Title: The role of the TRAF-interacting protein in proliferation and differentiation.

Authors: Chapard C, Hohl D, Huber M

Journal: Experimental dermatology

Year: 2012 May

Volume: 21

Issue: 5

Pages: 321-6

DOI: 10.1111/j.1600-0625.2012.01477.x

The role of the TRAF-interacting protein (TRAIP) in proliferation and differentiation

Christophe Chapard, Daniel Hohl, and Marcel Huber

Service of Dermatology, University Hospital Center and University of Lausanne, Lausanne, Switzerland

Correspondence:

Marcel Huber

CHUV

Service de dermatologie

1011 Lausanne

Switzerland

Email: marcel.huber@chuv.ch

Keywords: ubiquitination, RING-type E3 ubiquitin ligase, cell cycle

Abstract

Ubiquitination of proteins is a post-translational modification which decides on the cellular fate of the protein. Addition of ubiquitin moieties to proteins is carried out by the sequential action of 3 enzyme: E1-activating enzyme, E2-conjugating enzyme and E3 ubiquitin ligase. The TRAF-interacting protein (TRAIP, TRIP, RNF206) functions as RING-type E3 ubiquitin ligase but its physiological substrates are not yet known. TRAIP was reported to interact with TRAF (tumor necrosis factor (TNF) receptor-associated factors) and the two tumor suppressors CYLD and Syk (spleen tyrosine kinase). Ectopically expressed TRAIP was shown to inhibit nuclear factor-kappa B (NF-κB) signaling. However, recent results suggested a role for TRAIP in biological processes other than NF-κB regulation. Knock-down of TRAIP in human epidermal keratinocytes repressed cellular proliferation and induced a block in the G1/S phase of the cell cycle without affecting NF-κB signaling. TRAIP is necessary for embryonal development since mutations affecting the Drosophila homolog of TRAIP are maternal effect-lethal mutants and TRAIP knock-out mice die in utero due to aberrant regulation of cell proliferation and apoptosis. These findings underline the tight link between TRAIP and cell proliferation. In this review, we summarize the data on TRAIP and put them into a larger perspective regarding a role of TRAIP in the control of tissue homeostasis.

Ubiquitination of proteins

Ubiquitination, a conserved post-translational protein modification regulating the cellular fate of proteins, is involved in multiple biological functions such as proliferation, differentiation, apoptosis, or inflammation (1,2). Ubiquitins are covalently attached to lysine residues of target proteins or to a pre-existing ubiquitin chain through the sequential action of the E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme and E3 ubiquitin-ligase (Fig. 1). During mono-ubiquitination, the C-terminal glycine of one ubiquitin monomer is attached to one lysine residue in target proteins. During multi-ubiquitination, several ubiquitins are attached to different lysines in target proteins. During poly-ubiquitination, additional ubiquitin monomers are attached to one of the seven lysines (K) (K6, K11, K27, K29, K33, K48, K63) or the N-terminal methionine (M1) of the existing ubiquitin unit; thus generating polymers which have different structures and flexibilities (3,4). These ubiquitin signals are recognized by proteins carrying ubiquitin-binding domains and which decide on the cellular fate and function of ubiquitinated proteins (3-9). However, this simplistic view has been challenged by findings that the cellular metabolism of selected proteins does not always follow these paradigms (6). Recently, the formation of linear head-to-tail polyubiquitin chains (10) and there in vivo relevance for controlling inflammation and immune signaling (11-16) has been demonstrated. The physiological process of protein ubiquitination can be reversed by deubiquitinases (Fig. 1) which remove ubiquitin conjugates (17).

During the ubiquitination process, an ubiquitin monomer is activated in an ATP-dependent manner by an E1 ubiquitin-activating enzyme and subsequently transferred to an E2 ubiquitin-conjugating enzyme. Ubiquitin-charged E2 interacts with E3 ubiquitin ligases which determine the substrate specificity of the ubiquitination reaction (18). E3 ligases are classified into two major families: HECT (homologous to the E6-AP carboxyl-terminus)-type and RING (Really Interesting New Gene)-type E3 ligases (19). HECT-type E3 ligases function as true ubiquitination catalysts since the activated ubiquitin is covalently bound via a thioester bond to a conserved cysteine within the HECT domain before transfer to the substrate. In contrast, RING-type E3 ligases do not covalently bind ubiquitin but serve as a scaffold to bring the E2 and the substrate into close proximity to facilitate the ubiquitin transfer (20,21). The RING domain, highly conserved in eucaryotes, is composed of 40 to 60 amino

acids that are arranged in a "cross-brace" or interleaved manner [**C**-X_2-**C**-X_{9-39}-**C**-X_{1-3}-**H**-X_{2-3}-(**N/C/H**)-X_2-**C**-X_{4-48}-**C**-X_2-**C**] around two zinc ions (Zn^{2+}) (20). RING domains seem to be engaged exclusively in the recruitment and positioning of the E2 conjugating enzymes for allowing substrate ubiquitination (20,22,23). RING domain E3 ubiquitin ligases function either as single subunit or multisubunit proteins (20). Numerous RING variants have been described where cysteines and histidines swapped their positions or where aspartates or asparagines replaced cysteines to coordinate zinc ions (20). However, some of these variants, i.e. the LIM (Lin-11, Isl-1, Mec-3) (24) and the PHD (Plant Homology Domain) (25) domains fold differently and do not catalyze ubiquitination (20). The U-Box domain proteins, in which the zinc-binding amino acids are replaced with charged and polar residues forming hydrogen-bonds to stabilize the structure, have E3 ubiquitin ligase activity involved in quality control of intracellular proteins (26,27).

The knowledge about E3 ubiquitin ligase partner proteins (substrates and E2 enzymes) is extremely important to understand their physiological functions. However, the identification of molecular partners of E3 ligases is hampered by the inefficiency of biochemical screens due to low substrate levels and intrinsically weak interactions between E3s and their substrates (28,29), leading to the paucity of confirmed functional interactions.

The molecular structure and evolution of TRAIP protein

The E3 ubiquitin ligase TRAF-interacting protein TRAIP was identified as interactor of TRAF, and ectopically expressed TRAIP was shown to inhibit tumor necrosis factor-alpha (TNFα)-mediated NF-κB activation (30). TRAIP is a 53kDa protein that contains a 55 amino acids long RING domain at its N-terminal end which is followed by a putative coiled-coil domain and a leucine-zipper region (Fig. 2). The RING domain of TRAIP is of the $Cys_3His_2Cys_3$ (RING-H2) type. The Cys and His residues are highly conserved and serve to bind two functionally essential zinc ions. RING domains share a conserved 3-dimensional structure constituted principally of four elements built around the Cys/His residues: an N-loop, a first β-sheet region, a βα-region, and a C-loop (31). These structural conserved regions provide a scaffold to spatially position four hydrophobic and two polar residues which are

crucial for RING-E3 function. The second and third hydrophobic amino acids contribute to the hydrophobic core, while the two remaining hydrophobic and the two polar residues directly participate through hydrophobic and electrostatic interactions in E2/RING-E3 binding (31). The four hydrophobic (I9, F29, L34, P47) and two polar (Q36 and R50) amino acids (numbers refer to human TRAIP, Genebank NP_005870) have been highly conserved in the evolution of TRAIP proteins. In-vitro ubiquitination experiments suggested that TRAIP has the ability to undergo auto-ubiquitination, an activity that was dependent on an intact RING domain. Therefore, TRAIP can be considered at least in vitro as functional ubiquitin ligase (32). Until now, no validated in vivo substrates of TRAIP have been identified. Two publications reporting on large scale yeast two-hybrid screens to identify human E2/E3 interactions mentioned several TRAIP/E2 pairs which were not further validated by functional assays (33,34).

Structure prediction analysis (psiPRED V 3.0; (35)) of human TRAIP protein showed a high proportion of long α-helices in the region of amino acids 60 to 270 while only short α-helical structures and β-sheets were predicted for the remaining parts. The long α-helical structures correspond to the coiled-coil and leucine zipper region proposed previously (30) which are structural motives most likely implicated in protein-protein and/or protein-DNA interactions (36-38). These two regions of TRAIP are the only ones required to interact with the TRAF domain of TRAF1 or 2 in yeast two-hybrid assays (30).

The N-terminal region encompassing the RING, coiled-coil and leucine zipper domains of human TRAIP has 90, 68 or 28% sequence identity with TRAIP orthologues from mouse, Xenopus laevis or Drosophila melanogaster, respectively (Fig. 2). The conservation level is even higher for RING domain amino acids which are important for Zn^{2+} binding and E2 interaction (see above). While the conservation of the C-terminal half of TRAIP, which does not contain any known protein motif, is quite good for vertebrate proteins (68% for human vs. mouse; 37% for human vs. Xenopus), the C-terminal sequences of human and Drosophila TRAIP have practically no sequence identity anymore. There are two stretches (35 and 14 amino acids long) outside of the RING domain which share high sequence identities from H. sapiens to Xenopus (Fig. 2), indicating that they might be functionally important for TRAIP. The variable degree of sequence similarity for the domains of the TRAIP

protein suggests that the evolutionary pressure for sequence conservation has been varying. This might indicate that, except for the RING domain, these regions do not necessarily have similar functions in different species, i.e. substrates might be different.

Transcriptional regulation of the TRAIP gene

The human and mouse TRAIP genes are located on chromosome 3 and 9, respectively. Both genes have 15 exons and a total length of 20-30kbp. Annotation analysis of the human gene predicted 11 alternatively spliced transcripts (ENSEMBL data base and Havana project). Six of them do not encode proteins, four transcripts translate into proteins for which there is no experimental evidence so far that they are expressed in vivo. The longest transcript encodes the 53 kDa TRAIP protein, Little is known about the transcriptional regulation of the gene or the organization of its promoter. Microarray analysis revealed that TRAIP mRNA level was significantly upregulated 4 hours after treatment of human acute monocytic leukemia cells with lipopolysaccharides (39). The authors postulated a NF-κB site 1736bp upstream of the initiator ATG site without presenting experimental evidence. Similarly, TAp63α, a homologue of p53, upregulated TRAIP expression in Hep3B hepatoma cell line (40). Putative p53 binding sites were found in intron 1 by transcription factor binding site analysis. Small-hairpin RNA-mediated downregulation of β-catenin expression in a gastric cancer cell line suppressed cell proliferation and induced apoptosis. Microarray analysis revealed that TRAIP expression was reduced by more than 3-fold in β-catenin knockdown cells (41). Analysis of the 5'upstream sequence from the human TRAIP gene predicted a conserved E2F transcription factor binding site close to the transcription start site (42). This would be consistent with a report that an E2F repressor complex binds to the TRAIP gene promoter in serum-deprived T98G cells (43).

Identification of TRAIP interactors and their roles in molecular signaling pathways

a) TRAF proteins

TRAIP was identified to interact with the TRAF domain (amino acids 183-409) from TRAF1 in a yeast two-hybrid screen using a mouse thymocyte cDNA library (30). Subsequently, it was shown that GST-TRAIP transfected into human embryonic kidney 293 cells coprecipitated overexpressed TRAF1,

TRAF2, TRAF3, TRAF5 and TRAF6 (30,32). The coiled–coil and leucine zipper domains of TRAIP (amino acids 56-270) provide the full interaction with the TRAF domain of TRAF1 and TRAF2 (30) (Fig. 2). Glutathione-S-transferase pull-down experiments showed that TRAIP is recruited to the cytoplasmic domains of TNFR2 and CD30 receptors only in the presence of TRAF2 (30). Overexpression of TRAIP in 293 cells inhibited NF-κB activation mediated by TRAF2 and TNFα (30) and interleukin IL-1 (44). In contrast to this, stable expression of TRAIP in L929 mouse fibroblasts did not negatively regulate TNFα- or IL-1-mediated phosphorylation of IκBα (inhibitor of NF-κB alpha) (32). Furthermore, stable retroviral expression of TRAIP in the murine monocytic cell line RAW264.7 failed to repress phosphorylation of IκBα upon RANKL (Receptor Activator for NF-κB Ligand) treatment (32). However, expression of higher TRAIP levels in 293T cells was able to suppress RANKL-mediated NF-κB activation (45). These findings evoke the question whether inhibition of NF-κB signaling is not an experimental artifact rather than a physiological function of TRAIP. Interestingly, ectopic expression of mutant TRAIP composed of the coiled-coil and the leucine zipper only inhibited TRAF2-mediated NF-κB activation (30) as strongly as the full-length protein, suggesting that only TRAIP domains interacting with TRAF2 but not the RING domain are required for inhibition. Although it is difficult to compare the expression level of TRAIP from the different experiments, the observed discrepancies could be explained by presuming that only artificially high but not physiological amounts of TRAIP are capable to perturb the interaction between TRAFs and TNFR family members and to inhibit NF-κB signaling. The majority of endogenously and ectopically expressed C-terminally tagged TRAIP is localized to the nucleolus in breast epithelial cell lines (46). Regarding TRAIP/TRAF interaction, this would be inconsistent with the subcellular localization of TRAF proteins which are mainly found at the cell membrane and/or in the cytosol (47).

Several groups reported findings on the effect of either knock-down or overexpression of TRAIP on TNFα-induced cell death. Mouse embryonic fibroblasts (NIH3T3 cells) transfected with siRNA targeting TRAIP showed a marked dose-dependent induction of activated caspase-3 and a significant reduction in cell viability following TNFα/cycloheximide treatment (48). These findings suggested

that mouse TRAIP prevents TNFα/cycloheximide-mediated apoptosis. Without showing the data, the authors claimed that TNF-induced cell death was not affected by overexpression of TRAIP in NIH3T3 cells. TNFα-induced activation of caspase-8 in MCF7 breast cancer cells was decreased upon knock-down of TRAIP (46). Overexpression of TRAIP led to the loss of viable, adherent MCF7 cells in response to TNFα addition (46), similarly to a report that overexpression of TRAIP enhanced TNF-mediated cell death in HeLa cells (30). In the immortalized mouse hypothalamic N42 cell line, TRAIP knockdown promoted TNFα-induced NF-κB activity, decreased cell death and increased the expression of B-cell lymphoma 2 (Bcl2) mRNA (49).

b) CYLD

Yeast two-hybrid assays and coimmunoprecipitation in mammalian cells demonstrated that the C-terminal part of TRAIP interacts with the tumor suppressor CYLD (44). Mutational inactivation of the CYLD gene causes the formation of skin appendage tumors (Brooke-Spiegler Syndrome) such as cylindromas, trichoepitheliomas and spiradenomas (50). CYLD protein functions as K63-specific deubiquitinase that negatively regulates NF-κB (44,51-53), Jun N-terminal Kinase (JNK) (54) and β-catenin (55) signaling. Moreover, recombinant CYLD cleaved in vitro K63- and linear-linked but not K48-linked tetraubiquitin chains suggesting that CYLD prefers ubiquitin substrates adopting an open conformation (56). In keratinocytes, CYLD inhibits by deubiquitination the translocation of the proto-oncogene Bcl-3 into the nucleus, thus preventing the transcription of cyclin D1 and cell proliferation (57). TPA and UV activate CYLD in primary keratinocytes and augment the repressive interaction of CYLD with HDAC6 leading to increased levels of acetylated α-tubulin (58). CYLD binds through its CAP-Gly domains to acetylated α-tubulin in the perinuclear region of cells where CYLD negatively affects Bcl-3 nuclear entry (58). Mutational inactivation of the CYLD gene in Brooke-Spiegler Syndrome patients permits increased cyclin D1 transcription due to higher nuclear levels of Bcl-3. Interestingly, CYLD KO mice have no spontaneous skin phenotype but develop significantly higher number of and larger papillomas than wildtype or heterozygous mice after treatment with DMBA and TPA (57). In addition to this, three groups have reported on the implication of CYLD in the regulation of cell proliferation and mitosis (58-60), which is of interest in the light of our results regarding the

effect of TRAIP depletion on proliferation (see below) and their physical interaction (44). Whether this interaction means that CYLD and TRAIP control the ubiquitination levels of each other or of common substrates remains to be seen but this information will be crucial to understand their biological functions. With respect to the fate of common substrates, the linkage type of the ubiquitin chains formed by TRAIP would be important to know since CYLD appears to be specific for K63- or linear-linked ubiquitin chains. In vitro ubiquitination assays with bacterially purified protein showed that TRAIP undergoes autoubiquitination in the presence of UbcH5 but not with a range of other E2 proteins (32). However, UbcH5 is well known for its ability to promote autoubiquitination of numerous E3 ligases without being necessarily physiologically relevant. Furthermore, it is difficult to predict whether TRAIP works as K48 or K63 E3 ligase since UbcH5 has the capacity to introduce different linkage types, depending on the partner E3 ligase (20).

c) Syk

A yeast two-hybrid screen identified TRAIP as binding partner of spleen tyrosine kinase Syk, a non-receptor protein kinase which is thought to act as tumor suppressor in melanoma and breast cancer (46,61-63). Syk enhances the TNFα-dependent activation of NF-κB (64), an effect that is reversed by overexpressed TRAIP. TNFα activates Syk by phosphorylation at tyrosine residues promoting its binding to TRAIP (46). TRAIP becomes phosphorylated after binding of activated Syk (46). Ectopically co-expressed TRAIP and Syk in breast cell cancer lines co-localized in cytosolic punctate complexes in a small percentage of cells (46). TNFα increased the level of punctate complexes suggesting that Syk, previously reported to shuttle between the cytoplasma and the nucleus (65), might facilitate the nuclear export of TRAIP.

Interestingly, none of these described interactors has been reported to be ubiquitinated by TRAIP.

Implications of TRAIP in proliferation and differentiation

a) Mammalian cells

TRAIP is expressed at low levels in a large number of adult tissues such as intestine, lung, brain, skin, testes, thymus and spleen (30,66). The TRAIP mRNA level is strongly decreased in normal human

epidermal primary keratinocytes undergoing differentiation induced by high calcium concentration, high cell density or short phorbol ester treatment (42). TRAIP expression was decreased in the immortalized monocytic mouse cell line (RAW 264.7) induced to undergo differentiation (osteoclastogenesis) (32), and in mouse T lymphocytes upon stimulation with anti-TCR and anti-CD28 antibodies (30), suggesting that its expression is down-regulated in differentiating cells. In addition, TRAIP expression was described as a specific target of the mammalian target of rapamycin (mTOR) (42) signaling, a protein kinase regulating cell growth and starvation (67). In addition, mTOR inhibits autophagy, a conserved lysosomal degradation pathway which is highly active during tissue differentiation and organism development (68). Activation of autophagy was recently proposed to control the morphological reorganization occurring in human keratinocytes during the early steps of differentiation (69), indicating that mTOR-dependent-TRAIP-expression could be implicated in the regulation of the balance between proliferation and differentiation in keratinocytes, and perhaps in other cells.

Knock-down of TRAIP by lentiviral-transduced shRNAs in proliferating primary keratinocytes resulted in strong inhibition of cell proliferation and cell-cycle arrest in the G1/S phase (42). The morphology of keratinocytes after TRAIP knock-down was reminiscent of differentiated cells, consistent with increased expression of the differentiation markers keratin 1 and profilaggrin (42). However, TRAIP knock-down in keratinocytes was not accompanied by an increase in NF-κB activity at 72 hours post-transduction (42). Interestingly, TRAIP is required for early mice development since homozygous TRAIP knock-out mice died in utero at embryonic day 6.5/7.5 due to the failure to develop primitive embryonic tissues (48). This was consistent with decreased number of proliferative cells and excessive apoptosis in TRAIP-/- embryos (48).

b) NOPO, the Drosophila homologue of TRAIP

In a screen for cell cycle regulators, a Drosophila maternal effect-lethal mutant was identified that affected the ‘no poles’ (NOPO) gene (CG5140) (70). The nopo gene encodes a protein containing an N-terminal RING domain that has a 47% sequence identity with the RING domain of human TRAIP and an overall sequence identity of 20% (70). One of the mutant nopo alleles had a glutamic acid to

lysine change at position 11 in the RING domain of NOPO protein, a residue that is highly conserved (invariantly aspartic or glutamic acid) in TRAIP homologues. Mutant nopo embryos undergo mitotic arrest during the rapid S-M cycles of syncytial embryogenesis showing barrel-shaped acentrosomal mitotic spindles as most prominent feature (70). The authors proposed that NOPO is required for the preservation of genomic integrity during early embryogenesis (70). Absence of NOPO leads to the activation of a CHK2-mediated DNA checkpoint which causes mitotic arrest (70). Interestingly, co-expressed Drosophila NOPO and human TRAIP localized to nuclear punctae in interphase HeLa cells, underscoring their functional homology (70). Most likely, the nuclear punctae correspond to nucleoli since both endogenous and ectopically expressed TRAIP has been reported to be nucleolar in mammalian cell lines (46). In a high-throughput yeast two-hybrid screen (71), NOPO interacted with the E2 enzyme Bendless (BEN), the Drosophila homolog of yeast Ubc13 (72,73) and human UBE2N. Using yeast two-hybrid assays the interaction was confirmed using BEN as bait and shown to be abolished using either NOPO RING domain or BEN mutants (70). Drosophila BEN mutants have nopo-like defects (70) indicating that NOPO and BEN are functionally connected. BEN expressed in HeLa cells was found throughout the cells with a predominantly perinuclear staining; however, when co-expressed with TRAIP a large proportion of cells showed expression of BEN in nuclear punctae co-localized with TRAIP (70). These data suggest that TRAIP and BEN can interact, and therefore, one would expect that homologues of BEN would do similarly. However, bacterially purified TRAIP was not ubiquitinated in vitro by Ubc13/Uev1A (32). In addition, UBE2N was not identified as human TRAIP-binding protein in two large yeast two-hybrid screens (33,34). It remains to be seen whether the functional failure of TRAIP/Ubc13 autoubiquitination is due to species differences, to missing partner molecules, i.e. CYLD or Syk, or to the use of an in vitro assay where posttranslational modifications such as phosphorylation, important for some E2/E3 interactions, are missing.

Recently, it has been reported that NOPO modulates the Egr (Eiger, Drosophila TNF ortholog)-induced JNK-independent cell death in Drosophila through the transcriptional upregulation of two pro-apoptotic genes, reaper and hid (74). Genetic engineering experiments further showed that complex formation between NOPO and Ben/dUEV1a is required for NOPO-induced apoptosis.

These results indicate that TRAIP is tightly implicated in the regulation of cellular proliferation and survival consistent with the finding that TRAIP expression is down-regulated when cells leave the cell cycle and undergo differentiation. Putative substrates of TRAIP-mediated ubiquitination might therefore be involved in the control of cell cycle progression and cell cycle exit/differentiation. The balance between cell proliferation and growth arrest/differentiation in epidermis must be tightly controlled to ensure tissue homeostasis. Notch and p63 signaling pathways are important effectors governing epidermal stratification and differentiation after keratinocytes switched from symmetric to asymmetric cell divisions (75-77). It remains to be elucidated whether and how TRAIP affects these signaling pathways.

TRAIP and the nucleolus

As mentioned above, TRAIP is mainly found in the nucleolus in primary cells and cell lines. The nucleolus is a major nuclear substructure which has prominent functions in the synthesis of components required for the formation of ribosomes, and which impinges on the control of numerous cellular processes (78-81). Proteome studies showed that the nucleolus is a dynamic structure whose composition varies during the cell cycle and in response to metabolic conditions (82). Signals for nucleolar sequestration and release of proteins are ill defined and no consensus nucleolar localization sequence has been identified. It has been proposed that protein nucleolar localization may result from high-affinity interaction of specific regions with nucleolar building blocks such as rDNA, rRNA or protein components (83). Several motifs that can target proteins to the nucleolus are composed mostly of Arg and Lys residues ranging in size from 7 to about 30 amino acids (84,85). Inspection of the TRAIP amino acid sequence revealed several regions, mainly in the C-terminal part of the protein, which are moderately enriched in basic amino acids. Furthermore, in silico analysis of TRAIP did not identify nuclear localization sequences, well characterized and moderately conserved motifs predictable by suitable algorithms (86). Cellular localization studies of truncated TRAIP proteins are required to delineate the region(s) responsible for nuclear and nucleolar import. In addition, to fully understand the biological function of TRAIP it will be necessary to determine the impact of its knock-

down or forced expression on the structure and function of the nucleolus and to investigate the cellular fate of TRAIP after nucleolus disassembly during cell mitosis.

TRAIP in human diseases

As shown above, TRAIP interacts with CYLD and Syk which are tumor suppressors implicated in the formation of skin appendages tumors such as cylindroma, trichoepithelioma and spiradenoma for CYLD (50,87) and of melanomas and breast tumors for both CYLD and Syk (61-63,88,89). Interestingly, TRAIP expression is increased in basal cell carcinomas (42) and in multiple breast epithelial cell lines with oncogenic potentials ranging from nonmalignant to highly invasive (46) whereas, concomitantly, the tumor suppressor expression levels are decreased in basal cell carcinomas (CYLD) (90) and in breast epithelial cell lines (CYLD and Syk) (46,88). Thus, the question whether TRAIP plays a direct pathogenic role in tumorigenesis and whether CYLD and/or Syk suppress aberrant cell proliferation by controlling TRAIP activity through deubiquitination and/or phosphorylation are topics for future research.

A two-stage candidate gene association study of chromosome 3p21 with inflammatory bowel disease demonstrated an association between a single nucleotide polymorphism (SNP) in an intron of the human *TRAIP* gene and Crohn's disease (91). Because of the localization of this SNP in the 3'non-translated region of the gene the authors argue that *TRAIP* is unlikely to play an important role in disease pathogenesis but may be in linkage disequilibrium with two nonsynonymous SNPs in the macrophage stimulating 1 receptor gene. Furthermore, an association analysis of 3p21 with Crohn's disease using SNPs in the New Zealand population excluded *TRAIP* as candidate gene (92). Nevertheless, differential gene expression analysis showed a decrease of TRAIP gene expression in Crohn's disease patients (93).

Conclusions

The initially described interaction of TRAIP with TRAF proteins and the ensuing negative regulation of NF-κB signaling seem to be caused by the non-physiological binding of over-expressed TRAIP to the C-terminal region of TRAF proteins. Both the C-terminal part of TRAF proteins (TRAF domain)

and the region of TRAIP protein identified to bind TRAF form coiled-coils, secondary structure elements well known to provide protein-protein interactions. In fact, coiled-coils have been specifically used to engineer proteins so that they can interact with non-physiological partner proteins (94). Furthermore, the finding that suppression of TRAIP expression in keratinocytes did not increase the basal NF-κB activity (42) provided strong evidence that the physiological roles of TRAIP extend beyond the regulation of NF-κB activity.

Based on recently published data in keratinocytes (42) and the finding that TRAIP expression is down-regulated when cells leave the cell cycle and undergo differentiation in other cell types (30,32) we conclude that the E3 ubiquitin ligase TRAIP is required for cellular proliferation, most likely by controlling cell cycle progression from G1 to S. The two TRAIP-interacting tumor supressors CYLD and Syk negatively affect cellular proliferation which may suggest that all three act together to regulate various physiological processes as depicted in Fig. 3. Whether CYLD or Syk activity changes both TRAIP posttranslational modifications such as ubiquitination or phosphorylation and/or indirectly gene expression is a central question to understand the biological function of TRAIP. Another important challenge in the future will be the identification of proteins that are ubiquitinated by TRAIP and the E2 enzyme(s) collaborating with TRAIP. Identification of bona fide substrates of TRAIP will be rather difficult using current methodology due to the inherent weak interaction of most E3 ubiquitin ligases with their substrates. None of the currently available methods appears to work in a general and predictable way, so that several biochemical and genetically approaches will probably be required to identify TRAIP substrates.

The development of mouse models ectopically expressing TRAIP or in which TRAIP expression is ablated will be required to confirm the results obtained by in vitro experiments and to define more precisely physiological pathways that depend on TRAIP. Due to the embryonic lethality of TRAIP knock-out mice (48), inducible systems are required to study the effects of TRAIP ablation in different tissues. Efforts to generate these animals are underway in the laboratory. Combining the results of these approaches should improve our understanding of cell cycle control and may deliver new opportunities for therapeutic interventions in human diseases such as tumor formation.

Acknowledgements

This work has been supported by the Swiss National Science Foundation grant 3100A-118093 to MH.

C.C., D.H.and M.H. wrote the paper.

Conflict of interest

The authors declare no conflict of interest.

References

1. Dikic I, Wakatsuki S, Walters K J. Nat Rev Mol Cell Biol 2009: **10**: 659-671
2. Welchman R L, Gordon C, Mayer R J. Nat Rev Mol Cell Biol 2005: **6**: 599-609
3. Ikeda F, Dikic I. EMBO Rep 2008: **9**: 536-542
4. Iwai K, Tokunaga F. EMBO Rep 2009: **10**: 706-713
5. Clague M J, Urbe S. Cell 2010: **143**: 682-685
6. Ikeda F, Crosetto N, Dikic I. Cell 2010: **143**: 677-681
7. Matsumoto M L, Wickliffe K E, Dong K C *et al.* Mol Cell 2010: **39**: 477-484
8. Thrower J S, Hoffman L, Rechsteiner M *et al.* EMBO J 2000: **19**: 94-102
9. Wickliffe K, Williamson A, Jin L *et al.* Chem Rev 2009: **109**: 1537-1548
10. Kirisako T, Kamei K, Murata S *et al.* EMBO J 2006: **25**: 4877-4887
11. Gerlach B, Cordier S M, Schmukle A C *et al.* Nature 2011: **471**: 591-596
12. Haas T L, Emmerich C H, Gerlach B *et al.* Mol Cell 2009: **36**: 831-844
13. Ikeda F, Deribe Y L, Skanland S S *et al.* Nature 2011: **471**: 637-641
14. Rahighi S, Ikeda F, Kawasaki M *et al.* Cell 2009: **136**: 1098-1109
15. Tokunaga F, Nakagawa T, Nakahara M *et al.* Nature 2011: **471**: 633-636
16. Tokunaga F, Sakata S, Saeki Y *et al.* Nat Cell Biol 2009: **11**: 123-132
17. Komander D, Clague M J, Urbe S. Nat Rev Mol Cell Biol 2009: **10**: 550-563
18. Nagy V, Dikic I. Biol Chem 2010: **391**: 163-169
19. Liu Y C, Penninger J, Karin M. Nat Rev Immunol 2005: **5**: 941-952
20. Deshaies R J, Joazeiro C A. Annu Rev Biochem 2009: **78**: 399-434
21. Wertz I E, Dixit V M. Cell Death Differ 2010: **17**: 14-24
22. Matthews J M, Sunde M. IUBMB Life 2002: **54**: 351-355
23. Saurin A J, Borden K L, Boddy M N *et al.* Trends Biochem Sci 1996: **21**: 208-214
24. Zheng Q, Zhao Y. Biol Cell 2007: **99**: 489-502
25. Bienz M. Trends Biochem Sci 2006: **31**: 35-40
26. Hatakeyama S, Nakayama K I. Biochem Biophys Res Commun 2003: **302**: 635-645
27. Cyr D M, Hohfeld J, Patterson C. Trends Biochem Sci 2002: **27**: 368-375

28. Yen H C, Elledge S J. Science 2008: **322**: 923-929

29. Emanuele M J, Elia A E, Xu Q *et al.* Cell 2011: **147**: 459-474

30. Lee S Y, Choi Y. The Journal of experimental medicine 1997: **185**: 1275-1285

31. Ying M, Huang X, Zhao H *et al.* PLoS One 2011: **6**: e23863

32. Besse A, Campos A D, Webster W K *et al.* Biochem Biophys Res Commun 2007: **359**: 660-664

33. van Wijk S J, de Vries S J, Kemmeren P *et al.* Mol Syst Biol 2009: **5**: 295

34. Markson G, Kiel C, Hyde R *et al.* Genome Res 2009: **19**: 1905-1911

35. Jones D T. J Mol Biol 1999: **292**: 195-202

36. Burkhard P, Stetefeld J, Strelkov S V. Trends Cell Biol 2001: **11**: 82-88

37. Landschulz W H, Johnson P F, McKnight S L. Science 1988: **240**: 1759-1764

38. Lupas A N, Gruber M. Adv Protein Chem 2005: **70**: 37-78

39. Sharif O, Bolshakov V N, Raines S *et al.* BMC Immunol 2007: **8**: 1

40. Gressner O, Schilling T, Lorenz K *et al.* EMBO J 2005: **24**: 2458-2471

41. Jiang H, Xia J, Kang J *et al.* Scand J Gastroenterol 2009: **44**: 1452-1462

42. Almeida S, Ryser S, Obarzanek-Fojt M *et al.* J Invest Dermatol 2011: **131**: 349-357

43. Cam H, Balciunaite E, Blais A *et al.* Mol Cell 2004: **16**: 399-411

44. Regamey A, Hohl D, Liu J W *et al.* The Journal of experimental medicine 2003: **198**: 1959-1964

45. Wong B R, Josien R, Lee S Y *et al.* J Biol Chem 1998: **273**: 28355-28359

46. Zhou Q, Geahlen R L. Oncogene 2009: **28**: 1348-1356

47. Birbach A, Gold P, Binder B R *et al.* J Biol Chem 2002: **277**: 10842-10851

48. Park E S, Choi S, Kim J M *et al.* Biochem Biophys Res Commun 2007: **363**: 971-977

49. Krishnan S, Intlekofer K A, Aggison L K *et al.* Proc Natl Acad Sci U S A 2009: **106**: 16692-16697

50. Bignell G R, Warren W, Seal S *et al.* Nat Genet 2000: **25**: 160-165

51. Brummelkamp T R, Nijman S M, Dirac A M *et al.* Nature 2003: **424**: 797-801

52. Kovalenko A, Chable-Bessia C, Cantarella G *et al.* Nature 2003: **424**: 801-805

53. Trompouki E, Hatzivassiliou E, Tsichritzis T *et al.* Nature 2003: **424**: 793-796

54. Reiley W, Zhang M, Sun S C. J Biol Chem 2004: **279**: 55161-55167

55. Tauriello D V, Haegebarth A, Kuper I *et al.* Mol Cell 2010: **37**: 607-619

56. Komander D, Reyes-Turcu F, Licchesi J D *et al.* EMBO Rep 2009: **10**: 466-473

57. Massoumi R, Chmielarska K, Hennecke K *et al.* Cell 2006: **125**: 665-677

58. Wickstrom S A, Masoumi K C, Khochbin S *et al.* EMBO J 2010: **29**: 131-144

59. Stegmeier F, Sowa M E, Nalepa G *et al.* Proc Natl Acad Sci U S A 2007: **104**: 8869-8874

60. Sun L, Gao J, Huo L *et al.* J Pathol 2010: **221**: 425-432

61. Bailet O, Fenouille N, Abbe P *et al.* Cancer Res 2009: **69**: 2748-2756

62. Coopman P J, Do M T, Barth M *et al.* Nature 2000: **406**: 742-747

63. Moroni M, Soldatenkov V, Zhang L *et al.* Cancer Res 2004: **64**: 7346-7354

64. Takada Y, Aggarwal B B. J Immunol 2004: **173**: 1066-1077

65. Zhou F, Hu J, Ma H *et al.* Mol Cell Biol 2006: **26**: 3478-3491

66. Su A I, Wiltshire T, Batalov S *et al.* Proc Natl Acad Sci U S A 2004: **101**: 6062-6067

67. Zoncu R, Efeyan A, Sabatini D M. Nat Rev Mol Cell Biol 2011: **12**: 21-35

68. Mizushima N, Levine B. Nat Cell Biol 2010: **12**: 823-830

69. Aymard E, Barruche V, Naves T *et al.* Experimental dermatology 2011: **20**: 263-268

70. Merkle J A, Rickmyre J L, Garg A *et al.* Development 2009: **136**: 449-459

71. Giot L, Bader J S, Brouwer C *et al.* Science 2003: **302**: 1727-1736

72. Muralidhar M G, Thomas J B. Neuron 1993: **11**: 253-266

73. Oh C E, McMahon R, Benzer S *et al.* J Neurosci 1994: **14**: 3166-3179

74. Ma X, Huang J, Yang L *et al.* Cell research 2011:

75. Dotto G P. Nat Rev Cancer 2009: **9**: 587-595

76. Koster M I, Dai D, Roop D R. Cell Cycle 2007: **6**: 269-273

77. Williams S E, Beronja S, Pasolli H A *et al.* Nature 2011: **470**: 353-358

78. Boisvert F M, van Koningsbruggen S, Navascues J *et al.* Nat Rev Mol Cell Biol 2007: **8**: 574-585

79. Martindill D M, Riley P R. Cell Cycle 2008: **7**: 17-23

80. Mayer C, Grummt I. Cell Cycle 2005: **4**: 1036-1038

81. Raska I, Shaw P J, Cmarko D. Curr Opin Cell Biol 2006: **18**: 325-334

82. Lam Y W, Lamond A I, Mann M *et al.* Curr Biol 2007: **17**: 749-760

83. Carmo-Fonseca M, Mendes-Soares L, Campos I. Nat Cell Biol 2000: **2**: E107-112

84. Birbach A, Bailey S T, Ghosh S *et al.* J Cell Sci 2004: **117**: 3615-3624

85. Reed M L, Dove B K, Jackson R M *et al.* Traffic 2006: **7**: 833-848

86. Cokol M, Nair R, Rost B. EMBO Rep 2000: **1**: 411-415

87. Lee D A, Grossman M E, Schneiderman P *et al.* J Med Genet 2005: **42**: 811-819

88. Hutti J E, Shen R R, Abbott D W *et al.* Mol Cell 2009: **34**: 461-472

89. Massoumi R, Kuphal S, Hellerbrand C *et al.* The Journal of experimental medicine 2009: **206**: 221-232

90. Kuphal S, Shaw-Hallgren G, Eberl M *et al.* Oncogene 2011: **30**: 4523-4530

91. Beckly J B, Hancock L, Geremia A *et al.* Inflamm Bowel Dis 2008: **14**: 500-507

92. Morgan A R, Han D Y, Lam W J *et al.* Hum Immunol 2010: **71**: 602-609

93. Hughes A L. Immunogenetics 2005: **57**: 709-716

94. Nikolaev Y, Deillon C, Hoffmann S R *et al.* PLoS One 2010: **5**: e10765

95. Lupas A, Van Dyke M, Stock J. Science 1991: **252**: 1162-1164

96. McDonnell A V, Jiang T, Keating A E *et al.* Bioinformatics 2006: **22**: 356-358

97. Bornberg-Bauer E, Rivals E, Vingron M. Nucleic Acids Res 1998: **26**: 2740-2746

Figure Legends

Figure 1: Ubiquitin is activated in an ATP-dependant manner by the ubiquitin-activating enzyme E1. Activated ubiquitin is transferred to an ubiquitin-conjugating enzyme E2 and subsequently to an ubiquitin ligase E3 which catalyzes the transfer of ubiquitin to a lysine residue of the substrate (mono-ubiquitination). Linkage of additional ubiquitins either to different lysines of the substrate or to lysines (K6, K11, K27, K29, K33, K48 or K63) of the already attached ubiquitin monomer produces multi-ubiquitinated or poly-ubiquitinated proteins, respectively. Ubiquitin moieties can be removed in a reverse process carried out by deubiquitinating enzymes (DUB).

Figure 2: Schematic diagram of TRAIP showing the positions of the RING-finger, coiled-coil region and leucine zipper domains and locations of binding sites for TRAF proteins, and CYLD and SYK. The numbers below the domains indicate the percentage of sequence identity of mouse TRAIP (mTRAIP), Xenopus (xTRAIP) and Drosophila *melanogaster* homolog of TRAIP (dTRAIP) with human TRAIP (hTRAIP). The double arrows indicate regions with high sequence identities in vertebrate TRAIPs. The following GenBank sequences were used: hTRAIP NP_005870.2; mTRAIP NP_035764.2; xTRAIP NP_001084838.1; and dTRAIP NP-611305.1. Coiled-coil and leucine zipper domain annotations were based on predictive analysis of TRAIP sequences using the following programs: COILS version 2.2 (95), Paircoil2 (96) and 2ZIP (97).

Figure 3: The scheme illustrates the relationship of TRAIP with CYLD, Syk and TRAFs and the different cellular processes these TRAIP interactors impinge on. Double arrows means that the two proteins interact but that it is not yet clear in which way the activity of the partner protein is regulated. The following abbreviations have been used: NF-κB, nuclear factor κB; NFAT, nuclear factor of activated T cells; PI3K, phosphoinositide-3 kinase; PKC, protein kinase C; DAG, diacylglycerol; MAPK, mitogen activated protein kinase, PLCγ, phospholipase Cγ; PLK1, polo-like kinase 1; HDAC6, histone deacetylase 6; Bcl-3, B-cell lymphoma 3 protein.

Figure 1

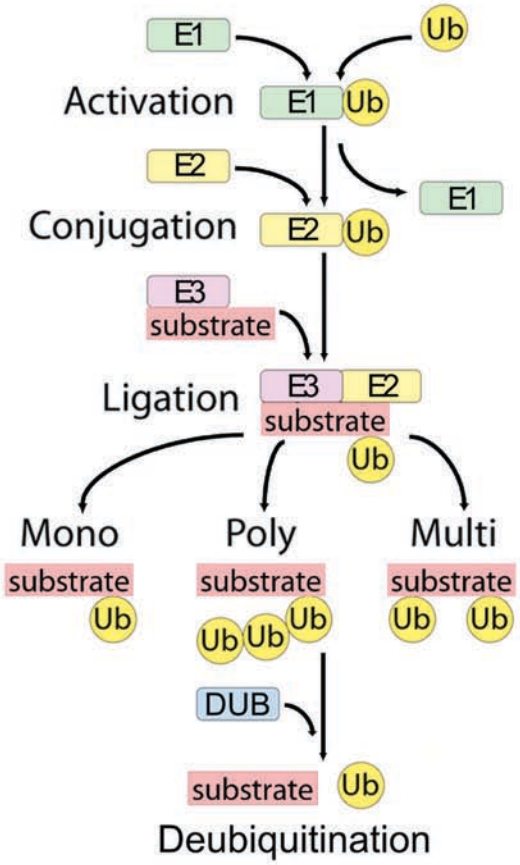

E1
Ub
Activation
E1
Ub
E2
E1
Conjugation
E2
Ub
E3
substrate
Ligation
E3
E2
substrate
Ub
Mono
Poly
Multi
substrate
Ub
substrate
Ub
Ub
Ub
substrate
Ub
Ub
DUB
substrate
Ub
Deubiquitination

Figure 2

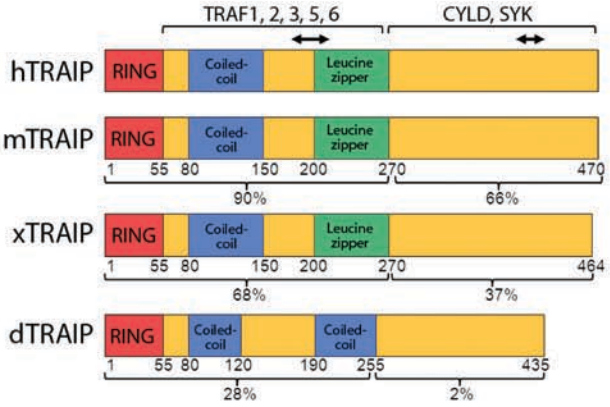
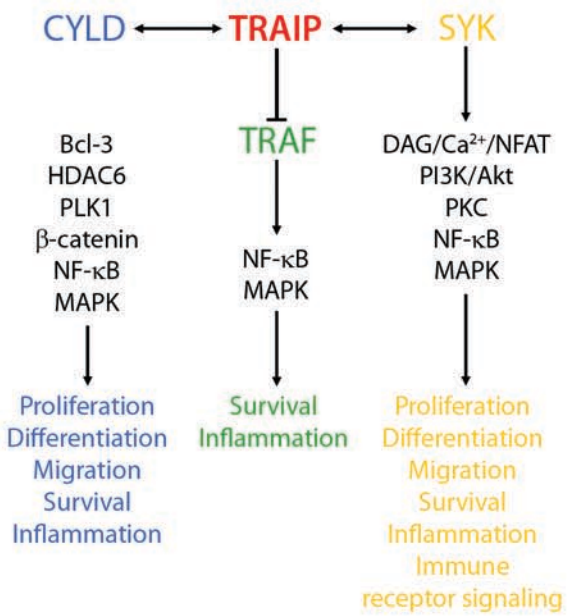

TRAF1, 2, 3, 5, 6
CYLD, SYK
hTRAIP
RING
Coiled-coil
Leucine zipper
mTRAIP
RING
Coiled-coil
Leucine zipper
1 55 80 150 200 270 470
90%
66%
xTRAIP
RING
Coiled-coil
Leucine zipper
1 55 80 150 200 270 464
68%
37%
dTRAIP
RING
Coiled-coil
Coiled-coil
1 55 80 120 190 255 435
28%
2%

Figure 3


CYLD
TRAIP
SYK
TRAF
Bcl-3
HDAC6
PLK1
β-catenin
NF-κB
MAPK
NF-κB
MAPK
DAG/Ca2+/NFAT
PI3K/Akt
PKC
NF-κB
MAPK
Proliferation
Differentiation
Migration
Survival
Inflammation
Survival
Inflammation
Proliferation
Differentiation
Migration
Survival
Inflammation
Immune
receptor signaling