Characterization of human torsinA and its dystonia-associated mutant form

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Deletion of a single glutamate in torsinA correlates with early-onset dystonia, the most severe form of a neurological disorder characterized by uncontrollable muscle contractions. TorsinA is targeted to the ER (endoplasmic reticulum) in eukaryotic cells. We investigated the processing and membrane association of torsinA and the dystonia-associated Glu-deletion mutant (torsinAΔE). We found that the signal sequence of torsinA (residues 1–20 from the 40 amino-acid long N-terminal hydrophobic region) is cleaved in *Drosophila* S2 cells, as shown by the N-terminal sequencing after partial protein purification. TorsinA is not secreted from S2 cells. Consistently, sodium carbonate extraction and Triton X-114 treatment showed that torsinA is associated with the ER membrane in CHO (Chinese-hamster ovary) cells. In contrast, a variant of torsinA that contains the native signal sequence without the hydrophobic region Ile^{24}–Pro^{40} does not associate with the membranes in CHO cells, and a truncated torsinA without the 40 N-terminal amino acids is secreted in the S2 culture. Thus the 20-amino-acid-long hydrophobic segment in torsinA, which remains at the N-terminus after signal-peptide cleavage, is responsible for the membrane anchoring of torsinA. TorsinAΔE showed similar cleavage of the 20 N-terminal amino acids and membrane association properties similar to wild-type torsinA but, unlike the wild-type, torsinAΔE was not secreted in the S2 culture even after deletion of the membrane-anchoring segment. This indicates that the dystonia-associated mutation produces a structurally distinct, possibly misfolded, form of torsinA, which cannot be properly processed in the secretory pathway of eukaryotic cells.

Key words: dystonia, endoplasmic reticulum, signal peptide, torsinA, transmembrane protein.

INTRODUCTION

Early-onset dystonia is the most severe and common form of hereditary dystonia, a neurological disorder manifesting as uncontrollable movements and abnormal twisted postures. Progress in understanding the etiology of this disease and developing therapies has been slow due to the absence of evident neuropathology in dystonia patients, in contrast with other movement disorders, such as Huntington's disease and Parkinson's disease [1]. The first clue about the molecular origins of early-onset dystonia came with the discovery of a mutation in the *DYT1* gene, which is associated with the majority of cases of the disease, with a minority of cases apparently not linked to this locus [2,3]. *DYT1* is expressed ubiquitously in human tissues and encodes a 37.8-kDa protein, torsinA. The dystonia-associated mutation is an in-frame GAG deletion that results in a loss of one glutamic acid residue from the Glu-Glu pair in the C-terminal region of torsinA. Protein homologues of torsinA have been found in mammalian genomes, *Drosophila*, nematodes, and zebrafish, but not in yeast or prokaryotic organisms, which suggests that function of the torsins may be related to the physiology of multicellular organisms [4].

Although the function of torsinA is unknown, several important clues about this protein can be deduced from its amino-acid sequence [2,5]. TorsinA belongs to the recently identified AAA^+ protein superfamily of ATPases associated with a variety of cellular activities [6]. AAA^+ ATPases are found in both prokaryotic and eukaryotic cells and are involved in a variety of functions, such as protein quality control (refolding, disaggregation and degradation), membrane fusion and vesicular transport, DNA replication and repair, and cytoskeletal regulation [7,8]. AAA^+ proteins can be described as 'molecular machines' that use energy from the hydrolysis of ATP to drive rearrangements in macromolecular structure or to assemble and disassemble multiprotein complexes. AAA^+ sequences contain either one or two conserved ATP-binding AAA^+ modules, as well as more variable N- and/or C-terminal extensions, which are believed to specify the activity of an AAA^+ protein by mediating its interactions with partner proteins [9]. Whereas most AAA^+ proteins are soluble, one group of AAA^+ proteases forms membrane-anchored oligomers [10].

TorsinA contains a single AAA^+ module including Walker A and B nucleotide binding motifs, as well as several other characteristic sequence motifs (see Figure 1). The interaction of torsinA with ATP has been confirmed experimentally [11]. Interestingly, the AAA^+ sensor-2 motif (located C-terminally to Box VII″), which supports the nucleotide binding and oligomerization of some AAA^+ proteins [12,13], is not easily recognizable in torsinA, and the sequence similarity between torsinA and other AAA^+ proteins is poor within the C-terminal region [6]. Thus it is possible that the C-terminal region of torsinA (where the dystonia-associated glutamate deletion occurs) contains a putative 'binding domain', which uniquely determines the function of the protein.

The amino-acid sequence of torsinA contains a 40-amino-acid-long N-terminal hydrophobic region (see Figure 1). Recent studies showed the targeting of torsinA to the lumen of the ER (endoplasmic reticulum) in several mammalian cell lines [14,15]. In contrast, the glutamate-deletion mutant of torsinA was found in cytosolic inclusions, possibly derived from the ER. The pattern of protein glycosylation indicates that torsinA does not progress in the secretory pathway beyond the ER and/or the *cis*-Golgi compartment [14].

Hydrophobic N-terminal sequences of lumenal proteins are often removed by signal peptidases [16,17]. It is not known, however, how the signal sequence of torsinA is processed, if any

Abbreviations used: CHO, Chinese-hamster ovary; Con A, Concanavalin A; ER, endoplasmic reticulum; ERAD, ER-associated degradation; PNS, post-nuclear supernatant.

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1   MKLGRAVLGL LLLAPSVVQA VEPISLGLAL AGVLTGYIYP RLYCLFAECC GQKRSLSREA
          Box II                                    Walker A
61  LQKDLDDNLF GQHLAKKIIL NAVFGFINNP KPKKPLTLSL HGWTGTGKNF VSKIIAENIY
          Box IV                          Box IV'     Walker B
121 EGGLNSDYVH LFVATLHFPH ASNITLYKDQ LQLWIRGNVS ACARSIFIFD EMDKMHAGLI
    Box VI              Sensor 1          Box VII             Box VII'
181 DAIKPFLDYY DLVDGVSYQK AMFIFLSNAG AERITDVALD FWRSGKQRED IKLKDIEHAL
                        Box VII''
241 SVSVFNNKNS GFWHSSLIDR NLIDYFVPFL PLEYKHLKMC IRVEMQSRGY EIDEDIVSRV

301 AEEMTFFPKE ERVFSDKGCK TVFTKLDYYY DD
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Figure 1 Amino-acid sequence of human torsinA

Characteristic AAA$^+$ sequence motifs are shown in bold (see [6]). The pair of glutamates, one of which is deleted in the dystonia-associated mutant torsinA, is marked with **. The cleaved signal sequence is shown in italics and the membrane-anchoring region is underlined (see the Results section).

part of the hydrophobic region remains in the mature torsinA, and, if this is the case, whether such a hydrophobic region anchors the protein in the ER membrane, as has been suggested previously [4]. In this study, we addressed the question of processing and membrane association of torsinA and the dystonia-associated Glu-deletion mutant (torsinAΔE). We found that the signal sequence of torsinA is cleaved upon the protein's import into the ER and that the remaining part of the hydrophobic sequence anchors torsinA in the ER membrane. Thus torsinA appears to be the only known membrane-anchored lumenal AAA$^+$ ATPase, which suggests its important role in the secretory machinery of eukaryotic cells. Our results may stimulate further research on the function of torsinA and its relation to dystonia by focusing future studies on the activities associated with the ER membrane.

EXPERIMENTAL

TorsinA variants

The variants of torsinA produced in the present study are shown in Figure 2. DNA constructs containing the coding regions of human torsinA and torsinAΔE in pcDNA3 vector [14] were kindly provided by Dr Xandra Breakefield (Massachusetts General Hospital, Harvard Medical School, Boston, MA, U.S.A.). These vectors (pcDNA3torA, pcDNA3torAΔE) were used for expression of the full-length TorA and TorAΔE in CHO (Chinese-hamster ovary) cells. For expression of TorA and TorAΔE in *Drosophila* S2 cells, the DNA constructs of torsinA or torsinAΔE, including STOP codons, were amplified by PCR using *PfuTurbo* DNA polymerase (Stratagene). The PCR products were subcloned into pMT/V5-His A vector (Invitrogen) between *Eco*RI and *Xba*I sites. For expression of Δ(24–40) and Δ(24–40)ΔE in CHO cells, fragments of pcDNA3torA and pcDNA3torAΔE that did not contain regions encoding the amino acids from positions 24–40 were produced by PCR using *PfuTurbo* DNA polymerase, followed by phosphorylation of blunt 5′-ends and DNA ligation. The N-terminally truncated variants (Δ40, Δ40ΔE) for expression in S2 cells were generated by PCR and cloned into pMT/Bip/V5-His A vector (Invitrogen) between *Sma*I and *Xba*I sites.

Antibodies

Anti-peptide antibodies recognizing the C-terminal sequence of torsinA [11] were a gift from Dr P. Shashidharan (Mount Sinai School of Medicine, New York, NY, U.S.A.). Rabbit anti-calnexin polyclonal antibody and mouse anti-KDEL monoclonal antibody were obtained from StressGen Biotechnologies, Victoria, BC, Canada.

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TorA            MKLGRAVLGL LLLAPSVVQA VEPISLGLAL AGVLTGYIYP RLYCLFAECC ...
TorAΔE

Δ(24-40)        MKLGRAVLGL LLLAPSVVQA VEP---     ----------  RLYCLFAECC ...
Δ(24-40)ΔE

Δ40                       MKLCILLAVV AFVGLSLGRS PWPGHHHHHH RLYCLFAECC ...
Δ40ΔE
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Figure 2 Variants of torsinA produced in this study

The N-terminal sequences are shown for the full-length TorA and TorAΔE constructs, the Δ(24–40) and Δ(24–40)ΔE constructs with a deletion within the hydrophobic region (Ile^{24}–Pro^{40}), and the Δ40 and Δ40ΔE constructs with a deletion of the hydrophobic leader sequence (Met^1–Pro^{40}) and an insertion of the BiP signal sequence (underlined) and a 6-His tag. The variants TorAΔE, Δ(24–40)ΔE, and Δ40ΔE contain deletions of a single glutamic acid residue in the Glu^{302}/Glu^{303} pair (see Figure 1).

CHO cell culture, transfection and cell extracts

CHO-K1 cells (ATCC) were maintained in the growth medium F-12K (Invitrogen) supplemented with 10% (v/v) fetal bovine serum (BioWhittaker) at 37 °C in the presence of 5% CO_2. Transfections with pcDNA3 vectors containing TorA, TorAΔE, Δ(24–40), or Δ(24–40)ΔE were performed using FuGene 6 transfection reagent (Roche) according to the manufacturer's instructions. Two days post-transfection, cells were collected and washed twice with PBS. Cells were then incubated on ice for 10 min in hypotonic buffer [10 mM Tris/HCl, pH 7.5, with protease inhibitor cocktail (Roche)] and disrupted using a Dounce homogenizer (20 × 3 strokes). Cell lysates were subjected to centrifugation (1000 ***g***, 5 min) and the resulting PNS (post-nuclear supernatants) were used in the membrane protein extractions.

Carbonate extraction

For the carbonate extraction [18], PNS from the CHO lysate was mixed with an equal volume of 0.2 M sodium carbonate, pH 11.5 and incubated at 4 °C for 30 min with gentle agitation. Soluble and membrane proteins were then separated by centrifugation at 100000 ***g*** (30000 r.p.m. in a Beckman 50.2Ti rotor) for 60 min at 4 °C. The supernatant and the pellet were dissolved in equal amounts of SDS sample loading buffer and subjected to SDS/PAGE.

Triton X-114 extraction

For the Triton X-114 partitioning [19], Triton X-114 (Sigma) was pre-condensed by washing three times in TBS [10 mM Tris/HCl, pH 7.5, 150 mM NaCl]. The concentration of the stock was then determined by spectrophotometry [$\varepsilon_{280} = 28$ for 1% (w/v) Triton X-114]. PNS from the CHO lysate was mixed with an equal volume of Triton X-114 buffer [2% (w/v) Triton X-114 in TBS] and incubated at 4 °C for 45 min with end-over-end mixing. The aqueous phase and the detergent-rich phase were separated by incubating at 37 °C for 10 min and centrifugation at 3000 ***g*** for 3 min. The extraction process was repeated three times. The resulting soluble protein fraction and membrane protein fraction were then dissolved in equivalent amounts of SDS sample loading buffer.

Drosophila S2 cell culture and transfection

Drosophila S2 cells (Invitrogen) were maintained in the complete medium [Schneider's *Drosophila* medium (Invitrogen) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Invitrogen)] at 23 °C. pMT/V5-His A vectors containing TorA

or TorAΔE and pMT/Bip/V5-His A vectors containing Δ40 and Δ40ΔE were maintained in *E. coli* strain DH5α (Stratagene) and purified using EndoFree Plasmid Maxi kit (Qiagen). *Drosophila* S2 cells were co-transfected with one of the expression vectors together with the blasticidin-resistance gene bearing vector pCoBlast (Invitrogen) using the calcium phosphate transfection method (Invitrogen). Stably transfected cell lines were selected after 2 weeks of culturing with 25 μg/ml blasticidin (Invitrogen) in the complete medium.

Protein expression in *Drosophila* S2 cells, partial protein purification and N-terminal sequencing

Expression of proteins in *Drosophila* S2 cells was induced by 0.5 mM $CuSO_4$ for 2 days. For the N-terminal sequencing of TorA and TorAΔE, cells were lysed in 25 mM Tris/HCl, pH 7.5, 0.5 M NaCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$, 1 % (w/v) Triton X-100 with protease inhibitor cocktail (Roche). Cell lysates were subjected to centrifugation (1000 ***g***, 5 min) and filtration (0.22 μm filter). Clear filtrates were incubated with Con A (Concanavalin A) Sepharose (Pharmacia) at 4 °C for 2 h. The Con A resin was washed first with the lysis buffer and then with 0.2 M methyl α-D-mannopyranoside (Sigma) in the lysis buffer. The fraction containing TorA or TorAΔE was eluted by an overnight incubation at 4 °C with 1 M methyl α-D-mannopyranoside in the lysis buffer. Proteins in the eluted fraction were separated by SDS/PAGE and transferred to PVDF membrane. The Coomassie-stained band (see Results and Figure 4A) present in the eluate containing TorA or TorAΔE, but not in the eluate from mock-transfected cells (empty expression vector), were cut from the PVDF membrane and sequenced by Edman degradation at the Biotechnology Microchemical Core Facility, Kansas State University.

Immunoblotting

Samples from the CHO cell extractions and S2 cells and media were separated by SDS/PAGE and transferred to nitrocellulose membrane. The following dilutions of antibody were used: anti-calnexin, 1:2000; anti-KDEL, 1:250; anti-torsinA, 1:2500; goat anti-rabbit secondary antibody conjugated to horseradish peroxidase (Southern Biotechnology Associates, Birmingham, AL), 1:4000; goat anti-mouse secondary antibody (Southern Biotechnology Associates), 1:2000. Blots were visualized by SuperSignal West Pico Chemiluminescent Substrate (Pierce).

RESULTS

First, we asked whether torsinA expressed in eukaryotic cells behaves as a membrane protein or a soluble one. After transient transfection of CHO cells with pcDNA3 vector containing torsinA or torsinAΔE DNA (full-length TorA or TorAΔE constructs, see Figure 2), the cells were lysed and the cell extracts were subjected to either high pH treatment in the carbonate buffer [18] or Triton X-114 extraction of membrane proteins [19]. The samples were subsequently separated by centrifugation into supernatant fractions, which contained soluble proteins, and pellet fractions, which contained transmembrane proteins. As shown in Figure 3, TorA, as well as TorAΔE, were found exclusively in the pellet fractions after the Triton X-114 extraction and were also predominantly found in the carbonate pellets. To test the efficiency of fractionation, we tracked calnexin, an ER transmembrane protein, as well as a group of soluble lumenal proteins that contain the C-terminal KDEL sequence. Results of the extraction experiments shown in Figure 3 indicated that torsinA is a transmembrane protein.

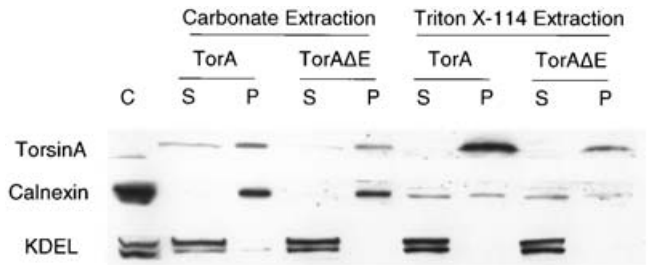

Figure 3 Fractionation of full-length torsinA

CHO cells were transiently transfected with TorA in pcDNA3 or with TorAΔE in pcDNA3. Post-nuclear fraction from the cells collected 2 days after the transfection was either incubated in 0.1 M sodium carbonate, pH 11.5, followed by ultracentrifugation, or extracted with 1 % Triton X-114 at 4 °C, followed by incubation at 37 °C and centrifugation (see the Experimental section). The supernatant (S) and pellet (P) fractions from both fractionation methods were subjected to SDS/PAGE and Western blotting analysis using either anti-torsinA antibodies (top), anti-calnexin (middle), or anti-KDEL antibodies (bottom). The two bands recognized by the anti-KDEL antibody correspond to ER-resident soluble proteins: Grp94 and BiP. C, control untransfected cells. A representative result of two independent experiments is shown.

Interestingly, extraction of torsinA from the membrane into the Triton X-114 detergent phase was more efficient than that of calnexin (see Figure 3). After a high pH-induced disruption of membranes, a small fraction of torsinA (but no detectable amounts of calnexin) was found among soluble proteins in the supernatant. Collectively, our data suggest that the mechanism of membrane anchoring and/or the lipid environment of transmembrane domains may be different for calnexin and torsinA. The dystonia-associated deletion of a glutamate in the C-terminal region of torsinA did not affect the membrane anchoring of the protein.

Next, we investigated the proteolytic processing of the signal sequence of torsinA. To obtain higher levels of protein synthesis than those shown in mammalian cells, we stably transfected *Drosophila* S2 cells with a vector containing the DNA sequence of TorA or TorAΔE. Accumulation of TorA and TorAΔE in insect cell lysates was confirmed by Western blotting (see Figure 5). Since torsinA is glycosylated *in vivo* [14,15], we attempted to achieve partial purification of the protein using a Con A Sepharose column. Indeed, the eluate from Con A Sepharose showed a strong Coomassie-stained band (∼35 kDa, marked with an arrow in Figure 4A) in cells transfected with TorA or TorAΔE, but not in those transfected with the empty vector. The Con A eluates were highly enriched in the 35-kDa protein, in comparison with the total cell lysates (Figure 4A). Western blotting of the Con A eluates with anti-torsinA antibodies showed a strong signal at the position of the 35 kDa band in either TorA or TorAΔE-transfected cells, but not in the control cells (Figure 4B).

Proteins separated by SDS/PAGE, as shown in Figure 4(A), were transferred on to a PVDF membrane, the 35-kDa bands were excised and subjected to N-terminal sequencing by Edman degradation. The N-terminal sequence of the protein band shown in Figure 4(A) was VEPISLG, which uniquely corresponds to the amino acids 21–27 in the sequence of torsinA (see Figure 1). We conclude that the torsinA N-terminal signal sequence is cleaved after Ala^{20} during or after import of the protein into the ER. The deletion of a glutamate in TorAΔE did not have any effect on the processing of the N-terminal leader sequence.

We then asked whether the remaining part of the hydrophobic leader sequence of torsinA (amino acids 21–40) was responsible

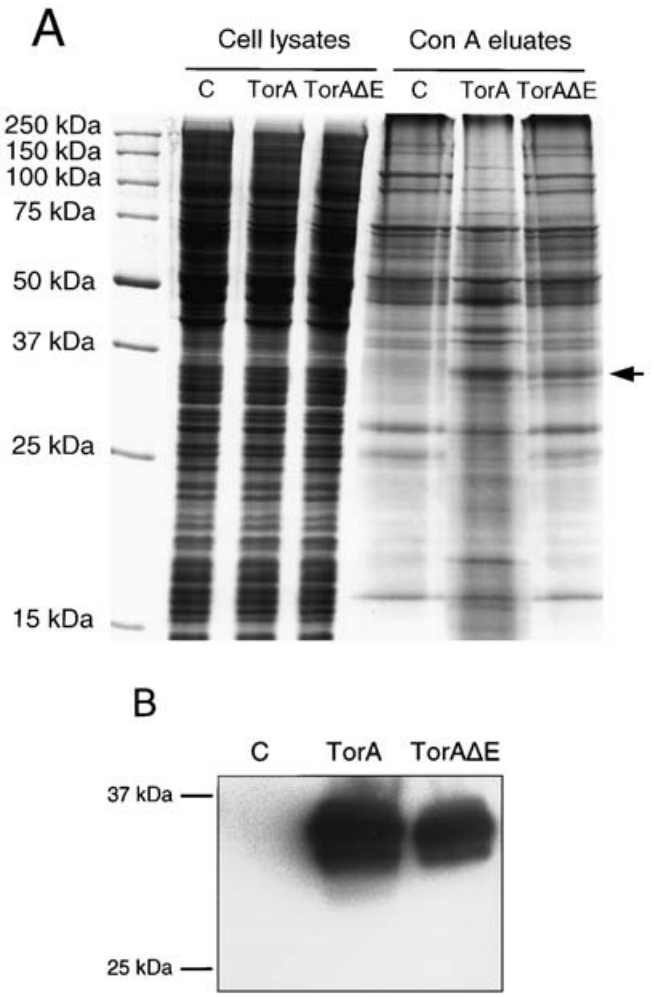

Figure 4 Partial purification of torsinA and torsinAΔE from *Drosophila* S2 cells

S2 cells were stably transfected with an empty vector (control, C) or with a vector containing the DNA sequence of TorA, or TorAΔE. Gene expression was induced with 0.5 mM $CuSO_4$ (see the Experimental section). Cells were lysed in the presence of 1 % Triton X-100. Cell lysates were precleared by centrifugation and were applied to Con A Sepharose beads. After extensive washing, glycosylated proteins were eluted by an overnight incubation with 1 M methyl-α-D-mannopyranoside. (**A**) SDS/PAGE analysis of cell lysates and Con A eluates with a Coomassie Blue stain. (**B**) Western blot analysis using anti-torsinA antibodies of the samples eluted from the Con A column.

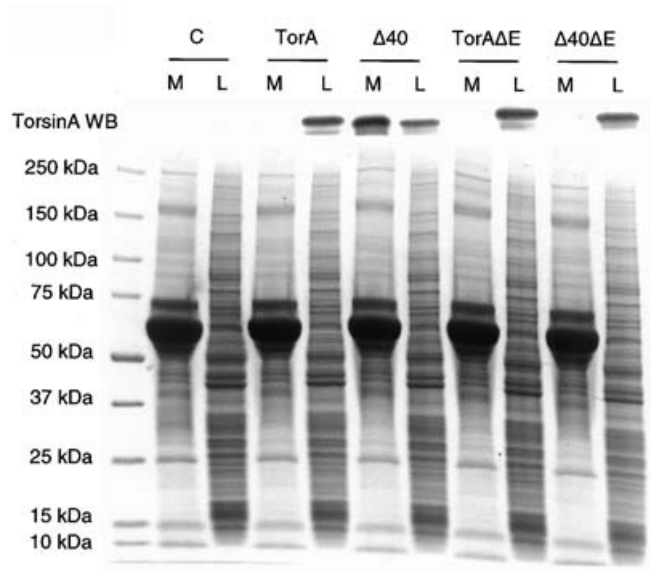

Figure 5 Expression of torsinA variants in *Drosophila* S2 cells

S2 cells were stably transfected with an empty vector (control, C), a vector containing the DNA sequence of TorA, Δ40, TorAΔE, or Δ40ΔE (see Figure 2). Cells were cultured in S2 medium supplemented with 10 % (v/v) heat-inactivated fetal bovine serum. Gene expression was induced with $CuSO_4$ (see the Experimental section). The cell-culture media (M) and cell lysates (L) were collected 2 days after induction and analysed by SDS/PAGE followed by Western blotting using anti-torsinA antibodies (upper panel) or Coomassie Blue staining (lower panel). A representative result of three independent experiments is shown.

for the apparent membrane anchoring of the protein, as shown in Figure 3. We produced DNA constructs of the N-terminally truncated torsinA and torsinAΔE without the hydrophobic leader sequences, i.e. starting at Arg^{41}. The resulting DNA constructs have been subcloned into an expression vector downstream from the sequence of the signal peptide from the *Drosophila* BiP gene and a 6-His affinity tag (constructs Δ40, Δ40ΔE, see Figure 2). As a result, we produced variants of torsinA and torsinAΔE, which are targeted to the secretory pathway, similar to the full-length torsinA, but do not contain the 40-amino-acid-long hydrophobic leader sequence.

We found that the torsinA variant Δ40, unlike the full-length TorA, is secreted in high amounts into the cell culture media and behaves as a soluble protein (Figure 5, torsinA Western blot). This result indicates that torsinA loses its amphipathic character upon deletion of the 40 N-terminal amino acids. The amount of the Δ40 variant in cell extracts is comparable to that of the full-length TorA, which suggests that Δ40 is not subject to intracellular degradation and implies that Δ40 is conformationally stable.

Notably, secretion of the variant Δ40ΔE was significantly less efficient than that of Δ40 (Figure 5). This indicates that the dystonia-associated glutamate deletion inhibits transport of torsinA through the secretory pathway. Our result is consistent with previous studies that showed an apparent removal of torsinAΔE from the ER and its accumulation in as yet uncharacterized inclusions [14,15].

To corroborate the results obtained in the S2 culture, we transiently transfected CHO cells with vectors containing truncated variants of torsinA. To test whether the amphipathic character of torsinA is indeed due to the 20-amino-acid-long segment, Val^{21}–Pro^{40}, which remains at the N-terminus of torsinA after cleavage of the signal peptide, we deleted the amino acids from positions 24–40 from the full-length torsinA [constructs Δ(24–40) and Δ(24–40)ΔE, see Figure 2]. The tripeptide Val^{21}–Pro^{23} was retained in the truncated torsinA variants in order to preserve the signal peptide cleavage site.

As shown in Figure 6, unlike the full-length TorA and TorAΔE, the truncated variants Δ(24–40) and Δ(24–40)ΔE were found predominantly in the supernatant fractions after either carbonate treatment or Triton X-114 extraction of membrane proteins (compare Figures 3 and 6). We conclude that the hydrophobic segment Ile^{24}–Pro^{40} is essential for the association of torsinA with membranes.

DISCUSSION

Although five years have passed since the dystonia-associated mutation in *DYT1* was identified, the biological function of

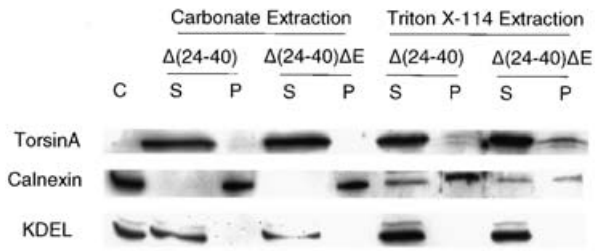

Figure 6 Fractionation of truncated torsinA

CHO cells were transiently transfected with the construct Δ(24–40) in pcDNA3 or with Δ(24–40)ΔE in pcDNA3 (see Figure 2). Post-nuclear fraction from the cells collected 2 days after the transfection was either incubated in 0.1 M sodium carbonate, pH 11.5, followed by ultracentrifugation, or extracted with 1 % Triton X-114 at 4 °C, followed by incubation at 37 °C and centrifugation (see the Experimental section). The supernatant (S) and pellet (P) fractions from both fractionation methods were subjected to SDS/PAGE and Western blotting analysis using either anti-torsinA antibodies (top), anti-calnexin (middle), or anti-KDEL antibodies (bottom). C, untransfected control cells.

torsinA remains unknown and no clear hypothesis has been proposed on the relation between the loss of a glutamic acid in torsinA and early-onset dystonia. In particular, fundamental biochemical properties of torsinA and its dystonia-associated mutant have not been explored yet. Our studies provide novel information on the biochemical properties and the topology of localization of torsinA. We have shown that torsinA is an amphipathic membrane-associated protein (see Figures 3 and 6).

The 20 N-terminal amino acids in torsinA form a signal sequence that targets the nascent protein to the translocation machinery in the ER membrane. Indeed, SignalP algorithm using neural networks and hidden Markov models trained on eukaryotic signal sequences [20] calculates a very high probability of the signal-peptide function for the 20 N-terminal amino acids in torsinA. The signal peptide cleavage site between Ala^{20} and Val^{21}, as predicted by SignalP, agrees with that determined experimentally (see the Results section). Two positively charged residues at the beginning of the signal sequence of torsinA (Lys^2, Arg^5) may preferentially stabilize the N-terminus at the cytoplasmic side of the translocon [16,17], which is consistent with the lumenal localization of the C-terminal part of torsinA.

After removal of the signal sequence (amino acids 1–20) during the protein import into the ER, the mature form of torsinA consists of at least two structural domains: the N-terminal hydrophobic domain (amino acids 21–40), which is responsible for the membrane association (see Figures 5 and 6) and the C-terminal lumenal domain (amino acids 41–332). The length of the membrane-association domain in torsinA is consistent with a single transmembrane polypeptide segment. We postulate, therefore, that the N-terminal domain of torsinA anchors the protein in the ER membrane with a type-II orientation [16]. Indeed, the TopPred algorithm [21] detects a single high-probability transmembrane segment in torsinA that spans the region Val^{21}–Pro^{40}.

The mechanism of ER-membrane insertion and signal-peptide processing in torsinA appears different from the conventional translocation mechanisms of many membrane-anchored proteins [17]. In torsinA, the signal-peptide cleavage site is adjacent to the putative transmembrane domain, which implies that the signal peptide and the transmembrane region may not orient themselves independently during topogenesis. It is possible that the signal-peptide cleavage occurs after the N-terminal amino acids of the transmembrane domain assume the type-II orientation within the translocon, which stabilizes the final topology of torsinA.

The transmembrane topology of torsinA in the ER is analogous to that of the yeast mitochondrial AAA^+ protease Yme1p, which is anchored in the inner membrane with a single N-terminal transmembrane segment and exposes its C-terminal catalytic domain into the inter-membrane space [10]. The C-terminal lumenal domain of torsinA is soluble and, most likely, conformationally stable (see Figure 5). In contrast, the low levels of secretion of the lumenal domain of torsinAΔE suggest that the dystonia-associated mutation induces a structural change in torsinA, possibly leading to protein misfolding, which blocks the processing of the lumenal domain within the secretory pathway. The mutation-induced structural changes are also likely to occur in the full-length membrane-anchored torsinA, which is retained in the ER [14]. The nature of such conformational changes will be explored once the purification of torsinA and torsinAΔE is achieved.

A number of ERSDs (ER storage diseases) are associated with a disruption of the processing of mutated proteins in the secretory pathway [22]. The source of pathology in such disorders may be associated with a loss of the native activity in a mutated protein or with an abnormal behaviour of the mutant. Misfolded lumenal proteins are usually retro-translocated into the cytosol and subjected to proteasomal degradation (ERAD, ER-associated degradation) [23]. In the case of torsinA, no apparent degradation of the glutamate-deletion mutant was observed (see Figures 3, 5 and 6). Instead of being degraded, torsinAΔE accumulates in cellular inclusions [14,15]. In the absence of an efficient ERAD, 'indigestion' associated with defective processing of ER proteins may manifest itself either as Russell bodies derived from the ER, cytoplasmic aggresomes assembled at the microtubule organizing centre, or cytoplasmic inclusion bodies [24]. Russell body formation is associated with misfolded/aggregated lumenal proteins, which cannot be retro-translocated into the cytosol for proteasomal degradation. In contrast, aggresomes and inclusion bodies are formed by proteins, whose degradation fails due to their (re)aggregation after translocation into the cytosol. Different cellular machineries may be involved in handling these different types of cellular deposits of defective proteins. In the context of dystonia, further studies are necessary to determine the origins and nature of the torsinAΔE-containing inclusions before hypotheses on their physiological effects can be proposed.

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