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# Control of NF-κB activation by the COP9 signalosome

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## Abstract

**The transcription factor NF-κB (nuclear factor κB) exerts crucial functions in the regulation of innate and adaptive immune responses, wound healing and tissue maintenance and in the development of immune cells. Tight control of NF-κB is essential for an efficient defence against pathogens and environmental stress to protect organisms from inflammatory diseases including cancer. An involvement of the CSN (COP9 signalosome) in the regulation of NF-κB has been discovered recently. The CSN is a conserved multiprotein complex, which mainly functions in the control of proteolysis. Here, we review recent observations indicating important roles of the CSN in the control of NF-κB in innate immunity, as well as T-cell activation and maturation.**

## Introduction

The CSN (COP9 signalosome) is an eight subunit (CSN1–CSN8) protein complex highly conserved in all eukaryotes. Up to now, its best characterized function is the control of proteolysis via the UPP [Ub (ubiquitin) proteasome pathway] [1]. Owing to a JAMM (JAB1/MPN/MOV34 metalloenzyme) motif located in CSN5, the CSN holocomplex, but not CSN5 alone, possesses intrinsic metalloprotease activity specific for the Ub-like molecule NEDD8 (neural-precursor-cell-expressed developmentally down-regulated 8) [2]. Reversible NEDDylation of cullins represents a critical step in the regulation of CRL (cullin–RING Ub ligase) assembly and CRL-dependent proteolysis [3,4]. CRLs are important contributors to the timely regulation of many cellular processes such as cell cycle progression, DNA repair and replication, as well as signal transduction in response to inflammatory and developmental stimuli [5]. Although NEDDylation facilitates both the recruitment of Ub-loaded Ub-conjugating enzymes to CRLs and their correct positioning towards CRL substrates to finally promote substrate ubiquitylation [6–8], this is counteracted by CSN deNEDDylase activity. However, cycles of NEDDylation and deNEDDylation might be required *in vivo* to allow efficient proteolysis via the UPP [3,4].

Apart from its deNEDDylase activity, the CSN exerts DUB (deubiquitinase) activity through its association with the USP (Ub-specific protease) USP15 [9,10]. USP15, by its DUB activity, contributes to both the protection of CRL subunits from degradation [11] and the fine-tuning of the degradation of CRL substrates [12–15]. Less well characterized activities of the CSN include the phosphorylation of target molecules by CSN-associated protein kinases [16] and direct transcriptional control by the CSN [17].

The transcription factor NF-κB (nuclear factor κB) is a crucial regulator of many physiological and pathophysiological processes, including control of the adaptive and innate immune responses, inflammation, proliferation and the decision between apoptosis and cell survival [18–20]. Therefore tight regulation of NF-κB is essential to allow, on the one hand, normal growth, development and tissue repair, but, on the other hand, to protect organisms from inflammatory diseases and cancer, as well as from severe harm by pathogens and other environmental or intracellular stressors [21–23]. Here, we review recently recognized roles of the CSN in the regulation of NF-κB in response to TNFR1 [TNF (tumour necrosis factor) receptor 1] ligation, as well as to antigenic T-cell stimulation.

## TNFR1 signalling to NF-κB

One crucial effector molecule of the innate immune response is the trimeric cytokine TNFα, which potently activates NF-κB, mainly through the ligation of TNFR1 (Figure 1). Ligation of TNFR1 induces receptor trimerization and the assembly of a TNFR1-associated signalling complex composed of the adaptor molecules TRADD (TNF-associated death domain) and RIP1 (receptor-interacting protein 1), as well as the TRAF (TNFR-associated factor) proteins TRAF2 and TRAF5. While RIP1 is a serine/threonine kinase of

**Key words:** Carma1–Bcl10–Malt1 complex (CBM), cullin–RING ubiquitin ligase (CRL), deubiquitinase (DUB), inhibitor of nuclear factor κB (IκB), neural-precursor-cell-expressed developmentally down-regulated 8 (NEDD8), tumour necrosis factor receptor 1 (TNFR1).

**Abbreviations used:** CARD, caspase recruitment domain; CBM, Carma1–Bcl10–Malt1 complex; CRL, cullin–RING ubiquitin ligase; CSN, COP9 signalosome; DUB, deubiquitinase; GSK-3β, glycogen synthase kinase 3β; NF-κB, nuclear factor κB; IκB, inhibitor of NF-κB; IKK, IκB kinase; IL-2, interleukin-2; JNK, c-Jun N-terminal kinase; NEDD8, neural-precursor-cell-expressed developmentally down-regulated 8; NEMO, NF-κB essential modulator; NUB, NEMO ubiquitin-binding domain; PKCθ, protein kinase C θ; PDK1, phosphoinositide-dependent kinase 1; RIP1, receptor-interacting protein 1; SCF, Skp1/cullin/F-box; TGF, transforming growth factor; TAK1, TGF-β-activated kinase; TCR, T-cell receptor; TNF, tumour necrosis factor; TRADD, TNF-associated death domain; TNFR1, TNF receptor 1; TRAF, TNFR-associated factor; β-TrCP, β-transducin repeat-containing protein; Ub, ubiquitin; UPP, Ub proteasome pathway; USP, Ub-specific protease.

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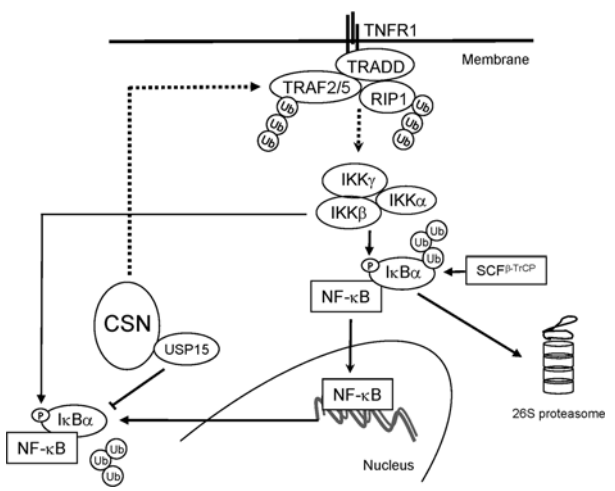
**Figure 1** | **Control of TNFR1-induced NF-κB by the CSN**

Ligation of TNFR1 by TNFα induces the formation of a receptor-bound signalling complex consisting of the adaptor molecules TRADD and RIP1 and the Ub ligases TRAF2 and TRAF5, which contribute to the modification of TRAF molecules and RIP1 with Lys$^{63}$-linked Ub chains. Recruitment and activation of the IKK complex result in the phosphorylation, Lys$^{48}$-linked ubiquitylation and proteasomal degradation of the NF-κB inhibitor IκBα and, in consequence, the release of active NF-κB. Among the NF-κB target genes is IκBα, which represents a major negative NF-κB feedback loop. The CSN might, on the one hand, facilitate IKK complex activation by supporting receptor recruitment and Lys$^{63}$-linked ubiquitylation of TRAF2/5 and RIP1 through an unknown mechanism. On the other hand, the CSN contributes to the down-regulation of persistent TNFR1 signalling via stabilization of re-synthesized IκBα by deubiquitylation, catalysed by CSN-associated USP15.




unknown substrate specificity, whose kinase activity seems to be dispensable for the activation of NF-κB, the TRAF proteins are Ub ligases, which become activated by receptor-induced oligomerization and are involved in the TNFα-induced regulatory (Lys$^{63}$-linked) ubiquitylation of both themselves and RIP1 [24]. This, furthermore, depends on the Lys$^{63}$ linkage-specific dimeric Ub-conjugating enzyme Ubc13/Uev1a [23]. Ubiquitylated RIP1 and/or TRAF proteins recruit the TAK1 [TGF (transforming growth factor)-β-activated kinase 1] kinase complex through the association of its regulatory subunits, TAB2 and TAB3, with Lys$^{63}$-linked Ub chains attached to target proteins. Ub binding of the TAB proteins might induce a conformational change within the TAK1 kinase complex, which might contribute to the catalytic autoactivation of TAK1, a major candidate kinase for the direct activation of the IKKs [IκB (inhibitor of NF-κB) kinases] IKKα and IKKβ, the catalytic subunits of the IKK complex, consisting of two catalytic subunits and the regulatory subunit IKKγ [NEMO (NF-κB essential modulator)] [24]. Additionally, RIP1 recruits the IKK complex through direct binding to NEMO, which might be supported by the NUB (NEMO Ub-binding domain), exhibiting affinity for Lys$^{63}$-linked Ub chains attached to RIP1. As a consequence, the IKK complex would come in close proximity to its upstream kinase TAK1 [24]. This model has been challenged, however, by the recent observation that the NEMO NUB preferentially binds to linear-linked Ub chains, attached to NEMO by the LUBAC (linear Ub assembly complex) in response to TNFα stimulation, which are supposed to be selectively required for the activation of the IKK complex, but not JNK (c-Jun N-terminal kinase) [25,26]. According to this model, binding of NEMO to linear-linked Ub chains induces oligomerization of the IKK complex, which might mediate a conformational change within the IKK complex to allow the *trans*-autoactivation of IKKs, a mechanism that has been proposed by others as well [27]. In this scenario however, the mode of IKK complex recruitment and the contributory role of TAK1 in its catalytic activation remain unclear.

The activated IKK complex phosphorylates the classical IκBs, e.g. IκBα, IkBβ and IκBε, at two N-terminal serine residues, which is a prerequisite for their subsequent recognition and Lys$^{48}$-linked polyubiquitylation by the CRL SCF$^{\beta-\mathrm{TrCP}}$ (SCF is Skp1/cullin/F-box and β-TrCP is β-transducin repeat-containing protein) and finally leads to their UPS-dependent degradation with different kinetics [24]. Degradation of IκBs, which associate with NF-κB to keep it inactive in the cytosol, releases active NF-κB dimers, which migrate into the nucleus to activate their target genes. Among the NF-κB target genes are cytokines and effector molecules involved in the inflammatory response, as well as IκBα and IκBε. Concomitant with their re-accumulation, IκBs, most prominently IκBα, bind to NF-κB in the nucleus and relocate it back to the cytoplasm, which represents one major negative NF-κB feedback loop. Another one is the NF-κB-induced up-regulation of the DUB A20, which down-regulates receptor-proximal signalling to NF-κB by the deubiquitylation of RIP1 and by its subsequent contribution to the Lys$^{48}$-linked ubiquitylation of RIP1. The latter targets RIP1 to the UPS and terminates sustained TNFR1-induced signalling [24].

## Roles of the CSN in TNFR1-induced NF-κB activation

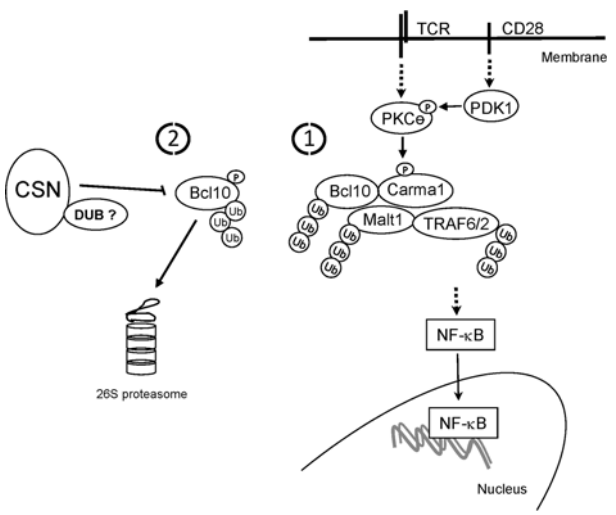
In fibroblast-like synoviocytes of patients with rheumatoid arthritis, the CSN was observed to shift the balance from receptor-induced apoptosis to NF-κB-dependent cell survival in response to TNFR1 ligation [28]. This was suggested to be due to a supportive role of the CSN in the activation of NF-κB upstream of the IKK complex. Through an interaction of CSN5 with TRAF2, the CSN might inducibly associate with TRAF2 in response to TNFα stimulation and support both TNFR1 recruitment and Lys$^{63}$-linked ubiquitylation of TRAF2 and RIP1. Knockdown of CSN5 interfered with the recruitment and ubiquitylation of both molecules and significantly retarded TNFα-induced degradation of IκBα, activation of NF-κB, as well as phosphorylation and activation of JNK [28]. How the CSN supports Lys$^{63}$-linked ubiquitylation of TRAF2 and RIP1 was not resolved. However, either enhancement of TRAF2

Ub ligase activity or inhibition/removal of an unknown DUB could be involved.

Downstream of the IKK complex, the CSN transiently associates with IκBα in response to TNFR1 ligation and supports its re-accumulation post induction. The CSN-dependent protection of IκBα from sustained CRL-dependent degradation is mediated by deubiquitylation, which depends on CSN-associated USP15 (Figure 1). Down-regulation of the CSN in epithelial cells results in sustained TNFα-induced nuclear accumulation of NF-κB and in sustained induction of selected NF-κB target genes [12]. Recently, the CRL substrate SNAIL (which is a short-half-life repressor of E-cadherin expression, supporting inflammation-induced tissue remodelling and cell migration, as well as cancer cell migration, invasion and metastasis) was reported to be post-translationally stabilized by the CSN as well. This, however, required TNFα-induced and NF-κB-dependent up-regulation of the expression of CSN subunits [13]. Whereas enhanced expression of CSN subunits resulted in reduced GSK-3β (glycogen synthase kinase 3β)-dependent phosphorylation and $SCF^{\beta-TrCP}$-dependent ubiquitylation of SNAIL and a decreased association of SNAIL with GSK-3β and $SCF^{\beta-TrCP}$, knockdown of CSN subunits prevented TNFα-induced and NF-κB-dependent SNAIL stabilization [13]. However, the exact mechanism of CSN-dependent SNAIL stabilization and whether it involves reduced ubiquitylation or enhanced deubiquitylation of SNAIL was not resolved.

**Figure 2 | Control of TCR/CD28-induced NF-κB by the CSN**

(1) Co-ligation of TCR and CD28 induces the formation of the CBM through the recruitment of PDK1 and PKCθ to the immunological synapse and the PKCθ-dependent phosphorylation of Carma1. Via the recruitment of TRAF2 and TRAF6 to Malt1 and the TRAF-dependent modification of the TRAF molecules, as well as Bcl10 and Malt1 with $Lys^{63}$-linked Ub chains, the IKK complex becomes activated, resulting in the release of active NF-κB from IκBs. (2) At late stimulation of TCR/CD28, Bcl10 becomes phosphorylated leading to destabilization of the CBM and $Lys^{48}$ ubiquitylation of Bcl10, which marks it for proteasomal or lysosomal degradation. The CSN stabilizes Bcl10 by either promoting the deubiquitylation of Bcl10 via an unknown CSN-associated DUB or suppressing the ubiquitylation of Bcl10, which might depend on CSN deNEDDylase activity.




## NF-κB activation in T-cells

Efficient T-cell activation via the TCR (T-cell receptor)–CD3 complex essentially depends on the simultaneous ligation of the TCR and the co-stimulatory receptor CD28 by their respective ligands expressed on the surface of antigen-presenting cells. The appropriate ligands for the induction of TCR/CD28-dependent signalling are antigen-derived peptides exposed on major histocompatibility complexes and B7-family molecules respectively [29]. The initiation of various signalling cascades then leads to the activation of mitogen-activated protein kinases and several transcription factors, e.g. AP-1 (activator protein 1), NFAT (nuclear factor of activated T-cells) and NF-κB, which ultimately results in the release of cytokines and the induction of target genes required for the co-ordination of T-cell expansion, differentiation and effector functions [29]. Activated T-cells are important actors in the adaptive immune response owing to their capability to selectively kill target cells and to regulate the B-cell-dependent as well as the global immune response [29].

NF-κB is essential for the activation and proliferation of mature T-cells [30] and for providing survival signals at many stages of T-cell development and maturation [31]. Regarding the CBM (Carma1–Bcl10–Malt1 complex)-dependent pathway of NF-κB activation (Figure 2), a first critical step downstream of a complex receptor-proximal signalling cascade largely based on tyrosine phosphorylation is the recruitment of PKCθ (protein kinase C θ) and PDK1 (phosphoinositide-dependent kinase 1) to the immunological synapse. In the immunological synapse, which assembles in lipid rafts upon TCR/CD28 co-ligation, both kinases become activated and placed in close proximity to each other and to Carma1 [29,30]. Phosphorylation of Carma1 by PKCθ induces a conformational change in Carma1, allowing it to interact with preformed Bcl10–Malt1 heterodimers to form the CBM [32]. Through the recruitment of TRAF6 and, eventually, TRAF2 to Malt1, a cascade of regulatory ubiquitylation is initiated, during which the TRAF proteins modify themselves, as well as Malt1, Bcl10 and IKKγ, with $Lys^{63}$-linked Ub chains [33–36]. $Lys^{63}$-linked Ub chains attached to target proteins provide the platform for the recruitment of proteins containing Ub-binding domains. Among those are NEMO and the TAB2 and TAB3 subunits of the TAK1 kinase complex. Whereas NEMO associates with ubiquitylated Bcl10–Malt1 [33,36], the TAK1 kinase complex is recruited by ubiquitylated TRAF6- or TRAF2-associated RIP1 [32]. As in TNFR1 signalling to NF-κB, TAK1 represents one major candidate kinase for the catalytic activation of the IKK complex [23,32]. Alternatively, binding of NEMO to $Lys^{63}$-linked Ub chains attached to Bcl10–Malt1 might induce a conformational change in the IKK complex,

which subsequently allows *trans*-autoactivation of the IKKs [24]. In the latter case, the observed critical role of TAK1 in the activation of IKK in primary but not effector T-cells [37] remains unclear.

Down-regulation of TCR/CD28-induced signalling to NF-$\kappa$B involves CBM-dependent, but NEMO-independent, C-terminal phosphorylation of Bcl10 by IKK$\beta$, resulting in destabilization of the CBM [38]. Additionally, CBM-dependent and either IKK-independent [39] or IKK-dependent phosphorylation of Bcl10 [40] within its N-terminal CARD (caspase recruitment domain) domain marks Bcl10 for lysosome- [39] or UPP-dependent [40] degradation, which efficiently terminates sustained CBM-dependent signalling. TCR-induced degradation of Bcl10 is preceded by its ubiquitylation within the CARD domain, suggested to be catalysed by several candidate Ub ligases, including the RING ligase cIAP2 (cellular inhibitor of apoptosis or cellular inhibitor of apoptosis protein 2) [41], the HECT (homologous with E6-associated protein C-terminus) ligases Itch and NEDD4 [39] and the CRL $SCF^{\beta-TrCP}$ [40].

Furthermore, the DUBs CYLD and A20 have been observed to contribute to the down-regulation of the TCR/CD28-induced activation of NF-$\kappa$B [42–45]. Reported DUB targets include (i) TAK1 [42] in the case of CYLD, and (ii) IKK$\gamma$, Malt1 and TRAF6, but not Bcl10, in the case of A20 [44–46]. A first study linking A20 to T-cell activation suggested that TCR/CD28-induced Malt1 paracaspase-dependent cleavage of A20, which is constitutively expressed in T-cells, where it inducibly associates with Malt1 in response to TCR/CD28 ligation [43], might be required for efficient TCR-induced activation of NF-$\kappa$B and the induction of IL-2 (interleukin-2) [43]. The Malt1 paracaspase-regulated step was hypothesized to be the CBM- and $Lys^{63}$-linked ubiquitylation-dependent activation of the IKK complex, which might be counteracted by $Lys^{63}$-specific A20 DUB activity and, in reverse, facilitated by TCR/CD28-induced cleavage and subsequent degradation of A20. This was, however, not supported by a recent study showing that activation of IKK and NF-$\kappa$B DNA binding was completely unaffected by inhibition of Malt1 paracaspase activity and the resulting (partial) cleavage of A20, although the inhibition resulted in strong reduction of IL-2 induction [45]. Reconstitution of Malt1-deficient T-cells with a catalytically inactive Malt1 mutant further confirmed that Malt1 paracaspase activity is dispensable for IKK complex activation by the CBM [45]. Therefore the induction of IL-2 in response to TCR/CD28 ligation might critically depend on either Malt1 paracaspase-dependent regulation of NF-$\kappa$B transactivation or an unknown MALT1 paracaspase-dependent, but NF-$\kappa$B-independent event. Nevertheless, TCR-induced (partially) UPS-dependent degradation of A20 was confirmed to be required for efficient activation of the IKK complex and NF-$\kappa$B DNA binding, both of which were blunted concomitant with the NF-$\kappa$B-induced re-accumulation of A20 [43,45], supporting an important role of A20 in shaping the TCR-induced NF-$\kappa$B response. While A20 might predominantly function in the down-regulation of TCR/CD28-induced NF-$\kappa$B signalling, CYLD, through the deubiquitylation of TAK1, might mainly keep under control the basal levels of active NF-$\kappa$B in mature T-cells [42]. During thymocyte development, CYLD exerts an additional function in the TCR-proximal activation of Lck [47].

## Roles of the CSN in the TCR-induced activation of NF-$\kappa$B

Initially, thymocytes with a knockout for CSN5 were observed to cause a defect in a late stage of thymocyte maturation, coinciding with arrested/delayed cell cycle progression and a severe reduction in cell survival [48]. At the molecular level, this defect was associated with a profound increase in the expression of p53 and the up-regulation of pro-apoptotic p53 target genes, as well as with a combined reduction in the expression of I$\kappa$B$\alpha$- and NF-$\kappa$B-regulated pro-survival genes. Based on the fact that thymocyte survival could not be rescued by the expression of a transgenic rearranged TCR, a functional role of the CSN directly downstream of the TCR was claimed, which was however not characterized in detail [48]. Defects in basal proliferation and long-term survival have been described for mature proliferating T-cells deficient in CSN8 as well [49].

Recently, an inducible association between CSN and CBM has been discovered in response to T-cell activation by TCR/CD28 ligation. This association was based on a direct interaction between CSN5 and the Malt1 and Carma1 subunits of the CBM [14]. However, the CSN holocomplex was required for an efficient CBM-dependent activation of the IKK complex and an optimal induction of IL-2 [14]. The CSN was shown to stabilize Bcl10, protecting it from rapid TCR-induced degradation and the CBM from premature dissociation (Figure 2). Regarding the mechanism of CSN-dependent Bcl10 stabilization, deubiquitylation of Bcl10 by an unknown CSN-associated DUB was suggested [14]. An involvement of USP15 or A20, which removes $Lys^{63}$-linked Ub chains from MALT1, IKK$\gamma$ and TRAF6 [44–46], but can disassemble $Lys^{48}$-linked Ub chains *in vitro* as well [50], was however not observed [14,45]. Apart from Bcl10 stabilization by deubiquitylation, a suppressive role of the CSN in the ubiquitylation of Bcl10, which might depend on the deNEDDylase activity of CSN5, remains possible. There is also urgent need for a careful characterization of the linkage type of Ub chains attached to Bcl10, which, in analogy to RIP1 in the TNFR1 signalling cascade [24], might switch from $Lys^{63}$-linked ubiquitylation at early time points after TCR/CD28 ligation, supporting IKK complex recruitment and activation, to $Lys^{48}$-linked ubiquitylation at later time points to promote Bcl10 degradation.

In quiescent T-cells, the CSN was shown to be required for cell cycle transition from the $G_0$ to the $G_1$ phase, namely the induction of proliferation [49]. Although quiescent T-cells lacking CSN8 appropriately responded to TCR/CD28 ligation with regard to MAK (male germ cell-associated kinase) kinase and I$\kappa$B phosphorylation, as well as I$\kappa$B degradation, they where unable to proliferate,

even in the presence of IL-2. Based on the observation that on the induction of proliferation, CSN1 and CSN8 inducibly associate with promotors of genes encoding cell cycle regulators in wild-type cells, a direct role of the CSN in transcriptional control was suggested [49].

## Conclusions

Evidence is accumulating for a regulatory role of the CSN at various stages of NF-$\kappa$B signal transduction cascades induced by different stimuli in various cell types. A clear picture of the functions and mechanisms of action of the CSN in the NF-$\kappa$B system will certainly be generated by future studies.

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