Truncation of the C Terminus of the Rat Brain Na\textsuperscript{+}-Ca\textsuperscript{2+} Exchanger RBE-1 (NCX1.4) Impairs Surface Expression of the Protein*

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The C terminus of the rat brain Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger (RBE-1; NCX1.4) (amino acids 875–903) is modeled to contain the last transmembrane \( \alpha \) helix (amino acids 875–894) and an intracellular extramembranous tail of 9 amino acids (895–903). Truncation of the last 9 C-terminal amino acids, Glu-895 to stop, did not significantly impair functional expression in HeLa or HEK 293 cells. Truncation, however, of 10 amino acids (Leu-894 to stop; mutant C10) reduced Na\textsuperscript{+} gradient-dependent Ca\textsuperscript{2+} uptake to 35–39% relative to the wild type parent exchanger, and further truncation of 13 or more amino acids resulted in loss of trace amounts of transport activity. Western analysis indicated that Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger protein was produced whether transfection was carried out with functional or non-functional mutants. Immunofluorescence studies of HEK 293 cells expressing wild type Na\textsuperscript{+}-Ca\textsuperscript{2+} exchangers revealed that transport activity in whole cells correlated with surface expression. All cells expressing the wild type exchanger or C9 exhibited surface expression of the protein. Only 39% of the cells expressing C10 exhibited surface expression, and none was detected in cells transfected with non-functional mutants C13 and C29. Since functional and non-functional mutants were glycosylated, the C terminus is not mandatory to translocation into the endoplasmic reticulum (ER). Endoglycosidase H digestion of \[^{35}S\]methionine-labeled protein derived from wild type Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger and from C10 indicated that resistance to the signal peptide was acquired after 1 and 5 h of chase, respectively. C29 did not acquire detectable resistance to endoglycosidase H digestion even after 10 h of chase. Taken together, these results suggest that the "cellular quality control machinery" can tolerate the structural change introduced by truncation of the C terminus up to Ser-893 albeit with reduced rate of ER to Golgi transfer and reduced surface expression of the truncated protein. Further truncation of C-terminal amino acids leads to retention of the truncated protein in the ER, no transfer to the Golgi, and no surface expression.

Hydropathy analysis of the cloned NCX1 (Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger) gene indicated that the protein can be organized into 12 transmembrane helices, the first of which was suggested to be a cleavable signal peptide (2). This was supported by microsequencing of the N terminus of the purified bovine heart Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger (3), indicating that the first amino acid of the mature protein corresponds to amino acid 33 of the cloned gene. By carrying out a series of deletions between the initiating methionine and Asp-33 (the first amino acid of the mature protein RBE-1), we have shown (4) that the signal peptide is not mandatory for functional expression of the protein in HeLa cells. Similar results have also been reported in HEK 293 cells (5) and in oocytes and SF9 cells (6). We have also shown that the signal peptide truncated protein is glycosylated in vitro when dog pancreatic microsomes are added to a reticulocyte lysate expression system (4), suggesting that the nascent protein translocates into the lumen of endoplasmic reticulum (ER) also in the absence of its signal peptide. To explore further the involvement of different domains of the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger in its functional expression, we have examined the role of its C terminus. To do so we have sequentially truncated 9–29 of its 903 amino acids, starting with mutant C9 (see Table I) in which the putative extramembranous tail of the protein was missing. Our results show that functional expression is severely impaired when 11 or more amino acids are truncated from the exchanger’s C terminus. Immunoreactive protein derived from the non-functional mutants translocates to the lumen of the ER, where it is glycosylated. However, unlike the wild type exchanger protein, it does not proceed further to the Golgi and is not expressed in the surface membrane.

EXPERIMENTAL PROCEDURES

Construction of C-terminal Truncated Mutants—The method of Kunkel (7) was used to prepare C-terminal truncated mutants of the rat brain clone rbe-1 (8). The antisense primers were planned to be composed at least 15 bases upstream and downstream from the point of the desired mutation. The following primers were used (5’ → 3’): C9, CTTGATTGTTCTTATTACAGGGAGAGAAGAAATG; C11, CTTGATTGTTCTTATTACAGGGAGAGAAGAGAT; C13, CTTGATTGTTCTTATTACAGGGAGAGAAGGATAC; C17, CTTGATTGTTCTTATTACAGGGAGAGAAGGATCC; C29, ATCTGTAGTTCTTACTGAGTCAGTCCGGGG.

Mutagenesis was confirmed by sequencing 300–600 bases upstream and downstream of the mutation. To ensure that no other mutation beyond the planned occurred, BglII-SacI fragments containing the desired mutation were subcloned into BglII-SacI-digested parent clone rbe-1. Subcloning into pcDNA3 involved preparation of HindIII-digested arms of pcDNA3 (Invitrogen) into which HindIII-digested clone rbe-1 (9) was ligated. Orientation was ensured by identification of appropriate restriction fragments. This construct was digested with Eco47III-AgeI and ligated with Eco47III-AgeI-digested rbe-1. C-termini

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Functional Expression of C-terminal Truncated Na\(^{+}\)-Ca\(^{2+}\) Exchanger Mutants

Transfection of HeLa cells was done as described (4, 8). Expression of the Cloned Na\(^{+}\)-Ca\(^{2+}\) Exchanger—HEK 293 cells—were transfected by the CaPi method and determination of Na\(^{+}\) transport was carried out ex-209-

**RESULTS**

Based on hydropathy analysis (14), the profile-fed neural network system (15), and the recently published revised topological models based on scanning cysteine accessibility method (16, 17), the 31 C-terminal amino acids (Table 1) of the NCX1 gene are modeled to contain the last transmembrane α helix (10) and an extramembranous tail. To examine the role of this protein segment in functional expression, sequential deletions

**The Effect of Tunicamycin on Na\(^{+}\)-Ca\(^{2+}\) Exchanger Protein Expression**—Tunicamycin at a final concentration of 10 μM was added to the methionine-free starvation medium and to the [\(\text{\textsuperscript{[35S]}methionine}\)]ml of medium was added. Unless stated otherwise, the cells were preincubated in fresh methionine-free DMEM to which 6 μCi [\(\text{\textsuperscript{[35S]}methionine}\)] was added. Treatment of the mutant Na\(^{+}\)-Ca\(^{2+}\) exchangers expressed in HEK 293 cells was carried out as described for HeLa cells in Ref. 10. Nuclei were stained in 1 μg/ml Hoechst 33342 (bisbenzimide, Sigma). Immunofluorescence microscopy was performed with epifluorescence optics (ZeissAxioScope) or confocal laser scanning microscopy (Zeiss LSM 410 system) attached to the Zeiss Axiovert 135M inverted microscope. The system was equipped with a 25-milliwatt air-cooled argon laser (488-nm excitation line with a 515-nm long pass barrier filter) for the excitation of green (fluorescein isothiocyanate) fluorescence. Blue fluorescence was excited with 364-nm line UV laser equipped with LP 397 barrier filter. Double-labeled specimens were excited with both laser lines and monitored simultaneously using dual detectors and the Zeiss-supplied filter block combination with dichroic beam splitter and emission filters. The fluorescence was collected by employing a 63×/1.2 C-Achromat water immersion lens (Zeiss). Autofluorescence of the specimens was set to background level. To reduce the visual noise, each image was processed in the laser confocal scanning microscopy acquisition mode (512 pixels/line) by averaging of 8 images, before the final image was produced on the monitor. Images simultaneously scanned on the two channels were automatically merged to produce false single multicolor profile. The image analysis of the confocal images including pseudo color representation, brightness, and contrast level were carried out using the standard Zeiss software package and the Adobe Photoshop 4.0 program (Adobe Systems, Inc., Mountain View, CA).

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starting with the last 9 amino acids, Glu-895 to stop (mutant C9), and ending with Leu-875 to stop (mutant C29) were carried out as described under "Experimental Procedures.”

Truncation of the last 9 C-terminal amino acids (C9) impaired only slightly functional expression (Fig. 1A or B) of the cloned exchanger. Its transport activity in HELa cells was 96.87% (S.D. = 11.37) and in HEK 293 cells 82.8% (S.D. = 15) when compared with that of the wild type exchanger RBE-1 (100%). Further truncation, however, of a single amino acid (mutant C10) resulted in a sharp decrease in transport activity, to about 35–39%, relative to that of the wild type exchanger.

Truncation beyond the last 11 amino acids resulted in expression of trace amounts of transport activity. The reduced transport activity of the C-terminal truncated mutants did not depend on mode of transfection, cell line, or the plasmid used for expression. In Fig. 1A, transport activity was determined in the DOTAP-mediated transfection of VTF-7-infected HELa cell expression system, using pBluescript (Stratagene) as the cloning vector. In Fig. 1B, the transport activity was measured in CaF2-mediated transfection of HEK 293 cells using pcDNA3 as the cloning vector. Similar results to those shown in Fig. 1 were obtained when proteins derived from HELa or HEK 293 cells transfected with RBE-1 or its C-terminal truncated mutants were reconstituted into exogenous brain phospholipids. Table II shows the relative transport activities of proteins derived from HELa and HEK 293 cells transfected with the parent Na+-Ca2+ exchanger (taken in each experiment as 100%) and that of each of its C-terminal truncated mutants (presented in relative values). It can be seen that reconstitution did not rescue the Na+ gradient-dependent Ca2+ uptake of the C-terminal truncated mutants, although following reconstitution the transport activity of C13 and C29 expressed in HEK 293 cells was somewhat higher than in HELa cells and than in whole cells. Moreover, the relative transport activities of C9 and C10 were lower than in whole cells.

To examine whether the decrease in transport activity of the C-terminal truncated mutants resulted from impaired Na+-Ca2+ exchanger protein synthesis, Western analysis was carried out. Fig. 2 shows that similar amounts of immunoreactive exchanger protein were detected whether HELa or HEK 293 cells were transfected with wild type DNA or with any of the C-terminal truncated mutant DNAs.

To study the cellular distribution of immunoreactive Na+-Ca2+ exchanger protein, the Flag epitope was inserted into the extracellular N terminus (10) of the wild type and C-terminal truncated clones. The transport activity of the N-Flag-tagged (FN) exchangers was similar to that of the corresponding parent clones (not shown).

FN-C10-expressing cells is immunofluorescence detected without permeabilizing the cells (panels 1 and 2 of Fig. 3A and B).

No such staining is detected in FN-C29-expressing cells (panels 1 and 2, Fig. 3C). When, however, cells were permeabilized, fluorescence was detected not only in RBE-1- and C10-expressing cells (panels 3 and 4 of Fig. 3A and B), but also in C29-expressing cells (panels 3 and 4, Fig. 3C). Studies with FN-C9-expressing cells were similar to those with FN-RBE-1-expressing ones and with FN-C13-expressing cells were similar to those with C29-expressing cells (data not shown).

These experiments suggest that only in cells expressing the parent exchanger RBE-1, C9, or its partially functional mutant C10 is the transporter in the surface membrane. Moreover, in RBE-1-expressing cells and in C9, similar numbers of cells were stained without and with permeabilization, whereas, in C10-expressing cells, considerably fewer cells stain in the absence of permeabilization than in its presence. Counting 10 different fields from each of three separate transfection experiments revealed that 23.33% (S.D. = 5.49) and 23.4% (S.D. = 3.32) of RBE-1-expressing HEK 293 cells light up without and with permeabilization, respectively, and 18.81% (S.D. = 2.71) and 17.43% (S.D. = 3.72) of the cells expressing C9. Hence, practically all the cells that express RBE-1 and C9 exhibit surface expression.

When the counting is carried out with C10-expressing cells, 9% (S.D. = 2.8) of the cells light up without permeabilization and 23.16% (S.D. = 5.87) light up with permeabilization. This suggests that, unlike in rbe-1- and C9-transfected cells, in only 39% of the C10-expressing cells can the exchanger protein be detected in the plasma membrane. In C29 (or C13)-expressing cells, no immunoreactive protein is detectable in the plasma membrane. Permeabilization reveals that 17.31% (S.D. = 4.5) of the FN-C29-transfected cells express the protein. The results obtained by indirect immunofluorescence of non-permeabilized cells are consistent with the expression of Na+-Ca2+ exchange activity in whole cells as shown in Fig. 1.

We have also tested the possibility that surface expression of N-Flag-tagged C29 was not detected since its topology was “scrambled” and its N-Flag-tagged N terminus projected into the cytoplasm rather than outside the cell. Were this the case, the epitope would not be accessible to the externally added antibody and immunoreactive C29 exchanger protein in the surface membrane would not be detected. We chose, therefore, an alternative method to test the surface expression of C29, by using the impermeant reagent NHS-SS-biotin (13) to covalently modify the amino residues in the surface membrane of transfected HEK 293 cells. The Na+-Ca2+ exchanger RBE-1 contains 46 lysine residues and C29 contains 45 lysines. Based on experimental evidence (10) and topological analysis (16, 17), 4 lysine residues are modeled to face the cell exterior. In addition, 2 lysines derived from the Flag epitope are external as well (10). All other lysines, except 2 that are intramembranous, face the cell interior. Hence, even if the topology of C29 is “scrambled” but the mutant protein is present in the plasma membrane, some of the lysines should be accessible to the biotinylating reagent. Fig. 4 shows an immunoblot of the biotinylated cell proteins and total cell proteins derived from rbe-1- and C29-transfected cells. Quantitative analysis indicates that in this experiment a 3-fold higher amount of total immunoreactive C29 was expressed than in parallel RBE-1-expressing cells. Biotinylated Na+-Ca2+ exchanger protein, however, is detected only in the surface of cells expressing the parent exchanger RBE-1, and none is detected in the surface of the cells expressing C29. Similar results were obtained in three additional experiments. It should be also noted that, for determination of surface expression, transfected cells derived from 1
Fig. 1. Na\(^+\) gradient-dependent Ca\(^{2+}\) uptake in transfected HeLa and HEK 293 cells. A, HeLa cells were transfected in parallel with the wild type Na\(^+-\)Ca\(^{2+}\) exchanger RBE-1 and with its C-terminal truncated mutants. 17 h after transfection, Na\(^+\) gradient-dependent 44Ca\(^{2+}\) influx was determined as described under "Experimental Procedures." The transport activity of RBE-1 in each experiment was defined as 100%, and that of the mutants was calculated in relative values. The data were compiled from seven separate transfection experiments; each measurement was carried out in triplicate. No transport activity was detected in mock-transfected (with pBluescript SK) cells. The numerical value of the Ca\(^{2+}\) gradient-dependent Na\(^+\) influx was determined as described under "Experimental Procedures." The transport activity of RBE-1 in each experiment was defined as 100% and that of the mutants was calculated in relative values. The data were compiled from six separate transfection experiments; each measurement was carried out in triplicate. The numerical value of the Ca\(^{2+}\) gradient-dependent Na\(^+\) influx was determined as described under "Experimental Procedures." The transport activity of RBE-1 in each experiment was defined as 100% and that of the mutants was calculated in relative values. The data are compiled from three separate transfection experiments.

**TABLE II**

| Name     | Relative Na\(^+\)-dependent Ca\(^{2+}\) uptake | HeLa cells | HEK 293 cells |
|----------|-----------------------------------------------|------------|---------------|
| RBE-1    | 100                                           | 100        |               |
| C9       | 49.33                                         | 38.2       |               |
| C10      | 20.0                                          | 31.4       |               |
| C11      | 2.11                                          | 9.4        |               |
| C29      | 2.1                                           | 7.2        |               |

A well of a 6-well plate (about 1.2 \(\times\) 10^6 cells at confluence) was used, whereas, for determination of total immunoreactive protein, about 1/10th of the total protein derived from 1 well out of a 12-well plate (0.4 \(\times\) 10^5 cells at confluence) was used.

In order to find out the reason that the C-terminal truncated mutants beyond C11 do not exhibit surface expression and in which cellular compartment the protein accumulates, we have studied their glycosylation pattern. Fig. 5 compares the protein profile of the \[^{125}\text{I}\]methionine-labeled immunoprecipitated exchanger derived from RBE-1- and from C29-expressing cells, grown without and with the glycosylation inhibitor tunicamycin (18). It can be seen (Fig. 5A) that, when tunicamycin is added to the labeling medium, the molecular mass of both immunoreactive exchangers RBE-1 and C29 is lower than the corresponding immunoreactive exchangers synthesized in the absence of tunicamycin. This finding suggests that RBE-1 and C29 are glycosylated in HEK 293 cells. For comparison, we are also showing a similar experiment, except that N-Flag-tagged rbe-1 and C29 were used to transfect the cells. It can be seen (Fig. 5B) that addition of tunicamycin does not result in expression of lower molecular mass immunoreactive protein. This is consistent with the fact that introduction of the N-Flag tag after Gly-8 involved also deletion of Asn-9 (10), the single glycosylation site of the NCX1, as suggested by the in vitro experiments of Hryshko et al. (19).

Transfer of membrane proteins from the ER to the Golgi is accompanied by modification of the oligosaccharide residues resulting in acquisition of resistance to endo H digestion. To determine whether the "non-functional" C-terminal truncated mutants accumulate in the ER or whether they are transferred
to the Golgi, we have studied their deglycosylation pattern by endo H. We have compared their protein profile before and after exposure to endo H and compared it to that of the parent clone RBE-1. In addition, we have determined the time required to acquire resistance to endo H for RBE-1 and its C-terminal truncated mutants by metabolically labeling them with \[^{35}S\]methionine and chasing the label with unlabeled methionine for different times.

Fig. 6 shows the protein profile of immunoprecipitated \[^{35}S\]methionine-labeled RBE-1 (Fig. 6A), C10 (Fig. 6B), and C29 (Fig. 6C) at different times after onset of chase with unlabeled methionine. Each sample was loaded on AbO-8 coated Protein A-Sepharose beads (see “Experimental Procedures”). The eluted proteins were divided into equal parts and incubated 90 min without and with endo H, after which they were analyzed by SDS-PAGE. It can be seen that RBE-1 acquires resistance to endo H digestion by 1 h after onset of the chase (Fig. 6A), C10 acquires resistance after 5 h (Fig. 6B), and C29 as well as C13 (data not shown) do not become resistant to endo H even after 10 h of chase (Fig. 6C).
DISCUSSION

Expression of transport proteins and their mutants in heterologous systems provides tools needed to study the role of structural elements that are important for functional expression. In this work we show that the C terminus of the Na\(^{+}\)-Ca\(^{2+}\) exchanger RBE-1 (NCX1.4) is important for assembly of a functionally apt form of the protein.

Secreted and surface membrane proteins follow a multistep, well coordinated and regulated pathway (20–23) to their final location. Defective proteins that translocate to the ER and fail to assemble or fold correctly are retained in that compartment and eventually become degraded without reaching their final cellular destination (24). Our experiments suggest that the C terminus (amino acids Leu-875 to stop) of the Na\(^{+}\)-Ca\(^{2+}\) exchanger is not mandatory for translocation of the nascent protein to the lumen of the ER, since functional and non-functional C-terminal truncated mutants including C29, the mutant in which the entire last transmembrane segment and the extramembrane tail were truncated, are glycosylated (Figs. 5 and 6).

Glycosylation pattern, however, can play an important role in correct folding and maturation of membrane proteins (25). Of the six consensus glycosylation sequences that are identified in the primary structure of the Na\(^{+}\)-Ca\(^{2+}\) exchanger, only Asn-9 and Asn-584 are glycosylation candidates based on distance from edges of transmembrane segments (26, 27). Of the two, only Asn-9 is extracellular (10) and hence faces the lumen of the ER. Both in vitro experiments and expression in oocytes indicated that only Asn-9 is glycosylated (19) in NCX1. This holds also for expression in HEK 293 cells, for functional and non-functional mutants (Fig. 5). Deletion of Asn-9 accompanied by insertion of the N-Flag epitope (10) leads to expression of a
non-glycosylated functional N-Flag-tagged RBE-1 and a non-glycosylated non-functional N-Flag-tagged C29. Hence, glycosylation of the Na\(^{+}-\)Ca\(^{2+}\) exchanger is not a prerequisite for acquisition of functional conformation and surface membrane expression in HEK293 cells. These experiments also suggest that the non-functional C29 translocated to the ER at least in part with similar orientation to that of the functional parent exchanger, since Asn-584 (or other consensus glycosylation candidate) did not become glycosylated.

Maturation of the protein and subsequent transfer from the ER to the Golgi is accompanied by trimming of high mannoside residues and terminal glycosylation, resulting in acquisition of resistance to endo H digestion (28). As can be seen in Fig. 6, sequential truncation of C-terminal amino acids impaired one of the processes that are mandatory for the maturation of a functional Na\(^{+}-\)Ca\(^{2+}\) exchanger, resulting in increased retention times of the impaired proteins in the ER, which led to either reduced surface expression as with C10 (Fig. 3B) or to no detectable surface expression as with C29 (Figs. 3C and 4).

Reconstitution of proteins derived from HeLa and HEK 293 cells transfected with the functionally impaired C-terminal truncated mutants into proteoliposomes did not rescue the Na\(^{+}\) gradient-dependent Ca\(^{2+}\) uptake (Table II). This suggests that these proteins are retained in the ER in a functionally impaired form. Moreover, reconstitution experiments suggest that even C9 is partially impaired, since its relative transport activity, which in whole cells is comparable to the parent exchanger, following reconstitution is much lower. Secretion is limited in overexpression systems by the cellular processing machinery (29). The plasma membrane incorporates only a small fraction of the overexpressed transporters. The cellular processing machinery suffices to “help” that small part of transporters to acquire mature conformation. Hence, in whole cells, the transport activity of C9 is similar to that of the wild type exchanger. During reconstitution of cells expressing C9, detergent treatment probably “destabilizes” further the impaired C9 protein. Unlike with the parent protein, removal of the detergent alone is not sufficient to restore the functional conformation of C9; therefore, we measure lower transport activity than in parallel reconstituted RBE-1-transfected cells. The consequences of the truncation of an additional amino acid and the limitations of the cellular processing machinery probably play a role in the reduced ER to Golgi transfer and reduced surface expression of mutant C10. Although we detect C10 protein only in the surface of about 40% of the expressing cells, it is possible that this is an underestimate due to limitation of surface detection in cells where the overall level of expression is not high. If such underestimate applies also to the 35–39% transport activity of C10 in whole cells, it would suggest that the “true” activity of the surface membrane C10 transporters is similar to that of the wild type exchanger and C9. Following reconstitution, the recovered transport activity of C10 reaches only 20–30% relative to the parent exchanger, probably since it is less apt than C9 and the results of detergent treatment are more detrimental.

Gabellini et al. (30) and recently also Li and Lytton (31) reported that expression of a Na\(^{+}-\)Ca\(^{2+}\) exchanger lacking the six C-terminal transmembrane segments exhibited considerable Na\(^{+}-\)Ca\(^{2+}\) exchange activity in HEK 293 cells. Immuno- blotting of protein derived from cells expressing the truncated isoform also revealed, in addition to the expected 70-kDa protein, higher molecular mass ones. It has been suggested (30, 31) that the truncated exchanger forms dimers that could be responsible for the transport activity detected. This option is presumably not open to mutants lacking only 9–29 amino acids.

Although the C terminus of the NCX1, NCX2, and NCX3 gene products is highly conserved and only one conservative substitution, K901R, is observed among the last 11 C-terminal amino acids (32, 33), we do not think that the conserved sequence itself is important. We have inserted the Flag epitope into the C terminus after Glu-895 instead of Ala-896 to Phe-903. The resulting C-Flag-tagged mutant exchanger, which had an extramembranous tail of 12 different amino acids instead of 9 present in NCX1, exhibited 139% (S.D. = 21) transport activity relative to that of the parent exchanger (10). We suggest that the presence of an extramembranous tail of at least 9–10 amino acids might be of importance. Sequential truncation of C-terminal amino acids of GLYT1 (34) resulted in progressively lower transport activities. Truncation of 64 out of a total of 76 intracellular extramembranous C-terminal amino acids of GLYT1 resulted in 9% of transport activity relative to the parent transporter and impaired surface expression. As with the C-terminal truncated mutants of the Na\(^{+}-\)Ca\(^{2+}\) exchanger, reconstitution did not rescue the transport activity of the impaired C-terminal truncated GLYT1 mutants (34).

One possible explanation could be that truncation of the C terminus led in both cases to exposure of hydrophobic sequences and formation in the ER of detergent-insoluble aggregates (24, 35) of misfolded overexpressed proteins, as in the case of ΔF508 mutant of the cystic fibrosis transmembrane conductance regulator or the overexpressed CFTR (24). It could also be that the presence of an extramembranous tail is important for acquisition of functional conformation of the protein since α helices are stabilized by residues flanking the helix termini (36, 37). Deletion of 9 or more C-terminal extramembranous amino acids of the Na\(^{+}-\)Ca\(^{2+}\) exchanger could destabilize the last transmembrane helix and impair the acquisition of functional conformation of the protein by exposure of hydrophobic sequences.

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