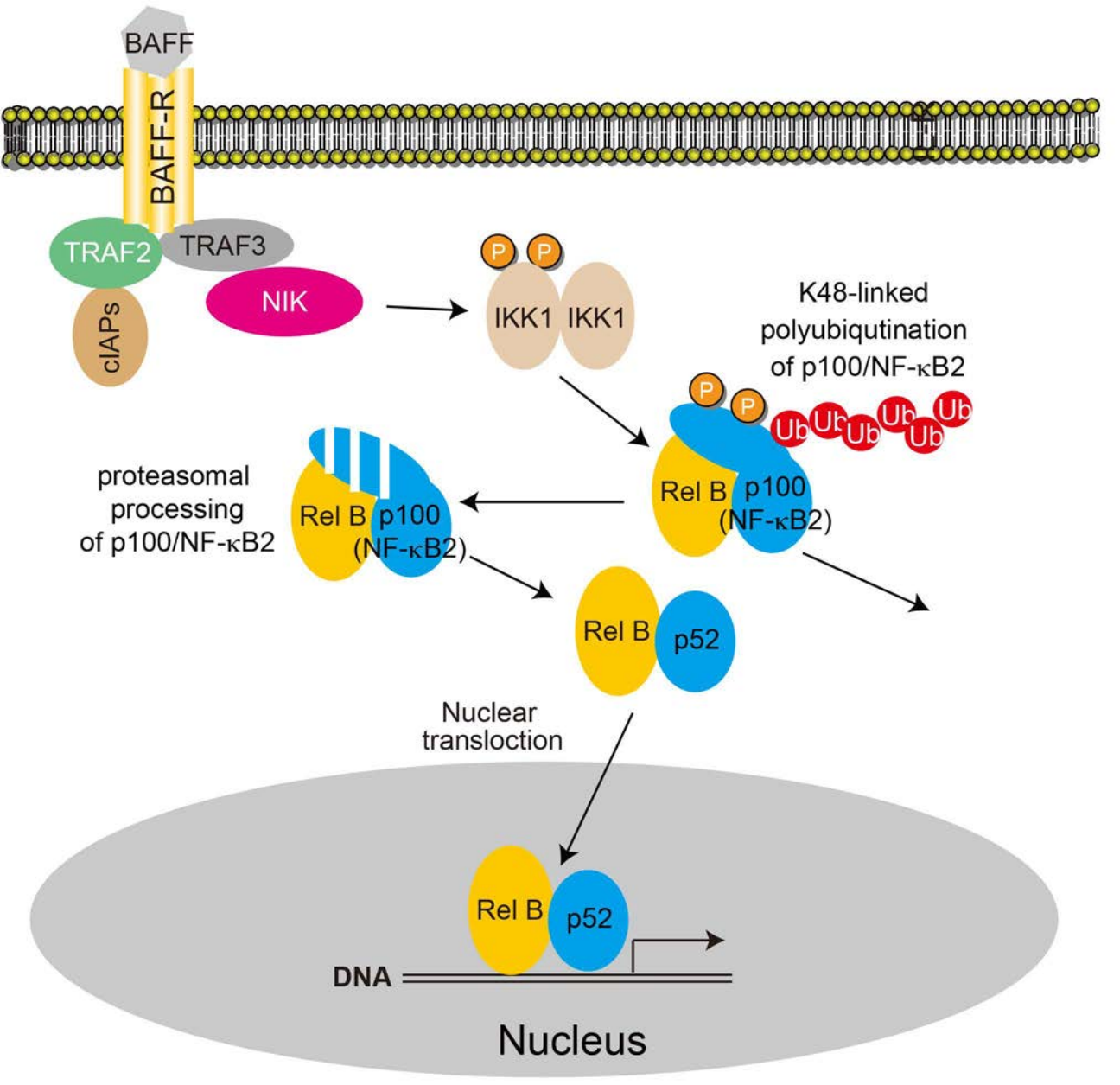
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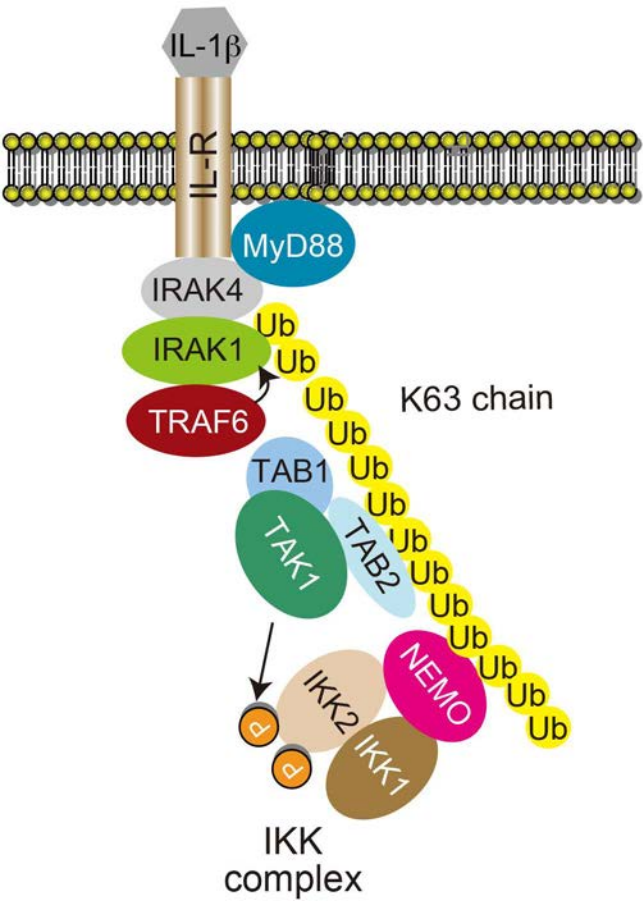
Fig. 2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MyD88
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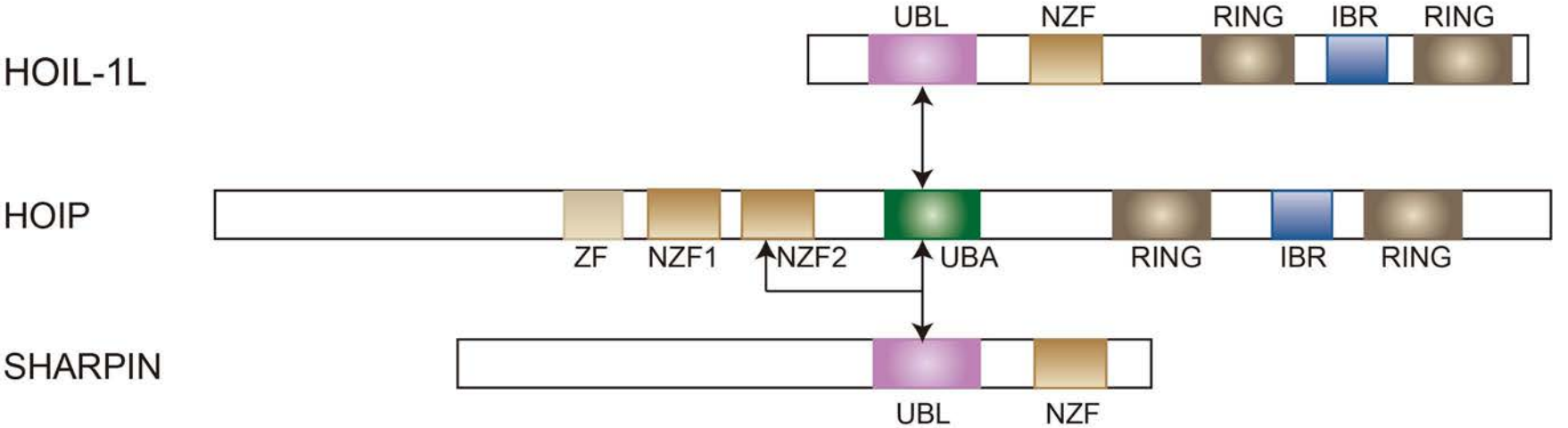
NEMO
IKK2
IKK1
P
IKK
complex

Fig. 3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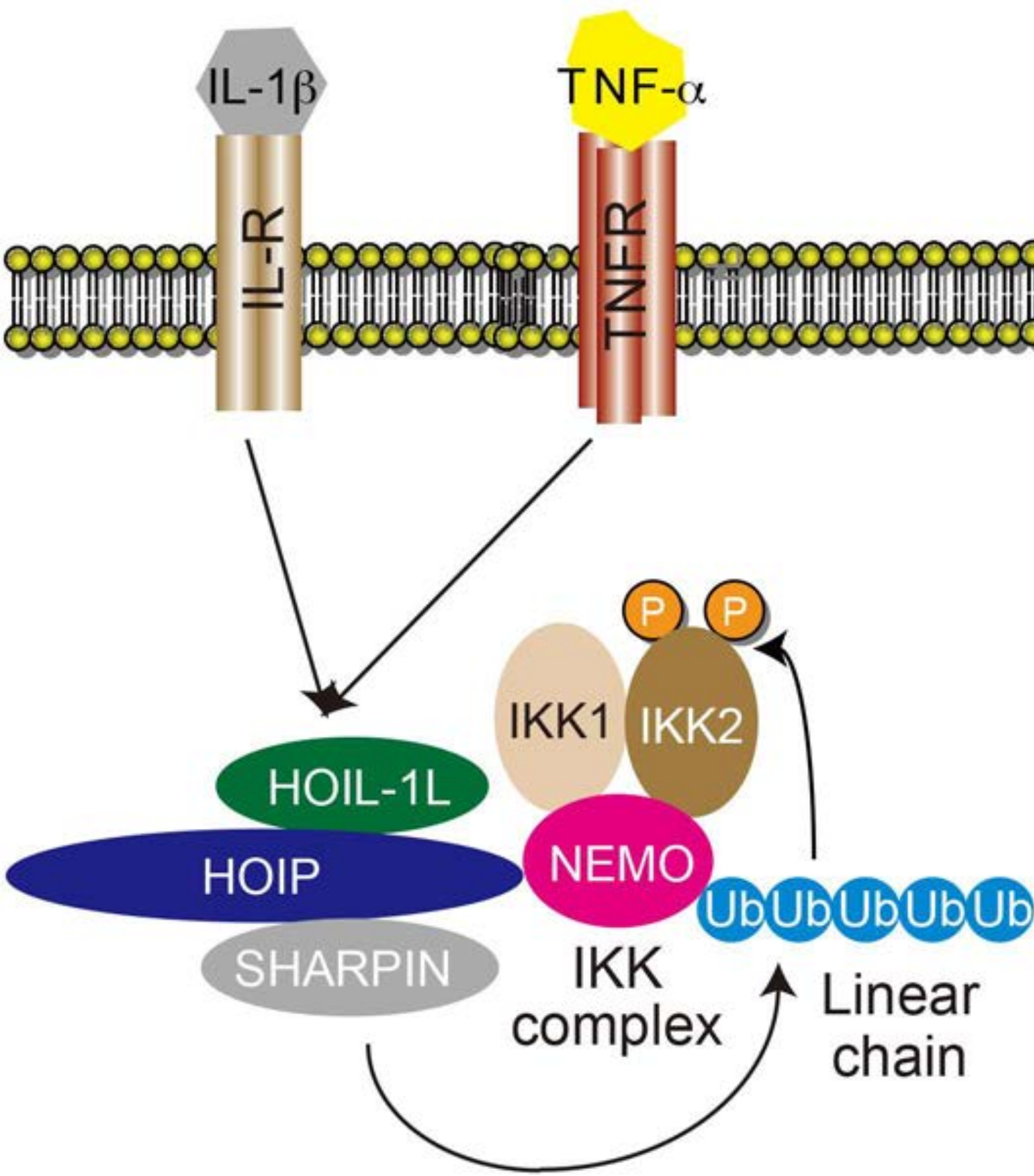
RING
SHARPIN
UBL
NZF

Fig. 4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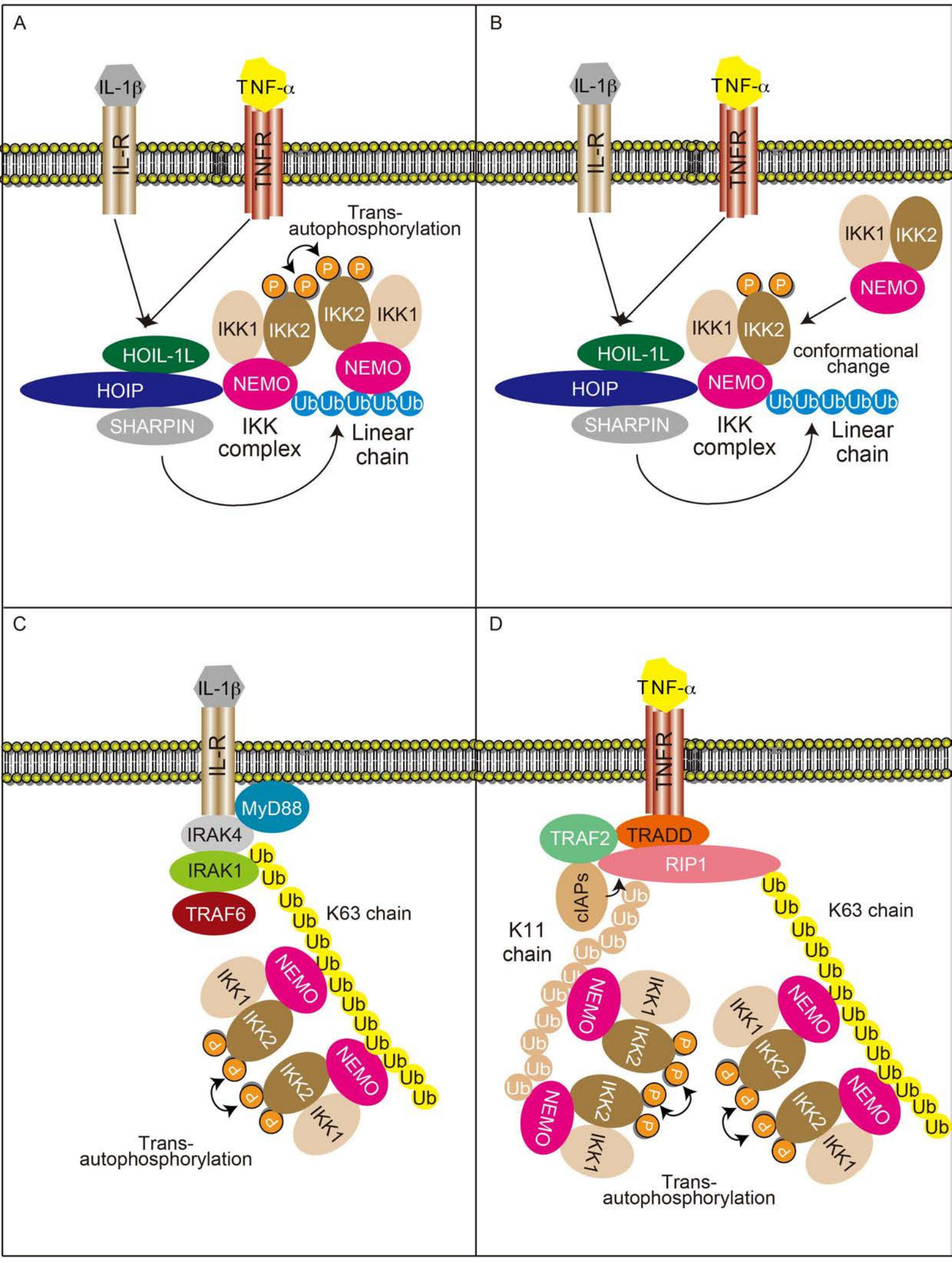
Ub
Ub
SHARPIN
IKK
complex
Linear
chain

Fig. 5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NEMO
Ub
IKK
complex
Linear
chain
B
conformational
change
C
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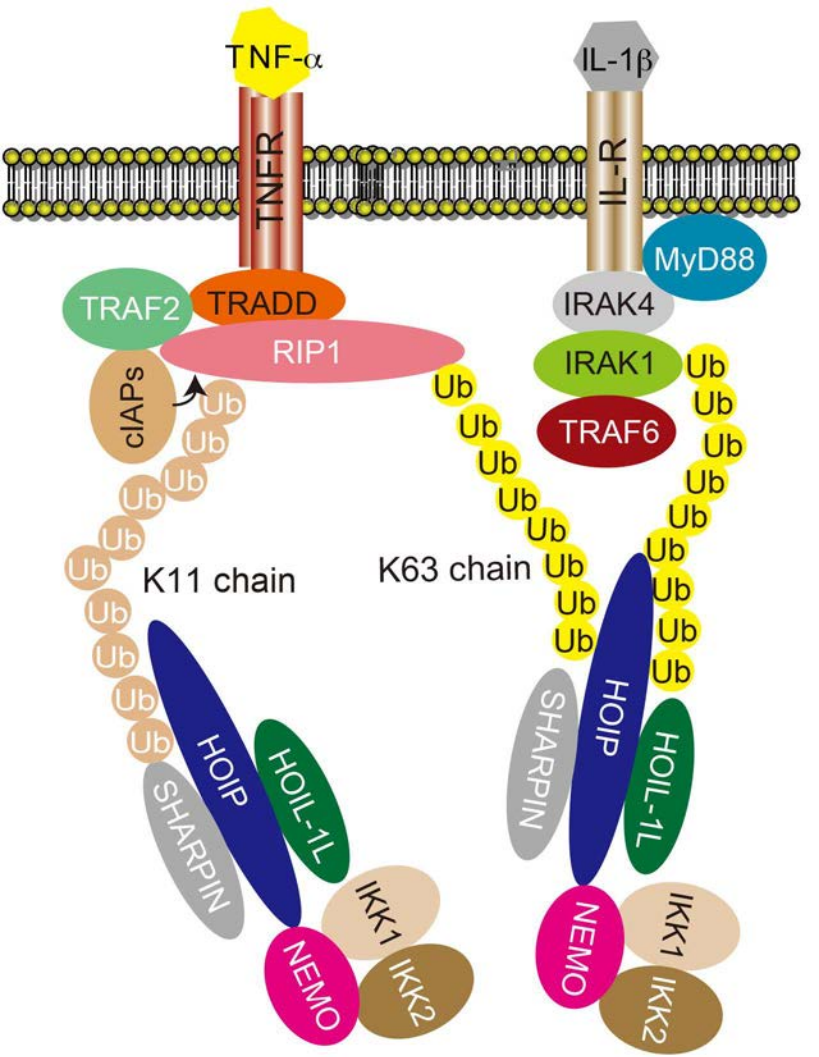
TRADD
RIP1
cIAPs
K11
chain

Fig. 6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K63 chain
HOIP
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IKK2
NEMO

Fig. 7