ROMANIAN ACADEMY
INSTITUTE OF BIOCHEMISTRY

Summary of the Ph.D. thesis:

The role of EDEM1 in glycoprotein degradation associated to the endoplasmic reticulum

SCIENTIFIC COORDINATOR:
DR. STEFANA MARIA PETRESCU

PH.D. CANDIDATE:
MARIOARA CHIRITOIU

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AIMS OF THESE STUDIES

Secretory and membrane proteins synthesized on ER-bound ribosomes, undergo folding within the ER with the assistance of molecular chaperones and are subsequently exported to the secretory pathway. Most of these proteins undergo glycosylation to specific sequences Asn-X-Ser/Thr immediately after entering the ER [1]. Proteins that fail to achieve their native conformation are targeted for proteolysis through the ER-associated protein degradation pathway (ERAD).

This thesis aims to contribute to the elucidation of the molecular mechanism of ERAD, with emphasis on EDEM1, an ER resident glycoprotein playing a crucial and yet not elucidated role in ERAD. To achieve this goal, I had to choose a well characterized protein that undergoes ERAD and further to dissect the effect and the interaction of EDEM1 with this protein. The thesis is therefore organized in two main parts: the *first part* aimed to characterize the ERAD substrate proposed to associate with EDEM1, further used to the second part of the thesis to elucidate the role of EDEM1 in ERAD. For this, I used a heavily glycosylated protein, tyrosinase, to elucidate the role of individual N-glycans in folding and degradation, thus completing the processes already described by our lab and others related to the subject. The *second part* aimed to elucidate the molecular mechanism by which EDEM1 functions in ERAD and to establish how EDEM1 domains define its function and modulate the interactions it engages to.

Tyrosinase is a glycoprotein transiting the ER on way to melanosomes where it functions as enzyme in melanin synthesis. Tyrosinase has important biological functions in pigmentation and melanoma and it is a typical ERAD substrate. Abnormalities in tyrosinase glycosylation have been associated with many pathologies (oculocutaneous albinism, melanoma) and it represents the basis for the studies presented here [2,3]. Since melanoma is one of the deadliest forms of cancer and tyrosinase is one of the proteins modified in cancer, it is important to understand how this protein is processed in melanoma cells.

My specific goals for this research project were to:

a) understand how individual glycans influence the folding process of tyrosinase and to what extent their deletion affects this process,
b) evaluate the role of individual glycans in tyrosinase association with molecular chaperones;
c) determine the impact of glycan deletion/sliding on tyrosinase traffic and assess the effect these mutations have on tyrosinase activity.

To achieve these goals, throughout this work, I used advanced molecular and cellular biology methods to assess tyrosinase folding and traffic. The studies were performed on melanoma cells to investigate the folding and traffic of endogenous tyrosinase and non-melanoma cells transfected with mutant tyrosinases to evaluate the mutated phenotype in comparison to wild type.

EDEM proteins were identified as ER stress expressed proteins and were proposed to be involved in misfolded proteins targeting to degradation and translocation to the cytosol for proteasomal degradation [4]. ER stress is commonly encountered in different pathologies including inflammation, neurodegenerative disorders and cancer. In many cases it is caused by the accumulation of misfolded proteins in the ER. To cope with protein overload, the cells overexpress molecular chaperones and proteins involved in ERAD, among them EDEM proteins.

It was found EDEM1 accelerates the degradation of ERAD substrates and interacts with them *en route* to retrotranslocation and proteasomal degradation; initially it was proposed to recognize high-mannose glycans exposed on misfolded proteins [5,6,7]. Recent studies have shown EDEM1 interacts with the ERAD substrates in a glycan-independent manner or both [8,9,10].

The specific aims of this second part were to:

a) investigate the role of EDEM1 in selection and transport of misfolded polypeptides from the ER using several ERAD substrates,
b) determine the function of EDEM1 domains for the association of misfolded proteins,
c) understand the molecular mechanism by which EDEM1 operates in ERAD.

In order to accomplish these objectives I employed specific biochemical, molecular and cellular biology methods to identify the mechanism by which EDEM1 recognizes misfolded proteins. The experiments were performed in recombinant system, overexpressing both EDEM1 and/or ERAD substrates, in normal and stress conditions, using specific inhibitors.

INTRODUCTION

EDEM function in ERAD

Membrane and secretory proteins are targeted to the endoplasmic reticulum (ER) for synthesis and folding, and subsequently transported to down-stream organelles through the secretory pathway. To ensure proper folding, the endoplasmic reticulum provides an environment filled with chaperones, which prevent illegitimate inter- and intra-molecular interactions, and protect hydrophobic amino acid side chains [11,12]. However protein misfolding and aggregation is a common feature in mammalian cells, causing ER stress and leading to various human pathologies. These pathologies are the result of a proteomic equilibrium perturbation at cellular level. Numerous disorders are caused by the retention of misfolded proteins in the ER of mammalian cells and, in this way, generating ER stress [13,14,15].

Immediately after entering the ER, newly synthesized proteins undergo an important post-translational modification: the addition of oligosaccharide structures to specific sequences Asn-X-Ser/Thr in the polypeptide backbone [1]. N-glycans exposed on proteins ensure their solubility, mediate the interaction with molecular chaperones and in many cases act as signals for sorting through the secretory pathway. Glycoproteins that fail to achieve their native conformation in the endoplasmic reticulum and don not pass the ERQC criteria are targeted for proteolysis through the ER-associated protein degradation pathway (ERAD) [16,17].

EDEM proteins were identified as ERAD components, up-regulated in ER stress and were proposed to mediate the selection and transport of misfolded proteins out of the ER for proteasomal degradation [4]. EDEM1, EDEM2, EDEM3 along with Golgi α1, 2 mannosidases IA, IB and IC, ER α-1, 2 mannosidase I are members of the GH47 (glycosyl hydrolase 47) family and were proposed to be involved in disposal of misfolded glycoproteins from the ER lumen [4,18].

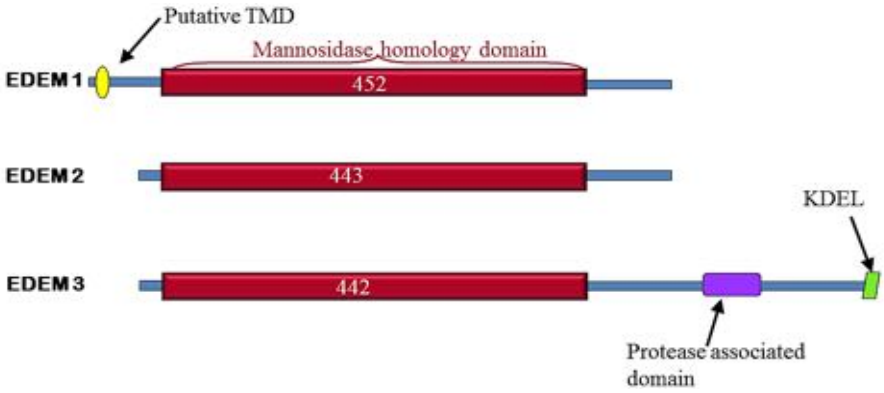

Figure 1. EDEMs schematic representation. In red the mannosidase-like domain is represented and the number of aminoacids it comprises for each protein is indicated in white, the putative transmembrane domain is represented in yellow, the protease associated domain in purple and the KDEL sequence in green

Several results suggested that EDEM1 accelerates the degradation of ERAD substrates and interacts with them after exiting the folding cycle and it was proposed to recognize high-mannose glycans

exposed on misfolded proteins [5,6,7]. However in the recent years this theory has been challenged since recent studies showed EDEM1 can interact with ERAD substrates in a glycan-independent manner or both [8,9,10]. The other two proteins with similar structures to EDEM1 were identified subsequently and named EDEM2 and EDEM3 [19,20,21]

The specific pathway from recognition to delivery is still under debate, but several proteins involved in this process and the interactions between them were identified. An interaction between EDEM1 and SEL1L, an adaptor of the export channel, has been demonstrated and proposed to have a functional role in ERAD substrates delivery to dislocation [8]. This would imply that EDEM1 is able to extract misfolded proteins from the folding cycles and deliver them directly to the export channel.

Recently the role of the three EDEMs was evaluated in the degradation process of Sonic hedgehog protein (SHH). EDEM2 was found to be required for the degradation of both wt and mutant SHH, EDEM3 was involved in the degradation of glycosylated SHH, and not of non-glycosylated protein and EDEM1 seemed to be dispensable for the degradation of both [22]. This implies that certain interactions in ERAD might be required for activation or for recruiting co-factors that might impose directionality to a process.

Model proteins employed as ERAD substrates

Tyrosinase is constitutively expressed in melanocytes and melanoma cells where it is synthesized in the ER and transported through the secretory pathway to melanosomes for melanin synthesis. It is a glycoprotein with six or seven glycans exposed to the lumen of melanosomes and requires the coordination of two cooper ions to fulfill its enzymatic function [23].

Mutations in tyrosinase, leading to enzyme inactivation and N-glycosylation abnormalities, were identified in oculocutaneous albinism IA (OCA IA) [24]. In melanoma cells tyrosinase is either hyperactivated (pigmented melanoma) or synthesized, but not active, due to deficient transport to melanosomes (amelanotic melanoma). In most cases tyrosinase mutations lead to ER retention and transport through ERAD for degradation, thus this protein is a good model to study glycoprotein degradation associated to the ER [25,26,27].

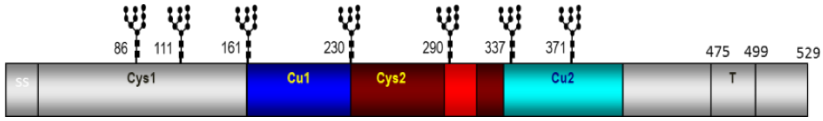

Figure 2. Tyrosinase structure-schematic representation

Alpha 1-antitrypsin (AT) is a monomeric glycoprotein predominantly synthesized in liver cells that functions as protease inhibitor in the lungs [28]. The degradation of AT from the ER was found to be ERAD dependent and considering the high number of studies conducted on alpha 1-antitrypsin it was

proposed as a good model for studying the role of N-glycans in folding and degradation of glycoproteins associated to the endoplasmic reticulum [29].

Two of the numerous mutations identified for AT were found to be secretion incompetent or secretion impaired, NHK (truncated AT) and ATZ (point mutation) respectively. Most NHK forms are terminally misfolded, implicitly retained in the endoplasmic reticulum and subjected to proteasomal degradation [28]. However, ATZ expression favors polymer formation and hinders the secretion of monomeric protein and is discarded both by proteasomal or lysosomal degradation, involving canonical autophagy [30].

Ribophorin 1 is type I transmembrane protein of 583 aminoacids, with one occupied glycosylation site in position 297 and identified as a very stable protein (T1/2~25h). It is a component of the OST complex responsible for N-glycosylation in Eukaryotic cells [31,32]. Two truncated variants of ribophorin 1: Ri-467 (membrane spanning) and Ri-332 (soluble form) were found to be retained intracellular and rapidly degraded by a non-lysosomal mechanism [33]. The degradation of truncated ribophorin Ri-332 was found to be ERManI dependent and to undergo proteasomal degradation in the cytosol [34].

BACE1 *(*beta-secretase1) is a type 1 transmembrane aspartic acid protease with 4 potential glycosylation sites, folded in the ER and stabilized by disulfide bonds [35]. It is involved in the processing of APP (amyloid precursor protein) to amyloid peptide (Aβ) in Alzheimer's disease. Alternative splicing of BACE1 mRNA resulted in generation of three variants that encoded truncated forms of the protein: BACE-501, BACE-476 and BACE-457, the latter two being retained in the ER and undergo proteasomal degradation directed by ERAD [36,37,38].

All these glycoproteins were employed here as ERAD substrates to elucidate the role of EDEM1 in ERAD.

RESULTS

Tyrosinase N-glycosylation and processing

To elucidate the role of glycans in tyrosinase function, several mutants with ablated glycans were constructed and the effect of these mutations on tyrosinase folding and traffic was analyzed. Primarily, the deletion of each individual glycans was targeted by site directed mutagenesis, replacing the N residue to Q in the N-X-T sequence to impede N-glycosylation (Δ1 to Δ7-TYR).

In the first step a DOPA-oxidase activity experiment was performed and it revealed wt-TYR and Δ5-TYR generated a similar level of oxidized DOPA (Figure 3A). The mutants without N-terminal glycans (Δ1-4) displayed a residual enzymatic activity and were able to oxidize a small amount of L-DOPA, whilst the C-terminal mutants (Δ6,7) had no enzymatic activity. This allowed the assumption that the fifth glycan is not required for tyrosinase functionality since Δ5-TYR can exhibit full oxidizing capacity. These results also raised a question concerning the requirement of the C-terminal glycans for tyrosinase activity [39].

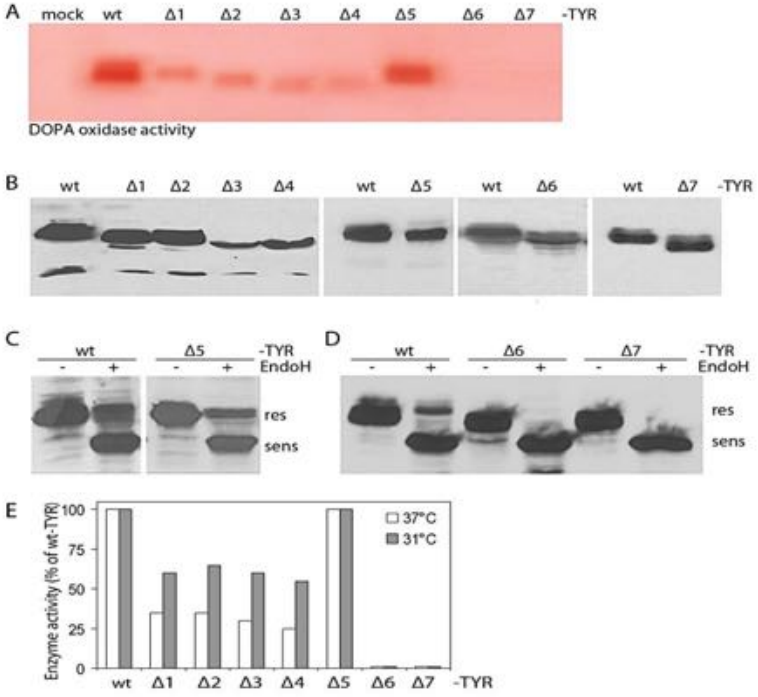

Figure 3. N-glycosylation site occupancy controls tyrosinase folding. (A) Single N-glycosylation mutants were overexpressed in HEK293T cells and their enzymatic activity was detected by *in gel* DOPA oxidase assay. **(B)** The expression of tyrosinase mutants in HEK293T cells was detected by Western blotting with mouse α-tyrosinase antibodies (T311). **(C and D)** The processing of tyrosinase mutants overexpressed in HEK293T cells was evaluated by EndoH digestion (*res*-complex glycans acquired in the Golgi, *sens*-high mannose ER-retained glycans) and Western blotting. **(E)** HEK293T cells were transfected with plasmids encoding for tyrosinase mutants and grown 24h at 37°C and 31°C respectively. The enzymatic activity of tyrosinase mutants was determined by evaluating the absorbance changes at 450nm

In figure 3B the expression of all tyrosinase N-glycosylation mutants transiently transfected in HEK293T, determined by Western blotting, is presented. The apparent molecular weight detected for each mutant differs according to the glycan presence, with the Δ5-TYR having the same apparent molecular weight as wt-TYR. By EndoH digestion it was revealed that deleting the N290 glycan din not affected tyrosinase processing and a correspondent fraction of complex glycans was detected for both wild type

and mutant protein (Figure 3C). The EndoH sensitivity of the other two mutants: Δ6-TYR and Δ7-TYR was also tested; as expected the glycans exposed on these mutants did not undergo Golgi processing (*res*) and possessed only high mannose/hybrid type EndoH sensitive forms (*sens*) (Figure 3D). This suggests they are retained in the ER and are probably sent to degradation by ERAD

N-glycosylation is an important process for tyrosinase and based on the previous observations, the effect of multiple glycan deletion was tested on tyrosinase. Tyrosinase mutants with three of the seven glycans deleted (Δ123-TYR, Δ567-TYR) and a tyrosinase with all the glycosylation sites mutated (Δall-TYR) were generated. The mutants obtained are depicted in figure 4A.

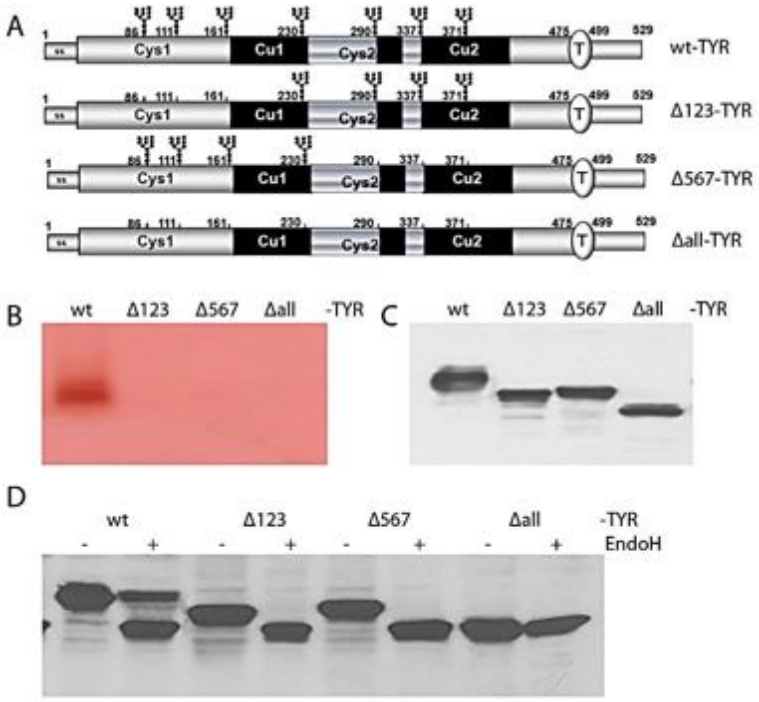

Figure 4. Characterization of tyrosinase mutants with multiple glycans deleted. (A) Graphical representation of the tyrosinase mutants. **(B)** Tyrosinase mutants *in gel* enzymatic activity assessment after transient transfection in HEK293T cells. **(C)** The expression of tyrosinase mutants transiently transfected in HEK293T cells was evaluated by Western blotting in reducing conditions. **(D)** EndoH sensitivity of tyrosinase mutants determined after overexpression in HEK293T cells and Western blotting for tyrosinase

In the first step the enzymatic activity of these mutants was tested and none of them could conserve enzymatic activity as observed in figure 4B. This result is explained by the previous ones when single N-glycans deletion had severe consequences on tyrosinase folding and enzymatic activity. The expression of these mutants, by transient transfection in HEK293T cells and Western blotting, was also assessed. As expected, the proteins were expressed at similar levels and migrated according to their specific molecular weight (Figure 4C). However, the Δ567-TYR had a slightly higher MW than Δ123-TYR possibly due to the lack of N290 glycan, not occupied in wt-TYR.

In terms of glycan processing, the EndoH digestion shown in figure 4D revealed no complex N-glycans forms for all three tyrosinase mutants. This indicates an ER retention of these proteins with no transport to the Golgi, supporting thus the enzymatic activity assay presented in A. All these results suggest that N-glycosylation is an important process for tyrosinase folding and processing and any glycan modification had a dramatic effect on its functionality.

EDEM1 structure and function

Using specific bioinformatics tools a tridimensional model of EDEM1 was constructed based on similarity with α-mannosidases. This model covered an extended sequence of EDEM1 with the exception of two regions: the N-terminal sequence of approximately 100 amino acids and the 54 aminoacids fragment from the C-terminus. These regions were predicted as mainly coiled and the N-terminal extension displays an increased disposition for intrinsic disorder in the region 40 to 119, with the highest values between aa 48–93 [40]. Analysis of the model and correlation with the aminoacid structure revealed a requirement for three highly conserved aminoacids in mannosidases as a prerequisite for binding mannose residues [40,41].

In order to investigate the role of different domains in EDEM1 function two mutants were constructed: the first mutant had the intrinsically disordered domain deleted (Δ-EDEM1) and the other mutant has three aminoacids mutated predicted to be critical for glycan binding in the mannosidase site of EDEM1 (AVV-EDEM1).

The expression of these mutants was verified by transient transfection in HEK293T cells. 24 h post-transfection the cells were lysed and subjected to SDS-PAGE in reducing conditions and Western blotting. EDEM1 mutants expression was verified by blotting with either rabbit α-EDEM1 and goat α-HA antibodies, since all EDEM1 constructs have an HA tag. As depicted in figure 5A, both antibodies recognized the EDEM1 mutants at their expected molecular weight, with similar levels detected. When the same antibodies were used for a pulse chase and immunoprecipitation experiment an improved capacity of the home-made antibodies to recognize the EDEM1 mutants was observed; therefore, for further immunoprecipitation experiments only these antibodies were used (Figure 6B).

In both Western blotting and immunoprecipitation experiments the EDEM1 mutants presented a band at lower molecular weight (Figure 5A and B); to test if this band is due to glycosylation discrepancies an EndoH digestion experiment was performed. Indeed as observed in figure 5C the band of lower molecular weight represented the non-glycosylated form of the EDEM1 mutants [40].

The half-life of EDEM1 mutants was determined by radioactive labeling and immunoprecipitation at different time points. The Δ-EDEM1 mutant degraded faster than the wt and AVV-EDEM1 (Figure 5D) and the correspondent half-lifes were determined at roughly 1.5h for the Δ-EDEM1 and around 2h for the wt-EDEM1 and AVV-EDEM1 [40].

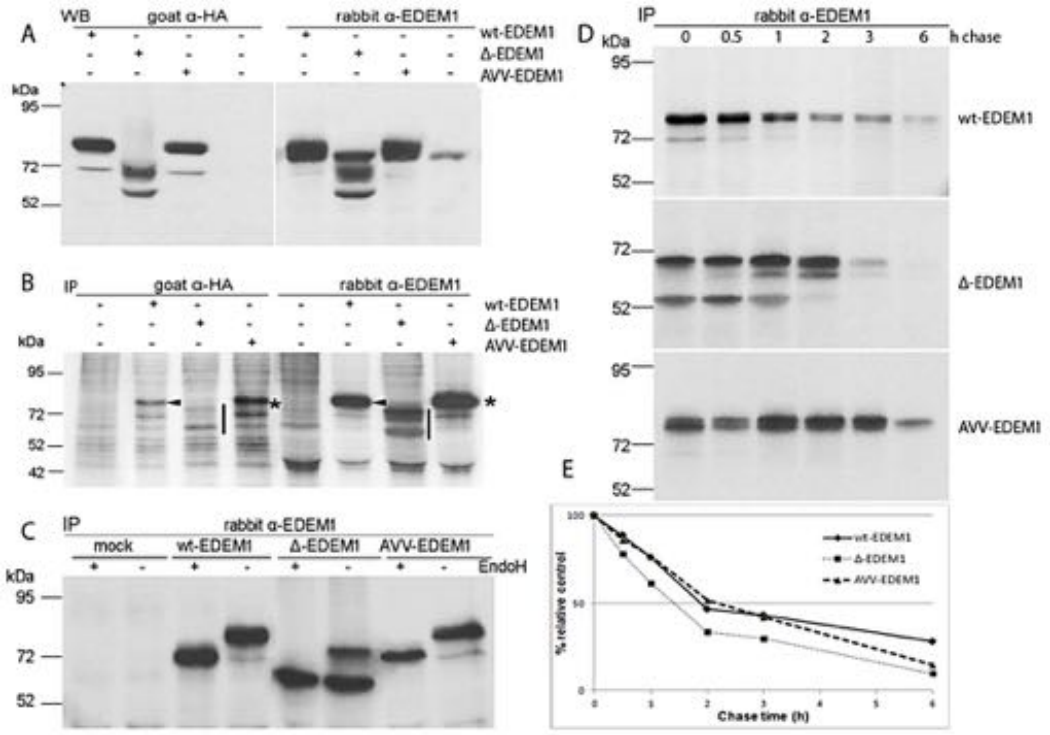

Figure 5. EDEM1 mutants characterization. (A) EDEM1 mutants were transfected in HEK 293T; 24h post-transfection the cells were harvested and lysed. Total lysates were used for SDS-PAGE and Western blotting with goat α-HA or rabbit polyclonal antiserum to EDEM1. **(B)** The transfection was performed as in (A) and the cells were pulse labeled for 30 min and harvested. Cell lysates were used for immunoprecipitation with HA or EDEM1 antibodies. **(C)** Cells overexpressing wt-EDEM1, Δ-EDEM1 and AVV-EDEM1 were used for pulse chase and immunoprecipitation and the eluates were subjected to EndoH digestion. The samples were separated by SDS-PAGE and visualized by autoradiography. **(D)** HEK 293T transiently transfected to express EDEM1 mutants were pulse labeled for 20 min and chased for 0 up to 6h. Cell lysates were immunoprecipitated with anti-EDEM1 antisera and the eluates were solved by SDS-PAGE; autoradiograms for all three mutants are presented here. **(E)** Band densitometry for the upper mentioned samples was represented here (mean of two independent experiments)

Previous results showed that EDEM1 knockdown impairs tyrosinase degradation and extends its half-life; next, I analyzed the effect of EDEM1 mutants overexpression on tyrosinase degradation. EDEM1 mutants were co-expressed with wild type, soluble and non-glycosylated tyrosinase respectively and the levels of tyrosinase were evaluated. 48h post transfection the cells were harvested and the proteins were separated by SDS-PAGE in denaturing conditions and subjected to Western blotting with tyrosinase, EDEM1 and calnexin antibodies respectively.

As observed in figure 6A, the degradation of wt-TYR and ST-TYR was accelerated when they were co-expressed with wt-EDEM1. The expression level of tyrosinases was lowered with ~80% compared to control samples (Figure 6C). Despite the mutation of the three aminoacids predicted to be critical for glycan recognition, the AVV-EDEM1 accelerated tyrosinase degradation similar to wt-EDEM1 (Figure 6A and C). However, Δ-EDEM1 had a less dramatic effect on tyrosinase degradation; the level of

tyrosinase detected in this case was lowered with 20-25%, but I estimated the decrease was irrelevant in comparison to the effect the other two EDEMs had (Figure 6A and C) [40].

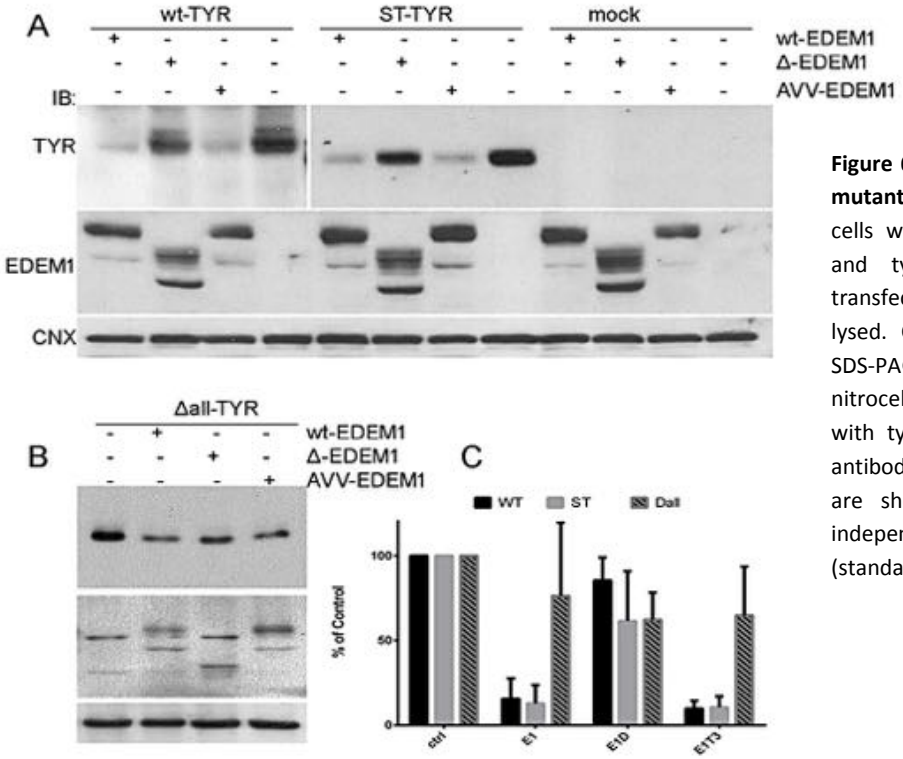

Figure 6. EDEM1 accelerates tyrosinase mutantsdegradation. (A and B) 293T cells were co-transfected with EDEM1 and tyrosinase mutants, 48h post transfection cells were harvested and lysed. Cell lysates were separated by SDS-PAGE, transferred onto nitrocellulose membrane and probed with tyrosinase or EDEM1 recognizing antibodies. **(C)** Band densitometry plots are shown here as mean of three independent experiments with SD (standard deviation).

The EDEM1 mutants have a less pronounced effect on the non-glycosylated tyrosinase (Δall-TYR), probably due to an overall reduced level of EDEM1 mutants in this case (middle panel of figure 6B). Moreover no significant differences could be detected for Δall-TYR levels when co-expressed with all EDEM1 mutants (Figure 6C).Overall these results sustain the hypothesis that the N-terminal domain of EDEM1 is important for its function in ERAD and as observed here, is imperative for accelerating the degradation of tyrosinase mutants [40].

Since EDEM1 mutants could affect tyrosinase degradation, in the next step the association between these proteins was tested. EDEM1 constructs (wt-EDEM1, AVV-EDEM1, Δ-EDEM1) and tyrosinase mutants (wt, ST, Δall-TYR) were co-transfected in HEK293T cells and used for a pulse chase and immunoprecipitation experiment with both tyrosinase and EDEM1 antibodies. To exclude the possibility that the absence of interaction between Δ-EDEM1 and tyrosinase mutants is not actually due to DTT sensitive conformational changes induced by deleting the IDD domain from EDEM1 the experiment was performed in reducing and non-reducing conditions. Controls are presented in figure 7A.

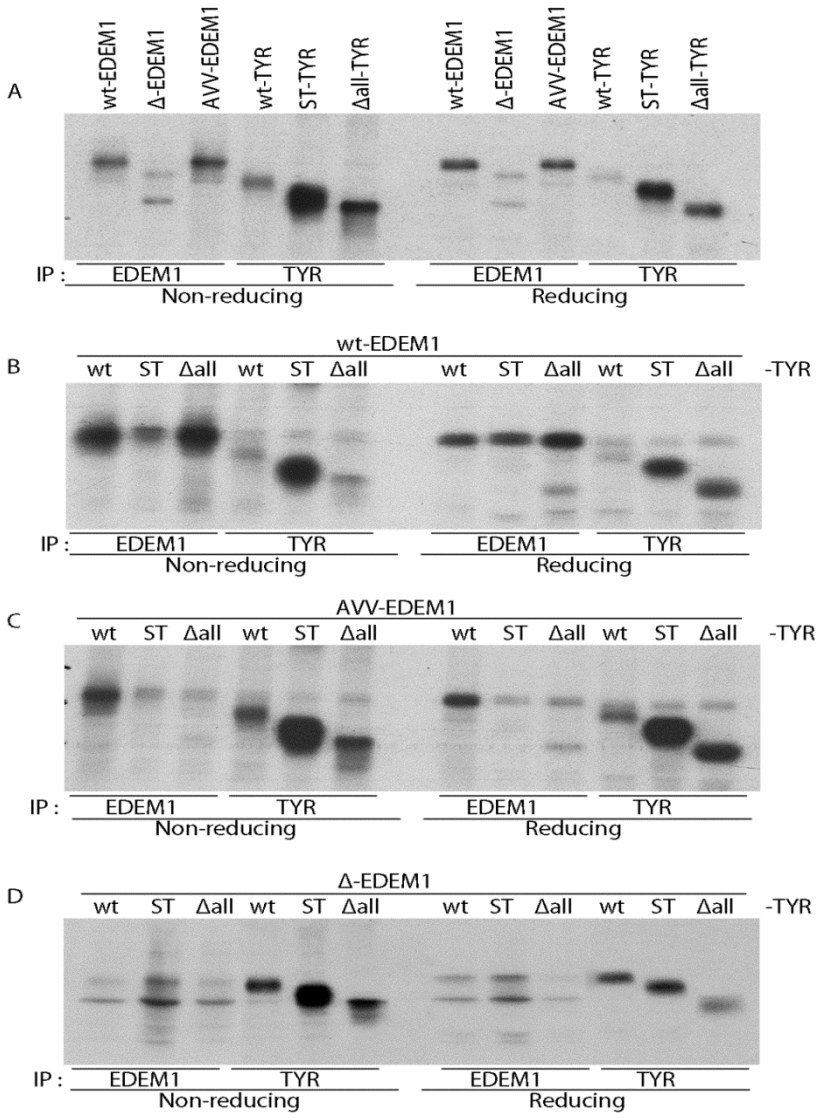

Figure 7. Tyrosinase mutants associate EDEM1 and its mutant forms and the interaction is not DTT sensitive. HEK293T cells were transiently transfected with tyrosinase and EDEM1 mutants, respectively, labeled with [S^{35}]-Met/Cys for 30 min and harvested. Cell lysates were immunoprecipitated with either tyrosinase or EDEM1 antisera. The eluates were split in two: half were boiled in non-reducing sample buffer and the other half was boiled in the presence of DTT respectively. The autoradiograms are presented in **(A)** for single transfected samples for both EDEM1 and tyrosinase mutants. In figures **(B)** wt-EDEM1 **(C)** AVV-EDEM1 and **(D)** Δ-EDEM1, respectively, the co-immunoprecipitation samples of EDEM1 with tyrosinase mutants are presented.

As observed in figure 7B wt-EDEM1 was co-precipitated with all tyrosinase mutants regardless of the reducing agent's presence. The AVV-EDEM1 mutant also co-precipitated with all tyrosinases, and similar levels were observed in reducing and non-reducing conditions (Figure 7C). When the same experiment was performed for cells co-expressing Δ-EDEM1 and tyrosinase mutants, no interaction between Δ-EDEM1 and tyrosinase mutants could be observed, even in non-reducing conditions (Figure 7D). Altogether this implies the IDD of EDEM1 is required for its interaction with tyrosinase and this interaction is not dependent on disulfide bond formation.

Previous studies indicated that EDEM1 directly interacts with SEL1L, an adaptor protein that scaffolds the ERAD substrates to the retrotranslocation channel [8,42,43]. Thus it was interesting to investigate the interaction of EDEM1 mutants to SEL1L; this interaction was assessed by two

complementary methods: pulse chase and immunoprecipitation and immunoprecipitation coupled with Western blotting.

EDEM1 mutants were transiently transfected in 293T cells and the cells were used for pulse chase and immunoprecipitation with EDEM1 or SEL1L antibodies and the eluted samples were separated by SDS-PAGE and visualized by autoradiography. EDEM1 mutants were co-precipitated with SEL1L, as observed in figure 8A, suggesting a direct interaction independent of the mutations generated here for EDEM1 [40].

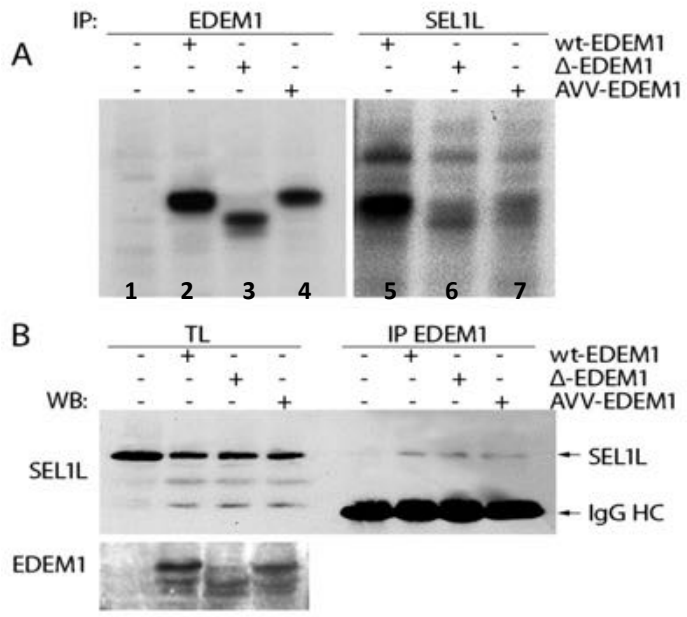

Figure 8. EDEM1 mutants associate with SEL1L. **(A)** 293T cells were transiently transfected with wt-EDEM1, Δ-EDEM1 and AVV-EDEM1, respectively, starved in Met/Cys free media, pulse labeled with [S^{35}]-Met/Cys for 20 min and harvested. Cell lysates were used for immunoprecipitation with antibodies for EDEM1 and SEL1L respectively. The samples were separated in SDS-PAGE gels and the dried gels were exposed to films. **(B)** Cells transfected as in (A) were used for immunoprecipitation with EDEM1 antibodies; the eluates were separated by SDS-PAGE in reducing conditions along with 5% of input (TL) and further subjected to Western blotting with SEL1L and EDEM1 antibodies.

The interaction between EDEM1 and SEL1L detected as previously described was confirmed with experiments of immunoprecipitation and Western blotting. HEK293T cells transiently transfected to overexpress EDEM1 mutants were harvested, lysed and immunoprecipitated with EDEM1 antibodies. The eluates were separated by SDS-PAGE, and used for Western blotting with antibodies for SEL1L. As observed in figure 8B SEL1L was co-precipitated with all EDEM1 mutants, suggesting a direct association between the two proteins, supporting the previous experiment. Altogether these data suggest that none of the mutations designed here for EDEM1 affected its capacity to associate with SEL1L [40].

CONCLUSIONS

In conclusion some of the connections established in ERAD were pointed out here with functional implications on protein homeostasis. The results presented here contribute to the elucidation and interpretation of the interatomic landscape of ERAD.

Tyrosinase N-glycosylation and processing

- ✓ N-glycosylation controls tyrosinase folding in the endoplasmic reticulum as both enzymatic activity assessment and folding experiments, indicate an important role for each glycan;
- ✓ The N290 site of tyrosinase is not occupied in wild type tyrosinase since deletion of this site had no impact on the traffic and activity of tyrosinase;
- ✓ The two C-terminal glycans are compulsory for folding and activity; in their absence tyrosinase in no longer efficiently transported from the ER and losses its enzymatic activity. Moreover, the Δ6-TYR and Δ7-TYR, unlike any other glycan deletion, were not able to recover enzymatic activity and implicitly fold correctly at lower temperature;
- ✓ Sliding the glycosylation site to proximal position in some cases recovers the wild type phenotype; thus glycan position is compulsory for tyrosinase structure and function;
- ✓ The glycans could modulate tyrosinase interaction with ER chaperones, as some of the mutants employed here showed disparate association kinetics with calnexin.

EDEM1 structure and function

- ✓ EDEM1 was found to accelerate the degradation of tyrosinase mutants in a glycan independent manner, as observed in Western blotting and pulse-chase and immunoprecipitation experiments;
- ✓ Inactivation of the glycan binding site had no effect on EDEM1 function since mutations of conserved aminoacids of this domain did not affect the association with ERAD substrates neither impede their degradation;
- ✓ The interaction between EDEM1 and tyrosinase mutants is mediated by its intrinsically disordered domain since the lack of the IDD abolished the association of EDEM1 with tyrosinase mutants;
- ✓ EDEM1 function in ERAD is controlled by its ID domain, since it seems the degradation of other misfolded proteins is dependent of its presence;
- ✓ The association of EDEM1 to ER chaperone and ERAD involved protein takes place independent of the presence of the ID domain or an active glycan binding site;
- ✓ EDEM1 interacts with EDEM3 and their association has functional implications since they could modulate one another's expression and traffic;
- ✓ EDEM1 undergoes active demannosylation in the ER since kifunensine treatment blocked this process.

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LIST OF PUBLICATIONS:

Published papers/patents:

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2. Dana Cioaca*, Simona Ghenea*, Laurentiu N Spiridon*, Marioara Marin*, Andrei-Jose Petrescu, Stefana M. Petrescu, "C-terminus glycans with critical role in the maturation of secretory glycoproteins", (*authors with equal contribution), PLoS ONE 2011;6(5):e19979. Epub 2011 May 18 *8 citations (Google Scholar)*
3. National OSIM patent,Marioara Marin, Ghenea S, Petrescu S.M. "Production of antibodies against EDEM1"- 2010