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Research update

ABINs: A20 binding inhibitors of NF-κB and apoptosis signaling

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ABSTRACT

ABINs have been described as three different proteins (ABIN-1, ABIN-2, ABIN-3) that bind the ubiquitin-editing nuclear factor-κB (NF-κB) inhibitor protein A20 and which show limited sequence homology. Overexpression of ABINs inhibits NF-κB activation by tumor necrosis factor (TNF) and several other stimuli. Similar to A20, ABIN-1 and ABIN-3 expression is NF-κB dependent, implicating a potential role for the A20/ABIN complex in the negative feedback regulation of NF-κB activation. Adenoviral gene transfer of ABIN-1 has been shown to reduce NF-κB activation in mouse liver and lungs. However, ABIN-1 as well as ABIN-2 deficient mice exhibit only slightly increased or normal NF-κB activation, respectively, possibly reflecting redundant NF-κB inhibitory activities of multiple ABINs. Other functions of ABINs might be non-redundant. For example, ABIN-1 shares with A20 the ability to inhibit TNF-induced apoptosis and as a result ABIN-1 deficient mice die during embryogenesis due to TNF-dependent fetal liver apoptosis. On the other hand, ABIN-2 is required for optimal TPL-2 dependent extracellularly regulated kinase activation in macrophages treated with TNF or Toll-like receptor ligands. ABINs have recently been shown to contain an ubiquitin-binding domain that is essential for their NF-κB inhibitory and anti-apoptotic activities. In this context, ABINs were proposed to function as adaptors between ubiquitinated proteins and other regulatory proteins. Alternatively, ABINs might disrupt signaling complexes by competing with other ubiquitin-binding proteins for the binding to specific ubiquitinated targets. Altogether, these findings implicate an important role for ABINs in the regulation of immunity and tissue homeostasis.

1. Introduction

Nuclear factor-κB (NF-κB) dependent gene expression plays a key role in development and immunity. The NF-κB family of transcription factors consists of five members: p50/p105, p52/p100, c-Rel, RelA (p65) and RelB, forming several homo- and heterodimers. In resting cells, NF-κB is kept inactive in the cytoplasm by binding to inhibitor of κB (IκB) proteins. In the classical NF-κB pathway, which is activated by tumor necrosis factor (TNF), interleukin-1 (IL-1), Toll-like receptors (TLRs) and other innate immune stimuli, receptor triggering stimulates an IκB kinase (IKK) complex consisting of the regulatory protein NEMO, also known as IKKγ, and the kinases IKKα and IKKβ. IKKβ mediates the phosphorylation of IκBα, followed by its K48-linked poly-ubiquitination and degradation by the proteasome. Free NF-κB then translocates to the nucleus where it can bind to κB elements in the promoters of responsive genes [1,2]. Importantly, unrestrained NF-κB activation is associated with several autoimmune diseases and sepsis [3]. Several proteins therefore negatively regulate NF-κB activation by affecting specific protein–protein interactions or posttranslational modifications of NF-κB signaling proteins [4]. The expression of many of these negative regulators is itself regulated by NF-κB and thus imposes a negative feedback mechanism. One of these negative feedback NF-κB inhibitors is the ubiquitin-editing protein A20, also known as tumor necrosis factor alpha induced protein 3 (TNFAIP3) [5]. Remarkably, in some cells A20 also exerts anti-apoptotic activities. A20 deficient mice die shortly after birth due to massive inflammation and tissue damage in multiple organs. Moreover, A20 deficient murine embryonic fibroblasts are hypersensitive to TNF-induced apoptosis and show prolonged IKK activity and IκBα phosphorylation, leading

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Abbreviations: aa, amino acids; ABIN, A20-binding inhibitor of NF-κB; AHD, ABIN homology domain; Ang, angiopoietin; AUF1, AU-rich element binding factor 1; EGF, epidermal growth factor; ERK, extracellularly regulated kinase; HIV, human immunodeficiency virus; IκB, inhibitor of κB; IKK, IκB kinase; IL, interleukin; LPS, lipopolysaccharide; LZ, leucine zipper; NBD, NEMO-binding domain; NF-κB, nuclear factor κB; PI3K, phosphoinositide-3 kinase; TLR, Toll-like receptor; PIC, pre-integration complex; PSGL-1, P-selectin glycoprotein ligand 1; TNF, tumor necrosis factor; TNFAIP3, tumor necrosis factor alpha induced protein 3; TPA, 12-O-tetradecanoylphorbol-13-acetate; UBD, ubiquitin-binding domain.

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to sustained NF-κB activation and enhanced cytokine production [6]. Mechanistically, A20 was shown to exert its NF-κB inhibitory function in TNF signaling by acting as a dual ubiquitin-editing protein on RIP1 [7]. The latter is normally K63-polyubiquitinated, which in contrast to K48-polyubiquitination does not trigger proteasome-mediated degradation but enables the binding of RIP1 to specific downstream signaling proteins such as the IKK adaptor protein NEMO that contains an ubiquitin-binding domain (UBD). The N-terminal de-ubiquitinating domain of A20 mediates the removal of K63-polyubiquitin chains from RIP1, whereas the C-terminal zinc finger domain catalyzes RIP1 K48-polyubiquitination, thereby targeting RIP1 for proteasomal degradation [7]. Similarly, A20 has been shown to inhibit lipopolysaccharide (LPS)-induced NF-κB activation by de-ubiquitinating K63-polyubiquitinated TRAF6 [8], but A20-mediated K48-ubiquitination of TRAF6 has not yet been reported.

2. Identification of ABINs

Yeast two-hybrid screening of a mouse fibroblast L929r2 cDNA library with A20 as bait originally led to the identification of A20-binding inhibitor of NF-κB (ABIN)-1 and ABIN-2 [9]. A TBLASTN search in the non-redundant and expressed sequence tag database identified ABIN-3 as another related protein [10]. Based on their identification in other independent studies, several alternative names have been given to ABINs: ABIN-1/TNIP-1/NAF1/VAN, ABIN-2/TNIP-2/FLIP1, ABIN-3/TNIP-3/LIND (see below). Although TNIP-1, -2 and -3 (*TNF*AIP3 *i*nteracting *p*rotein) are proposed as the official symbols, most literature still refers to these proteins as ABINs and we will therefore also use the latter in the rest of this review. ABINs are defined based on three different parameters: (1) their ability to bind A20; (2) their ability to inhibit NF-κB activation upon overexpression; (3) the presence of specific short

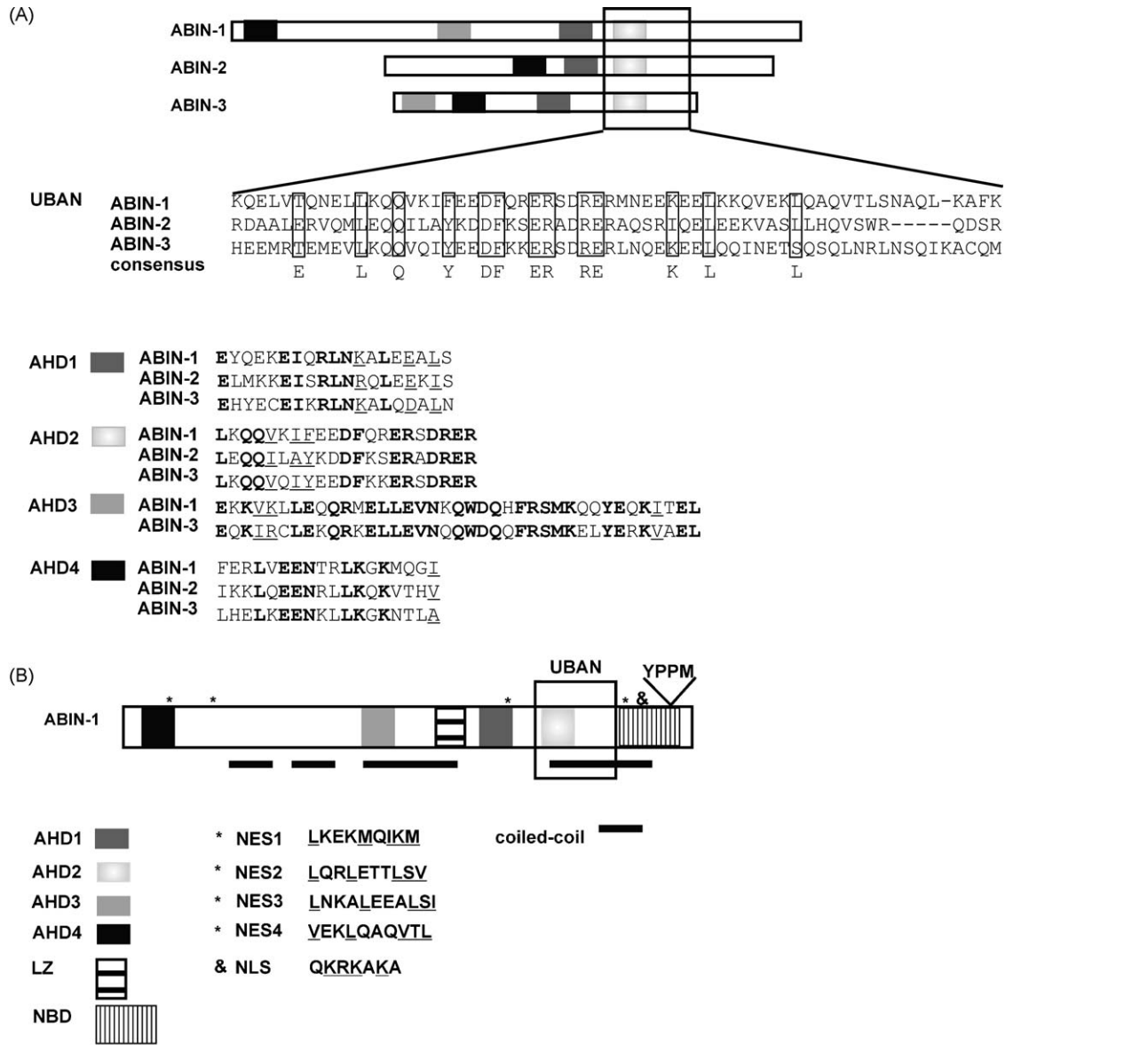

Fig. 1. Structural domains of ABINs. (A) Localization and sequence alignment of AHD and UBAN domains in ABIN-1, -2 and -3. Identical and homologous aa residues that are present in the AHD of different ABINs are indicated in bold or underlined, respectively. (B) ABIN-1 contains, next to the four AHDs and the UBAN domain, four coiled-coil regions, a leucine zipper (LZ) structure, and a NEMO binding domain (NBD). Moreover, ABIN-1 has four putative nuclear export signals (NES) and one nuclear localization signal (NLS). A Src kinase phosphorylation motif is present in the ABIN-1 C-terminus.

amino acid (aa) regions of strong homology, designated ABIN homology domains (AHD)1–4. All human ABINs share AHD1, AHD2 and AHD4, but AHD3 is not present in ABIN-2 (Fig. 1A) [10,11]. Moreover, AHD2 is missing specifically in mouse ABIN-3. AHD1 is necessary for A20 binding, whereas AHD2 is responsible for NF-κB inhibition. No function is attributed yet to AHD3 and AHD4. All three ABINs also contain a UBD that is referred as UBAN (*UB*D in *A*BIN proteins and *N*EMO) domain, comprising AHD2 [12]. In contrast to A20, ABINs do not exert enzymatic activity, but most likely function as adaptor proteins. In the following sections, the expression and function of each ABIN protein will be discussed in more detail.

3. Expression of ABINs

3.1. ABIN-1

The human *ABIN-1* gene is situated on chromosome 5q32–33.1 and consists of 18 exons. The first exon remains untranslated, whereas exons 2 till 18 contain the coding sequence. Cloning of the ABIN-1 cDNA revealed the existence of two isoforms of 72 kDa, ABIN-1α and ABIN-1β, which only differ in their C-terminus. Genomic structure analysis indicated that ABIN-1α and ABIN-1β are produced by alternative splicing, with ABIN-1β mRNA being synthesized when an alternative splice acceptor site within exon 18 is used [13]. Moreover, eight other splice variants of ABIN-1 were described (Fig. 2) [14,15]. In silico analysis of the *ABIN-1* gene led to the identification of three of these splice variants, one emerging from a deletion of exon 2 (ABIN-1Δ2), and two by the use of alternative promoters, resulting in two different types of exon 1 [14]. These splice variants show intercellular variation in expression. Recently, Shiote et al. identified five other splice variants: ABIN-1α and β which lack exon 16 (ABIN-1α2 and ABIN-1β2) or which lack exon 16 with an insertion of 100 bp between exons 15 and 17 (ABIN-1α3 and ABIN-1β3), and a variant which lacks exons 16 and 17 (ABIN-1α4) [15]. The insertion in ABIN-1α3 and ABIN-1β3 leads to premature termination. In most leukemia–lymphoma cell lines as well as in cells derived from acute myeloid leukemia patients, ABIN-1 (full length), ABIN-1α3 and ABIN-1β3 show higher expression, while ABIN-1α4 shows lower expression compared to peripheral blood mononuclear cells from healthy persons. Furthermore, ABIN-1α2 is the dominant transcript in cells from healthy donors, while ABIN-1 (full length) is the main transcript in leukemia–lymphoma, solid tumor cell lines and cells from acute myeloid leukemia patients. Interestingly, upon chemotherapy, the expression levels of ABIN-1 (full length) and ABIN-1α3 decreased. However, how these different splice variants relate to the formation of tumors is currently still unknown.

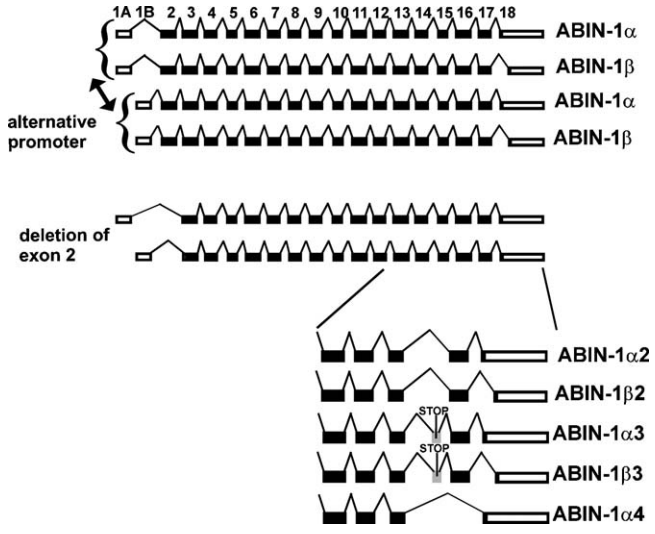

Fig. 2. Schematic representation of different splice variants of ABIN-1. ABIN-1α and ABIN-1β result from the alternative use of a splice acceptor site in exon 18 and can both be transcribed from alternative promoters (1A and 1B). ABIN-1Δ2 represents splice variants of the former variants in which exon 2 has been deleted. ABIN-1α2 and ABIN-1β2 lack exon 16, while ABIN-1α3 and ABIN-1β3 lack exon 16, but contain an additional insert, resulting in premature termination of the transcripts. ABIN-1α4 lacks both exons 16 and 17.

The murine *ABIN-1* gene is located on chromosome 11 and consists of 18 exons, of which the first exon is not translated. Two different splice variants of approximately 2800 and 2600 nucleotides long were found, containing open reading frames of respectively 1941 and 1792 nucleotides and encoding proteins of 72 and 68 kDa, respectively [9]. In contrast to the human ABIN-1 isoforms, the two murine splice variants differ at their N-terminus due to the initiation from two different methionines.

ABIN-1 mRNA is ubiquitously expressed in several tissues and cells. Strong expression was reported in human peripheral blood lymphocytes, spleen and skeletal muscle, but weak expression in brain. Whereas resting T cells express low amounts of ABIN-1, activated $CD4^+$ and $CD8^+$ T cells and activated T-cell lines such as Jurkat and H-9 express high levels of ABIN-1 [16], indicating that T-cell activation is associated with the upregulation of ABIN-1. Several reports suggest that the expression of ABIN-1 is regulated by NF-κB. Indeed, an NF-κB responsive element has been identified in the human *ABIN-1* gene promoter and chromatin immunoprecipitation analysis showed binding of p65 to this NF-κB site in TNF-treated HeLa cells. Furthermore, overexpression of p65 led to an increase of ABIN-1 mRNA expression in these cells [17]. Similarly, retroviral overexpression of p50 and p65 upregulates ABIN-1β mRNA in primary human keratinocytes, whereas ABIN-1 expression in fibroblasts is unaffected, indicative for a cell type specific NF-κB dependent inducibility of ABIN-1 [18]. Also expression of mouse ABIN-1 seems to be regulated by NF-κB activation, as ABIN-1 mRNA is less abundant in NF-κB-deficient cells than in wild type cells [19]. The NF-κB dependent expression of ABIN-1 is further supported by studies showing augmented ABIN-1 expression in various cell types upon stimulation with NF-κB activating agents. For instance, ABIN-1 mRNA is upregulated after TNF stimulation of HeLa cells, primary human synoviocytes and human umbilical vein endothelial cells [20,21,22], as well as upon LPS stimulation of RAW264.7 macrophages and the precursor B cell line 70/Z3 [23,24,25]. Furthermore, ABIN-1 mRNA expression is augmented in tissue biopsies from patients with inflammatory arthritis, in contrast to patients suffering from non-inflammatory arthritis [21]. Higher levels of ABIN-1 were also found in Hodgkin lymphoma compared to non-Hodgkin lymphoma [24]. The elevated expression of ABIN-1 mRNA in these disease conditions correlates with the constitutive activation of NF-κB in the cell types studied. Finally, ABIN-1 expression is also induced in an NF-κB-dependent manner upon infection of epithelial cells with *Yersinia enterocolitica* [26].

At the protein level, ABIN-1 contains four AHDs and a UBAN domain that is characteristic for all ABINs as described above. In addition, ABIN-1 contains a NEMO binding domain (NBD) in its C-terminus [27], a leucine zipper (LZ) structure, and four coiled-coil structures [9,16]. Furthermore, ABIN-1 also has four putative leucine-rich nuclear export signals distributed throughout its sequence, as well as a nuclear localization signal in its C-terminal part. Finally, ABIN-1 contains an 'YPPM' motif, with Y552 being a target for phosphorylation by Src kinases (Fig. 1B) [28]. Whereas ABIN-1 is predominantly cytoplasmic, treatment of HeLa cells with an inhibitor of Crm-1 mediated nuclear export (leptomycin B) results in its nuclear accumulation, suggesting that ABIN-1

constitutively shuttles between the cytosol and the nucleus in a Crm-1 dependent way [16].

3.2. ABIN-2

The human *ABIN-2* gene is situated on chromosome 4p16.3 and contains six exons, whereas the mouse *ABIN-2* gene is located on chromosome 7 and comprises 7 exons. Both the mouse and human ABIN-2 cDNA encode a protein with a molecular weight of approximately 49 kDa [29,30]. Human ABIN-2 shows 78% aa identity with mouse ABIN-2 and contains four putative coiled-coil domains at the N-terminus [30,31]. ABIN-2 is localized in the cytoplasm, but deletion of the first 195 aa allows ABIN-2 to enter the nucleus, indicating that the N-terminus is involved in the retention of ABIN-2 in the cytosol [30].

ABIN-2 mRNA is constitutively expressed in several murine and human tissues or cell lines [29,30,31]. In contrast with ABIN-1 and A20, ABIN-2 mRNA expression is not modulated upon stimulation of cells with TNF, LPS or interferon-γ [29], and is therefore believed to be NF-κB independent. However, ABIN-2 mRNA expression is augmented in adult mouse liver after partial hepatectomy, and like A20, in progesterone-stimulated progesterone receptor B expressing cells [32,33]. Furthermore, the 3′-untranslated region of ABIN-2 mRNA contains two binding sequences for 'AU-rich element binding factor 1' (AUF1), which has been suggested to regulate mRNA turnover [34]. In the case of rat uterus ABIN-2 mRNA, these sequences were indeed shown to bind AUF1, and ABIN-2 mRNA was upregulated with the same kinetics as cytoplasmic AUF1 protein isoforms in ovariectomized rat uteri stimulated with 17β-estradiol [35]. Altogether, these findings support a role for AUF1 in the stabilization of ABIN-2 mRNA.

3.3. ABIN-3

ABIN-3 was originally identified as a novel protein (referred as LIND) that is induced in human mononuclear phagocytes infected with *Listeria* [36], as well as by in silico cloning of ABIN-1 and ABIN-2 related proteins [10]. The human *ABIN-3* gene is located on chromosome 4q27, and encodes a protein of approximately 39 kDa. Northern blot analysis of human tissues revealed a restricted ABIN-3 mRNA expression pattern with high expression levels in brain, spleen, liver, colon, lung, small intestine, muscle, stomach, testis, placenta, thyroid, uterus, prostate, skin, peripheral blood lymphocytes and fetal liver, but low expression in kidney and bone marrow, and no expression in heart, salivary gland, adrenal gland, pancreas, ovary and fetal brain. In addition, constitutive ABIN-3 mRNA or protein expression is not detectable in several cell lines, but is strongly induced upon treatment of human monocytes with LPS as well as upon infection of human mononuclear phagocytes with *Listeria* [10,36]. Furthermore, deactivation of mononuclear phagocytes with IL-4 prior to *Listeria* infection did not influence ABIN-3 mRNA expression, whereas prior treatment with IL-10 or dexamethasone led to increased or decreased ABIN-3 mRNA levels, respectively [36]. Interestingly, ABIN-3 is also upregulated in monocytes isolated from septic shock patients, and decreased again after low dose corticotherapy [37]. Analysis of the human ABIN-3 promoter revealed an NF-κB responsive element that is necessary for the induction of ABIN-3 expression by LPS and TNF, suggesting an important role for NF-κB in the regulation of ABIN-3 expression [37].

The mouse *ABIN-3* gene is located on chromosome 6 and comprises 8 exons. Similar to human ABIN-3, mouse ABIN-3 is induced in macrophages and monocytes by IL-1, TNF, and several TLR stimuli, and is further superinduced by costimulation with IL-10 [38]. Three ABIN-3-related transcripts are generated by way of either alternative splicing (in the case of the smallest form, referred to as ABIN-3β) or alternative polyadenylation (in the case of the largest form, referred to as ABIN-3L). The mouse ABIN-3 and ABIN-3L transcripts encode an identical 208 aa ABIN-3 protein isoform. In contrast, the ABIN-3β splice variant encodes a 197 aa protein that has a unique 57 aa C-terminus. Interestingly, both mouse ABIN-3 isoforms lack AHD2 that is present in human ABIN-3 and all other ABINs and which is necessary for their NF-κB inhibitory function (see below). Comparison of the full length protein sequences of all three human ABINs reveals that ABIN-3 shows more homology with ABIN-1 than with ABIN-2, with ABIN-1 and ABIN-3 sharing a third region of homology (AHD3) that is absent in ABIN-2 (Fig. 1A).

4. Biological activities of ABINs (Table 1)

4.1. ABIN-1

Several functions have been suggested for ABIN-1, mostly depending on the identity and function of its protein interaction partners. As already mentioned before, all ABINs bind to the ubiquitin-editing protein A20, which is known for its NF-κB inhibiting and anti-apoptotic activities. Similar to A20, ABIN-1 overexpression in HEK293T cells inhibits TNF-, IL-1-, and LPS-induced NF-κB activation [9]. Moreover, RNA interference of A20 also impairs the NF-κB inhibitory effect of ABIN-1 overexpression [27]. A role for ABIN-1 in the regulation of NF-κB activation and apoptosis signaling by A20 has therefore been suggested. A20 and ABIN-1 can also inhibit NF-κB activation that is triggered by overexpression of the TNF-receptor signaling proteins TRADD, RIP1 and TRAF2, but not that induced by IKKβ or p65 overexpression, suggesting that A20 and ABIN-1 act upstream of IKKβ but downstream of RIP1 or TRAF2 [9,27]. In line with these observations, ABIN-1 was shown to interact with NEMO via a specific NBD that is located C-terminal from AHD2, and which is required for A20-mediated de-ubiquitination of NEMO [27] (Fig. 3A). Interestingly, we could demonstrate that ABIN-1 also contains a UBD (referred as UBAN) that overlaps with AHD2 and which is involved in NEMO binding [12]. Altogether, the above-mentioned findings led to the suggestion that ABIN-1 physically links A20 to ubiquitinated NEMO, thus facilitating A20-mediated de-ubiquitination of NEMO and NF-κB inhibition. Interestingly, a similar adaptor function has been described for the UBD containing protein TAX1BP1, which recruits A20 to ubiquitinated RIP1 and TRAF6 [39] (Fig. 3A). It is very likely that ABIN-1 and TAX1BP1 still bind other ubiquitinated signaling proteins than those that have been described so far, thus increasing the number of targets of A20. One could question why A20 uses multiple adaptors to de-ubiquitinate specific signaling molecules. Some redundancy could be expected, which is also suggested by the fact that TAX1BP1 deficient mice show a less severe phenotype than ABIN-1 deficient mice [39]. Moreover, one could expect cell-specific functions of each protein. Whereas the model that assumes an A20-adaptor function for ABIN-1 and TAX1BP1 is quite attractive, there is also evidence for an A20-independent NF-κB inhibitory function of ABIN-1. For example, co-expression of ubiquitin-binding deficient mutants of ABIN-1 does not exert a dominant-negative effect on the NF-κB inhibitory function of A20 [10]. Moreover, A20-binding deficient ABIN-1 mutants (by deletion of AHD1) can still inhibit NF-κB [10]. In this context, it is interesting to note that the UBD of ABINs is also present in the C-terminal part of NEMO and the NEMO-related protein optineurin [10,12]. The UBD of NEMO mediates its interaction with K63-polyubiquitinated signaling proteins such as RIP1, which is essential for TNF-induced NF-κB signaling [40]. Overexpression of optineurin, which also binds ubiquitin via a similar UBD, was recently shown to inhibit TNF-induced NF-κB activation by competing with NEMO for RIP1 binding [41]. We therefore hypothesize that also ABIN-1 might compete with NEMO

Table 1
Biological activities of ABINs and involvement of different ABIN-binding proteins.

	Function	Mechanism	Interaction partner	Interacting domain in human ABIN	Interacting domain in binding partner	Reference
ABIN-1	Inhibition of NF-κB	Recruitment of A20 to NEMO	A20	AHD1	Zn-finger containing C-terminus	[9,11,27]
			NEMO	aa 500–588	aa 50–91	
	Transcriptional regulation	?	p105, p100			[49]
	Regulation of HIV-1 infection	Prevent CD4 downregulation by HIV-Nef	Nef			[13]
		Nuclear shuttling together with HIV-Gag and Matrix + incorporation into virions	Matrix			[16]
	Inhibition of EGF signaling	Blocking ERK1/2 nuclear translocation	ERK2			[43]
	Anti-apoptotic	?	A20	AHD1		[9,46]
	Leukocyte adhesion	Recruitment of PI3K to PSGL-1 receptor	PSGL-1	AHD2	aa 334–351	[28]
			PI3K	Y^{552}PPM		
ABIN-2	Inhibition of NF-κB	Compete with RIP1 for NEMO binding	NEMO	aa 253–346 (containing AHD1 and AHD2)	aa 174–306	[51]
		?	A20	AHD1	Zn-finger containing C-terminus	[29]
		?	Tie2	aa 229–272 (containing AHD1)	Tie2 (tyrosine phosphorylated)	[31]
	Transcriptional regulation	?	p50, p52, RelA, cRel			[45]
		?	BAF60a			[64]
	Anti-apoptotic on growth factor-deprived HUVEC	?	Tie2	aa 229–272 (containing AHD1)	Tie2 (tyrosine phosphorylated)	[31,60]
		PI3K/Akt dependent				[60]
	Promote RIP1-induced apoptosis	Inhibition of NF-κB dependent survival genes				[51]
	Promote ERK1/2 activation by TLR-2, -3, -4, -9 and CD40	Stabilization of TPL-2	TPL-2	aa 194–250	aa 398–468	[45,53,61]
			p105	aa 1–250	Death domain + aa 497–538 + PEST region	
	?	?	LKB1	340 C-terminal aa	aa 88–135 + 46 C-terminal aa (human)	[30]
ABIN-3	Inhibition of NF-κB	?	A20	AHD1		[10]

? = not known.

or other ubiquitin-binding proteins for binding to polyubiquitinated signaling proteins in the NF-κB pathway (Fig. 3B). The latter must be different from RIP1 as we were unable to demonstrate the binding of ABIN-1 to polyubiquitinated RIP1 [12].

ABIN-1 overexpression was recently shown to also prevent the constitutive and EGF-induced NF-κB activation in epidermal growth factor (EGF) receptor overexpressing tumor cell lines [42]. This was associated with a decrease in EGF-induced cyclin D1 expression and reduced tumor cell proliferation. EGF has also been shown to induce ABIN-1 phosphorylation by extracellularly regulated kinase (ERK)2 that binds ABIN-1 [43]. In addition, overexpression of ABIN-1 prevents ERK2 nuclear entry as well as ERK2-dependent Elk1 transactivation upon EGF treatment. Another study showed that during mitosis ABIN-1 is also phosphorylated by an unknown kinase and that the phosphorylated form of ABIN-1 is degraded upon exit of the cells from M phase [44]. Whether these effects also contribute to the observed antiproliferative effect of ABIN-1 overexpression in EGF receptor overexpressing tumor cells remains to be determined.

Tandem affinity purification of ABIN-1 interacting proteins revealed the binding of ABIN-1 with p100 and p105 NF-κB, but not of their p52 and p50 processing products [45]. Furthermore, the interaction with p100 was dependent on NIK stimulation, which is a kinase that is known for its role in the non-canonical NF-κB signaling pathway that triggers RelB-dependent gene expression. However, no functional significance has been attributed yet to the ABIN-1/p100/p105 interactions.

Similar to A20, ABIN-1 has been shown to also exert anti-apoptotic activities. Initial evidence for this was again based on overexpression of ABIN-1, showing that it prevents TNF-induced apoptosis of hepatocytes both in vitro and in vivo [46]. Recently, these findings were confirmed with the generation of ABIN-1 deficient mice, which die during embryogenesis with fetal liver apoptosis, anemia and hypoplasia that can be rescued by additional TNF deletion [47]. The embryonic lethality of ABIN-1 deficient mice contrasts with the phenotype of A20 deficient mice that do not show any defects in embryonic development but die shortly after birth due to cachexia and severe inflammation. This further suggests the existence of other A20-independent functions of ABIN-1 during embryonic development. Moreover, ABIN-1 still blocks TNF-induced apoptosis in A20 deficient cells, showing that ABIN-1 does not require A20 to exert its anti-apoptotic activities. In contrast to the clear NF-κB inhibitory effect of ABIN-1 overexpression, cells derived from ABIN-1 deficient mice only show a

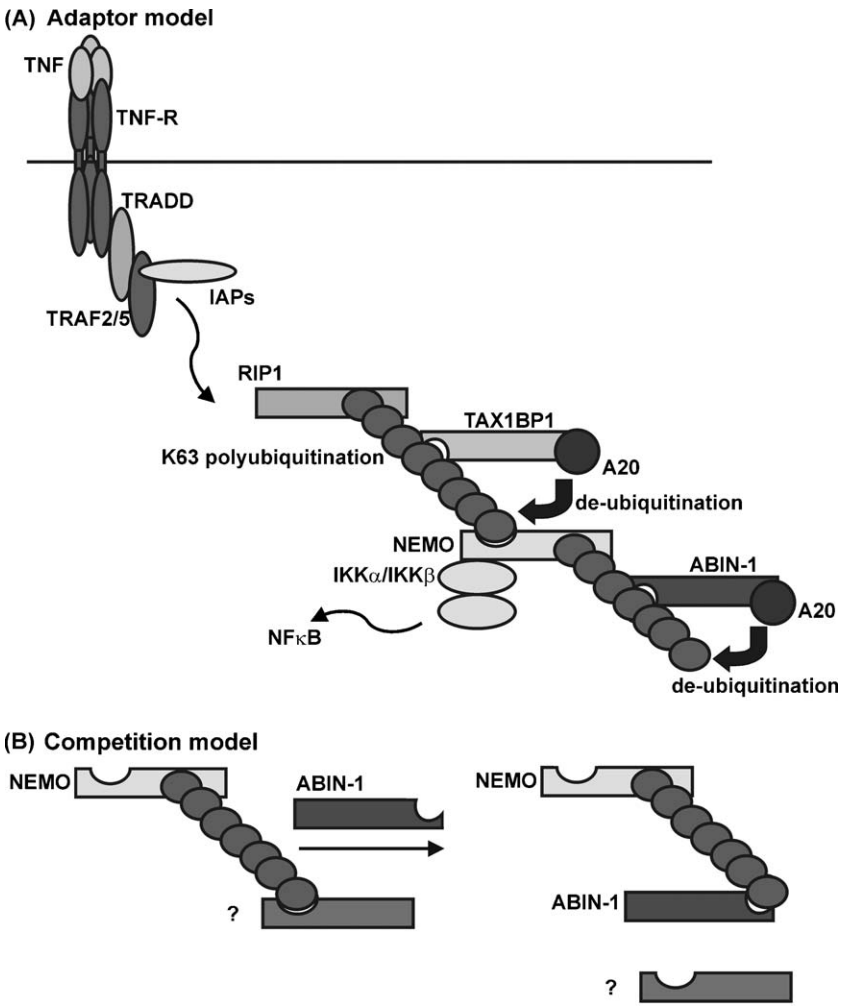

Fig. 3. Models for NF-κB inhibition by ABIN-1. (A) Adaptor model. Specific ubiquitin binding proteins such as ABIN-1 and TAX1BP1 recruit the ubiquitin-editing A20 protein to K63-polyubiquitinated signaling proteins such as RIP1 or NEMO. (B) Competition model. ABIN-1 inhibits NF-κB activation by competing with another protein for binding to polyubiquitinated NEMO or other signaling proteins, thus disrupting crucial protein–protein interactions.

slightly increased NF-κB response compared to control cells [47], possibly reflecting the redundant NF-κB inhibitory effect of other ABINs. Interestingly, similar to its NF-κB inhibitory effect, also the anti-apoptotic effect of ABIN-1 was dependent on its ubiquitin-binding properties, suggesting an important role for ubiquitination and its sensing by negative regulatory proteins in cell death signaling. ABIN-1 was shown to inhibit caspase-8 recruitment to FADD, thus preventing caspase-8 activation and apoptosis in response to TNF. However, whether caspase-8 or FADD are regulated by ubiquitination still needs to be investigated.

In addition to its role as a regulator of NF-κB dependent gene expression and cell death, ABIN-1 has also been implicated in the regulation of human immunodeficiency virus (HIV) infection. In this context, ABIN-1 was found to bind the HIV protein Nef and was therefore also named 'Nef-associated factor 1' (NAF1) [13]. Nef is known to enhance HIV replication and infectivity in T cells by down-regulating cell surface expression of CD4 and major histocompatibility complex class I molecules as a consequence of accelerated cellular endocytosis through clathrin-coated pits [48]. In contrast, ABIN-1 was shown to increase CD4 cell surface expression in 293T cells transfected with CD4. Cotransfection of Nef diminished the ABIN-1-induced CD4 upregulation, indicating that the ratio of Nef and ABIN-1 expression can affect cell surface CD4 expression levels [13]. Furthermore, another group identified ABIN-1 as an interaction partner for the HIV-1 protein Matrix, which led to the alternative naming of ABIN-1 as 'virion-associated nuclear matrix-interacting protein' (VAN) [16]. Matrix is a key component of the HIV pre-integration complex (PIC) [49]. Like Matrix, ABIN-1 constantly shuttles between the nucleus and the cytoplasm in a Crm1-dependent manner. Therefore, ABIN-1 was suggested to regulate nuclear import of the PIC as well as nuclear export of the gag precursor polyprotein and viral genomic RNA during virion production [16]. Although both studies clearly show a role for ABIN-1 in HIV infection, single nucleotide polymorphisms in the ABIN-1 gene leading to the permissiveness of CD4 T cells for HIV replication could not be found [50].

Finally, ABIN-1 also constitutively binds to the cytoplasmic domain of P-selectin glycoprotein ligand 1 (PSGL-1) in human neutrophils [28]. ABIN-1 becomes phosphorylated by Src kinases on Y552 upon P-selectin binding to PSGL-1, leading to the recruitment of phosphoinositide-3 kinase (PI3K) p85-p110δ heterodimer and the activation of $\alpha_M\beta_2$ integrin-mediated leukocyte adhesion. Preventing the formation of the PSGL-1/ABIN-1 signaling complex also inhibited leukocyte adhesion in a mouse model for acute peritonitis, demonstrating its physiological relevance.

4.2. ABIN-2

Based on its ability to bind A20 and to prevent TNF-, IL-1- as well as 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced NF-κB activation upon overexpression, ABIN-2 was originally described as a negative regulator of NF-κB dependent gene expression [29].

ABIN-2 overexpression also inhibits EGF-induced NF-κB activation [42]. Similar to ABIN-1, ABIN-2 was shown to interfere with NF-κB activation downstream of the signaling proteins RIP1, TRAF2, IRAK1 and TRAF6, but upstream of IKKβ [29]. ABIN-2 also shares with ABIN-1 the ability to form a complex with NEMO [51]. Binding of NEMO involves a 50 aa long stretch in ABIN-2 that is homologous to a NEMO-binding sequence in RIP1 [52]. Consequently, ABIN-2 was shown to compete with RIP1 for binding to NEMO, suggesting that ABIN-2 inhibits NF-κB activation by preventing RIP1-NEMO complex formation [51]. Although the above mentioned findings pointed to a role for ABIN-2 in the regulation of NF-κB activation in response to different stimuli, primary macrophages of ABIN-2 deficient mice show no difference in IKK activity, nuclear NF-κB DNA binding, IκBα degradation or expression of several NF-κB dependent genes in response to LPS or TNF when compared to wild type cells [53]. Furthermore, LPS-induced and IKK-mediated p105 NF-κB phosphorylation in bone marrow-derived macrophages, as well as B- or T-cell receptor-induced NF-κB activation, are not affected by the absence of ABIN-2. Possibly, redundancy with ABIN-1 is responsible for the absence of any defect in NF-κB signaling in ABIN-2 deficient mice.

ABIN-2 has also been proposed as a regulator of Tie2-induced signaling. The receptor tyrosine kinase Tie2 is expressed predominantly on endothelial cells and is essential for vessel formation and maintenance [54]. The natural ligands for Tie2 are the angiopoietins, of which angiopoietin-1 (Ang1) has both angiogenic and anti-inflammatory properties [55,56,57]. For example, binding of Ang1 to Tie2 inhibits the NF-κB-dependent expression of adhesion molecules, decreases the permeability of endothelial monolayers and impedes TNF-induced transmigration of leukocytes [58,59]. ABIN-2 was found to interact with the cytoplasmic tail of Tie2 via a domain encompassing AHD1. This interaction depends on Tie2 autophosphorylation and is further enhanced by Ang1 stimulation. Because overexpression of a mutant form of ABIN-2 that no longer inhibits NF-κB but still binds Tie2 destroyed the NF-κB inhibitory effect of Tie2 [31], a role for ABIN-2 in the NF-κB inhibitory and anti-inflammatory effect of Tie2 was suggested. In addition, ABIN-2 was also proposed to be involved in the anti-apoptotic effects of Tie2, since the ABIN-2 mutant described above also abolishes the Ang1-promoted survival of growth factor-deprived human umbilical vein endothelial cells. This anti-apoptotic effect of ABIN-2 might be dependent on the PI3K/Akt survival pathway, as expression of ABIN-2 increases Akt S473 phosphorylation [60]. It should be noted that in contrast to its survival role in endothelial cells, ABIN-2 has been shown to promote apoptosis in RIP1-overexpressing HEK-293 cells [51]. This pro-apoptotic effect of ABIN-2 is however most likely due to its NF-κB inhibitory properties, preventing the induction of anti-apoptotic proteins by RIP1. Further experiments with cells derived from ABIN-2 deficient mice will hopefully provide more conclusive data for the role of ABIN-2 in the regulation of Tie2-induced responses.

Besides its potential role as a regulator of NF-κB activation and apoptosis, ABIN-2 was also described as a modulator of the ERK signaling pathway. Initial data based on ABIN-2 overexpression already pointed to a positive effect of ABIN-2 on serum responsive element-dependent gene expression [29,30]. Later on, ABIN-2 was shown to form a ternary complex with the ERK kinase TPL-2 and the NF-κB precursor p105 [61]. Both p105 and ABIN-2 are required for TPL-2 protein stabilization as demonstrated in p105 deficient [62,63] as well as in ABIN-2 deficient cells [53,61], respectively. Conversely, ABIN-2 itself is stabilized by its binding to p105. Upon LPS stimulation of bone marrow derived macrophages, ABIN-2 dissociates from the ABIN-2/TPL-2/p105 ternary complex, ERK becomes activated and all components of the ternary complex are proteolyzed by the proteasome. The importance of TPL-2 release from ABIN-2 is unclear but, in contrast to p105, ABIN-2 does not appear to function as an inhibitor of TPL-2 MEK kinase activity [61]. ABIN-2 mediated TPL-2 stabilization enables the TPL-2-mediated activation of ERK in response to TLR-2, -3, -4 and -9 stimulation in macrophages, as well as in response to TNF receptor or CD40 stimulation in dendritic cells or B-cells, respectively. However, ABIN-2 deficient macrophages do not show impaired expression of all TPL-2 dependent genes, indicating a role for different thresholds of ERK activity [53].

Tandem affinity purification revealed that ABIN-2 not only binds to p105 NF-κB, but also forms a constitutive complex with other NF-κB subunits such as p50, p52 and p65, whereas c-Rel associates with ABIN-2 in response to TNF [45]. The functional role of these interactions is still unclear, but might reflect other transcriptional regulatory functions of ABIN-2. In this context, it is worth mentioning that ABIN-2 has also been predicted to function as a transcriptional co-activator in the nucleus because expression of a fusion protein of ABIN-2 and the DNA-binding domain of Gal4 led to the expression of a Gal4-dependent reporter gene in yeast. In a mammalian expression system, however, only the C-terminal fragment of ABIN-2 could enter the nucleus and exert transactivating activity [64]. This led to the identification of the N-terminal 195 aa of ABIN-2 as a regulatory domain that keeps ABIN-2 in the cytoplasm. Two regions (aa 196–253 and aa 346–429) in ABIN-2 were shown to mediate its transactivation activity, with the former one being able to interact with BAF60a, which is a component of the SWI-SNF chromatin remodeling complex [64]. Whether the observed binding of ABIN-2 to NF-κB subunits plays a role in the transactivation of NF-κB in the nucleus remains an interesting hypothesis.

Finally, ABIN-2 has also been identified as 'fetal liver LKB1-interacting protein 1' (FLIP1) [30]. LKB1 is a tumor suppressor serine/threonine protein kinase that has been linked to different human disorders, including Peutz–Jeghers syndrome that is characterized by hamartomatous polyps and an elevated risk for cancer [65,66]. However, the functional significance of the ABIN-2/LKB1 interaction is still elusive.

4.3. ABIN-3

Similar to the other ABINs, human ABIN-3 binds to A20 and inhibits NF-κB activation induced by TNF, IL-1, LPS and TPA upon overexpression [10]. A20-binding is again mediated by AHD1, whereas AHD2 is essential for NF-κB inhibition. Whether A20-binding is involved in its NF-κB inhibitory function remains unclear. In contrast to human ABIN-3, both murine splice variants are unable to prevent NF-κB activation [38], which is due to the absence of AHD2 in murine ABIN-3. In this respect it is not surprising that ABIN-3 deficient mice do not display any phenotype related to inflammation or deregulated NF-κB activation [38]. Human ABIN-3 also inhibits NF-κB activation induced by overexpression of the signaling proteins MyD88, IRAK1 and TRAF6, but not in response to IKKβ overexpression, suggesting that similar to ABIN-1 and ABIN-2, also ABIN-3 acts upstream of IKKβ [10]. Because treatment of human monocytic cells with LPS strongly induces ABIN-3 in an NF-κB dependent manner [37], ABIN-3 has been proposed as a negative feedback regulator of LPS-induced NF-κB activation. Moreover, as IL-10 costimulation further increases LPS-induced ABIN-3 expression, it has been suggested that ABIN-3 induction might contribute to the anti-inflammatory features of IL-10 observed in humans [10,38].

5. ABINs in disease

Given the central role of NF-κB in several inflammatory diseases, NF-κB inhibition has been proposed as a novel therapeutic

strategy. Using a murine model of allergen-induced asthma, we demonstrated that adenovirus-mediated delivery of ABIN-1 to the lung results in considerable inhibition of allergen-induced NF-κB activity and eosinophil infiltration [67]. Furthermore, ABIN-1 decreases allergen-specific immunoglobulin E levels in the serum, as well as the levels of eotaxin, IL-1, IL-4, IL-5, and IL-13 in bronchoalveolar lavage fluid. These findings not only prove that NF-κB plays a critical role in the pathogenesis of allergic inflammation, but also illustrate that inhibiting NF-κB could have therapeutic value in the treatment of asthma and other chronic inflammatory lung diseases.

Recently, a genome-wide association scan for 1409 psoriasis patients and 1436 controls revealed a strong association of psoriasis with single nucleotide polymorphisms in loci including *ABIN-1* and *A20* [68]. Moreover, this study also revealed altered expression of ABIN-1 in the skin of psoriasis patients, indicating that changes in the expression of ABIN-1 may be a key event in the initiation and progression of psoriasis.

ABIN-1 has also been shown to protect against TNF-induced liver failure [46]. TNF plays a central role in several liver diseases by inducing parenchymal cell apoptosis and inflammation [69]. NF-κB is believed to mediate at least part of these pro-inflammatory effects of TNF. However, NF-κB also suppresses TNF-mediated hepatocyte apoptosis [70,71], complicating the use of NF-κB inhibitors for therapeutic purposes. Interestingly, adenovirus-mediated expression of ABIN-1 in the liver, but not of an IκBα superrepressor in which the IKK phosphorylation sites have been mutated [72], completely prevented lethality in the TNF/galactosamine-induced murine model of acute liver failure, which was associated with a significant decrease in TNF-induced leukocyte infiltration and hepatocyte apoptosis [45]. This indicates that the anti-apoptotic effect of ABIN-1 is dominant compared to its potential apoptosis sensitizing effect due to the inhibition of NF-κB dependent expression of survival genes in the liver. The dual NF-κB inhibitory and anti-apoptotic activity of ABIN-1 overexpression in the liver might thus be of considerable interest for the development of novel therapeutic approaches for inflammatory liver disease. Similarly, adenoviral gene transfer of human ABIN-3 was shown to partially protect mice against LPS/galactosamine-induced mortality [10], which is known to be TNF-dependent. Because in contrast to ABIN-1, ABIN-3 does not directly protect against TNF-induced apoptosis, the protective effect of ABIN-3 is most likely due to the ABIN-3 mediated inhibition of LPS-induced TNF expression.

It has also been shown that partial hepatectomy induces a rapid and transient upregulation of ABIN-2 in the liver [73,74]. Hepatectomy-induced p65 NF-κB nuclear localization in hepatocytes was markedly reduced in ABIN-2 transgenic mice versus wild type mice. Furthermore, liver regeneration was delayed in ABIN-2 transgenic mice due to impairment of G1/S transition [32]. Both events thus suggest a regulatory role for ABIN-2 mediated NF-κB inhibition in the regenerating liver.

6. Conclusion and perspectives

From the above-mentioned findings it is clear that ABINs fulfill important roles in the regulation of immunity and normal tissue homeostasis. Much information is still based on results obtained by overexpression of specific ABINs but data from ABIN deficient mice have recently become available [47,53]. These illustrate that each ABIN family member has specific non-redundant functions but that their originally described NF-κB inhibitory function might be redundant. The generation and analysis of double deficient ABIN-1/ABIN-2 knockout mice could further clarify the role of ABINs as NF-κB regulatory proteins. The molecular mechanisms by which ABINs exert their different biological activities remain unclear although the recent identification of an ubiquitin-binding domain in all ABINs might shed some light on this [12]. The role of ubiquitination in the regulation of protein degradation and protein–protein interactions, as well as its implications for different cellular processes such as endocytosis and intracellular signaling, provide interesting potential points of interference by ABINs. For example, ABINs might function as adaptors between ubiquitinated signaling proteins and other regulatory proteins such as the ubiquitin-editing protein A20. Alternatively, they might also compete with other ubiquitin-binding proteins for binding to a common ubiquitinated target. Evidence for a role of ubiquitin-binding by ABINs in the regulation of NF-κB and cell death signaling has already been provided [12,47], but the specific targets still need to be identified. Moreover, it is not unlikely that the ubiquitin-binding potential of ABINs might also provide ABINs the potential to regulate endocytosis and intracellular trafficking of specific receptors and signaling proteins. This is also suggested by the already described effects of ABINs on CD4 cell surface expression and nuclear shuttling of specific HIV proteins. Finally, nuclear shuttling of ABINs also suggests a nuclear function of ABINs and the existence of ubiquitinated targets for ABINs in the nucleus. These possibilities as well as the mechanisms that provide specificity for distinct ubiquitinated targets of ABINs will most likely be the topic of future research.

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