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MASTERARBEIT

Titel der Masterarbeit

„The role of the ubiquitin ligase TRIM9 in immune signalling“

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Abstract

The tri-partite motif (TRIM) family is an E3 ligase family with over 70 members in humans, of which several have already been shown to have functions in regulating the innate immune system. The focus of this thesis is on TRIM9 and TRIM67, two closely related members of this family, whose functions are yet largely unknown. Both TRIM9 and TRIM67 are known to be mainly expressed in the brain, but previous experiments have shown that TRIM9 was also expressed in macrophages and interacts with p47^{phox}, a member of the NADPH oxidase complex, which is important for Reactive Oxygen Species (ROS) production. Recently it has also been shown that TRIM9 interacts with β-TrCP (F-box/WD repeat-containing protein 11) and that this interaction regulates the expression of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and therefore regulates the immune response upon infection. In this thesis the interaction of TRIM9 and p47^{phox} has been further supported by experiments using immunofluorescence. Further, the interaction of TRIM9 and β-TrCP has been confirmed, while it was also observed that TRIM67 and β-TrCP interact. Additionally, it was observed that TRIM9 and β-TrCP influence the amount of protein expression of each other, however the mechanism remains unknown.

Introduction

The innate immune system is necessary for the detection of pathogens, ensures survival during infection and activates the slower but more specific adaptive immune system. Once pathogens are detected by pattern recognition receptors (TLRs, RLRs, NLRs), proinflammatory cytokines and interferons are released to activate the adaptive immune system. In addition, anti-inflammatory cytokines are synthesized, which in turn are essential once the infection has been fought off to enable the cells to return to their homeostatic state. To ensure a sufficient but not excessive immune response the immune system needs to be tightly, but also quickly regulated. Posttranslational modifications such as phosphorylation, acetylation and ubiquitination allow swift changes in the immune response. Ubiquitination is already well-known to have a significant role in degrading proteins, but its immunological impact has not been as well studied [1,2,5].

During the ubiquitination process, E3 ubiquitin ligases recognize substrates and catalyze the transfer of a ubiquitin protein to the substrate, resulting in the specificity of the modification. This thesis focuses on the E3 ligase family called the tripartite motif (TRIM) family [1, 2]. The tripartite motif family is a protein family with over 70 members in humans and is suggested to have several functions in cell proliferation, differentiation, oncogenesis, apoptosis, immune signaling, autoimmune disease and anti-viral activity, but the mechanisms of action are mostly unknown [3, 6]. Nevertheless, for those that are already well studied, major roles in immune regulation could be found, for example that TRIM25 is critical for RIG-I activation after RNA-virus infection [2]. All TRIM family members contain a RING (Really Interesting New Gene) finger domain with potential E3 ubiquitin ligase function, one or two B-Box domains and a coiled-coil domain (CC). In addition, some TRIMs have other C-terminal domains, e.g. the SPRY domain, enabling protein-protein interaction [3, 6, 7].

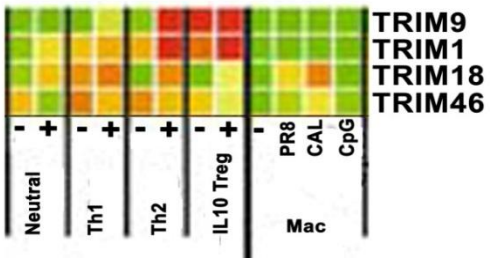

Figure 1: TRIM9 has a unique mRNA expression profile. Heat map of a mRNA expression profile of TRIM family members in CD4+ T cells, Macrophages and dendritic cells. TRIM9 is upregulated in uninduced and induced IL-10 expressing T-regulatory cells and in induced Th2-cells. Image from *Rajsbaum, R. et al* [3].

An mRNA expression profile of TRIMs that was performed in $CD4^+$ cells, macrophages and dendritic cells (DCs) revealed a unique pattern of TRIM9 expression (Fig.1). TRIM9 showed low to no mRNA expression in unstimulated and stimulated macrophages and DCs, but high mRNA expression in $CD4^+$ cells, especially in the IL-10 producing subsets: stimulated Th2 and both unstimulated and stimulated IL-10 T-regulatory cells [3]. Until now TRIM9 was thought to have only brain-specific expression and functions in repressing Parkinson's disease and dementia, but the results of this experiment suggest that TRIM9 is expressed in these specific $CD4^+$ Th cells and may play a biological role in the induction of IL-10 or the response of these cells to IL-10 [3,8].

The phylogenetically closest relative to TRIM9 within the TRIM family is TRIM67 (Fig.2b). Both TRIMs belong to the same subgroup of TRIMs that contains the variable domains COS, FN3 and PRY/SPRY (Fig.2a). The biological role of TRIM67 is unknown, but, like TRIM9, it is thought to be mainly expressed in the brain [16].

Recent research has shown that stimulation of TRIM9 leads to an increase of IFNβ and a decrease of NF-κB which are both well known for their immunological role. The regulatory function of TRIM9 on NF-κB has been recently described as a result of its interaction with β-TrCP [15].

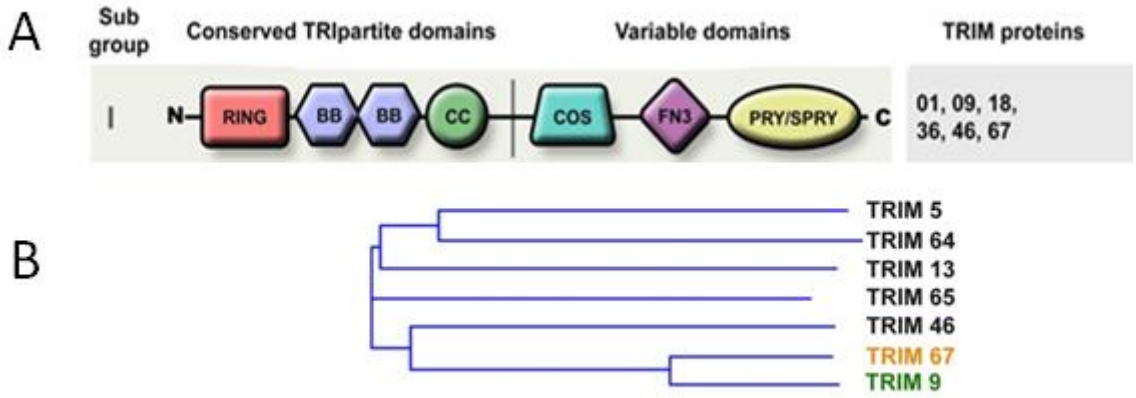

Figure 2: TRIM9 and TRIM67 are very similar to each other. (A) TRIM9 and TRIM67 are both members of the TRIM subgroup containing a COS-, FN3- and PRY/SPRY-domain. (B) Phylogenetic tree of part of the TRIM family.

Preliminary data

To determine interactors of TRIM9 our group previously performed a pull-down assay of TRIM9. The pulled down proteins were analyzed by mass spectrometry and revealed proteins with immune regulatory properties as possible interactors for TRIM9. This encouraged further research (Table.1). Co-immunoprecipitation, performed by Esther Drent in our lab, has further supported that TRIM9 interacts with $p47^{phox}$ and PP2A and the site of interaction between TRIM9 and $p47^{phox}$ has been narrowed down to the B-Box domain and the COS-domain (Fig.3). Nevertheless, it should be mentioned that these previous experiments have been performed in HEK-293T cells using overexpression of TRIM9 and $p47^{phox}$. In future it will be important to confirm these results with endogenous proteins in relevant primary cells.

#peptides	Gene symbol	Gene name
48	TRIM9	Tripartite motif-containing protein 9
20	2AAA	Serine/threonine-protein phosphatase 2A 65kDa regulatory subunit A
17	2ABA	Serine/threonine-protein phosphatase 2A 55kDa regulatory subunit B
13	NCF1	Neutrophil cytosol factor1 (p47phox)
10	PP2AA	Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform
10	PP2AB	Serine/threonine-protein phosphatase 2A catalytic subunit beta isoform
4	PABP4	Polyadenylate-binding protein 4
4	CUL1	Cullin-1
3	FBW1B	F-box/WD repeat-containing protein 11

Table1: The interaction partners of TRIM9. A pull-down assay on TRIM9 has been performed and the interacting proteins have been determined using mass spectrometry.

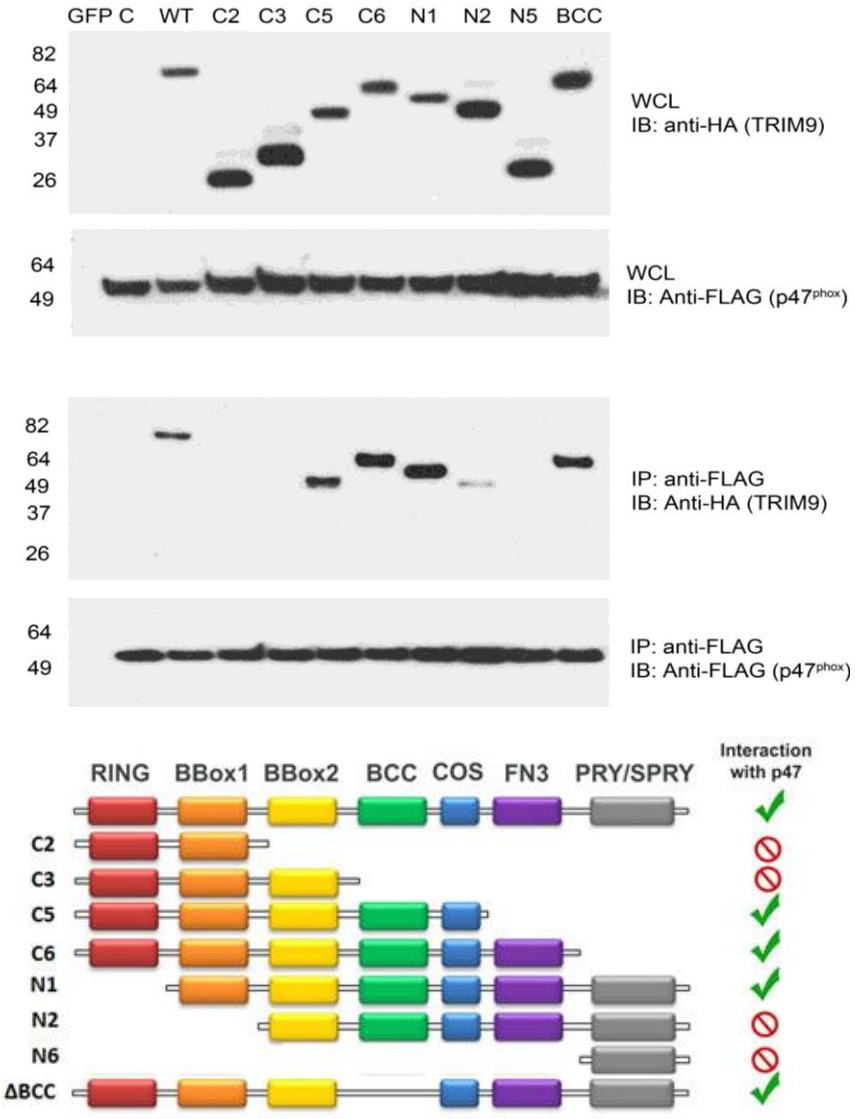

Figure 3: TRIM9 interacts with p47^{phox} at its BBox1 & COS-domain. HA-tagged TRIM9 mutants of different lengths (see schematic image below) have been expressed in 293T cell line together with Flag-tagged p47^{phox}. A pull-down assay on the Flag-tag was performed. The TRIM9 mutants that interact with p47^{phox} were pulled down.Below is a schematic display of the results on interaction between TRIM9 and p47^{phox}. Ticks on the right side indicate a positive pull-down of both interaction partners when pulling only down one using co-immunoprecipitation.

P47^{phox} is one of the subunits of the NADPH oxidase with functions in macrophages as well as T-lymphocytes. P47^{phox} deficient mice are known to be very prone to bacterial as well as fungal infections and inflammatory diseases [10, 11].

In macrophages, p47^{phox} is involved in the regulation of cytokine- and protein expression, especially in IL-4 signaling which leads to alternatively activated macrophages. Alternatively activated macrophages stimulate towards resolution of inflammation and produce anti-inflammatory cytokines and metabolic factors such as arginase (Fig.4). In contrast, classically activated macrophages drive inflammatory cytokines and produce ROS [10, 12]. Therefore, p47^{phox} has the ability to not only upregulate the immune system but also to downregulate it and needs to be regulated tightly itself. It is tempting to speculate that TRIM9 could be part of this regulation.

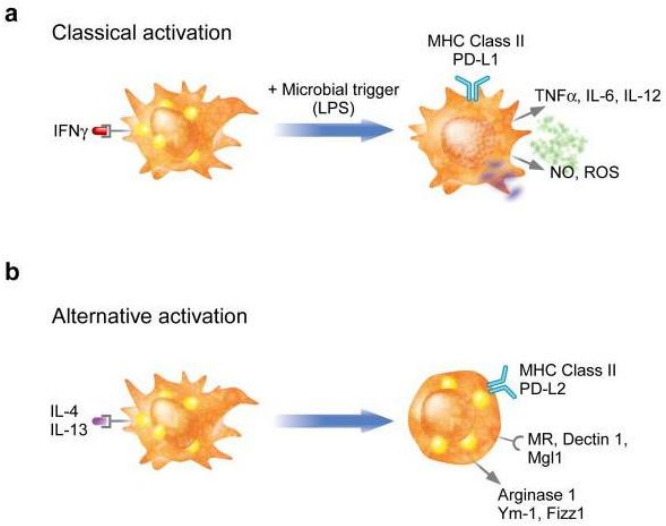

Figure 4: Schematic display of the activation pathways of macrophages. Image from Odegaard, JI et al. [4,5]

Interestingly, p47^{phox} has also been reported to play an additional role in non-phagocytic cells. In CD8^{+} cells, it was found to regulate the interactions between PP2A and FOXO3a [9]. The increased incidence of autoimmune diseases in p47^{phox} deficient mice could suggest a function in T lymphocytes such as regulatory T-cells (Treg). Tregs are known to suppress autoimmune T-cell response and are important for the downregulation of immune responses. It is suggested that macrophages induce Tregs in a ROS-dependent way [11]. P47^{phox} deficient mice also show an increase in B-cell and memory CD8^{+} cell divisions as well as increased accumulation of naïve B- and T-lymphocytes. This is thought to be due to an intrinsic survival defect of apoptotic pathways [11, 13, 14].

Hypothesis

TRIM9 interacts with the immunological relevant proteins p47^{phox} and β-TrCP. If I transfect both HA-tagged TRIM9 and Flag-tagged p47^{phox} / β-TrCP into HeLa cells and use immunofluorescence against the tags to detect the proteins within the cells, they will co-localize. Even though TRIM67 has a very similar structure to TRIM9 they are not completely abundant.

The aims of this thesis were to **i)** confirm the interaction between TRIM9 and p47^{phox} by a third independent method (immunofluorescence) and to detect if TRIM9 and p47^{phox} co-localize; **ii)** to confirm the interaction of TRIM9 and β-TrCP by co-immunoprecipitation and to detect if they co-localize; **iii)** to analyze if TRIM67 interacts with either p47^{phox} and/or β-TrCP by co-immunoprecipitation and to detect if they co-localize after overexpression in HeLa cell line; **iv)** to determine differences and similarities between TRIM9 and TRIM67.

Results

TRIM9 and p47^{phox} co-localize upon overexpression

In past experiments, the interaction partners of TRIM9 were determined by using a mass spectrometry screening method. Transfected HA-TRIM9 was pulled down using antibodies against the HA-tag and the proteins that were interacting with TRIM9 were detected by mass spectrometry (Table 1). One of these hits, p47^{phox}, a protein which is already known to have immunological functions, encouraged further research. A co-immunoprecipitation assay between HA-TRIM9 and Flag-p47^{phox} suggested further that TRIM9 and p47^{phox} do interact. To provide further confirmation for these results an immunofluorescene assay with overexpressed HA-TRIM9 and Flag-p47^{phox} in HeLa Kyoto cells was performed. In order to interact with each other two proteins need to be in immediate proximity and their localization can be detected by using antibodies against the tags of the proteins coupled to fluorescent dyes. p47^{phox} and TRIM9 transfected HeLa Kyoto cells were fixed, permeabilized and incubated with antibodies (rabbit-α-Flag, mouse-α-HA) and DAPI to visualize the DNA, p47^{phox} and TRIM9 within the cells. As negative controls single stains had been performed (data not shown) and HA-GST was used to determine the localization of non-interacting proteins which displayed a dispersed localization throughout the cytoplasm for both GST and p47^{phox} (Fig.5a). In contrast, TRIM9 and p47^{phox} accumulated in either filaments around the nucleus (Fig.5b) and/or in dots throughout the cytoplasm (Fig.5c). Together, these results suggest that TRIM9 and p47^{phox} interact upon overexpression.

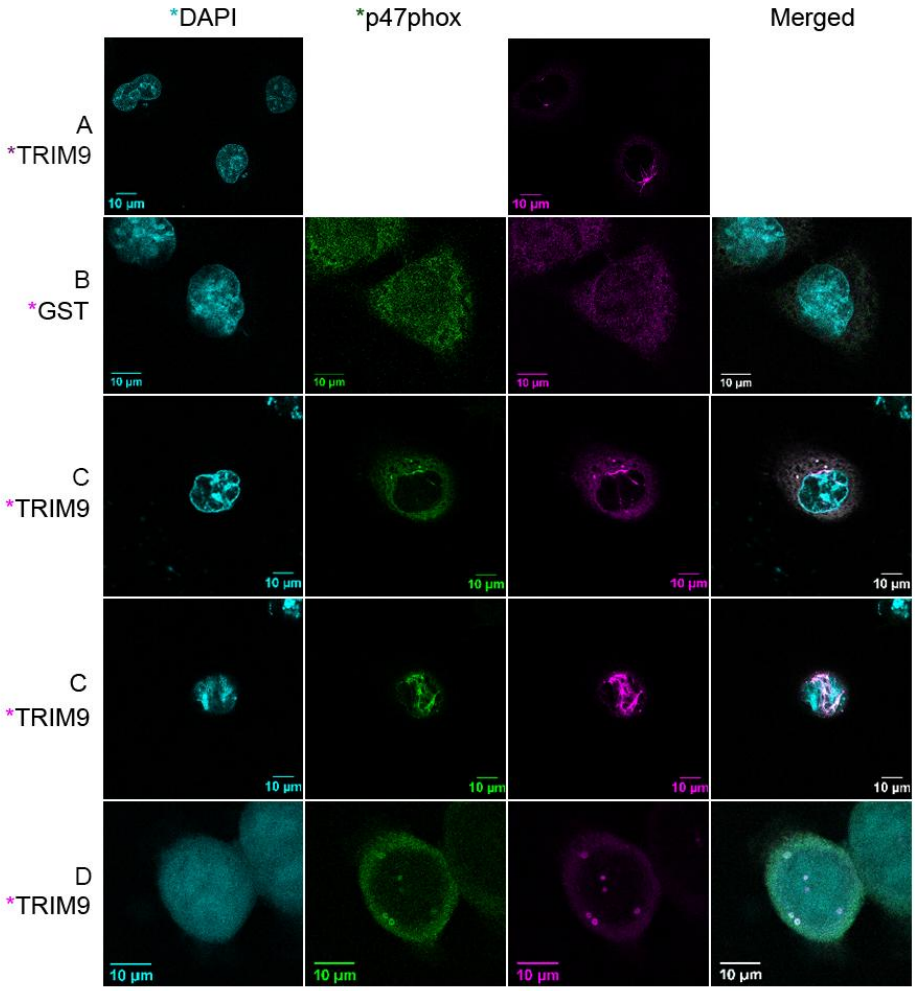

Figure 5: TRIM9 and p47phox co-localize in dots and filaments. HeLa Kyotos were transfected with (A) HA-TRIM9, (B) HA-GST & p47phox (C) & (D) HA-TRIM9 & p47phox, each 1µg and analyzed via immune fluorescence against the Flag- & HA-tags. (A) TRIM9 localizes in ball-like looking filaments as well as in dots (not shown). (B) GST and p47phox both are localized dispersed in the cytoplasm. (C) Both the upper and the lower images display the same cell but at a different focus in the z-layer. TRIM9 and p47phox co-localize in ball-like looking filaments around the nucleus (DAPI). (D) TRIM9 and p47phox co-localize in dots.

TRIM9 expression influences β-TrCP expression while TRIM9-SA-mutant does not

Initially, a screening for TRIM9 interaction partners was performed by using a pull-down method together with mass spectrometry which revealed β-TrCP as a possible interaction partner (Table1). β-TrCP is known to be a part of the NF-kB signaling pathway, which is very crucial for regulating the innate immune system and therefore an interesting target to study. To further study the relationship between TRIM9 and β-TrCP, co-immunoprecipitation was used but during the preparations of this experiment we observed that TRIM9 expression influenced the expression of β-TrCP. First, different amounts of DNA (HA-TRIM9 and Flag-β-TrCP) were co-transfected into HEK 293T cell line (human embryonic kidney) and the protein expression was quantified in relation to one another via Western blots, to ensure an equal amount of protein throughout the experiment. Interestingly, while TRIM9 expression matches the amount of DNA that was transfected, it seems that β-TrCP expression decreases upon greater TRIM9 expression (Fig.6). It is known, that β-TrCP interacts with Inhibitor of NF-κB α (IκBα) by detecting its phosphorylated degron motif (DSGXXS) and that TRIM9 contains the sequence DSGYGS, by which the two proteins can interact. To detect if the motif was necessary for the interaction, a TRIM9 mutant was used in which the serines within the degron motif were substituted with alanines (TRIM9-SA-mutant) [15]. Co-transfection between β-TrCP and TRIM9-SA-mutant revealed that β-TrCP expression did not decrease upon increasing amounts of TRIM9-SA-mutant (Fig.6). These data suggest that TRIM9 expression has a regulatory effect on β-TrCP expression and that the degron motif is necessary for this effect.

Co-expression of β-TrCP with TRIM67 did not decrease β-TrCP expression. However, since the overall expression of TRIM67 is very low in relation to TRIM9 and TRIM9-SA protein expression the result could change upon higher TRIM67 expression.

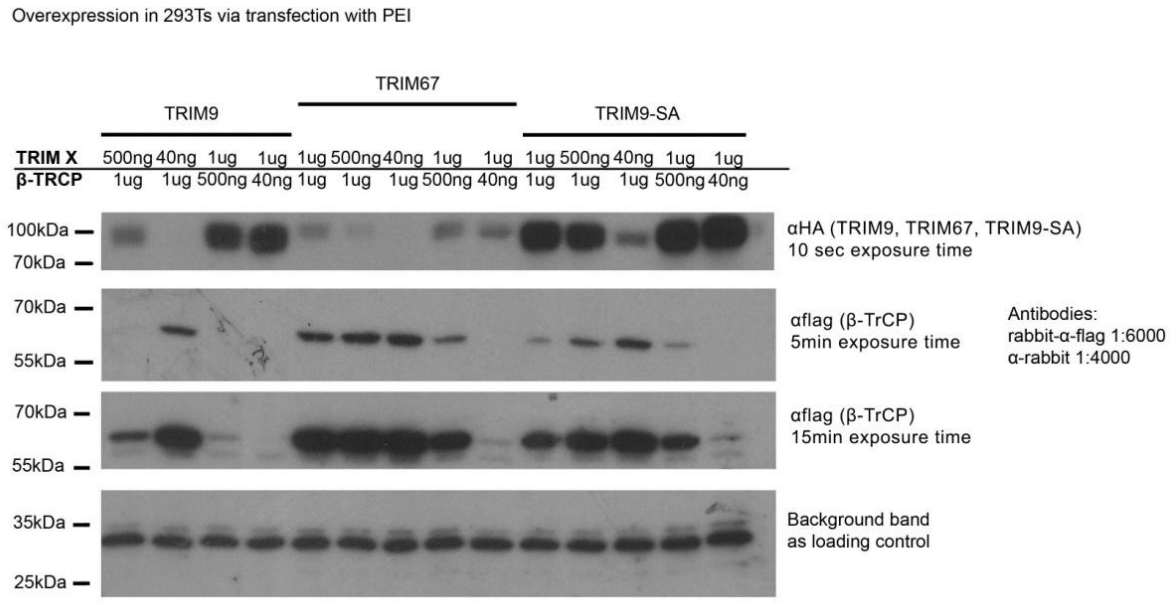

Figure 6: TRIM9 influences the expression levels of β-TrCP. The displayed amounts of Flag-β-TrCP and either HA-TRIM9,HA-TRIM67 or HA-TRIM9-SA mutant were transfected into HEK 293Ts, harvested 72h later, lysed and analyzed by western blot using antibodies against the tags. Two different exposure times of β-TrCP were used to better illustrate the expression levels. A background band was used as loading control together with PonceauS staining (not shown). Increasing amounts of TRIM9 expression lead to decreasing expression of β-TrCP, while TRIM9-SA mutant does not lead to this effect.

TRIM9 and TRIM67 interact with β-TrCP

To investigate whether TRIM9 and β-TrCP interact, we performed co-immunoprecipitation analysis. Since TRIM67 is very similar to TRIM9 in terms of domains and phylogenetic origin, it was included on one hand as a control and on the other to detect differences and similarities between those two very similar TRIMs. TRIM9-SA mutant was used as negative control for interaction. Flag-β-TrCP and HA-TRIM9/TRIM67/TRIM9-SA were co-transfected into HEK 293T cell line, lysed and co-immunoprecipitated for Flag-β-TrCP. Constant expression of β-TrCP can be appreciated both in the WCE (whole cell extract) and in the IP blot. TRIM9 and TRIM67 were detected relatively weak in the WCE, especially when co-expressed with β-TrCP, but, nevertheless, both TRIM9 and TRIM67 were pulled down efficiently by β-TrCP (Fig.7). TRIM9-SA signal was present both in the pull-down samples with and without β-TrCP. A possible explanation could be that TRIM9-SA was not washed away sufficiently during the washing steps since the expression of TRIM9-SA in the WCE was about 10x as strong compared to TRIM9 and TRIM67 expression. Nevertheless, it is obvious that TRIM9-SA was pulled down less efficiently compared to TRIM9 and TRIM67 which can lead to the conclusion that TRIM9-SA and β-TrCP do not, or only very inefficiently, interact.

To investigate the interaction between β-TrCP and TRIM9/TRIM67 we performed immunofluorescence analysis in HeLa cells. When expressed alone or with GST, β-TrCP localized throughout the cell being more prominent in the nucleus in some cells but evenly distributed in others (Fig.8a). In contrast, when co-expressed β-TrCP and TRIM9/TRIM67 seem to co-localize in dots throughout the cytoplasm (Fig.8 b, Fig.8d). These data further support that β-TrCP is interacting with TRIM9 as well as TRIM67.

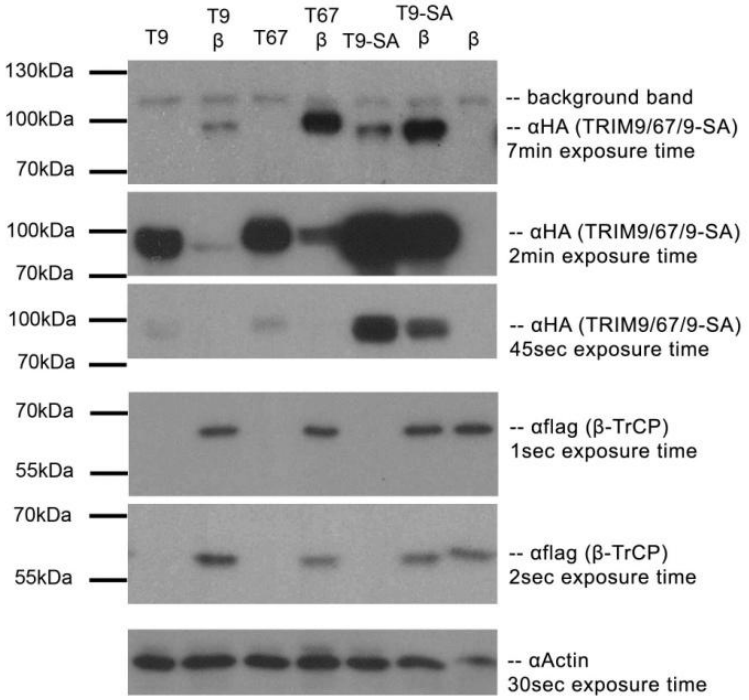

Figure 7: β-TrCP pulls down TRIM9 and TRIM67 in a co-immunoprecipitation. Flag-β-TrCP and either HA-TRIM9, HA-TRIM67 or HA-TRIM9-SA mutant have been transfected into HEK 293Ts. The cells have been harvested after 72h, lysed and treated with α-flag-beads. After washing the beads several times to remove non-binding proteins the beads have been boiled and the proteins western blotted with antibodies against their tags. The WCE displayed constant expression for β-TrCP but varying expression for TRIM9 and TRIM67. To show that TRIM9 and TRIM67 are present in with β-TrCP co-transfected cells two exposure times are shown.

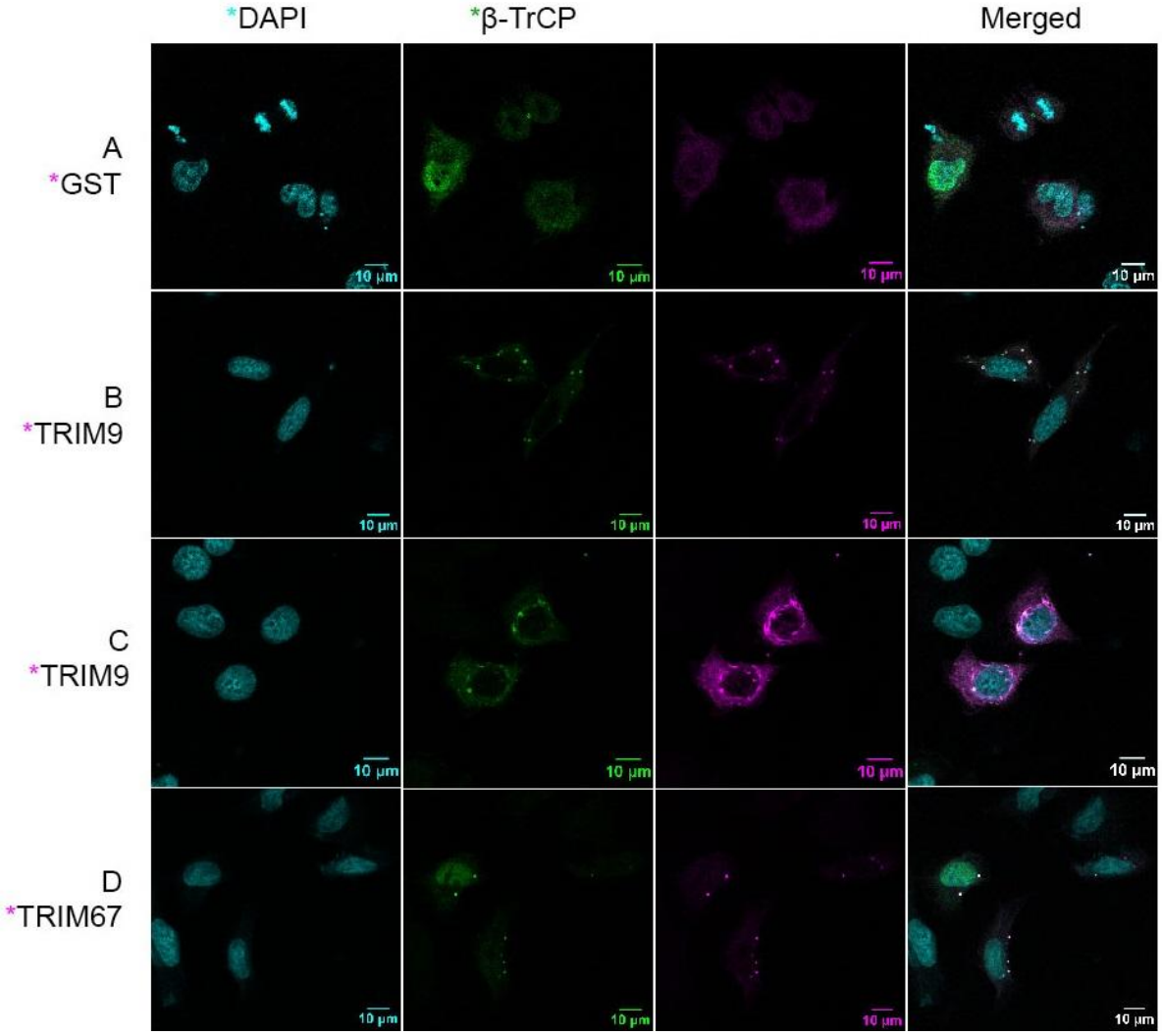

Figure 8: Both TRIM9 and TRIM67 colocalize in dots with β-TrCP in HeLa Kyoto cells and TRIM9 colocalizes in filaments with β-TrCP. HeLa Kyoto cells were transfected with expression plasmids encoding Flag-β-TrCP and (A) HA-GST, (B,C) HA-TRIM9 or (D) HA-TRIM67, each 1µg and analyzed via immune fluorescence against the Flag- & HA-tags.

Discussion

Aim 1 of this thesis was to investigate the hypothesis that TRIM9 and p47^{phox} interact, as it has been previously seen when pulling-down TRIM9 with following mass-spectrometry of all interactors. Immunofluorescence visualized a cytoplasmic localization for p47^{phox} when expressed alone or with a negative control (GST), while it was found accumulated in either dots or filaments when co-expressed with TRIM9. TRIM9 however was already found in the aforementioned dots and filaments when expressed alone or with a negative control (p40^{phox}, which is also a member of the NADPH oxidase but was not found to interact). However, actin co-localizes with TRIM9 in the filaments around the nucleus. Since TRIM9 can already be found early in evolution, it is possible that it has some function in essential cellular processes.

Together with the previous results, this may suggest that p47^{phox} and TRIM9 can interact. It is tempting to hypothesize that TRIM9 may recruit p47^{phox} to a site of action and is necessary for downstream reactions of p47^{phox}-dependent pathways. Nevertheless, it must be mentioned that until now the experiments have been done in an artificial setup in cell lines and it is not yet known if TRIM9 and p47^{phox} are expressed in the same cell types in biological environment. To gain further insight into this possibly important relationship, further experiments in primary cells have to be performed.

Aim 2 of this thesis was to investigate the interaction between TRIM9 and β-TrCP. The pull-down assay of TRIM9 with following mass-spectrometry displayed β-TrCP as another strong candidate for interaction. In this thesis, the interaction between TRIM9 and β-TrCP has been further supported by Co-immunoprecipitation and immunofluorescence. In Co-immunoprecipitation, the pull-down of TRIM9 and TRIM67 after pulling on β-TrCP was very efficient, while the pull-down of the negative control TRIM9-SA was inefficient. Furthermore, TRIM9-SA was expressed more efficiently which can be already seen in the WCE and TRIM9-SA could also be seen in the IP in the samples containing no β-TrCP. Including more stringent washing steps and/or expressing less protein could be the solution for more comparable expression levels. Nevertheless, comparison of the amount of protein in the WCE and the weak band in the IP leads to the conclusion that β-TrCP and TRIM9-SA either interact only very inefficiently or not at all. It is important to mention that TRIM9 and β-TrCP seem to regulate the expression of one another, even though the mechanism remains to be unknown. When co-expressing TRIM9 and β-TrCP at each 1µg, it seems that either β-TrCP or TRIM9 is always expressed a lot less compared to the single-expressed controls (also 1µg). One hypothesis could be that they compete in targeting each other for degradation and that the lower expressed one gets degraded. To test this hypothesis proteasome inhibitors could be used.

Aim 3 of this thesis was to analyze if TRIM67, the most similar protein to TRIM9, interacts with $p47^{phox}$ and/or with β-TrCP. It could not be shown that TRIM67 and $p47^{phox}$ co-localize but TRIM67 seems to interact with β-TrCP when using co-immunoprecipitation and immunofluorescence. Immunofluorescence assays detected co-localization of β-TrCP in dots with both TRIM9 and TRIM67 but not with the negative control GST. Until now, it is not known if these accumulations are peroxisomes, lysosomes or other intracellular vesicles, but the identification of these dots could give further insight into the functions of TRIM9 and TRIM67. It is important to mention that when looking closely the accumulations look like rings rather than dots. It would be interesting to see if the localization changes upon infection. TRIM9 and β-TrCP have also been detected to co-localize in filaments around the nucleus (Fig.8c), while this pattern has never been detected for TRIM67. This may suggest that some biological functions of TRIM9 and TRIM67 may be not or at least not completely redundant.

Aim 4 of this thesis was to determine differences and similarities between TRIM9 and TRIM67.

<u>Similarities</u>	
	Both TRIM9 and TRIM67 seem to interact with β-TrCP.
	Both TRIM9 and TRIM67 localize in dots within the cytoplasm of HeLa Kyoto cells.
<u>Differences</u>	
	No influence of TRIM67 on β-TrCP expression could be detected.
	TRIM67 does not localize in filaments around the nucleus in HeLa Kyoto cells.
	No interaction between TRIM67 and $p47^{phox}$ could be shown.

The next steps in research would be to create knock-down cell lines with shRNAs and knock-out cell lines using the CRISPR/Cas9 system to knock down/out TRIM9 and TRIM67. These cell lines could be used to do qPCRs of several immunological relevant factors as IL-6 and check if the knock-down/out influences the immunological response of the cells to different stimuli as PMA (Phorbol myristate acetate) or IFN-β. These experiments may also reveal if TRIM9 and TRIM67 are redundant for regulating the immunological response.

Material and Methods

Cell culture maintenance

HEK (human embryonic kidney) 293T and HeLa Kyoto (human epithelial cervix adenocarcinoma) were maintained in Dulbecco's Modified Eagle's Medium with additionally added 10% fetal calf serum (FCS) and 100U/mL penicillin-streptomycin. The cells were regularly tested for mycoplasma.

Transfection

Transfection was used to transfer plasmid DNA into HEK293T and HeLa Kyoto cells. The day before transfecting HEK293T they were seeded to reach 60% confluency at the time of transfection. HeLa Kyotos were seeded to reach 30% confluency at the time of transfection. In a 6-well format a total of 2µg DNA were transfected while in 10cmØ dish format a total of 15,6µg DNA were transfected. Poly-ethyleneimine (PEI) was used as transfection reagent in a 1:3 ratio (DNA : PEI) in serum-free OptiMEM. Once the DNA, OptiMEM and PEI were mixed it was incubated for 30 minutes at room temperature and then slowly dropped onto the seeded cells.

Harvesting lysates

48h after transfection the cells were trypsinized, harvested and centrifuged at 250xg for 5min at 4°C. Excess medium was aspirated and the cells were washed 1x with cold, sterile PBS. After centrifuging again for 5min at 250xg the cells were lysed in ice-cold IP-buffer (0.2% NP-40, 150mM NaCl, 50mM Tris pH 8.0, phosphatase inhibitor, protease inhibitor) and either incubated on ice for 10min, if they were used directly for western blot, or incubated on the shaker at 4°C for 30min, if they were used for Co-immunoprecipitation.

Western Blot

Protein samples were standardized to 1µg/uL using Bradford assay and boiled at 95°C for 10min in Laemmli sample buffer containing a final concentration of 1.6% β-Mercaptoethanol. The samples were run on 10% Polyacrylamide gels and transferred to PVDF membranes using semi-dry western blotting. The membranes were usually incubated in 2% Non-fat milk powder dissolved in PBS overnight at 4°C. Primary and secondary antibodies were each incubated for 1h at 4°C between 3x 10min washing steps with PBS-Tween (0.5%). Antibodies were usually diluted in 0.2% non-fat milk powder dissolved in PBS. Enhanced chemiluminescence (ECL) was used for detection.
Antibodies used:

Antibody	Dilution	Company / #Catalognumber
Rabbit-α-Flag	1 : 6000	Sigma Aldrich #F7425
Mouse-α-HA	1 : 6000	Sigma Aldrich #H9658
α-Rabbit	1 : 4000	NEB #7074
α-Mouse	1 : 4000	NEB #7076

Co-immunoprecipitation

After lysing the cells a whole cell extract (WCE) of each protein sample was taken and boiled at 95°C for 10min in Laemmli sample buffer containing a final concentration of 1.6% β-Mercaptoethanol. The rest of the protein was used for co-immunoprecipitation. The protein was incubated on a shaker with αflag-beads for 3h at 4°C, then washed 6x with cold IP-buffer (0.2% NP-40, 150mM NaCl, 50mM Tris pH 8.0) and afterwards boiled at 95°C for 10min in Laemmli sample buffer containing a final concentration of 1.6% β-Mercaptoethanol and separated from the beads. Both WCE- and IP-samples were analyzed by western blot.

Immunofluorescence

HeLa Kyoto cell line was seeded and transfected onto coverslips. 48h after transfection the cells were fixated with ice-cold methanol (3min, RT), permeabilized with 0.25% TritonX-100 for 15min, blocked with 1% BSA for 1h and incubated with primary and secondary antibodies for 1h each at RT. Additionally the cells were treated with DAPI or Hoechst for 10min to stain the DNA and stored on microscope slides with non-hardening VECTASHIELD mounting medium in the dark until further used.

For detection the ZEISS 710 confocal microscope with ZEN software was used.

Dye	**Excitation**	**Emission**
DAPI/Hoechst	345nm	455nm
Alexa Fluor 488	499nm	519nm
Alexa Fluor 647	652nm	668nm

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Eidesstattliche Erklärung

Ich erkläre hiermit an Eides Statt, dass ich die vorliegende Arbeit selbständig und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht.

Die Arbeit wurde bisher in gleicher oder ähnlicher Form keiner anderen Prüfungsbehörde vorgelegt und auch noch nicht veröffentlicht.

Wien, am 31.3.2015

Tamara Engelmaier

Anhang

Abstract (deutsch)

Die „tri-partite motif“ (TRIM) Familie besteht aus E3 Ligasen, von welchen über 70 Mitglieder im Menschen vertreten sind. Bei vielen von Ihnen wurde bereits festgestellt, dass sie das natürliche Immunsystem regulieren können. Die vorliegende Arbeit beschäftigt sich mit TRIM9 und TRIM67, zwei sehr nah verwandten TRIMs, deren Funktionen bisher weitestgehend unbekannt sind. Der bisherige Stand des Wissens ist, dass TRIM9 und TRIM67 hauptsächlich im Gehirn exprimiert werden, jedoch konnten wir in jüngsten Experimenten feststellen, dass TRIM9 auch in Makrophagen exprimiert wird. Weitere Experimente haben außerdem ergeben, dass TRIM9 mit $p47^{phox}$, einem Mitglied des NADPH Oxidasen Komplexes, welcher entscheidend für die Produktion von Reaktive Sauerstoffspezies (ROS) ist, interagiert. Vor kurzem wurde veröffentlicht, dass TRIM9 mit β-TrCP (F-box/WD repeat-containing protein 11) interagiert, dies die Expression von NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) reguliert und damit auch die Reaktion des Immunsystems auf Infektionen. In der vorliegenden Arbeit wurde die Vermutung auf eine Interaktion zwischen TRIM9 und $p47^{phox}$, durch Experimente mit Immunfluoreszenz, weiter unterstützt. Die Interaktion zwischen TRIM9 und β-TrCP konnte bestätigt werden, außerdem wurde beobachtet, dass auch TRIM67 mit β-TrCP interagiert. Zusätzlich konnte festgestellt werden, dass sich TRIM9 und β-TrCP gegenseitig in ihrer Expression beeinflussen, die Gründe und Abläufe dahinter sind jedoch noch unbekannt.

Curriculum Vitae (deutsch)

Beruflicher Werdegang

01/09/2013 – 31/08/2014 Max F. Perutz Laboratories (MFPL), Wien, Österreich	Masterpraktikum – Eigenständiges arbeiten an dem Projekt *“The role of the ubiquitin ligase TRIM9 in immune signalling“*, Betreuer: Dr. Gijs Versteeg
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Ausbildung

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