Interplay between BCL10, MALT1 and IκBα during T-cell-receptor-mediated NFκB activation

Gabrielle Carvalho[1,2], Armelle Le Guelte[3,4], Catherine Demian[1,2,5], Aimé Vazquez[1,2], Julie Gavard[3,4] and Nicolas Bidère[1,2,*]

[1]INSERM UMR_S 1014, Hôpital Paul Brousse, Villejuif 94800, France
[2]Université Paris-Sud P11, Orsay 91400, France
[3]Institut Cochin, Université Paris Descartes, CNRS (UMR 8104), Paris 75014, France
[4]INSERM, U1016, 22 rue Méchain, Paris 75014, France
[5]ENCPB, 11 rue Pirandello, Paris 75013, France
*Author for correspondence (nicolas.bidere@inserm.fr)

Accepted 23 April 2010
Journal of Cell Science 123, 2375-2380

doi:10.1242/jcs.069476

Summary

T-cell-receptor (TCR) signalling to NFκB requires the assembly of a large multiprotein complex containing the serine/threonine kinase CK1α, the scaffold protein CARMA1, the heterodimer BCL10-MALT1 (the CBM complex) and the IκB kinase complex (IKK). Although the mechanisms regulating recruitment and activation of IKK within the CBM microenvironment have been extensively studied, there is little understanding of how IKK subsequently binds and phosphorylates IκBα, the inhibitor of NFκB, to promote IκBα ubiquitylation and proteasomal degradation. Here, we show that BCL10, MALT1 and IKK inducibly associate with IκBα in a complex that is physically distinct from the early CK1α-CBM signalosome. This IκBα-containing complex probably maturates from the CBM, because siRNA-based knockdown of CARMA1, CK1α and BCL10 hampered its assembly, leading to a reduction in NFκB activation. By contrast, CK1α normally recruited both BCL10 and ubiquitylated species of MALT1 when IκBα levels were reduced. However, knockdown of IκBα led to an altered ubiquitylation profile of BCL10-MALT1 combined with a defect in MALT1 reorganisation within large cytoplasmic structures, suggesting that, following stimulation, IκBα might also participate in MALT1 recycling. Altogether, our data suggest a two-step mechanism to connect active IKK to IκBα, and further unveil a potential role for IκBα in resetting TCR-mediated signalling.

Key words: NFκB, Lymphocyte, Signalling, Ubiquitylation

Introduction

The transcription factor NFκB has a vital role in cellular development, homeostasis and survival in the immune system, as well as in the propagation of certain lymphoma cells (Ghosh and Hayden, 2008). The NFκB heterodimer of Rel-family proteins, typically p50 and RelA (p65), is tethered to its main cognate inhibitors, IκB proteins, in the cytosol of the cells. In T cells, T-cell-receptor (TCR) engagement nucleates a multiprotein complex within the lipid-raft microdomains to recruit and activate the inhibitor of NFκB kinase (IKK) (Rawlings et al., 2006). IKK then phosphorylates and marks IκBα for proteasomal degradation, thereby unleashing NFκB, which translocates to the nucleus and initiates transcription of specific target genes (Wan and Lenardo, 2010).

Although this IKK-activating signalosome contains several proteins that are yet to be identified, the scaffold protein CARMA1, together with the heterodimer BCL10-MALT1 (CBM complex) and the serine threonine kinase CK1α are essential to convey NFκB signalling (Bidere et al., 2009; Egawa et al., 2003; Gaide et al., 2002; Ruland et al., 2001; Ruland et al., 2003; Wang et al., 2002). Non-degradative ubiquitylation of BCL10-MALT1 oligomers was proposed to authorise IKK recruitment and activation within the CBM (Oeckinghaus et al., 2007; Sun et al., 2004; Wu and Ashwell, 2008). To restrain signalling to a single pass, MALT1-associated ubiquitin chains are further targeted by deubiquitylating enzymes (DUBs), whereas BCL10 phosphorylation and ubiquitylation promote its turnover (Duwel et al., 2009; Lobry et al., 2007; Wegener et al., 2006; Zeng et al., 2007).

How the CBM links IKK to IκBα is an open conundrum. Although a fraction of BCL10, MALT1 and IKK is quickly redistributed in raft microdomains upon stimulation (Bidere et al., 2006; Che et al., 2004; Wang et al., 2004), NFκB, IκBα and phosphorylated IκBα remain cytosolic (Sebald et al., 2005). Here, we show that BCL10, MALT1 and IKK first assemble the early IKK-activating CK1α-CBM signalosome, and subsequently associate with IκBα in a physically distinct complex. This atypical BCL10-MALT1-IκBα complex maturates from the CK1α-CBM and probably serves as an essential scaffold to bridge IKK to IκBα and therefore expel NFκB heterodimers. Furthermore, we provide evidence that IκBα participates in the optimal ubiquitylation of BCL10 and in deubiquitylation and redistribution of MALT1 into large cytosplasmic structures.

Results and Discussion

To better understand how the CBM links IKK to NFκB, IκBα was immunoprecipitated from cell lysates of Jurkat T cells stimulated with PMA and ionomycin to mimic TCR activation. As expected, IKKβ and phosphorylated species of IKKβ did bind to IκBα shortly after stimulation (Fig. 1A). Surprisingly, we also observed a robust association between IκBα, BCL10 and MALT1. This complex appeared stable despite IκBα degradation, and contrasted with the fast and transient binding of the same set of molecules to CK1α (Fig. 1A). Indeed, CK1α recruited BCL10, MALT1 and IKKβ to reach a maximal interaction between 15-30 minutes after stimulation, before dismantling after 45-60 minutes (Fig. 1A). Of

Journal of Cell Science

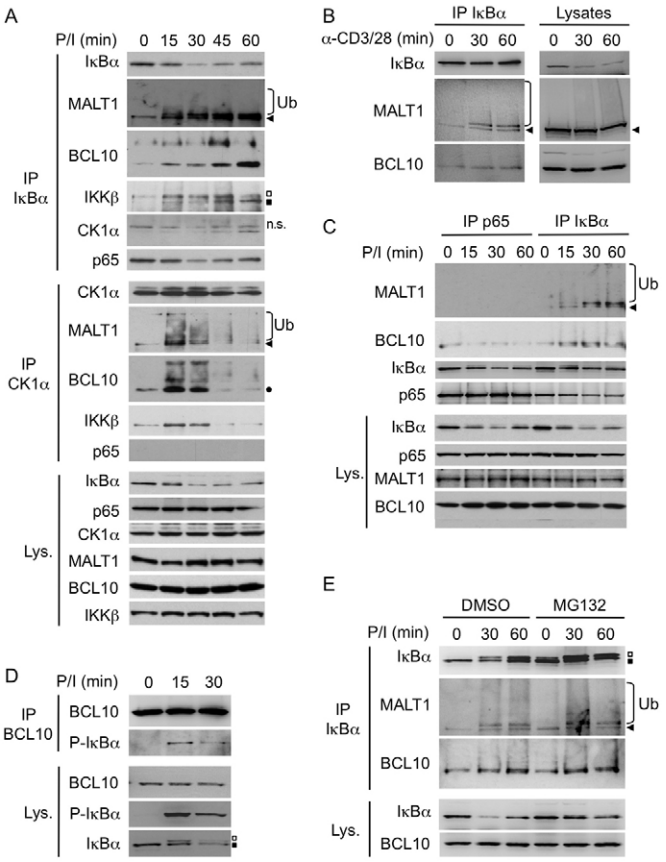

Fig. 1. Association of BCL10 and MALT1 with IκBα and CK1α during TCR activation. (**A**) Jurkat T cells were stimulated with 20 ng/ml PMA and 300 ng/ml ionomycin (P/I). Cell extracts were immunoprecipitated (IP) with antibodies against IκBα or CK1α, and immunoblots (IB) were performed as indicated. Closed and open squares indicate IKKβ and phosphorylated-IKKβ, respectively. Triangle and circle show unmodified MALT1 and BCL10, respectively. Ub, Ubiquitin; Lys, lysates. (**B**) Jurkat T cells stimulated with 1 μg/ml anti-CD3 and anti-CD28. (**C**,**D**) Lysates from cells stimulated as in A. Closed and open squares indicate IκBα and phosphorylated IκBα. (**E**) Cells pretreated with 25 μM MG132 or DMSO for 30 minutes were stimulated as in A.

note, a mixture of MALT1 and ubiquitylated species of MALT1 associated with IκBα, whereas it was essentially ubiquitylated when bound to CK1α. IκBα association with BCL10 and MALT1 was also detected in cells stimulated with antibodies against CD3 and CD28 (Fig. 1B). Interestingly, we did not detect significant binding between IκBα and CK1α, suggesting that these two proteins might segregate in distinct complexes (Fig. 1A, and supplementary material Fig. S1). Since the association of BCL10 and MALT1 with CK1α and IκBα differed in their duration and dynamics, these complexes were termed complex I and complex II, respectively.

Because IκBα phosphorylation and degradation coincided with recruitment of BCL10-MALT1, we further investigated whether NFκB p65 could also be part of complex II. However, no BCL10 or MALT1 associated with p65, although they significantly bound to IκBα (Fig. 1C). It is thus likely that BCL10-MALT1 accumulates with phosphorylated species of IκBα as soon as it disengages from NFκB. Accordingly, phosphorylated species of IκBα were efficiently found together with BCL10 upon stimulation (Fig. 1D). Finally, blockade of IκBα degradation with the proteasome inhibitor MG132 only slightly increased its association with BCL10-MALT1, suggesting that BCL10 and MALT1 further dissociate from IκBα regardless of its state of degradation (Fig. 1E). Altogether, our results suggest that BCL10 and MALT1 selectively and transiently associate with IκBα during TCR stimulation.

To determine whether complexes I and II segregate in two distinct structures, IκBα-containing units were precleared from cell lysates, and the association of CK1α with MALT1 was investigated in the remaining extracts (Fig. 2A). If IκBα, CK1α and MALT1 assemble in a unique complex, then immunodepletion of IκBα should also remove CK1α-MALT1 interaction. Although IκBα and IκBα-associated proteins were significantly removed after two rounds of immunodepletion, MALT1 still efficiently co-precipitated with CK1α in the remaining lysates (Fig. 2B-D). This suggests that MALT1 can partition between CK1α- and IκBα-associated complexes. Accordingly, removal of the CK1α-containing complex only slightly affected the IκBα association with BCL10-MALT1 (Fig. 2E,F). By contrast, immunodepletion of BCL10, which associates with both CK1α and IκBα, removed IκBα binding to MALT1 (Fig. 2F). Collectively, our data suggest that BCL10 and MALT1 nucleate two separate complexes: an upstream CK1α-containing platform and a downstream IκBα-containing complex.

To further dissect interconnections between complexes I and II, BCL10-MALT1 recruitment to IκBα was analysed in the absence of the upstream CK1α and CARMA1, or BCL10. As expected, Jurkat T cells stably expressing a doxycyclin-dependent shRNA to knock down CK1α exhibited decreased IκBα degradation. This was accompanied by a reduced association of BCL10-MALT1 with IκBα (Fig. 3A). Knockdown of CARMA1 or BCL10 with specific siRNA efficiently reduced TCR-mediated NFκB and markedly diminished binding of BCL10-MALT1 to IκBα (Fig. 3B,C,D). Similarly, BCL10, MALT1 and IKKβ were recruited to IκBα at a lower level in the CARMA1-deficient Jurkat-T-cell line JPM50.6 (Wang et al., 2002) (not shown). Altogether, our data suggest that complex I upstream components are required for bridging BCL10-MALT1-IKK to IκBα, thereby driving its phosphorylation and degradation.

Several core components of the CBM exert contrasting function within the same complex. For example, CK1α, PKCθ and IKKβ positively convey NFκB signalling, but also secondarily phosphorylate CARMA1 and BCL10 in a negative-feedback loop to inactivate the CBM (Bidere et al., 2009; Matsumoto et al., 2005; Moreno-Garcia et al., 2009; Sommer et al., 2005; Wegener et al., 2006; Zeng et al., 2007). To determine its impact on NFκB and CBM formation, we used siRNA to knock down IκBα. This led to a 60% knockdown, as estimated by densitometric analysis (not shown), which probably accounts for the lack of increased NFκB baseline activity in our system. By contrast, TCR-induced NFκB was significantly decreased (Fig. 4A), reinforcing the concept that IκB proteins actively participate to stimulus responsiveness (Chen et al., 2000; Tergaonkar et al., 2005). This was, however, not due to defective binding of ubiquitylated MALT1 or BCL10 to CK1α, because complex I assembled normally without IκBα (Fig. 4B).

We further investigated whether IκBα participates in the ubiquitylation of BCL10 and MALT1 that occurs during TCR signalling (Duwel et al., 2009; Scharschmidt et al., 2004). Subcellular-fractionation experiments revealed that BCL10 was essentially ubiquitylated in heavy membrane fractions, which

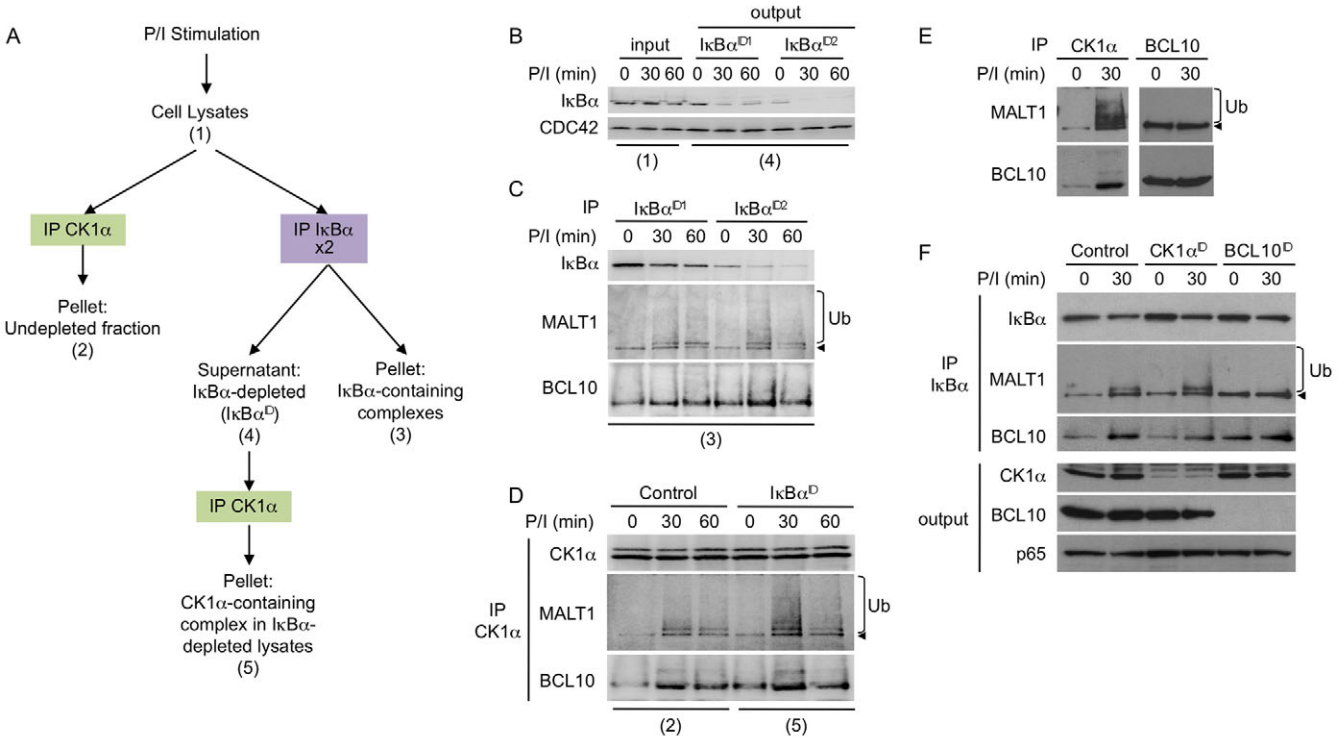

Fig. 2. BCL10 and MALT1 integrate distinct complexes. (**A**) Scheme of IκBα depletion in lysates from Jurkat T cells. (**B-D**) Jurkat T cells were stimulated with 20 ng/ml PMA and 300 ng/ml ionomycin (P/I) as indicated. Lysates were then precleared twice by immunoprecipitation with antibodies against IκBα ($IκBα^{ID1}$ and $IκBα^{ID2}$) to remove all the IκBα-associated proteins. ID, Immunodepleted. Identical volumes were saved before (input) and after IκBα immunodepletion (output) and analysed by immunoblot (IB). CDC42 served as loading control. The remaining lysates (Fraction $IκBα^{ID}$) were immunoprecipitated with an anti-CK1α, and its interaction with BCL10 and MALT1 was evaluated by immunoblotting (D). Numbers indicate the different steps, as depicted in A. (**E**) Immunoprecipitation of CK1α or BCL10 and immunoblotting. (**F**) Lysates in which CK1α or BCL10 were removed, as in E, immunoprecipitated with anti-IκBα antibodies. BCL10-MALT1 binding was assessed by immunoblotting. Output lysates show the immunodepletion efficiency.

contain lysosomes, mitochondria and endoplasmic reticulum (supplementary material Fig. S2). To determine the nature of ubiquitin chains bound to BCL10, linkage-specific ubiquitin antibodies were used. We found that antibodies raised against K63-specific and not against K48-specific ubiquitin chains recognised ubiquitylated species of BCL10 in stimulated cells, suggesting a non-degradative regulation of BCL10 (supplementary material Fig. S2). Interestingly, knockdown of IκBα slightly, but consistently, reduced BCL10 ubiquitylation, unveiling a possible role of IκBα on BCL10 function (Fig. 4C). In sharp contrast, MALT1 ubiquitylation was enhanced in lysates from IκBα-knockdown cells (Fig. 4D), suggesting that IκBα participates in regulation of MALT1 deubiquitylation processes. In addition, confocal microscopy experiments revealed that MALT1 integrated discrete filamentous and vesicular structures that further coalesced into larger signalosomes upon stimulation (Fig. 4E), possibly POLKADOTS (Rossman et al., 2006). Importantly, reduction of

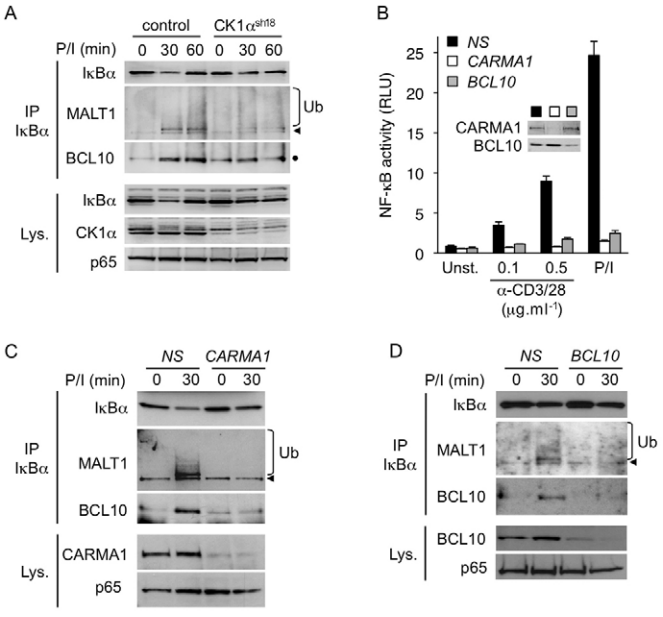

Fig. 3. Association of IκBα with BCL10-MALT1 requires CK1α, CARMA1 and BCL10. (**A**) Jurkat cells stably expressing CK1α shRNA ($CK1α^{sh18}$) were stimulated with 20 ng/ml PMA and 300 ng/ml ionomycin (P/I). Cell extracts were immunoprecipitated (IP) with antibodies against IκBα and immunoblots (IB) were performed as indicated. Lys., lysates; Ub, ubiquitin. (**B**) Jurkat were transfected with siRNA to knock down CARMA1 or BCL10, or with a nonsilencing (NS) control. After 3 days, cells were retransfected with siRNA together with an NFκB-dependent luciferase reporter gene and a *Renilla* luciferase control. Cells were then stimulated with anti-CD3 and anti-CD28, or with 20 ng/ml PMA and 300 ng/ml ionomycin. Graph shows the mean ± s.d. of triplicate experiments. Knockdown efficiency was assessed by immunoblotting. (**C**,**D**) Cells transfected with siRNA to knock down CARMA1 or BCL10 were stimulated as in A for 30 minutes.

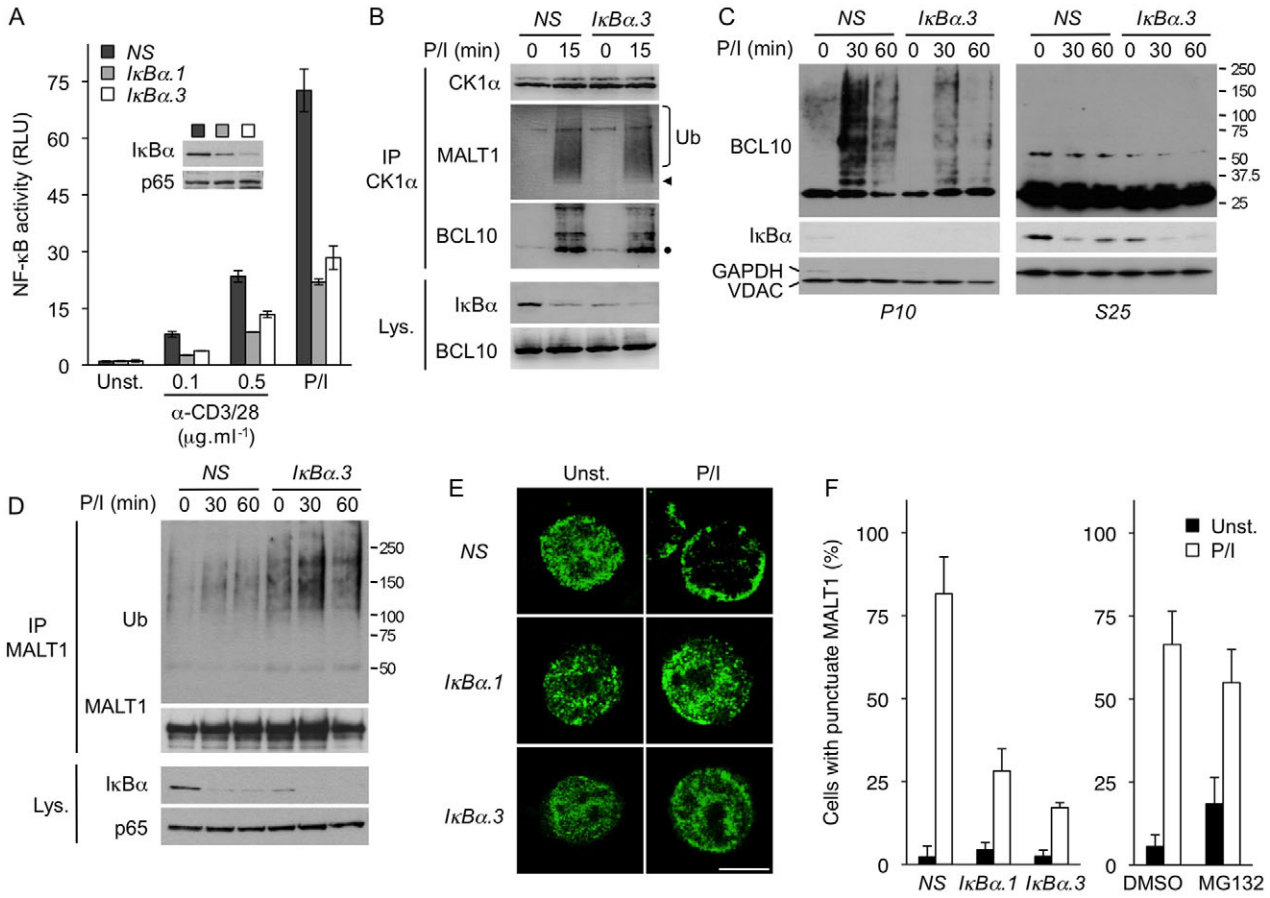

Fig. 4. Impact of knockdown of IκBα on NFκB activation and ubiquitylation and relocation of MALT1. (**A**) Jurkat cells were transfected with two individual siRNAs to knock down IκBα (IκBα.1 and IκBα.3) or with a nonsilencing (NS) control for 3 days. Cells were then transfected with siRNA together with an NFκB-dependent luciferase reporter gene and a *Renilla* luciferase control for an additional 24 hours, before stimulation with anti-CD3 and anti-CD28, or with 20 ng/ml PMA and 300 ng/ml ionomycin (P/I). Histograms represent the mean ± s.d. of triplicate experiments. Knockdown efficiency was assessed by immunoblot (IB). (**B**) Jurkat T cells transfected with siRNA to knock down IκBα were stimulated for 15 minutes as in A. Triangle and circle indicate unmodified MALT1 and BCL10, respectively. Ub, Ubiquitin. (**C**) Examination of BCL10 in heavy membrane (P10) and cytosolic fractions (S25). GAPDH and VDAC were used as purity control for cytosolic and membrane fractions, respectively. (**D**) MALT1 ubiquitylation profile. (**E**) siRNA-transfected Jurkat cells were stimulated with P/I for 60 minutes or left unstimulated (Unst.) and subcellular localisation of MALT1was analysed by confocal microscopy. Scale bars: 10 μm. (**F**) Number of cells exhibiting punctuate MALT1 staining was counted. Similar quantification was performed in cells pretreated with 25 μM MG132 or with DMSO. *n*=200 in two independent experiments.

IκBα levels significantly altered redistribution of MALT1, which remained mostly diffuse with discrete foci (Fig. 4E,F). By contrast, MG132 treatment did not affect relocation of MALT1, suggesting that IκBα degradation is not a prerequisite for MALT1 redistribution upon stimulation (Fig. 4F). Altogether, our data show that IκBα does not directly influence complex I assembly, but rather controls optimal ubiquitylation levels of BCL10-MALT1 and its redistribution upon stimulation.

In summary, we propose a model in which IKK is first activated within the CBM microenvironment until BCL10-MALT1 guide its subsequent release into the cytosol to target IκBα in a distinct complex. Such a phenomenon might allow an 'all-or-nothing' threshold of activation, therefore avoiding any potentially harmful activation. This two-step system is reminiscent of CD40-induced IKK-MEKK1 activation, which triggers the recruitment at the membrane of a large multi-protein complex before its release into the cytosol through TRAF3 proteasomal degradation (Matsuzawa et al., 2008). Because MG132 did not affect the association of IκBα with BCL10-MALT1, another molecular switch probably exists. In this scenario, IKKβ-driven serine phosphorylation of BCL10 is an appealing candidate for triggering expulsion of IκBα-activating structures into the cytosol, because it destabilises the CBM and ultimately favours BCL10 turnover (Wegener et al., 2006; Welteke et al., 2009; Zeng et al., 2007). In addition to NFκB, CARMA1 also controls TCR-induced JNK2 activation through the BCL10-MALT1 association with JNK2, TAK1 and MKK7 (Blonska et al., 2007). It will therefore be interesting to determine whether this MAPK-activating platform is different from the IκBα-activating structure we describe here.

How IκBα positively participates in activation of NFκB in addition to its inhibitory function remains unclear. IκBα probably bridges IKK to NFκB and helps to ensure stimulus responsiveness (Tergaonkar et al., 2005). Although CK1α efficiently assembled the CBM, ubiquitylation patterns of BCL10 and MALT1 were altered, and MALT1 no longer reorganised into large cytosolic structures without IκBα. Such structures might favour removal of MALT1-associated ubiquitin chains by DUBs, which thus resets MALT1. Because A20 removes K63-linked ubiquitin chains from

MALT1 at the CBM level to ensure IKK fine-tuning (Duwel et al., 2009), other DUBs are probably involved downstream of IκBα. In addition to blocking MALT1 enzyme activity (Ferch et al., 2009; Hailfinger et al., 2009; Rebeaud et al., 2008), regulation of its location or post-translational modifications might be a relevant strategy to treat a subset of activated B-cell-like diffuse large B-cell lymphoma (ABC DLBCL) lines, which rely on the CBM for their survival (Bidere et al., 2009; Compagno et al., 2009; Lenz et al., 2008; Ngo et al., 2006).

Collectively, our data provide mechanistic insights on how IKK connects to IκBα during TCR stimulation. Our findings reinforce the idea that TCR-mediated activation of NFκB is a dynamic network of multi-protein complexes, which further mature into separate units.

Materials and Methods

Cell culture and reagents

Jurkat T cells E6.1 were purchased from ATCC, and cells stably expressing a doxycycline-dependent shRNA to knock down CK1α were previously described (Bidere et al., 2009). Cells were activated with a combination of soluble anti-CD3ε and anti-CD28 (BD Biosciences), or with phorbol 12-myristate 13-acetate (PMA, Sigma) together with ionomycin (Calbiochem). MG132 was from Merck.

Cell lysates, immunoprecipitation and immunoblotting

25-50×10^6 cells per condition were sequentially stimulated to collect the samples simultaneously. Reactions were stopped with ice-cold PBS and cell pellets were further lysed with TNT buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% NP-40, 2 mM EDTA) supplemented with complete protease inhibitors (Roche), and samples were cleared by a centrifugation at 10,000 ***g*** at 4°C. Protein concentration was determined with a micro BCA kit (Pierce). Samples (1-2 mg) were precleared with protein-G-Sepharose beads (Roche) for 1 hour before immunoprecipitation with 5 μg antibodies and additional protein-G-Sepharose beads at 4°C for 2 hours. For immunodepletion experiments, lysates were precleared once with 5 μg antibodies and protein-G-Sepharose beads for 2 hours, and twice with protein-G-Sepharose for 1 hour at 4°C to remove the remaining immunocomplexes. Depleted lysates were then subjected to another round of immunoprecipitation for 2 hours. MALT1 ubiquitylation assays were performed as described (Oeckinghaus et al., 2007). For subcellular fractionation experiments, cells were mechanically permeabilised with $27G^{1/2}$ syringe in 20 mM HEPES pH 7.9, 1.5 mM $MgCl_2$, 60 mM KCl, and protease inhibitors. Nuclei and unbroken cells were removed at 2000 ***g***. Supernatants were spun at 10,000 ***g*** to obtain heavy membrane pellets, whereas cytosol was further collected after a 25,000 ***g*** centrifugation. Antibodies against BCL10 (A-6; 331.3), CK1α (C-19), LAMP2 (H4B4), IκBα (C-21; 6A920), p65 (C-20), MALT1 (H-300; B-12), and ubiquitin (P4D1) were from Santa Cruz. Antibodies against phosphorylated IκBα (5A5) and CARMA1 (Cell Signaling Technologies), IKKβ and calreticulin (BD), VDAC (Calbiochem) and GAPDH (Sigma) were also used. Anti-K48- (Apu2) and anti-K63-ubiquitin (HWA4C4) were from Millipore. Secondary antibodies were HRP-conjugated (Southern Biotechnology). Immobilon (Millipore) chemiluminescent substrates were used for illumination of immunoblots.

Luciferase assays

For luciferase reporter assays, firefly luciferase constructs downstream of promoters for NFκB or driven by NFκB-binding sequences were co-transfected with *Renilla* luciferase pRL-TK (Int^-) expression plasmids (Promega). Lysates were analysed using the Dual-Luciferase Kit (Promega), with firefly fluorescence units normalised to *Renilla* luciferase fluorescence units.

siRNA and transfections

siRNA (Invitrogen) targeted *BCL10*, 5′-GCCACGAACAACCUCUCCAGAUCAA-3′ or 5′-UCAGAUGAGAGUAAUUUCUCUGAAA-3′; *IκBα.1*, 5′-ACGAGGAGUACGAGCAGAUGGUCAA-3′; *IκBα.3*, 5′-GACACAGAGUCAGAGUUCACGGAGU-3′; and *CARMA1*, 5′- UGUCCCGUUGGACACAUGCACCAAA-3′.

Confocal microscopy

Stimulated cells were resuspended in 1% PBS-FBS to adhere onto poly-L-lysine-coated slides for 10 minutes, fixed in 4% PBS-formaldehyde for 15 minutes and were permeabilised in 0.05% Triton X-100 in PBS for 5 minutes. Rabbit polyclonal or mouse monoclonal anti-MALT1 were used (Santa Cruz). All samples were analysed using a Leica SP2 confocal microscope (Imagery core facility, Institut Cochin, France).

This work was supported by grants from ANR, Fondation de France, Association pour la Recherche contre le Cancer, and Ligue Nationale contre le Cancer (Equipe Labellisée et comité Val de Marne). J.G. is supported by a Marie Curie International Reintegration Grant within the 7th European Community Framework Program (PIRG04-GA-2008-239126) and by Ligue Nationale contre le Cancer comité Paris. G.C. is supported by a postdoctoral fellowship from la Ligue contre le Cancer, and A.L.G. by a Ph.D. fellowship from University Paris Descartes. We thank D. Arnoult for helpful discussions.

Supplementary material available online at
http://jcs.biologists.org/cgi/content/full/123/14/2375/DC1

References

Bidere, N., Snow, A. L., Sakai, K., Zheng, L. and Lenardo, M. J. (2006). Caspase-8 regulation by direct interaction with TRAF6 in T cell receptor-induced NF-kappaB activation. *Curr. Biol.* **16**, 1666-1671.

Bidere, N., Ngo, V. N., Lee, J., Collins, C., Zheng, L., Wan, F., Davis, R. E., Lenz, G., Anderson, D. E., Arnoult, D. et al. (2009). Casein kinase 1alpha governs antigen-receptor-induced NF-kappaB activation and human lymphoma cell survival. *Nature* **458**, 92-96.

Blonska, M., Pappu, B. P., Matsumoto, R., Li, H., Su, B., Wang, D. and Lin, X. (2007). The CARMA1-Bcl10 signaling complex selectively regulates JNK2 kinase in the T cell receptor-signaling pathway. *Immunity* **26**, 55-66.

Che, T., You, Y., Wang, D., Tanner, M. J., Dixit, V. M. and Lin, X. (2004). MALT1/paracaspase is a signaling component downstream of CARMA1 and mediates T cell receptor-induced NF-kappaB activation. *J. Biol. Chem.* **279**, 15870-15876.

Chen, C. L., Singh, N., Yull, F. E., Strayhorn, D., Van Kaer, L. and Kerr, L. D. (2000). Lymphocytes lacking I kappa B-alpha develop normally, but have selective defects in proliferation and function. *J. Immunol.* **165**, 5418-5427.

Compagno, M., Lim, W. K., Grunn, A., Nandula, S. V., Brahmachary, M., Shen, Q., Bertoni, F., Ponzoni, M., Scandurra, M., Califano, A. et al. (2009). Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. *Nature* **459**, 717-721.

Duwel, M., Welteke, V., Oeckinghaus, A., Baens, M., Kloo, B., Ferch, U., Darnay, B. G., Ruland, J., Marynen, P. and Krappmann, D. (2009). A20 negatively regulates T cell receptor signaling to NF-kappaB by cleaving Malt1 ubiquitin chains. *J. Immunol.* **182**, 7718-7728.

Egawa, T., Albrecht, B., Favier, B., Sunshine, M. J., Mirchandani, K., O'Brien, W., Thome, M. and Littman, D. R. (2003). Requirement for CARMA1 in antigen receptor-induced NF-kappa B activation and lymphocyte proliferation. *Curr. Biol.* **13**, 1252-1258.

Ferch, U., Kloo, B., Gewies, A., Pfander, V., Duwel, M., Peschel, C., Krappmann, D. and Ruland, J. (2009). Inhibition of MALT1 protease activity is selectively toxic for activated B cell-like diffuse large B cell lymphoma cells. *J. Exp. Med.* **206**, 2313-2320.

Gaide, O., Favier, B., Legler, D. F., Bonnet, D., Brissoni, B., Valitutti, S., Bron, C., Tschopp, J. and Thome, M. (2002). CARMA1 is a critical lipid raft-associated regulator of TCR-induced NF-kappa B activation. *Nat. Immunol.* **3**, 836-843.

Ghosh, S. and Hayden, M. S. (2008). New regulators of NF-kappaB in inflammation. *Nat. Rev Immunol*. **8**, 837-848.

Hailfinger, S., Lenz, G., Ngo, V., Posvitz-Fejfar, A., Rebeaud, F., Guzzardi, M., Penas, E. M., Dierlamm, J., Chan, W. C., Staudt, L. M. et al. (2009). Essential role of MALT1 protease activity in activated B cell-like diffuse large B-cell lymphoma. *Proc. Natl. Acad. Sci. USA* **106**, 19946-19951.

Lenz, G., Davis, R. E., Ngo, V. N., Lam, L., George, T. C., Wright, G. W., Dave, S. S., Zhao, H., Xu, W., Rosenwald, A. et al. (2008). Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. *Science* **319**, 1676-1679.

Lobry, C., Lopez, T., Israel, A. and Weil, R. (2007). Negative feedback loop in T cell activation through IkappaB kinase-induced phosphorylation and degradation of Bcl10. *Proc. Natl. Acad. Sci. USA* **104**, 908-913.

Matsumoto, R., Wang, D., Blonska, M., Li, H., Kobayashi, M., Pappu, B., Chen, Y., Wang, D. and Lin, X. (2005). Phosphorylation of CARMA1 plays a critical role in T Cell receptor-mediated NF-kappaB activation. *Immunity* **23**, 575-585.

Matsuzawa, A., Tseng, P. H., Vallabhapurapu, S., Luo, J. L., Zhang, W., Wang, H., Vignali, D. A., Gallagher, E. and Karin, M. (2008). Essential cytoplasmic translocation of a cytokine receptor-assembled signaling complex. *Science* **321**, 663-668.

Moreno-Garcia, M. E., Sommer, K., Haftmann, C., Sontheimer, C., Andrews, S. F. and Rawlings, D. J. (2009). Serine 649 phosphorylation within the protein kinase C-regulated domain down-regulates CARMA1 activity in lymphocytes. *J. Immunol*. **183**, 7362-7370.

Ngo, V. N., Davis, R. E., Lamy, L., Yu, X., Zhao, H., Lenz, G., Lam, L. T., Dave, S., Yang, L., Powell, J. et al. (2006). A loss-of-function RNA interference screen for molecular targets in cancer. *Nature* **441**, 106-110.

Oeckinghaus, A., Wegener, E., Welteke, V., Ferch, U., Arslan, S. C., Ruland, J., Scheidereit, C. and Krappmann, D. (2007). Malt1 ubiquitination triggers NF-kappaB signaling upon T-cell activation. *EMBO J.* **26**, 4634-4645.

Rawlings, D. J., Sommer, K. and Moreno-Garcia, M. E. (2006). The CARMA1 signalosome links the signalling machinery of adaptive and innate immunity in lymphocytes. *Nat. Rev. Immunol.* **6**, 799-812.

Rebeaud, F., Hailfinger, S., Posevitz-Fejfar, A., Tapernoux, M., Moser, R., Rueda, D., Gaide, O., Guzzardi, M., Iancu, E. M., Rufer, N. et al. (2008). The proteolytic activity of the paracaspase MALT1 is key in T cell activation. *Nat. Immunol.* **9**, 272-281.

Rossman, J. S., Stoicheva, N. G., Langel, F. D., Patterson, G. H., Lippincott-Schwartz, J. and Schaefer, B. C. (2006). POLKADOTS are foci of functional interactions in T-Cell receptor-mediated signaling to NF-kappaB. *Mol. Biol. Cell* **17**, 2166-2176.

Ruland, J., Duncan, G. S., Elia, A., del Barco Barrantes, I., Nguyen, L., Plyte, S., Millar, D. G., Bouchard, D., Wakeham, A., Ohashi, P. S. et al. (2001). Bcl10 is a positive regulator of antigen receptor-induced activation of NF-kappaB and neural tube closure. *Cell* **104**, 33-42.

Ruland, J., Duncan, G. S., Wakeham, A. and Mak, T. W. (2003). Differential requirement for Malt1 in T and B cell antigen receptor signaling. *Immunity* **19**, 749-758.

Scharschmidt, E., Wegener, E., Heissmeyer, V., Rao, A. and Krappmann, D. (2004). Degradation of Bcl10 induced by T-cell activation negatively regulates NF-kappa B signaling. *Mol. Cell. Biol.* **24**, 3860-3873.

Sebald, A., Mattioli, I. and Schmitz, M. L. (2005). T cell receptor-induced lipid raft recruitment of the I kappa B kinase complex is necessary and sufficient for NF-kappa B activation occurring in the cytosol. *Eur J. Immunol.* **35**, 318-325.

Sommer, K., Guo, B., Pomerantz, J. L., Bandaranayake, A. D., Moreno-Garcia, M. E., Ovechkina, Y. L. and Rawlings, D. J. (2005). Phosphorylation of the CARMA1 linker controls NF-kappaB activation. *Immunity* **23**, 561-574.

Sun, L., Deng, L., Ea, C. K., Xia, Z. P. and Chen, Z. J. (2004). The TRAF6 ubiquitin ligase and TAK1 kinase mediate IKK activation by BCL10 and MALT1 in T lymphocytes. *Mol. Cell* **14**, 289-301.

Tergaonkar, V., Correa, R. G., Ikawa, M. and Verma, I. M. (2005). Distinct roles of IkappaB proteins in regulating constitutive NF-kappaB activity. *Nat. Cell Biol.* **7**, 921-923.

Wan, F. and Lenardo, M. J. (2010). The nuclear signaling of NF-kappaB: current knowledge, new insights, and future perspectives. *Cell Res.* **20**, 24-33.

Wang, D., You, Y., Case, S. M., McAllister-Lucas, L. M., Wang, L., DiStefano, P. S., Nunez, G., Bertin, J. and Lin, X. (2002). A requirement for CARMA1 in TCR-induced NF-kappa B activation. *Nat. Immunol.* **3**, 830-835.

Wang, D., Matsumoto, R., You, Y., Che, T., Lin, X. Y., Gaffen, S. L. and Lin, X. (2004). CD3/CD28 costimulation-induced NF-kappaB activation is mediated by recruitment of protein kinase C-theta, Bcl10, and IkappaB kinase beta to the immunological synapse through CARMA1. *Mol. Cell. Biol.* **24**, 164-171.

Wegener, E., Oeckinghaus, A., Papadopoulou, N., Lavitas, L., Schmidt-Supprian, M., Ferch, U., Mak, T. W., Ruland, J., Heissmeyer, V. and Krappmann, D. (2006). Essential role for IkappaB kinase beta in remodeling Carma1-Bcl10-Malt1 complexes upon T cell activation. *Mol. Cell* **23**, 13-23.

Welteke, V., Eitelhuber, A., Duwel, M., Schweitzer, K., Naumann, M. and Krappmann, D. (2009). COP9 signalosome controls the Carma1-Bcl10-Malt1 complex upon T-cell stimulation. *EMBO Rep.* **10**, 642-648.

Wu, C. J. and Ashwell, J. D. (2008). NEMO recognition of ubiquitinated Bcl10 is required for T cell receptor-mediated NF-kappaB activation. *Proc. Natl. Acad. Sci. USA* **105**, 3023-3028.

Zeng, H., Di, L., Fu, G., Chen, Y., Gao, X., Xu, L., Lin, X. and Wen, R. (2007). Phosphorylation of Bcl10 negatively regulates T-cell receptor-mediated NF-kappaB activation. *Mol. Cell. Biol.* **27**, 5235-5245.