

Review

FAT10ylation as a signal for proteasomal degradation

Gunter Schmidtke ^a, Annette Aichem ^{a,b}, Marcus Groettrup ^{a,b,*}

^a Division of Immunology, Department of Biology, University of Konstanz, D-78457 Konstanz, Germany

^b Biotechnology Institute Thurgau at the University of Konstanz, CH-8280 Kreuzlingen, Switzerland

A B S T R A C T

The Nobel prize has been awarded for the discovery of ubiquitin as a transferable signal for the degradation of proteins by the 26S proteasome. While isopeptide linkage of a protein with a single ubiquitin does not serve as a degradation signal for the proteasome, poly-ubiquitylation via several different lysine residues within ubiquitin leads to efficient proteasomal degradation. Ubiquitin-like modifiers have not been shown to directly mediate proteasomal degradation except for the cytokine inducible modifier HLA-F adjacent transcript 10 (FAT10), which consists of two ubiquitin-like domains. FAT10 ends with a free diglycine motif at its C-terminus which is required for isopeptide linkage to hundreds of different substrates. In contrast to ubiquitin, a single FAT10 suffices to bind to the 26S proteasome and to efficiently mediate proteasomal degradation in a ubiquitin-independent manner. Here we review the data on ubiquitin-independent degradation by FAT10, on how FAT10 is conjugated to its substrates, how FAT10 binds to the 26S proteasome, and how the ubiquitin-like (UBL)-ubiquitin-associated (UBA) protein NUB1L accelerates FAT10 mediated proteolysis. Finally, with a glimpse on recently identified substrates, we will discuss the currently emerging knowledge about the biological functions of FAT10. This article is part of a Special Issue entitled: Ubiquitin-Proteasome System. Guest Editors: Thomas Sommer and Dieter H. Wolf.

1. Introduction

The 'ubiquitin-proteasome system' (UPS) is in charge of the degradation of most short lived and long lived proteins outside the lysosome. Since neither the central proteolytic unit of the UPS, the cylindrical 20S proteasome, nor the 26S proteasome degrades unmodified proteins to a significant degree, protein substrates of the UPS need to be modified with a transferable signal in order to enable tight proteasome binding and targeting for degradation. The impressive discovery of the post-translational modification of proteins with ubiquitin as a small protein tag which enables proteasomal degradation was honored with the Nobel prize for Avram Hershko, Aaron Ciechanover, and Erwin Rose in 2004 [1,2]. Ubiquitin becomes isopeptide linked to lysine residues within substrates and this requires the activation of ubiquitin at its C-terminal diglycine residue by one of the two E1 type enzymes UBE1 [3] and UBA6 [4-7]. The activated ubiquitin can then be transferred onto dozens of different E2 type enzymes which will be brought in close contact with substrates by hundreds of different E3 ligases to enable their ubiquitylation usually on lysine residues [8,9]. While many of the UPS substrates are short lived, ubiquitin itself is fairly long lived

with an approximate half-life of 9 h [10,11] because it is removed from its substrates by de-ubiquitylating enzymes within the 19S regulator of the 26S proteasome before the substrate gets degraded [12]. Therefore ubiquitin can be reused for further degradation cycles and is metabolically stable.

An important paradigm of the UPS system is that modification of a substrate protein with a single ubiquitin is not enough to mediate degradation by the 26S proteasome. Ubiquitin chains have to be assembled upon the substrate, or transferred as preformed chains, which need to contain four ubiquitin molecules or more [13]. Ubiquitin contains 7 lysines (at positions 6, 11, 27, 29, 33, 48, and 63) all of which can themselves become ubiquitylated to form poly-ubiquitin chains and all these linkages contribute to proteasomal degradation with the apparent exception of K63 [14]. The reason for the need to assemble poly-ubiquitin chains for proteasomal targeting is probably that a single ubiquitin binds with insufficient affinity to the validated ubiquitin receptors within the 19S regulator of the 26S proteasome which are S5a (RPN10) [15] and ARM1 (RPN13) [16].

In this article we review the current knowledge on a second transferable signal for degradation by the proteasome named FAT10. The interest in research on FAT10 is steadily increasing because it is the only ubiquitin-like modifier which directly targets its substrates for degradation by the 26S proteasome. The modification of substrates with the modifier SUMO-2/3 can lead to recognition and poly-ubiquitylation by the ubiquitin E3 ligase RNF4 and, as a consequence of this secondary poly-ubiquitylation, to degradation by the proteasome [17-19]. FAT10, in contrast, directly binds to the proteasome and according to our data

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* Corresponding author at: Division of Immunology, Department of Biology, University of Konstanz, Universitaetsstr. 10, D-78457 Konstanz, Germany. Tel.: +49 7531 88 2130; fax: +49 7531 88 3102.

E-mail address: marcus.groettrup@uni-konstanz.de (M. Groettrup).

does not rely on secondary poly-ubiquitylation in order to serve as a degradation tag [20–22]. However, this conception has recently been challenged [23] and we will review the data which support and which question this notion. In addition, we will outline in which respects FAT10 differs from ubiquitin as a degradation signal (Fig. 1), how FAT10 is conjugated to substrates, and finally we will surface what these substrates are and what they may tell us about the biological function of FAT10. In a nutshell: we will review the ‘FAT10-proteasome system’ (FPS).

2. The FAT10 facts paragraph

‘FAT10’ is an abbreviation for Human Leukocyte Antigen (HLA)-F adjacent transcript 10 as the FAT10 open reading frame consisting of two ubiquitin-like domains in tandem array was discovered during genomic sequencing of the human major histocompatibility complex (MHC) by Sherman Weissman and colleagues in 1996 [24]. FAT10 was initially named ‘diubiquitin’ or ‘ubiquitin D’ but these names are not in frequent use any longer. The two N- and C-terminal ubiquitin-like domains of the 18 kD FAT10 protein are 29% and 36% identical to ubiquitin. The FAT10 protein ends with a free GG motif which is immediately available for activation and conjugation in contrast to ubiquitin and other modifiers which need to be C-terminally processed from precursor proteins in order to act as modifiers. The high resolution three-dimensional structure of FAT10 has not been reported to date, most likely because FAT10 is poorly soluble at higher concentrations, both when produced in *Escherichia coli* or mammalian expression systems, but structure predictions hold that FAT10 folds quite similarly to ISG15 [25] with two typical β -grasp fold domains connected by a short linker [7].

In contrast to ubiquitin, which – as its name tells – is ubiquitously expressed, FAT10 expression is largely restricted to tissues of the immune system like thymus, lymph nodes and spleen in the human and mouse [26–28]. However, under stimulation with the pro-inflammatory cytokines interferon (IFN)- γ and tumor necrosis factor (TNF)- α , FAT10 is strongly and synergistically induced on mRNA and protein level in virtually all tissues [29,30]. A synergism between IFN- γ and TNF- α is observed with several MHC encoded genes but it is especially pronounced for

FAT10. We recently investigated the kinetics of FAT10 mRNA and protein induction following the stimulation of the human embryonic kidney cell line HEK293 with IFN- γ and TNF- α and found that while FAT10 mRNA is already detectable by qRT-PCR 2–3 h after induction [29] higher amounts of FAT10 mRNA and FAT10 protein are only detected 24 h after induction [31]. Moreover, FAT10 is induced during the maturation of dendritic cells triggered by different toll-like receptor ligands [27,32] which enhances their potential to present antigens and to stimulate T cells. Taken together, the localization of the FAT10 gene in the MHC locus, the tissue expression profile, the cytokine inducibility, and the up-regulation in mature dendritic cells all point at a function of FAT10 in the immune system. Consistent with a function of FAT10 in the immune system is the very mild phenotype of FAT10 knockout mice kept under specific pathogen free conditions. Only when these mice were systemically treated with a sublethal dose of lipopolysaccharide, an endotoxin hypersensitivity phenotype became apparent [28]. However, while most genes involved in the adaptive immune response evolved with sharks and bony fish, FAT10 has only been detected in mammals [7] arguing for a more specialized function in the immune defense as for instance the response to a certain group of mammalian pathogens.

3. The E1 and E2 enzymes of the FAT10 conjugation cascade

It is a hallmark of ubiquitin-like modifiers like SUMO, NEDD8, or ISG15 that their activation and transfer onto substrates are mediated by ‘private’ cascades of E1, E2, and E3 enzymes. The E1 enzyme of FAT10 conjugation, designated UBA6 (MOP-4, E1L2, and UBE1L2), in contrast, activates both ubiquitin and FAT10 [4–7]. While FAT10 binds to UBA6 with higher affinity than ubiquitin, the adenylation and transthiolation reaction is much slower for FAT10 than for ubiquitin [33]. The activation of FAT10 by UBA6 and the formation of a UBA6–FAT10 thioester have been shown in vitro. SiRNA mediated silencing of UBA6 expression interferes with the formation of FAT10 conjugates in HEK293 cells, indicating that no other E1 type enzymes activate FAT10 [6,34]. Interestingly, also the E2 enzyme in charge of FAT10 conjugation is bi-specific for ubiquitin and FAT10. This enzyme, first named UBE2Z [35] and then renamed to ‘UBA6 specific

Differences between ubiquitin and FAT10 as degradation signals

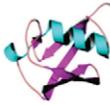
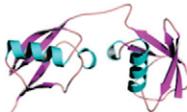
	 Ubiquitin	 FAT10
Degradation rate	slow (t1/2 ~ 9h)	fast (t1/2 ~ 1h)
Recycling	yes	no
Proteasome receptors	RPN10, RPN13	RPN10
Proteasome binding	polymer (>4)	monomer
RPN10 binding site	UIM1, UIM2	VWA
E1 enzyme(s)	UBE1, UBA6	UBA6
E2 enzyme(s)	~ 50	USE1
Tissue expression profile	ubiquitous	immune system
Inducibility	heat shock	IFN- γ /TNF- α

Fig. 1. Differences between ubiquitin and FAT10 as degradation signals.

E2 enzyme' (USE1) [5] is the only E2 enzyme known which can accept activated ubiquitin exclusively from UBA6 but not from UBE1. USE1 was shown to interact with FAT10 in yeast two hybrid assays and forms a thioester with FAT10 in vitro [34]. The knock down of USE1 interferes with the formation of bulk FAT10 conjugates both under endogenous and overexpression conditions, suggesting that USE1 is the main if not the only E2 enzyme for FAT10. Remarkably, USE1 auto-FAT10ylates itself in *cis* i.e. it transfers the thioester-linked FAT10 onto one of its lysines to form a stable isopeptide linkage both in vitro and in intact cells which renders USE1 both an E2 enzyme and a substrate of FAT10ylation [34]. It will be interesting to study the structural and functional consequences of this quite efficient auto-FAT10ylation of USE1.

Not yet discovered have been the putative E3 ligases and de-conjugating enzymes of the FAT10 cascade. While E3 enzymes are probably required to allow substrate specific FAT10ylations, it is less clear whether de-FAT10ylating enzymes exist at all. We have shown in HEK293 and HeLa cells, both before and after IFN- γ stimulation, that while linear ubiquitin-GFP fusions are rapidly cleaved by de-ubiquitylating enzymes, FAT10-GFP fusions are not [20]. This does not exclude that de-FAT10ylating enzymes may be expressed elsewhere e.g. in certain cell types of the immune system. Moreover, the assumption that a putative de-FAT10ylating enzyme is able to cleave linear FAT10-fusion proteins may not be valid. However, FAT10 – in contrast to other modifiers – does not need to be liberated from C-terminally extended precursors. In contrast to the fairly long lived ubiquitin, which is readily removed from substrates at the 26S proteasome before degradation, pulse chase experiments with a so far unidentified FAT10 substrate have not provided any evidence for a de-FAT10ylation reaction. Rather, the FAT10-conjugated substrate was degraded at the same rate as FAT10 itself with a half-life of approx. 1 h [20]. It is therefore quite possible that FAT10 is not de-conjugated at the 26S proteasome but instead degraded along with its substrate.

4. FAT10 as a transferable signal for degradation by the 26S proteasome

The first evidence that FAT10 and FAT10 conjugates are degraded by the proteasome emerged from the observation that they accumulated when the proteasome was inhibited [36]. Next it was shown that the N-terminal fusion of FAT10 to long lived proteins like green fluorescent protein (GFP), dihydrofolate reductase (DHFR) or the nucleoprotein of Lymphocytic Choriomeningitis Virus (LCMV) reduced their half-lives as potently as fusion with ubiquitin did, while fusion of SUMO1 to GFP did not have such an effect [20,37]. Recently, we have shown that a natural, isopeptide linked substrate of FAT10 conjugation in cytokine treated HEK293 cells i.e. the autophagy adaptor p62/Sequestosome-1, strongly accumulated under proteasome inhibition in cycloheximide chase experiments, while the unconjugated p62 protein did not [31]. Moreover, fusions of FAT10 to the LCMV nucleoprotein or the human cytomegalovirus derived protein pp65 enhanced their presentation along the proteasome dependent MHC class I presentation pathway [37,38].

Another line of evidence that FAT10 targets substrates to the proteasome emerged from the identification of the ubiquitin-like (UBL)/ubiquitin-associated (UBA) domain protein NEDD8 ultimate buster-1 long (NUB1L) [39] as a non-covalent interaction partner of FAT10. The coexpression of the likewise interferon inducible protein NUB1L [40] accelerated the degradation of FAT10 by the proteasome approx. fourfold. NUB1L binds to the proteasome via its N-terminal UBL domain and to FAT10 via its three C-terminal UBA domains suggesting that it may serve as a linker between the proteasome and FAT10 [41]. However, subsequently it was found that even a Δ UBA deletion variant of NUB1L was able to accelerate FAT10 degradation while the deletion of the UBL domain of NUB1L abolished this effect. Moreover, we found that FAT10 itself can bind to the

proteasome in the absence of NUB1L. Therefore, we postulated that docking of the UBL domain of NUB1L to the 26S proteasome would 'facilitate' the degradation of FAT10 by the proteasome [42] as it had been similarly postulated for RPN10 in the ubiquitin system [43] (Fig. 2). These interesting findings raised the question how and to which subunit FAT10 binds at the 26S proteasome and how NUB1L accelerates its degradation. We recently identified the ubiquitin receptor RPN10 (S5a) as the subunit of the 26S proteasome where FAT10 binds while NUB1L can bind both to RPN10 as well as the 19S regulator subunit RPN1 (S2) [22,44]. Surprisingly, neither FAT10 nor NUB1L bound to the two C-terminal ubiquitin interacting motifs (UIM) of RPN10, where poly-ubiquitin chains bind, but to the N-terminal von Willebrand A (VWA) domain of RPN10 which appears to be a novel docking site for ubiquitin-like proteins. In order to investigate whether this interaction with the VWA domain of RPN10 is functionally important, we reconstituted the NUB1L dependent FAT10 degradation in *S. cerevisiae* which survives the deletion of *rpn10*. We found that FAT10 degradation and its acceleration by NUB1L could be reconstituted in *rpn10*-deficient yeast just by expressing the VWA domain of RPN10 alone, thus demonstrating its functional relevance [22].

5. FAT10 – a ubiquitin independent signal for degradation by the proteasome: the Pros and Cons

It is an important question whether FAT10 can target substrate proteins to the proteasome independently of poly-ubiquitylation of FAT10 or whether it relies on secondary poly-ubiquitylation as has been shown for SUMO [17–19]. The finding that FAT10 directly interacts with the VWA domain of RPN10 [22] suggests that at least for binding to the proteasome no ubiquitylation is required. Apparently the two ubiquitin-like domains of FAT10 are sufficient for this interaction while ubiquitin requires four or more units to efficiently mediate degradation by the proteasome [13]. We have investigated the ubiquitin-dependence of FAT10-mediated degradation by the proteasome with three approaches [20].

- 1) All 17 lysines within FAT10 were mutated to arginines (an amino acid that cannot be ubiquitylated), which completely eliminated the marked mono-ubiquitylation and weak poly-ubiquitylation of FAT10 in HEK293 cells. In radioactive pulse-chase experiments wt and lysine-deficient (K0) FAT10 were degraded at the same rate and coexpression of NUB1L accelerated this proteasome dependent degradation even more as compared to wt FAT10, strongly suggesting that ubiquitylation of FAT10 was not required. The same conclusion was also reached when the degradation of wt FAT10 and FAT10-K0 was monitored in cycloheximide chase experiments in *Saccharomyces cerevisiae* [22].
- 2) The degradation of a FAT10-GFP fusion protein was monitored after radioactive pulse labeling over a time period of 5 h in the UBE1 temperature sensitive mutant E36-ts20 and the UBE1 reconstituted transfectant E36-ts20/E1 at the restrictive temperature. The degradation of FAT10-GFP was not changed after inactivation of the ubiquitin activating enzyme UBE1 while the ubiquitin-dependent degradation of the N-end rule substrate Arg- β -galactosidase was arrested under the same conditions. While both aforementioned experiments in cells were strongly suggestive for an ubiquitin independent FAT10-mediated degradation, a third experiment was performed in vitro which we consider the most conclusive.
- 3) A radiolabeled FAT10-DHFR fusion protein was incubated with highly purified 26S proteasome and the degradation was monitored in the presence and absence of purified recombinant NUB1L. It was shown that FAT10-DHFR was efficiently degraded by the 26S but not the 20S proteasome and the degradation was promoted by NUB1L in a dose dependent manner [21]. Because the generation of acid soluble radioactivity was used to measure

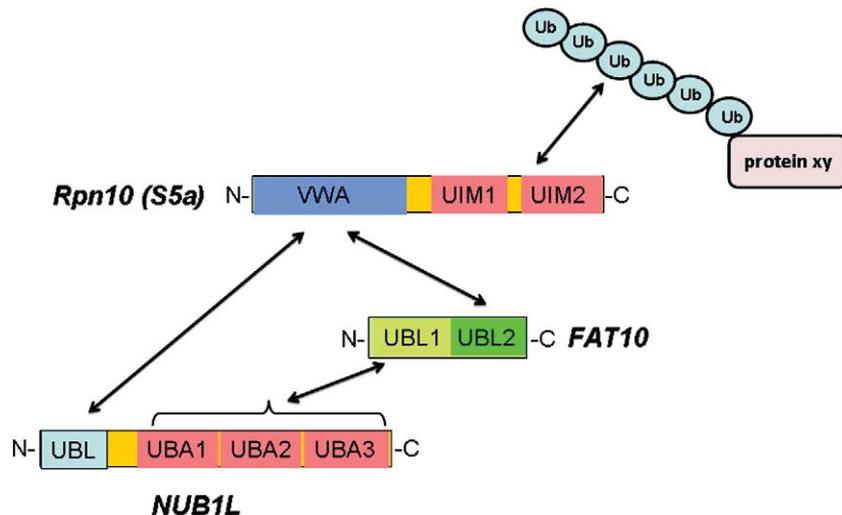


Fig. 2. The 26S proteasome subunit Rpn10 (S5a) as a docking site for FAT10, NUB1L, and poly-ubiquitin. FAT10 binds with its C-terminal ubiquitin-like (UBL) domain to the von Willebrand A (VWA) domain of Rpn10 and with its N-terminal UBL domain to the ubiquitin-associated (UBA) domains of NUB1L. NUB1L, in turn, binds with its N-terminal UBL domain to the VWA domain of Rpn10. Therefore FAT10 can dock to the 26S proteasome either directly or via NUB1L. Lysine 48-linked poly-ubiquitin chains, in contrast, bind to the ubiquitin interaction motifs (UIM) 1 and 2 of Rpn10.

degradation, any possible precipitation of the substrate cannot account for this result. Since in the same experiment a radiolabeled ubiquitin–DHFR fusion protein was not degraded we could be sure that no contaminating poly-ubiquitylation enzymes were present because otherwise ubiquitin–DHFR would have been further ubiquitylated and degraded by the 26S proteasome.

The concept that FAT10 functions as a degradation tag independently of poly-ubiquitylation has recently been called into question by a study by A. Ciechanover and colleagues which reports that ubiquitylation of FAT10 enhances the rate of FAT10-mediated degradation [23]. These authors also performed a pulse chase experiment in HEK293 cells with wt FAT10 and a lysine-deficient KO mutant of FAT10 but they ascribe the diminution of the FAT10-KO protein over time to progressive precipitation of this protein rather than degradation by the proteasome. We agree with Buchsbaum et al. that upon massive (but not moderate) overexpression the KO mutant has a slightly higher tendency to precipitate than wt FAT10. However, precipitation is unlikely to contribute to the loss of signal of the soluble FAT10 or FAT10 KO in our system. As proteasome inhibition, which usually promotes protein aggregation in cells, blocked degradation of soluble FAT10-KO, we very likely monitored degradation by the proteasome rather than precipitation. Even more compelling, the coexpression of NUB1L in our experiments strongly accelerated the degradation of FAT10-KO again arguing in favor of proteasomal degradation. In their discussion Buchsbaum et al. agree with us that binding of FAT10 is sufficient to promote the proteasomal degradation. What has remained controversial is whether ubiquitylation of FAT10 is able to accelerate FAT10-mediated protein destruction or not. Further experiment will hopefully settle this remaining issue.

6. The substrates of FAT10 conjugation – some clues to FAT10 biology

While considerable progress has been made throughout the past years with respect to the activation and conjugation of FAT10 and proteasome targeting via FAT10, the biological function of FAT10 is still poorly understood. The inapparent phenotype of FAT10^{-/-} mice kept under specific pathogen free conditions suggests that the phenotype will only become visible after infection with a certain pathogen which induces an IFN- γ /TNF- α response and maturation of dendritic cells and by this means switches on FAT10 expression. However, overexpression

of FAT10 has been shown to induce apoptosis in mouse fibroblasts [36], HeLa cells [30], and renal tubular epithelial cells [45]. The mechanism of apoptosis induction by FAT10 is not clear but it does not seem to be a mere overexpression artefact since immortalized human proximal tubular cells which had been infected with HIV-1 in vitro were protected from apoptosis when FAT10 expression was knocked down [45]. A role for FAT10 in the induction of apoptosis would be consistent with its inducibility by the pro-apoptotic cytokines IFN- γ and TNF- α . Recently, it has been shown that the inflammatory mediator leucine-rich repeat Fli-1-interacting protein 2 (LRRFIP2), which is a positive regulator of NF- κ B activity in the toll-like receptor (TLR)4-mediated inflammatory response, is covalently modified by FAT10 [46]. FAT10ylation of LRRFIP2 hinders its recruitment to the membrane leading to an inhibition of LPS/TLR4 mediated NF- κ B activation. Since NF- κ B activation transcriptionally induces apoptosis inhibitors, this finding may explain why the overexpression of FAT10 can induce apoptosis and why FAT10^{-/-} mice are hypersensitive to LPS challenge [28]. However, when spontaneous apoptosis was monitored in leukocytes from spleen, thymus and bone marrow of FAT10-deficient versus wt mice, FAT10 seemed to protect these cells from apoptosis rather than inducing it [28]. Moreover, the colon cancer cell line HCT116 was partially protected from TNF- α induced apoptosis in the presence of FAT10 [47]. It is possible that an apoptosis modulating function of FAT10 is cell type specific but the mechanisms involved have remained elusive and further investigations are warranted.

A second hint towards FAT10 function stems from the identification of the spindle assembly checkpoint protein MAD2 as a non-covalent binding partner of FAT10 [30]. Overexpression of FAT10 in HCT116 cells reduced the localization of MAD2 at the kinetochore during prometaphase and resulted in a missegregation of chromosomes after 100 cell divisions [47]. Conversely, TNF- α induced effects in the same cell line like the delocalization of MAD2 from kinetochores, acceleration of mitosis, and the missegregation of chromosomes were all abolished when FAT10 was knocked down [48]. An infidelity of chromosome segregation during mitosis is a hallmark of cancer cells and it is therefore striking that FAT10 was found to be highly overexpressed in hepatocellular, colorectal, ovarian, and uterus carcinoma which led to the proposal that FAT10 expression may promote oncogenesis [26]. However, we found that 72% of the analyzed hepatocellular carcinoma and 53% of colon carcinoma tissues, which overexpressed FAT10, also expressed the IFN- γ /TNF- α -dependent immunoproteasome subunit LMP2. This suggested that FAT10 overexpression in carcinoma tissues may be a

consequence of the pro-inflammatory cytokine response in these cancer samples rather than an indicator that FAT10 is oncogenic [27]. In support of this notion, FAT10 was found to be expressed in hepatocellular carcinoma cells in a chemically induced mouse model for liver cancer but aberrations of chromosome numbers in these cells could not be confirmed [49].

In order to gain better mechanistic insights into these and other proposed functions of FAT10, it is important to identify the substrates of FAT10 conjugation as well as further non-covalent interaction partners of FAT10. The overexpression of wt FAT10 but not a Δ GG mutant of FAT10 in HEK293 cells resulted in the formation of a high molecular weight smear of FAT10 conjugates which looked similar to classical ubiquitin smears [6,34]. Using a high affinity monoclonal antibody to human FAT10, designated 4F1, we could confirm the formation of such FAT10 smears also under endogenous conditions in IFN- γ /TNF- α stimulated HEK293 cells [34]. We have recently used this antibody to immunopurify endogenous FAT10 substrates and interaction partners from IFN- γ /TNF- α stimulated HEK293 cells and identified 569 FAT10 binding proteins by mass spectrometric analysis, none of which were detected in unstimulated HEK293 cells [31]. Of these 569 proteins, 169 were scored as putative covalent FAT10 conjugation substrates based on their apparent molecular mass being at least 18 kDa (i.e. the molecular weight of FAT10) higher than their predicted molecular mass. 14/15 of these proteins have been confirmed to be FAT10 conjugates by combined immunoprecipitation/western blotting experiments in our laboratory to date. We had hoped that the putative FAT10 conjugates would belong to one or at least a few cell biological pathways, which could have directed the focus of FAT10 research onto such a pathway. However, similarly to the hundreds of conjugates of ubiquitin, SUMO, or ISG15, also the many putative and confirmed substrates of FAT10 conjugation must be assigned to many different areas of cell biology and do not provide such a clue.

Therefore, we first focused on one interesting FAT10 conjugate, the autophagy adaptor p62/sequestosome-1. We were especially fascinated by this protein because we had shown before that FAT10, just like ubiquitin, is transported by histone deacetylase 6 along microtubules into juxtannuclear protein aggregates called 'aggresomes' when proteasome activity is inhibited in cells [50]. P62, which is also localized in aggresomes as well as protein aggregates of neuronal protein aggregation diseases, binds non-covalently to ubiquitin as well as to FAT10 but it is also a substrate of FAT10ylation under overexpression and endogenous conditions. We were expecting that p62, which binds to LC3 proteins involved in autophagosome formation, would target FAT10 in aggresomes for degradation in autophagolysosomes, but so far no evidence for degradation of FAT10 and its conjugates in acidifying autophagosomes could be obtained [31]. Based on these first data it appears that FAT10, in contrast to ubiquitin, does not target for autophagic but only for proteasomal degradation.

7. The 'FAT10 proteasome system' (FPS) — concluding remarks and outlook

Although the number of groups investigating FAT10 is constantly increasing, FAT10 research is still in an early stage especially with respect to understanding the biological consequences of FAT10ylation, but also with respect to the control of FAT10 conjugation. It is an intriguing question how the E1 and E2 enzymes UBA6 and USE1, which both are bispecific for ubiquitin and FAT10, discriminate which substrates are to be ubiquitylated and which substrates are to be FAT10ylated. The putative E3 enzymes of FAT10ylation, which remain to be identified, may offer a solution to this enigma. It is doubtful whether de-FAT10ylating enzymes exist, but if they do they would be worthwhile to be identified as well, given that deubiquitylating enzymes are very actively pursued as drug targets in the pharmaceutical industry these days and given that a number of putative FAT10 targets are oncogenes or apoptosis inhibitors. The most burning question, however, is why has the FAT10

Proteasome System (FPS) evolved so late? Why is it cytokine inducible and why is it apparently irreversible? Our perhaps biased opinion follows FAT10's chromosomal localization in the MHC locus and its tissue expression profile in thymus, lymph nodes and spleen: FAT10 is the immune system's back up for ubiquitin.

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