

Multifunctional roles for MALT1 in T-cell activation

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Abstract | The activation of T cells is vital to the successful elimination of pathogens, but can also have a deleterious role in autoimmunity and transplant rejection. Various signalling pathways are triggered by the T-cell receptor; these have key roles in the control of the T-cell response and represent interesting targets for therapeutic immunomodulation. Recent findings define MALT1 (mucosa-associated-lymphoid-tissue lymphoma-translocation gene 1) as a protein with proteolytic activity that controls T-cell activation by regulating key molecules in T-cell-receptor-induced signalling pathways.

MALT1 (mucosa-associated-lymphoid-tissue (MALT) lymphoma-translocation gene 1) has recently emerged as an important point of convergence of various signalling pathways in both innate and adaptive immune cells. MALT1 has an essential role in the activation of nuclear factor- κ B (NF- κ B) by antigen receptors and in the control of lymphocyte activation and proliferation, but also in NF- κ B regulation downstream of other receptors with immunoreceptor tyrosine-based activation motifs (ITAMs) and some G-protein coupled receptors (GPCRs) expressed by both immune and non-immune cells^{1,2,3}. Recent studies have shed new light on the molecular function of MALT1 by identifying its arginine-directed proteolytic activity. Here, I briefly review the biological functions of MALT1, and highlight recent advances in the understanding of its molecular functions.

The biological functions of MALT1

Lymphocyte activation and B-cell lymphoma. The engagement of antigen receptors triggers the activation of multiple signalling pathways that ultimately regulate changes in gene transcription and in cell shape and adhesion — processes that coordinately control the activation of lymphocytes during the adaptive immune response. Initial evidence of a role for MALT1 in the adaptive immune response was provided by the generation of MALT1-deficient mice,

which have defects in T-cell activation by antigen receptors^{1,2}. These defects specifically affect activation of the transcription factor NF- κ B and possibly of the JUN N-terminal kinase (JNK) pathway, but not extracellular-signal-regulated kinase (ERK) activation or Ca²⁺ release^{1,2}.

The T-cell activation defect of MALT1-deficient mice closely resembles the phenotype of B-cell lymphoma 10 (BCL-10)-deficient mice⁴, which is consistent with a previously reported physical and functional interaction between these two proteins (FIG. 1a)^{5,6}. Both MALT1- and BCL-10-deficient mice also have defects in the development of B-1 cells and marginal-zone B cells, suggesting an additional role for MALT1 and BCL-10 in signals that are specifically required for the generation of these B-cell subtypes. However, conflicting findings have been reported concerning the role of MALT1 in the activation of conventional B-2 cells. In one knockout model, MALT1 was required², whereas in another model, it was largely dispensable for B-cell receptor (BCR)-induced activation of IKK (inhibitor of NF- κ B kinase) and proliferation¹. Recently, it was proposed that in B cells, MALT1 selectively regulates activation of the NF- κ B subunit c-REL to control B-cell survival⁷.

In addition to having a role in lymphocyte activation, MALT1 and BCL-10 are important targets of genetic alterations that lead to the formation of B-cell lymphomas.

Indeed, the *MALT1* gene was originally identified from a chromosomal translocation, t(11;18)(q21;q21), found in B-cell lymphomas of MALT^{8–10}. As a result of this translocation, the *MALT1* gene is fused to the gene encoding cellular apoptosis inhibitor-2 (cIAP2). The resulting transcript variants all encode in-frame cIAP2–MALT1 fusion proteins that contain the three N-terminal baculovirus IAP repeats (BIR domains) of cIAP2 and the C-terminal portion of MALT1 including the caspase-like domain, and that constitutively activate NF- κ B^{11,12}. Additional chromosomal translocations of both BCL-10 and MALT1 have been shown to be involved in the development of MALT lymphomas, which are derived from chronically stimulated, extranodal marginal-zone B cells in the MALT¹¹. Moreover, an NF- κ B-dependent subtype of diffuse, large B-cell lymphoma (DLBCL), known as activated B-cell (ABC)-type DLBCL, has been shown to be clearly dependent on BCL-10 and MALT1 for proliferation¹³.

Together, these data support the idea that MALT1 cooperates with BCL-10 in the activation and proliferation of T and B cells, and that a dysregulation of MALT1- and BCL-10-dependent pathways contributes to the formation of particular subsets of B-cell lymphomas^{11,12,14}.

Role in innate immune and non-immune cells. New findings have revealed that the biological role of MALT1 extends to a function in NF- κ B regulation that is downstream of ITAM-containing receptors expressed by natural killer (NK), myeloid and mast cells, and of various GPCRs expressed by immune and non-immune cells^{3,15,16,17,18,19} (FIG. 1b–d). Although the exact molecular mechanisms that govern these pathways are not yet known, it is likely that MALT1 is recruited and activated through a common mechanism that involves BCL-10 and a protein containing a caspase-recruitment domain (CARD) and a coiled-coil domain, such as *CARMA1* (also known as CARD11), *CARMA3* (also known as CARD10) or *CARD9*. These molecules are thought to function as specific scaffold proteins that recruit multiple signal transducers on stimulation of individual subtypes of

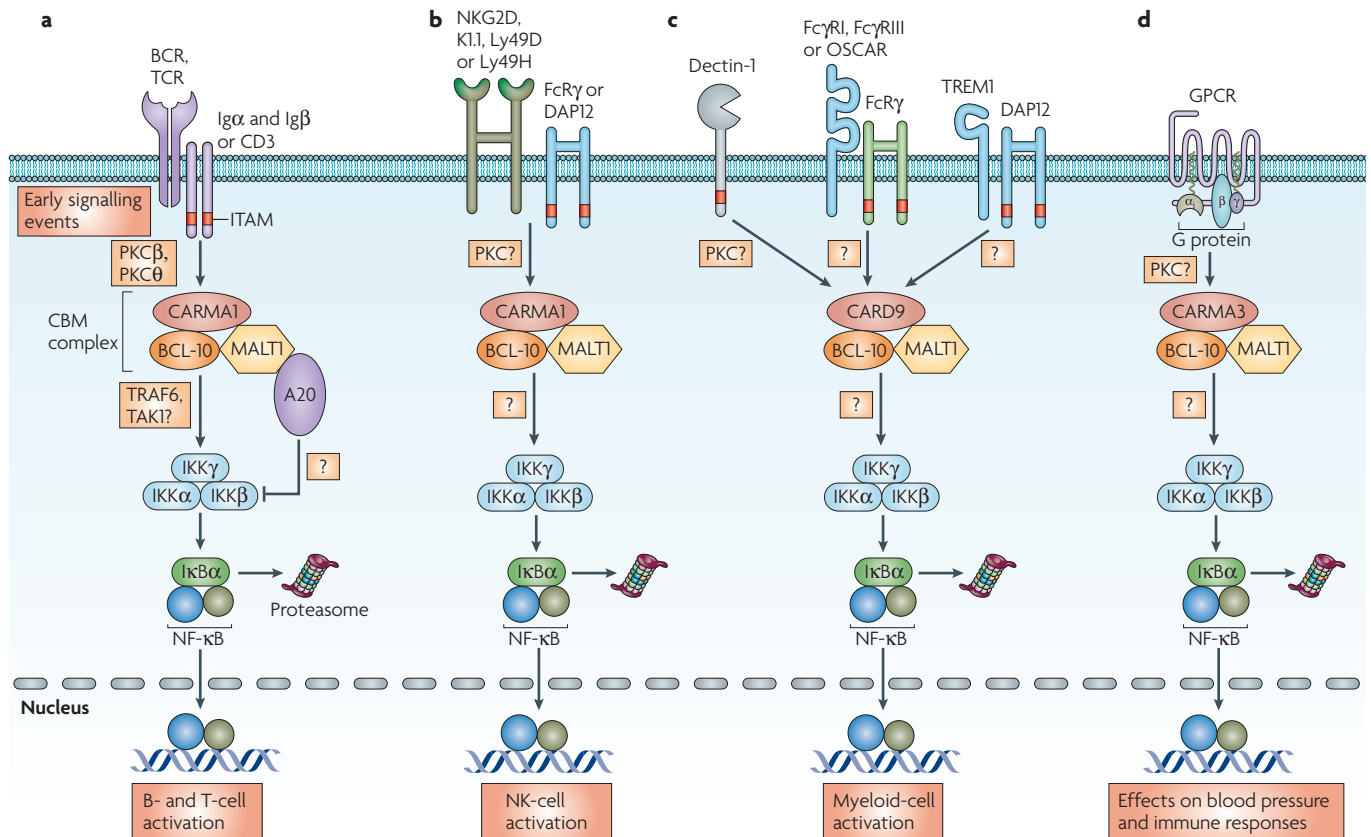


Figure 1 | Roles of MALT1 in receptor-induced NF- κ B activation. B-cell lymphoma 10 (BCL-10) and mucosa-associated-lymphoid-tissue lymphoma-translocation gene 1 (MALT1) act together in the activation of nuclear factor- κ B (NF- κ B) downstream of receptors containing immunoreceptor tyrosine-based activation motifs (ITAMs) and of G-protein coupled receptors (GPCRs) that use a $G\alpha_{12/13}$, $G\alpha_q$ or $G\alpha_i$ subunit. NF- κ B activation downstream of the B- and T-cell receptor (BCR and TCR, respectively) and by natural killer (NK)-cell receptors such as NKG2D (natural-killer group 2, member D), K1.1, Ly49D and Ly49H mediate signals through CARMA1 (caspase-recruitment domain (CARD)-containing membrane-associated guanylate kinase protein 1) (a,b), whereas ITAM-containing receptors expressed by myeloid cells, such as dectin-1, Fc γ R (Fc receptors for IgG), OSCAR (osteoclast associated, immunoglobulin-like receptor) and TREM1 (triggering receptor expressed by myeloid cells 1) mediate signals through CARD9 (c), and GPCRs signal through CARMA3 (d) for the activation of the BCL-10–MALT1 module. It is thought that a complex comprising a protein containing a CARD and a coiled-coil domain, BCL-10 and MALT1 (referred

to here as the CBM complex) mediates the activation of the inhibitor of NF- κ B (I κ B) kinase complex (IKK) by ubiquitylation and phosphorylation events that depend on TRAF6 (tumour-necrosis-factor-receptor-associated factor 6) and TAK1 (transforming-growth-factor- β -activated kinase 1), respectively, but it remains uncertain whether this is the case for all receptors signalling through BCL-10 and MALT1. IKK-mediated phosphorylation of I κ B α targets the inhibitor for proteasomal degradation and allows nuclear translocation of NF- κ B. Antigen-receptor triggering also leads to the activation of the proteolytic activity of MALT1, which is thought to contribute to NF- κ B activation through the proteolytic degradation of the NF- κ B inhibitor A20. Whether the proteolytic activity of MALT1 also contributes to NF- κ B activation downstream of other ITAM-containing receptors or of GPCRs is unknown. MALT1-dependent signalling by ITAM-containing receptors leads to leukocyte activation, whereas activation of MALT1-dependent GPCRs might control various biological responses, including angiotensin-mediated regulation of blood pressure and immune responses. Fc γ R, Fc receptor γ -chain; PKC, protein kinase C.

NF- κ B-activating receptors. CARMA1 is specifically required for lymphocyte activation by antigen receptors (FIG. 1a) and for NK-cell activation by receptors such as NKG2D (natural-killer group 2, member D), K1.1, Ly49H and Ly49D, which associate with the ITAM-containing signalling chains Fc γ R (Fc receptor γ -chain) or DAP12 (REFS 15,16) (FIG. 1b). CARD9, conversely, transduces signals from monocyte-activating receptors such as dectin-1, which recognizes β -glucan, a component of the yeast cell wall^{17,18}, and from Fc γ R (Fc receptors for IgG) and other Fc γ - or DAP12-associated receptors

such as OSCAR (osteoclast-associated, immunoglobulin-like receptor) and TREM1 (triggering receptor expressed by myeloid cells 1)¹⁷ (FIG. 1c). Finally, CARMA3 couples to GPCRs such as the receptors for angiotensin II or lysophosphatidic acid³ that are expressed by immune and non-immune cells (FIG. 1d). Whether a CARMA homologue also controls Fc ϵ R-mediated, BCL-10- and MALT1-dependent NF- κ B activation in mast cells¹⁹ still remains to be determined.

Some studies have also suggested a potential role for CARD9, BCL-10 and MALT1 in regulating Toll-like receptor (TLR)-mediated innate immune responses.

A role for BCL-10 in lipopolysaccharide (LPS)-mediated NF- κ B activation was suggested based on a biochemical study that showed a physical association between BCL-10 and the LPS receptor TLR4 (REF. 20). Moreover, genetic studies from some groups^{2,18,21}, but not others^{1,4,17}, have suggested a role for CARD9, BCL-10 and MALT1 downstream of other TLRs and the intracellular pathogen sensor nucleotide-binding oligomerization domain protein 2 (NOD2). Therefore, it remains highly controversial whether MALT1, together with BCL-10 and CARD9, has a relevant role in TLR-induced innate immune responses.

Molecular mechanisms of MALT1

Over the past few years we have witnessed impressive progress in our understanding of how MALT1 activates the NF- κ B pathway. An important step was the identification of BCL-10 as a binding partner for MALT1, and the demonstration that MALT1 synergized with BCL-10 to activate NF- κ B^{6,22}. Most additional insights into the underlying molecular mechanisms come from the study of T-cell receptor (TCR)-induced NF- κ B activation, which depends on the incorporation of MALT1 into an oligomeric CARMA1–BCL-10–MALT1 complex (which I will refer to as the CBM complex in all subsequent parts of the text) (FIG. 1a)²³. The CBM complex is thought to relay the activity of TCR-proximal kinases to the activation of the IKK complex. In this so-called canonical pathway of NF- κ B activation, the IKK complex mediates phosphorylation of inhibitor of NF- κ B (I κ B) which results in the transient I κ B degradation that allows nuclear translocation of NF- κ B²⁴. Target genes of NF- κ B in T cells include genes that control essential aspects of T-cell proliferation, differentiation and survival. Recent studies suggest that MALT1 acts both as a scaffold protein and a proteolytically active enzyme in the CBM-dependent signalling pathways that lead to T-cell activation.

MALT1 as a scaffold protein. MALT1 contains an N-terminal death domain followed by two immunoglobulin-like domains, a caspase-like domain and a third, immunoglobulin-like domain located at the C-terminal^{5,25} (FIG. 2). Several binding partners for MALT1 have been identified, which associate with it in either an inducible or constitutive manner. BCL-10 (the best-characterized interaction partner of MALT1) binds directly and constitutively to the region of MALT1 that comprises the two first immunoglobulin-like domains⁵. It is thought that, following antigen-receptor triggering, MALT1 binds its upstream regulator CARMA1 through BCL-10 to form the oligomeric CBM complex²³. It is possible that a direct interaction between MALT1 and the coiled-coil domain of CARMA1 (demonstrated using a yeast two-hybrid approach²⁶) also contributes to the formation or stabilization of the CBM complex. One way by which MALT1 links the CBM complex to IKK activation is by the recruitment of TRAF6 (tumour-necrosis factor (TNF) receptor-associated factor 6)^{27,28}, a ring-finger-containing ubiquitin ligase that has a central role in the IKK-dependent canonical NF- κ B pathway downstream of various types of NF- κ B-inducing stimuli.

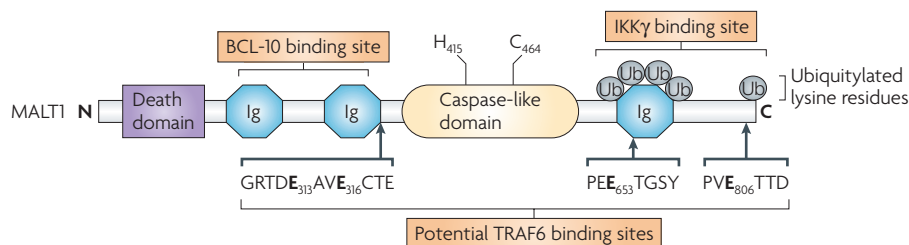


Figure 2 | Structural and functional elements of MALT1. The mucosa-associated-lymphoid-tissue lymphoma-translocation gene 1 (MALT1) protein contains an N-terminal death domain, followed by two immunoglobulin-like domains (Ig), a caspase-like domain and a C-terminal region that contains another immunoglobulin-like domain. MALT1-dependent nuclear factor- κ B (NF- κ B) activation requires an interaction between MALT1 and B-cell lymphoma 10 (BCL-10) through the two first immunoglobulin-like domains of MALT1. The MALT1–BCL-10 interaction is constitutive but increases following antigen-receptor triggering. The active site residues His415 and Cys464 are crucial for the proteolytic activity of MALT1. MALT1 contains three potential binding sites for TRAF6 (tumour-necrosis-factor-receptor-associated factor 6), which may regulate in coordination the recruitment and activation of TRAF6. TRAF6 then ubiquitylates itself and MALT1 on multiple C-terminal lysine residues that can in turn recruit the inhibitor of NF- κ B kinase (IKK) complex through the ubiquitin-binding IKK γ subunit (also known as NEMO). The first TRAF6 binding site is located within amino acids 309–319. This stretch of amino acids is encoded by exon 7 that is absent in some MALT1 isoforms, leading to differences in the amino-acid numbering and the total length of MALT1 constructs reported in different studies^{5,6,25,27,28,35,36,43}. Amino-acid numbering in the figure refers to human MALT1. Ub, ubiquitin.

It was initially proposed that the interaction between TRAF6 and MALT1 was dependent on the binding of TRAF6 to two putative consensus sequences (Pro-X-Glu-X-X-aromatic/acidic; where X denotes any amino acid) in the C-terminal region of MALT1 that is adjacent to the caspase-like domain²⁷. However, a third binding site within the second immunoglobulin-like domain of MALT1 has recently been identified in cIAP2–MALT1 fusion proteins²⁸ (FIG. 2). In this recent study, mutation of either the binding site closest to the C-terminal or the binding site within the second immunoglobulin-like domain impaired NF- κ B activation, whereas mutation or deletion of all three putative TRAF6 binding sites completely abolished the capacity of a MALT lymphoma-derived cIAP2–MALT1 fusion protein to activate NF- κ B²⁸. Interestingly, it has been suggested that TRAF2, similar to TRAF6, can also bind to MALT1 (REF. 27) to contribute to NF- κ B activation induced by cIAP2–MALT1 constructs⁵ or by TCR stimulation²⁷. However, the absence of an interaction of TRAF2 with MALT1 in activated Jurkat cells²⁸ suggests that the interaction might be of low stoichiometry or that it occurs indirectly, possibly through BCL-10 (REF. 29).

In conclusion, an important function of MALT1 is to act as a scaffold protein that physically bridges activated CARMA1 with BCL-10 and TRAF6 to activate the NF- κ B-regulating IKK complex.

Orchestration of ubiquitylation events. The recruitment of the ubiquitin ligase TRAF6 by MALT1 points towards an important role for ubiquitylation events in MALT1-dependent IKK activation (FIG. 3a–c). Based on the reconstitution of the BCL-10-dependent IKK activation pathway using recombinant, purified proteins in combination with HeLa cell extracts, TRAF6 and the serine/threonine kinase TAK1 (transforming-growth-factor- β -activated kinase 1) have been identified as proteins that mediate IKK activation by BCL-10 and MALT1 (REF. 27) (FIG. 3a). It was proposed that BCL-10 and MALT1 form oligomers that bind to and induce oligomerization and activation of TRAF6, which might in turn activate the IKK complex through ubiquitylation of its catalytically inactive subunit IKK γ (also known as NEMO)²⁷. Moreover, this study suggested that MALT1 also activates the IKK complex by promoting TRAF6 self-ubiquitylation. Ubiquitylated TRAF6 might in turn recruit TAB2 (TAK-binding protein 2) and TAK1, which is thought to activate the IKK complex by phosphorylation of the catalytically active IKK α and IKK β subunits in T cells²⁷.

Interestingly, MALT1 itself has been recently identified as a target for ubiquitylation^{25,30}. Although one study has attributed this to an intrinsic ubiquitin ligase activity of MALT1 (REF. 25), a more recent study has identified TRAF6 as the ubiquitin ligase that acts on MALT1 (REF. 30). The resulting C-terminal ubiquitylation of

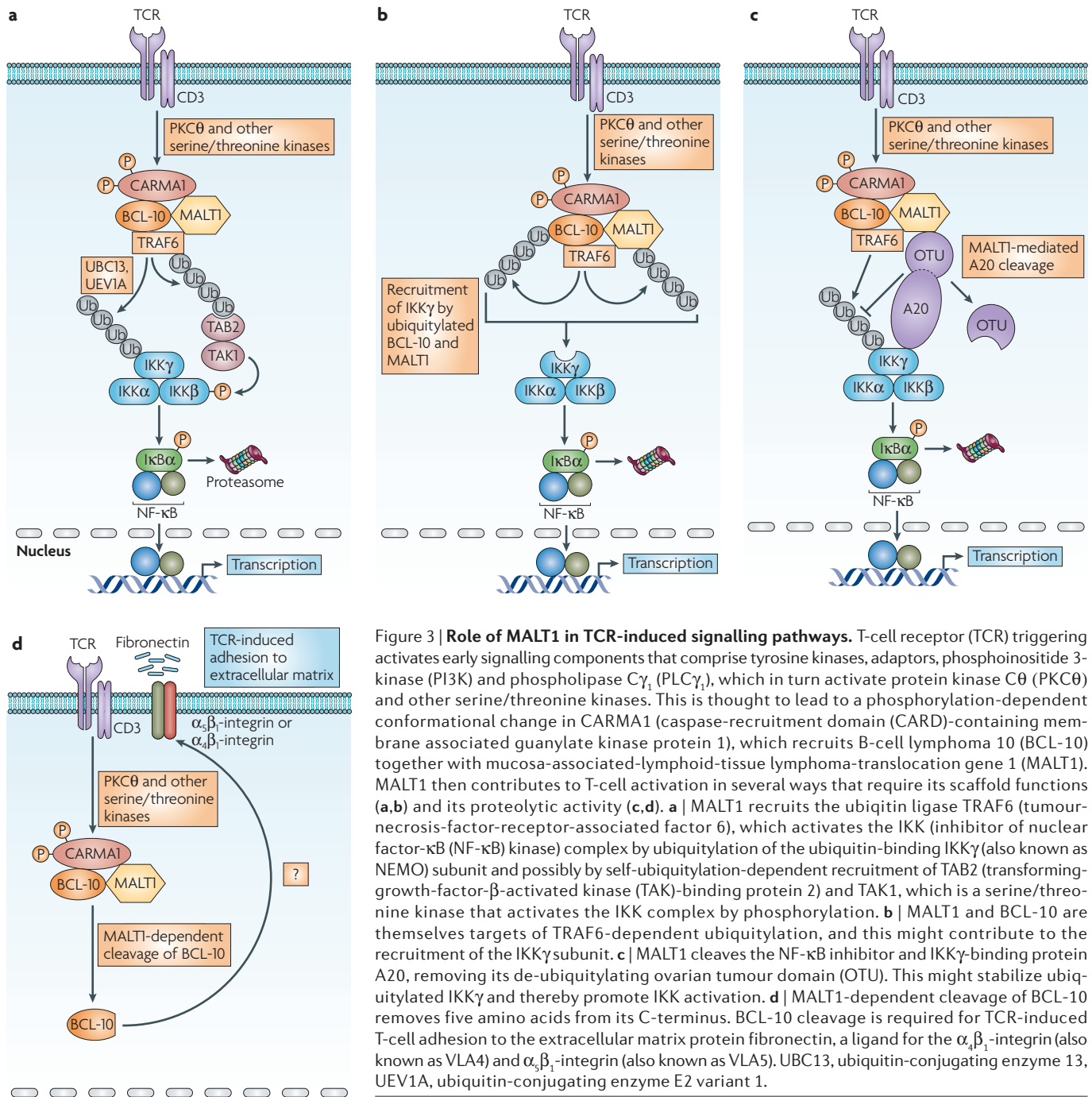


Figure 3 | Role of MALT1 in TCR-induced signalling pathways. T-cell receptor (TCR) triggering activates early signalling components that comprise tyrosine kinases, adaptors, phosphoinositide 3-kinase (PI3K) and phospholipase γ_1 (PLC γ_1), which in turn activate protein kinase C θ (PKC θ) and other serine/threonine kinases. This is thought to lead to a phosphorylation-dependent conformational change in CARMA1 (caspase-recruitment domain (CARD)-containing membrane associated guanylate kinase protein 1), which recruits B-cell lymphoma 10 (BCL-10) together with mucosa-associated-lymphoid-tissue lymphoma-translocation gene 1 (MALT1). MALT1 then contributes to T-cell activation in several ways that require its scaffold functions (**a,b**) and its proteolytic activity (**c,d**). **a** | MALT1 recruits the ubiquitin ligase TRAF6 (tumour-necrosis-factor-receptor-associated factor 6), which activates the IKK (inhibitor of nuclear factor- κ B (NF- κ B) kinase) complex by ubiquitylation of the ubiquitin-binding IKK γ (also known as NEMO) subunit and possibly by self-ubiquitylation-dependent recruitment of TAB2 (transforming-growth-factor- β -activated kinase (TAK)-binding protein 2) and TAK1, which is a serine/threonine kinase that activates the IKK complex by phosphorylation. **b** | MALT1 and BCL-10 are themselves targets of TRAF6-dependent ubiquitylation, and this might contribute to the recruitment of the IKK γ subunit. **c** | MALT1 cleaves the NF- κ B inhibitor and IKK γ -binding protein A20, removing its de-ubiquitylating ovarian tumour domain (OTU). This might stabilize ubiquitylated IKK γ and thereby promote IKK activation. **d** | MALT1-dependent cleavage of BCL-10 removes five amino acids from its C-terminus. BCL-10 cleavage is required for TCR-induced T-cell adhesion to the extracellular matrix protein fibronectin, a ligand for the $\alpha_5\beta_1$ -integrin (also known as VLA4) and $\alpha_4\beta_1$ -integrin (also known as VLA5). UBC13, ubiquitin-conjugating enzyme 13, UEV1A, ubiquitin-conjugating enzyme E2 variant 1.

MALT1 might provide a docking site for IKK γ and thereby mediate the recruitment of the IKK complex (FIGS 2,3b). Finally, an interesting new twist comes from a recent study that identified a requirement for MALT1 in the ubiquitylation of BCL-10 on lysine residues 31 and 63 as a mechanism to physically recruit IKK γ through its recently identified ubiquitin-sensing domain³¹. Therefore, in T cells, ubiquitylation of both MALT1 and BCL-10 may contribute to IKK recruitment (FIG. 3b).

In conclusion, the current data suggest that MALT1 acts as a scaffold protein in TCR-induced NF- κ B activation by binding to CARMA1, BCL-10 and TRAF6 and by promoting the ubiquitylation-dependent recruitment and activation of the IKK complex (FIG. 3a,b). However, questions remain about the extent to which CARMA1, BCL-10 and MALT1 contribute to the physical recruitment of the IKK complex and TAK1 and/or to the catalytic activation of these serine/threonine kinases^{30–33}

and contradictory views remain about the exact role of MALT1 in B-cell activation^{1,2,7}. Clearly, further work is required to establish the detailed function(s) of the described ubiquitylation events in T cells, and to assess their potential relevance for B-cell activation.

Protease activity and NF- κ B activation. In the first study that addressed its function, MALT1 was identified by a bioinformatic search for new caspase homologues⁵, and was named a paracaspase to account for its

homology to mammalian caspases and to an additional family of caspase-like proteins known as metacaspases⁵. The initial idea that MALT1 had caspase-like proteolytic activity was supported by the presence of two conserved residues predicted to be important for catalytic activity in various caspases and MALT1 proteins from multiple species; these correspond to Cys464 and His415 in the caspase-like domain of human MALT1 (REF. 5) (FIG. 2). Indeed, MALT1 constructs mutated in the predicted active site Cys464 showed a significant reduction in their capacity to activate NF- κ B in transfected human embryonic kidney 293 cells^{5,6}. However, initial attempts to demonstrate either a caspase-like or distinct proteolytic activity of MALT1 were not successful^{5,34}, possibly because of sub-optimal buffers, sequences of selected target substrates or experimental conditions used to promote MALT1 oligomerization. Optimal conditions for inducing MALT1 oligomerization were indeed crucial for the formal demonstration of an arginine-directed proteolytic activity of MALT1 in two recent studies^{35,36}. These studies were supported by the fact that metacaspases, which have variable biological functions in yeast, plants and parasites, have recently been recognized as cysteine-dependent, arginine- or lysine-specific proteases. In addition, MALT1 shares with metacaspases the presence of amino-acid residues that are responsible for arginine or lysine specificity³⁷.

But how does the proteolytic activity of MALT1 affect T-cell activation? Rebeaud *et al.* used a cell-permeable MALT1 inhibitor to show that the proteolytic activity of MALT1 is required for optimal NF- κ B (but not ERK or JNK) activation in T cells³⁶. Insight into the identity of the MALT1 substrate underlying this effect came from a study by Coornaert *et al.*, which revealed that MALT1 cleaves A20 (also known as TNFAIP3)³⁵, a protein previously identified as an inhibitor of TNF receptor and TLR-induced NF- κ B activation³⁸. In contrast to other cell types, lymphoid cells constitutively express the NF- κ B inhibitor A20 (REF. 35). Antigen-receptor-induced activation of T and B cells leads to the cleavage of a small percentage of A20 molecules, most likely those that physically associate with MALT1 following lymphocyte activation³⁵. A20 has an N-terminal ovarian tumour (OTU) domain with de-ubiquitylation activity, which is thought to remove activating, Lys63-mediated polyubiquitin chains from the serine/threonine kinase receptor-interacting protein 1 (RIP1), the ubiquitin ligase TRAF6 and the IKK γ subunit³⁸. As the C-terminal

Box1 | Outstanding questions

Several open questions remain regarding the exact mechanisms of action of mucosa-associated-lymphoid-tissue (MALT) lymphoma-translocation gene 1 (MALT1) in immune-cell activation. A fundamental question is whether the proteolytic activity of MALT1 is relevant for signalling pathways other than those induced by T-cell activation. It is likely that the protease activity of MALT1 has a role in B-cell activation, as B-cell lymphoma 10 (BCL-10) and A20 cleavage are also detected in activated B cells^{35,36}. Moreover, the protease activity of MALT1 might contribute to B-cell transformation, as cellular apoptosis inhibitor-2 (cIAP2)–MALT1 fusion proteins found in specific MALT lymphomas depend on the active site Cys464 residue for optimal nuclear factor- κ B (NF- κ B) activation⁵. However, it remains unclear if the proteolytic activity of MALT1 is also essential for signal transduction mediated by other receptors containing immunoreceptor tyrosine-based activation motif (ITAM) and by G-protein-coupled receptors (GPCRs).

Other issues to address include identifying exactly how cleavage of the MALT1 substrates A20 and BCL-10 interferes with their function, and investigating whether there are additional substrates for MALT1 proteolytic activity, which could reveal as-yet-unknown additional functions of MALT1.

Finally, several questions remain concerning the molecular regulation of MALT1. How does oligomerization control the enzymatic activation of MALT1? And how is MALT1, once activated, controlled? Rebeaud *et al.* showed that MALT1 activation in T cells is transient, peaking around 30 minutes after activation and returning to unstimulated levels after 60 minutes³⁶. This suggests that both the activation and inactivation of the paracaspase MALT1 are tightly regulated by mechanisms that merit further exploration.

region of A20 can bind to IKK γ (REF. 39), Coornaert *et al.* speculated that A20 cleavage might serve to remove the de-ubiquitylating OTU domain from A20 molecules associated with IKK γ , thereby preserving activating IKK γ ubiquitylation events (FIG. 3c). However, it remains possible that other ubiquitylated proteins, such as BCL-10, MALT1 itself or TRAF6, are targets of the inhibitory action of A20 in the TCR-induced NF- κ B pathway. Another open question is whether the cleavage of mouse A20, which occurs at a site much closer to the C-terminus and distinct from the cleavage site of human A20, has similar consequences on lymphocyte activation in mice. Although the detailed role of MALT1-dependent A20 cleavage will have to be worked out in future studies, the current data clearly support a regulatory role for the proteolytic activity of MALT1 in controlling the amplitude of the NF- κ B response.

BCL-10 cleavage controls T-cell adhesion

An unexpected function of MALT1 was revealed with the recent identification of BCL-10 as a MALT1 substrate. In a study by Rebeaud *et al.*, BCL-10 was shown to be cleaved close to its C-terminus following TCR ligation, without affecting its capacity to activate NF- κ B or JNK³⁶. Instead, MALT1-dependent BCL-10 cleavage was required for the optimal adhesion of activated T cells to the extracellular matrix protein fibronectin³⁶, which is bound by activated integrins of the β_1 -subtype. These findings suggest that, by cleaving five amino acids from the C-terminus of BCL-10, MALT1 positively regulates β_1 -integrin-dependent T-cell adhesion (FIG. 3d).

Integrin-dependent adhesion is thought to be physiologically important for the formation of contacts between antigen-presenting cells and T cells and for T-cell co-stimulation, but also for certain aspects of lymphocyte migration and extravasation⁴⁰. In this context, it is interesting to note that marginal-zone B cells, which are absent in both BCL-10- and MALT1-deficient mice, require integrin-mediated adhesion for their localization to the marginal zone⁴¹. Moreover, it is possible that integrin-dependent homing properties contribute to the tendency of gastric MALT lymphomas to disseminate to other parts of the gastrointestinal mucosa and the splenic marginal zone. But how exactly BCL-10 cleavage by MALT1 interferes with the molecular mechanisms of integrin activation and adhesion⁴⁰ remains unknown at this point. It is possible that cytoskeleton-dependent mechanisms are involved, as BCL-10 is also known to control TCR-induced actin polymerization and actin-dependent morphological changes of T cells required for T-cell spreading and immune synapse formation⁴². As BCL-10 phosphorylation, which is required for the actin-regulating function of BCL-10, precedes BCL-10 cleavage, it is tempting to speculate that the two events are linked, although further experiments are required to test this hypothesis.

Therapeutic implications

Collectively, the present data suggest that MALT1 controls T-cell activation through both its adaptor and protease functions. The discovery of the latter function has opened new perspectives for the understanding of the

mechanisms that control T- and B-cell activation. The proteolytic activity of MALT1 appears to fine-tune NF- κ B activation by the transient release of an A20-dependent 'brake' on the pathway³⁵, and to affect β_1 -integrin-mediated T-cell adhesion by the cleavage of BCL-10 (REF. 36). A peptide-based, cell-permeable MALT1 inhibitor has already been proved to effectively inhibit T-cell activation *in vitro* at micromolar concentrations³⁶. Although many questions remain (BOX 1), therapeutic targeting of the protease activity of MALT1 might become a useful approach for the treatment of autoimmune or inflammatory diseases, for the prevention of transplant rejection and the treatment of B-cell lymphomas such as MALT lymphomas and ABC-type DLBCLs associated with MALT1 dysfunction. The potential role for MALT1 in the signalling pathways induced by other immune receptors and GPCRs raises concerns about potential side effects of such therapies, but also offers additional possibilities for MALT1 inhibitors. In summary, the stage is set for the development of highly specific MALT1 inhibitors, which holds interesting new perspectives for research and therapy.

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- Ruland, J., Duncan, G. S., Wakeham, A. & Mak, T. W. Differential requirement for Malt1 in T and B cell antigen receptor signaling. *Immunity* **19**, 749–758 (2003).
- Rueffli-Brasse, A. A., French, D. M. & Dixit, V. M. Regulation of NF- κ B-dependent lymphocyte activation and development by paracaspase. *Science* **302**, 1581–1584 (2003).
- Wegener, E. & Krappmann, D. CARD–Bcl10–Malt1 signalosomes: missing link to NF- κ B. *Sci. STKE* **2007**, pe21 (2007).
- Ruland, J. *et al.* Bcl10 is a positive regulator of antigen receptor-induced activation of NF- κ B and neural tube closure. *Cell* **104**, 33–42 (2001).
- Uren, A. G. *et al.* Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. *Mol. Cell* **6**, 961–967 (2000).
- Lucas, P. C. *et al.* Bcl10 and MALT1, independent targets of chromosomal translocation in MALT lymphoma, cooperate in a novel NF- κ B signaling pathway. *J. Biol. Chem.* **276**, 19012–19019 (2001).
- Ferch, U. *et al.* MALT1 directs B cell receptor-induced canonical nuclear factor- κ B signaling selectively to the c-Rel subunit. *Nature Immunol.* **8**, 984–991 (2007).
- Akagi, T. *et al.* A novel gene, MALT1 at 18q21, is involved in t(11;18)(q21;q21) found in low-grade B-cell lymphoma of mucosa-associated lymphoid tissue. *Oncogene* **18**, 5785–5794 (1999).
- Dierlamm, J. *et al.* The apoptosis inhibitor gene API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21) associated with mucosa-associated lymphoid tissue lymphomas. *Blood* **93**, 3601–3609 (1999).
- Morgan, J. A. *et al.* Breakpoints of the t(11;18)(q21;q21) in mucosa-associated lymphoid tissue (MALT) lymphoma lie within or near the previously undescribed gene MALT1 on chromosome 18. *Cancer Res.* **59**, 6205–6213 (1999).
- Isaacson, P. G. & Du, M. Q. MALT lymphoma: from morphology to molecules. *Nature Rev. Cancer* **4**, 644–653 (2004).
- Lucas, P. C., McAllister-Lucas, L. M. & Nunez, G. NF- κ B signaling in lymphocytes: a new cast of characters. *J. Cell. Sci.* **117**, 31–39 (2004).
- Ngo, V. N. *et al.* A loss-of-function RNA interference screen for molecular targets in cancer. *Nature* **441**, 106–110 (2006).
- Jost, P., Peschel, C. & Ruland, J. The Bcl10/Malt1 signaling pathway as a drug target in lymphoma. *Curr. Drug Targets* **7**, 1335–1340 (2006).
- Thome, M. CARMA1, BCL-10 and MALT1 in lymphocyte development and activation. *Nature Rev. Immunol.* **4**, 348–359 (2004).
- Gross, O. *et al.* Multiple ITAM-coupled NK cell receptors engage the Bcl10/Malt1 complex via Carma1 for NF- κ B and MAPK activation to selectively control cytokine production. *Blood* 11 January 2008 (doi:10.1182/blood-2007-11-123513).
- Gross, O. *et al.* Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. *Nature* **442**, 651–656 (2006).
- Hara, H. *et al.* The adaptor protein CARD9 is essential for the activation of myeloid cells through ITAM-associated and Toll-like receptors. *Nature Immunol.* **8**, 619–629 (2007).
- Klemm, S. *et al.* The Bcl10–Malt1 complex segregates Fc ϵ R1-mediated nuclear factor κ B activation and cytokine production from mast cell degranulation. *J. Exp. Med.* **203**, 337–347 (2006).
- Dong, W. *et al.* The IRAK-1–BCL10–MALT1–TRAF6–TAK1 cascade mediates signaling to NF- κ B from Toll-like receptor 4. *J. Biol. Chem.* **281**, 26029–26040 (2006).
- Hsu, Y.-M. S. *et al.* The adaptor protein CARD9 is required for innate immune responses to intracellular pathogens. *Nature Immunol.* **8**, 198–205 (2007).
- Uren, A. G. *et al.* Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. *Mol. Cell* **6**, 961–967 (2000).
- Rawlings, D. J., Sommer, K. & Moreno-García, M. E. The CARMA1 signalosome links the signalling machinery of adaptive and innate immunity in lymphocytes. *Nature Rev. Immunol.* **6**, 799–812 (2006).
- Hacker, H. & Karin, M. Regulation and function of IKK and IKK-related kinases. *Sci. STKE* **2006**, re13 (2006).
- Zhou, H., Du, M. Q. & Dixit, V. M. Constitutive NF- κ B activation by the t(11;18)(q21;q21) product in MALT lymphoma is linked to deregulated ubiquitin ligase activity. *Cancer Cell* **7**, 425–431 (2005).
- Che, T. *et al.* MALT1/paracaspase is a signaling component downstream of CARMA1 and mediates T cell receptor-induced NF- κ B activation. *J. Biol. Chem.* **279**, 15870–15876 (2004).
- Sun, L., Deng, L., Ea, C. K., Xia, Z. P. & Chen, Z. J. The TRAF6 ubiquitin ligase and TAK1 kinase mediate IKK activation by BCL10 and MALT1 in T lymphocytes. *Mol. Cell* **14**, 289–301 (2004).
- Noels, H. *et al.* A Novel TRAF6 binding site in MALT1 defines distinct mechanisms of NF- κ B activation by API2–MALT1 fusions. *J. Biol. Chem.* **282**, 10180–10189 (2007).
- Yoneda, T. *et al.* Regulatory mechanisms of TRAF2-mediated signal transduction by Bcl10, a MALT lymphoma-associated protein. *J. Biol. Chem.* **275**, 11114–11120 (2000).
- Oeckinghaus, A. *et al.* Malt1 ubiquitination triggers NF- κ B signaling upon T-cell activation. *EMBO J.* **26**, 4634–4645 (2007).
- Wu, C. J. & Ashwell, J. D. NEMO recognition of ubiquitinated Bcl10 is required for T cell receptor-mediated NF- κ B activation. *Proc. Natl Acad. Sci. USA* **105**, 3023–3028 (2008).
- Stilo, R. *et al.* Physical and functional interaction of CARMA1 and CARMA3 with Ikk kinase–NF- κ B essential modulator. *J. Biol. Chem.* **279**, 34323–34331 (2004).
- Shambharkar, P. B. *et al.* Phosphorylation and ubiquitination of the I κ B kinase complex by two distinct signaling pathways. *EMBO J.* **26**, 1794–1805 (2007).
- Snipas, S. J. *et al.* Characteristics of the caspase-like catalytic domain of human paracaspase. *Biol. Chem.* **385**, 1093–1098 (2004).
- Coornaert, B. *et al.* T cell antigen receptor stimulation induces MALT1 paracaspase-mediated cleavage of the NF- κ B inhibitor A20. *Nature Immunol.* **9**, 263–271 (2008).
- Rebeaud, F. *et al.* The proteolytic activity of the paracaspase MALT1 is key in T cell activation. *Nature Immunol.* **9**, 272–281 (2008).
- Vercammen, D., Declercq, W., Vandenaabee, P. & Van Breusegem, F. Are metacaspases caspases? *J. Cell Biol.* **179**, 375–380 (2007).
- Heyninck, K. & Beyaert, R. A20 inhibits NF- κ B activation by dual ubiquitin-editing functions. *Trends Biochem. Sci.* **30**, 1–4 (2005).
- Klinkenberg, M., Van Huffel, S., Heyninck, K. & Beyaert, R. Functional redundancy of the zinc fingers of A20 for inhibition of NF- κ B activation and protein–protein interactions. *FEBS Lett.* **498**, 93–97 (2001).
- Kinashi, T. Intracellular signalling controlling integrin activation in lymphocytes. *Nature Rev. Immunol.* **5**, 546–559 (2005).
- Lu, T. T. & Cyster, J. G. Integrin-mediated long-term B cell retention in the splenic marginal zone. *Science* **297**, 409–412 (2002).
- Rueda, D. *et al.* Bcl10 controls TCR- and Fc γ R-induced actin polymerization. *J. Immunol.* **178**, 4373–4384 (2007).
- Zhou, H. *et al.* Bcl10 activates the NF- κ B pathway through ubiquitination of NEMO. *Nature* **427**, 167–171 (2004).

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DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
A20 | BCL-10 | CARMA1 | MALT1 | TRAF6

FURTHER INFORMATION

Margot Thome's homepage:
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