Identification of a COOH-terminal Segment Involved in Maturation and Stability of Human Ether-a-go-go-related Gene Potassium Channels*

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Mutations in the potassium channel encoded by the human ether-a-go-go-related gene (HERG) have been linked to the congenital long QT syndrome (LQTS), a cardiac disease associated with an increased predisposition of ventricular arrhythmias and sudden death. The COOH terminus of HERG harbors a large number of LQTS mutations and its removal prevents functional expression for reasons that remain unknown. In this study, we show that the COOH terminus of HERG is required for normal trafficking of the ion channel. We have identified a region critical for trafficking between residues 860 and 899 that includes a novel missense mutation at amino acid 861 (HERG_N861I). Truncations or deletion of residues 860–899, characterized in six different expression systems including a cardiac cell line, resulted in decreased expression levels and an absence of the mature glycosylated form of the HERG protein. Deletion of this region did not interfere with the formation of tetramers but caused retention of the assembled ion channels within the endoplasmic reticulum. Consequently, removal of residues 860–899 resulted in the absence of the ion channels from the cell surface and a more rapid turnover rate than the wild type channels, which was evident very early in biogenesis. This study reveals a novel role of the COOH terminus in the normal biogenesis of HERG channels and suggests defective trafficking as a common mechanism for abnormal channel function resulting from mutations of critical COOH-terminal residues, including the LQTS mutant HERG_N861I.

The long QT syndrome (LQTS) 1 is a congenital heart disorder characterized by delayed cardiac action potential repolarization and a prolongation of the QT interval. This leads to an increased susceptibility of the heart to potentially sustained ventricular tachyarrhythmias that cause syncope and sudden death. Molecular genetic studies have identified five genes linked to LQTS including the human ether-a-go-go-related gene (HERG) (1). HERG encodes the alpha-subunit of the rapidly activating delayed rectifier current I_Kr and consists of six transmembrane domains as well as NH2- and COOH-terminal cytoplasmic tails (2, 3). Over 90 mutations distributed throughout HERG have been linked to the LQTS, most of which reside in the intracellular tail regions of the channel protein (4, 5). Earlier electrophysiological and structural analysis have emphasized abnormal HERG function as a common manifestation of mutations in the NH2 terminus (6–8). In contrast, studies of COOH-terminal mutations suggest that the pathology is because of the absence of HERG channels from the cell surface (9–12). This phenotype may be caused by improper folding of newly synthesized HERG polypeptides, in a fashion similar to that described for the ΔF508 allele of CFTR (CFTR ΔF508). CFTR ΔF508 is recognized by the endoplasmic reticulum (ER) quality control machinery and is rapidly degraded before being processed in the Golgi apparatus (13). As a consequence, these molecules are prevented from journeying through the secretory pathway and are not deployed to the plasma membrane. Whereas little is known about the role of COOH-terminal signals in the biogenesis of HERG, studies of other ion channels support the importance of the COOH-terminal signals in protein stability, (14) maturation (15), surface delivery (16), and ER export (17).

We undertook the current study to investigate the role of the COOH terminus in the biogenesis of HERG and to explore possible mechanisms implicated in LQTS. The results demonstrate the importance of the COOH terminus in normal maturation of HERG channels and identify residues 860–899 as a critical region for trafficking. By exploiting the differential migration pattern of mature and immature HERG species on immunoblots, we demonstrate that this region is indispensable for HERG exit from the ER and hence the maturation and stability of the channel protein. The resulting defective trafficking of HERG mutants, including premature terminations and a novel point mutation, explains the absence of functional channels from the cell surface. Therefore, we propose abnormal trafficking as a common LQTS mechanism associated with a subset of mutations in the COOH terminus of HERG.

EXPERIMENTAL PROCEDURES

Common Reagents and Methods—All constructs were subcloned into the cytomegalovirus-based eukaryotic expression vectors and purified using the QiAfilter Plasmid Midi kit (Qiagen). PCR purification and gel extractions were done using QiAquick gel extraction kit (Qiagen). To ensure the fidelity of PCR products, amplifications were performed using a proofreading enzyme (Vent DNA Polymerase, New England Biolabs). Restriction enzymes and primers were purchased from New England Biolabs and Invitrogen, respectively. The fidelity of all constructs was confirmed by capillary electrophoresis based sequencing (Sheldon Biotechnology Center, McGill University).

Wild Type Constructs—Myc and HA tag wild type HERG (HERG_wt) were generated using vectors that allow the addition of the tag to the amino terminus of the target protein. Briefly, HERG cdNA was amplified using 5’ and 3’ primers with BamHI and EcoRI recognition se-

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1 The abbreviations used are: LQTS, long Q-T syndrome; HERG, human ether-a-go-go-related gene; ER, endoplasmic reticulum; HERG-C, COOH terminus of HERG; HA, hemagglutinin; PBS, phosphate-buffered saline; HEK, human embryonic kidney; GFP, green fluorescent protein; EndoH, endoglycosidase H; PNGase, N-glycosidase F.
quences, respectively. The amplified product was digested with BamHI and EcoRI restriction enzymes and subcloned in-frame into the Myc fusion vector pCMV-Tag 3 (Stratagene). HA-HERG<sub>wt</sub> was generated by amplifying HERG using EcoRI sites for both forward and reverse primers followed by an in-frame ligation into the pHA-CMV (Clontech). Nomenclature and Generation of Mutant Variants—Truncations are named according to the last residue truncated; e.g. in HERG<sub>Δ814</sub>, all residues downstream of amino acid 814 were removed. Deletions were numbered according to the residues that were deleted; e.g. in HERG<sub>Δ814-999</sub> residues between 860 and 899 were removed. To eliminate undesirable mutations associated with long PCR fragments, a cDNA cassette comprising the entire COOH terminus of HERG (HERG-C) was created by excising the BglII fragment from HA-HERG-C and ligating it back into pHa-CMV (3). All truncations were made by amplifying the HERG-C template using a single forward primer and an appropriate reverse primer containing a BglII restriction site. The amplified products were then digested with BglII and religated into BglII-digested HA-Tagged HERG<sub>wt</sub>. Deletion and point mutations were engineered using QuikChange site-directed mutagenesis kit (Stratagene) and HERG-C as the PCR template. Myc- and HA-tagged mutant constructs were interchanged either by a single XhoI digest or an XhoI and SalI double digest followed by religation into Myc- or HA-tagged HERG<sub>wt</sub>.

**Cell Culture and Transfection**—All reagents were purchased from Invitrogen unless indicated otherwise. Human embryonic kidney (HEK293) cells were maintained in Dulbecco modified Eagle’s medium containing 25 mM EDTA and protease inhibitor mixture. Cells were washed in ice-cold PBS and incubated in PK buffer (10 mM HEPES, 150 mM NaCl, and 95% O<sub>2</sub> incubator at 37°C) pre-coated with a monoclonal anti-CD8 antibody. To obtain an estimate of selection efficiency, sister cells transfected with HERG<sub>wt</sub> were always recorded prior to analysis of mutant variants. Selection efficiency varied between 70 and 90%.

The whole cell patch clamp technique was used to record membrane currents from M2 cells using an Axopatch amplifier (as described in Ref. 10). Cells were placed on the stage of an inverted microscope (Zeiss IM35) at room temperature. Patch clamp electrodes were filled with medium containing (mM): 130 KCl, 1 MgCl<sub>2</sub>, 5 MgATP, and 10 Hepes (pH 7.2). The external medium contained (mM): 137 NaCl, 4 KCl, 1.8 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 10 glucose, and 10 Hepes (pH 7.4). A two-step voltage clamp protocol was imposed from a holding potential of −60 mV to assess the presence of the HERG current. The first step, which served to activate HERG currents, consisted of a 4-s depolarizing pulse to potentials between −60 and +50 mV in increments of 10 mV. To assess HERG tail currents, at the end of the first step the membrane was lysed and the supernatants were analyzed by immunoblotting as described above. Surface Limited Proteolysis—The protocol for detection of surface membrane proteins based on their sensitivity to proteolysis has been previously described (9). Briefly, live unpermeabilized cells were washed in ice-cold PBS and incubated in PK buffer (10 mM HEPES, 150 mM NaCl, and 2 mM CaCl<sub>2</sub>) with or without 20 μg/ml proteinase K (BioShop, McGill University) at 37°C for 30 min. Cells were harvested in PK buffer containing 1 mg/ml proteinase K and visualized by autoradiography. Membranes were blocked in 5% dry milk and incubated with monoclonal anti-HA/Myc/CASK (Babco) or polyclonal anti-HERG/OPF (Chemicon and Clontech, respectively). After extensive washes, membranes were incubated with goat anti-mouse/rabbit IgG conjugated to horseradish peroxidase (Jackson ImmunoResearch Labs) and visualized on x-ray films by ECL Plus (Amersham Biosciences). All immunoblots were performed with anti-HA antibody unless otherwise indicated.

**RESULTS**

**HERG<sub>wt</sub> Glycosylation and Expression**—Immunoblot analysis of cells transfected with HERG<sub>wt</sub> reveals two bands with apparent molecular masses of ∼135 and 155 kDa, corresponding to the core and complex-glycosylated forms of HERG, respectively (9). As shown in Fig. 1a both bands are sensitive to N-glycosidase F, indicating that they both contain N-linked glycan chains. Fig. 1a also shows that only the faster migrating
band was sensitive to EndoH treatment indicating that this band represents the immature high-mannose core-glycosylated form. The more slowly migrating EndoH-insensitive band represents the mature glycosylated species produced in the Golgi complex.

Before questions related to biogenesis could be addressed, it was first necessary to select a careful transfection method that would prevent the escape of improperly folded molecules from the ER. Fig. 1b demonstrates that the core and complex-glycosylated species are differentially partitioned in an expression-dependent fashion between detergent-soluble and -insoluble fractions. At low expression levels, HERGwt showed two predominant bands in the detergent-soluble fraction but only a relatively faint core-glycosylated band in the detergent-insoluble fraction. In contrast, at high expression levels, there was a substantial increase in the abundance of the detergent-insoluble core-glycosylated species. These data suggests that at high expression levels the capacity of the ER to process immature HERGwt is overwhelmed resulting in the impairment of the 26 S proteasome and formation of insoluble aggregates (19). To test this possibility cells expressing low levels of HERGwt were exposed to the 26 S proteasome inhibitor, lactacystin. The result is consistent with the idea that high expression levels result in the impairment of the 26 S proteasome. To avoid associated artifacts, all experiments were performed using the low expression approach and HEK cells were not used beyond 30 passages.

HERG COOH-terminal Removal Impairs Maturation—Using immunoblot analysis we observed that the removal of the COOH-terminal tail of HERG, which starts at residue 814 (3) (HERG814), results in a shift in the molecular weight of the recombinant protein and the complete abolition of the mature complex-glycosylated band (Fig. 2a). This suggests that HERG814 failed to exit the ER and transit through the Golgi complex. To corroborate this point we examined the steady-state localization of HERGwt and HERG814. Fig. 2b shows confocal images of cells co-transfected with HA- and Myc-tagged HERGwt or with HA- and Myc-tagged HERG814. HERGwt displays punctate staining distributed throughout the cell and, as expected for heterologous expression systems, a fraction of HERGwt is also present as perinuclear staining. With the HERG814 mutant, a fainter and exclusively circum-scribed perinuclear pattern of staining was observed, consistent with the faint band seen in immunoblots (note the loading amounts in Fig. 2a).

To ensure that the immunoreactive band of HERG814 is indeed the immature species produced and retained in the ER, we evaluated its sensitivity to glycosidase digest. As shown in Fig. 2c, both PNGase and EndoH produced a shift in the apparent molecular mass of this band suggesting that HERG814 has a high mannos N-linked glycan of the type acquired in the ER. Together with immunolocalization experiments, these data suggest that HERG814 was prevented from advancing beyond the ER into the secretory pathway and that the COOH terminus of HERG is an important determinant of the channel protein maturation.

HERG860–899 Disrupts a Trafficking Element—To determine the region of the HERG-C that is necessary for its maturation, we analyzed the biochemical phenotypes of a series of progressively larger COOH-terminal truncations (Fig. 3a). The immunoblot analysis in Fig. 3b demonstrates that truncation mutants with more than 899 residues expressed normal amounts of the immature and mature glycosylated forms of HERG. Conversely, mutants with less than 860 residues were lacking complex oligosaccharides (Fig. 3b) and showed reduced levels of expression (Fig. 3d). This suggests that these mutants failed to exit the ER and were more rapidly degraded. To eliminate the remote possibility that downstream signals within HERG-C may restore the differential migration pattern of HERG899 and HERG860, we deleted these residues (HERG860–899) in an otherwise intact HERG-C. As indicated in Fig. 3c, HERG860–899 was unable to acquire complex oligosaccharides and showed a significant decrease in expression levels (Fig. 3d). Equal loading and transfection efficiency were verified by CASK and GFP immunoblotting.

Because HERG is normally expressed in cardiac myocytes, maturation of the HERG mutants might be facilitated by unidentified components unique to cardiac cells. To test this possibility, we undertook studies using a mouse cardiac cell line (HL-1) that has been shown to express delayed rectifier currents, ostensibly encoded by HERG (20). We confirmed the endogenous expression of HERG in these cells by immunoblot analysis conducted with an anti-HERG antibody. As shown in Fig. 4a (first lane) the antibody identifies an intense band at ~155 kDa and a fainter band at ~135 kDa (see longer exposure). We found that transfection of HA-tagged HERGwt into HL-1 cells increased the intensity of both bands (Fig. 4a, second lane), confirming that endogenous immunoreactive species are indeed HERG species. Fig. 4b shows that HERG860–899 transfected into HL-1 cells failed to acquire mature oligosaccharides suggesting that this mutant cannot be rescued by an unknown cardiac component. In support of this contention, we found that coexpression of HERG860–899 with HERGwt or MiRP, a puta-
The cytoplasmic COOH-terminal tail of HERG controls its maturation. (a) steady state expression of HERG wt was compared with HERG 899. Increasing amounts of total protein were analyzed by SDS-PAGE and Western blotting. (b) Immunolocalization in HEK cells co-transfected with HA- and Myc-tagged HERG wt (1 and 2, respectively) or HERG 899 (3 and 4, respectively). Immunolocalization experiments were performed using a monoclonal anti-HA and polyclonal anti-Myc primary antibodies followed by Oregon Green/Cy3-conjugated secondary antibodies, respectively. Co-assembled Myc- and HA-tagged subunits were detected independent of the primary or secondary antibodies indicating the specificity of the fluorescent signals. Scale bar indicates 10 μm. (c) Soluble fraction of cells transfected with HERG wt was treated (+) or not treated (−) with EndoH or PNGase and analyzed by immunoblots.

HERG 860–899 Tetramers Are Absent from the Cell Surface—To investigate the impact of the 860–899 deletion on the functional properties of HERG mutants the whole cell patch clamp technique was used. Membrane currents were recorded from M2 cells, a mouse melanoma cell line that is readily amenable to patch clamp experiments. Fig. 5a shows current traces recorded during a series of depolarizing voltage clamp steps from a −80 mV holding potential as well as the tail currents observed upon repolarization to −60 mV at the end of the step. The current traces in Fig. 5a and the current-voltage (IV) relationship in Fig. 5b, obtained from cells transfected with HERG wt or HERG 899, reflect a prominent expression of functional HERG currents. In contrast, the time-dependent currents were completely absent in cells transfected with HERG 860 or HERG 860–899 (Fig. 5a), and in untransfected control cells (not shown). To determine whether this was the result of a defect in tetrameric channel assembly we compared the fractional distribution of HERG wt and HERG 860–899 in a non-denaturing continuous sucrose gradient. As shown in Fig. 5c, the fractional distribution of the wild type and mutant channels were similar and the associated sedimentation profiles (Fig. 5d) show a single peak that corresponds closely to the apparent molecular masses of the tetrameric channel (−540 kDa); the slight shift between HERG wt and HERG 860–899 may be because of a difference in their molecular masses. Artifactual protein aggregation causing similar sedimentation profiles is unlikely because the bulk of endogenous HERG (HERGNative) appears in the same fraction as the recombinant protein (Fig. 5c).

The lack of functional currents observed following transfection with HERG 860 and HERG 860–899 is most likely because of the absence of recombinant proteins from the plasma mem-
brane. To test this possibility live and nonpermeabilized cells were treated with proteinase K, which is known to cleave extracellular exposed ectodomains (9, 22). To ensure that the proteinase K cleavage is surface limited, all immunoblots were stripped and reprobed for an endogenous intracellular protein, CASK. As indicated in Fig. 6a, exposure of cells transfected with HERG wt to the proteinase K resulted in the complete disappearance of the mature immunoreactive species and the appearance of a degradation product of \( \sim 62 \) kDa. In contrast, HERG860 and HERG899 were found to be completely insensitive to proteinase K proteolysis (Fig. 6a and b) suggesting a total absence of these mutants from the cell surface. As expected, HERG899 mutant, which displays significant time-dependent currents, was highly sensitive to proteinase K treatment, with about half of the protein present at the cell surface (Fig. 6b). Taken together, these data indicate that the lack of currents associated with HERG860–899 is because of the absence of channels from the cell surface.

**ER Retention Phenotypes Associated with HERG860–899 and HERGN861I**—The subcellular distribution of HERG mutants was investigated using confocal microscopy. As shown in Fig. 7a, a punctate staining pattern was detected for HERG899 distributed in a fashion similar to that of HERG wt. In contrast, cells transfected with HERG860 and HERG860–899 show a strong perinuclear staining pattern consistent with retention in the ER. In some cells, large perinuclear aggregate-like structures (not shown) were found that are similar to those reported for other misfolded proteins (23, 24). To determine a direct biochemical correlation associated with ER retention, we investigated the sensitivity of recombinant proteins to treatment with EndoH. The results in Fig. 7b show that treatment with EndoH generates a shift in the apparent molecular weight of both HERG860 and HERG860–899, as well as the core-glycosylated bands of HERG wt and HERG899. Sensitivity to EndoH, together with immunolocalization experiments, confirms that residues 860–899 are indispensable for HERG exit from the ER.

A recent report has identified a clinically relevant asparagine to isoleucine mutation at residue 861 (HERG N861I) (4). Because this mutation resides between residues 860 and 899, we reasoned that the trafficking abnormalities described above might be associated with this mutant. To address this possibil-
ity, we first examined the steady state expression pattern of HERGN861I in immunoblot experiments. As shown in Fig. 7b, HERGN861I is primarily synthesized as a single EndoH-sensitive immunoreactive species, indicating that it was unable to exit the ER. Consistent with this observation, immunolocalization experiments using confocal microscopy show that HERGN861I exhibits an ER-like distribution pattern (Fig. 7a). These results indicate that similar to deletion of residues 860–899, a point mutation in this segment can result in defective maturation of the ion channel.

Removal of Residues 860–899 Destabilizes HERG—ER retention of membrane proteins is often associated with their dislocation into the cytosol and subsequent degradation resulting in an overall high turnover rate of the retained proteins. To compare the relative stability of HERGΔ860-899 with HERGwt, pulse-chase experiments were performed for the radiolabeled recombinant proteins. As shown in Fig. 8a, HERGwt is synthesized as an immature precursor that acquires complex oligosaccharides as early as 2–4 h after synthesis and remains remarkably stable, with almost half of the immature species
recovered throughout the 24 h of chase time (Fig. 8b). Conversely, HERG<sub>860–899</sub> was unable to mature and acquire complex sugars at any of the indicated time intervals (Fig. 8a). In addition, the immature species of HERG<sub>860–899</sub> showed more rapid turnover kinetics, indicated by its rapid rate of disappearance compared with HERG<sub>wt</sub>. Subtle but consistent decreases were found in the amount of HERG<sub>860–899</sub> compared with HERG<sub>wt</sub> at time 0 (1 h of labeling pulse) and consequently shorter pulse experiments were conducted. As indicated in Fig. 8c, the reduced abundance of HERG<sub>860–899</sub> became apparent as early as 15 min of pulse time indicating either that HERG<sub>860–899</sub> was inefficiently synthesized or was very rapidly degraded. This pulse-chase analysis indicates that removal of residues 860–899 results in a decreased stability of the ion channel and an associated trafficking defect that appears very early during biogenesis of the nascent polypeptide.

**DISCUSSION**

In this study we show that the cytoplasmic COOH-terminal tail of HERG controls the maturation of the channel protein. Additional molecular, cellular, and functional analyses support two major conclusions. First, residues 860–899 are indispensable for ER exit and normal stability of the channel protein. Second, naturally occurring point mutations or premature truncations at residue 860 interfere with this domain can compromise surface delivery of the ion channel through defective trafficking.

HERG<sub>860–899</sub> was detected exclusively within the ER even when expressed at high levels or in the presence of 26 S proteasome inhibitors. Therefore, ER localization of this mutant appears to be because of an inability of the protein to exit the ER rather than rapid degradation of the mature protein. Consistent with this conclusion, pulse-chase analysis shows a decrease in the amount of HERG<sub>860–899</sub> as early as 15 min of pulse time. This indicates that removal of residues 860–899 interferes with an early step in the biogenesis of HERG rather than a late Golgi maturation step. A number of mechanisms, described below, have been implicated in ER retention of ion channels and membrane receptors including: failure of subunit assembly (29, 30), masking/unmasking of trafficking signals (27, 28) as well as protein misfolding and consequent binding to ER resident chaperones (29).

**Basis of ER Retention**—Biochemical analysis indicates that residues 860–899 are not implicated in subunit oligomerization and suggests that ER retention is not because of a major defect in homomeric assembly of the ion channel. We cannot completely eliminate the possibility that HERG<sub>860–899</sub> is unable to assemble with a putative auxiliary subunit that is pivotal for cell surface expression of the ion channel. However, this seems unlikely because such an auxiliary subunit would need to be ubiquitously present in a variety of heterologous systems used to express recombinant HERG. In addition, previous studies have indicated that multiple regions throughout the subunits are required for assembly of ion channels and it is unlikely that deletion or a point mutation of selected residues of HERG would completely abolish its interaction with an auxiliary subunit (30, 31). Interestingly, residues 860–899 fall between two non-contiguous regions of HERG involved in the interaction between HERG and GM130, proposed to facilitate HERG transport (32). It is possible that residues 860–899 eliminate the interaction between HERG and GM130. However, dissociation of HERG<sub>860–899</sub> and GM130 cannot explain the phenotypes reported here because this interaction is not required for the perfunctory delivery of HERG channels to the plasma membrane (32).

A number of COOH-terminal signals that regulate ER exit have been identified (17, 27, 28). HERG contains an ER retention motif between residues 1005 and 1007 that when exposed reduces cell surface expression of the ion channel (27). This signal, however, cannot explain the retention of our mutants because it is completely absent in channels that harbor large truncations at residue 860. Another possibility is that deletion of residues 860–899 may result in removal of ER export signals (17, 33). However, there are no identifiable ER export signals in this region. In addition, without these signals, membrane proteins exhibit only poor or delayed surface expression kinetics, whereas HERG<sub>860–899</sub> is completely absent from the cell surface (17).

In the absence of evidence for other mechanisms, the ER retention of HERG<sub>860–899</sub> may be the result of a conformational defect, similar to that described for ΔF508CFTR (13). Structural defects of misfolded membrane proteins, including ΔF508CFTR, are often rescued in the presence of glycerol (34). However, Golgi transit of HERG<sub>860–899</sub> could not be restored by glycerol (not shown), indicating that the structural defect of this mutant is distinct from that of the ΔF508CFTR. It has been proposed that it is primarily temperature-sensitive protein folding mutants that are amenable to correction via the chemical chaperons such as glycerol (35). Consistent with this view, we found that Golgi transit of HERG<sub>860–899</sub> could not be restored by incubating the cells at room temperature for 12 h (not shown). Similar insensitivity to glycerol and temperature have been described for other HERG mutants (36) as well as for distinct membrane proteins and ion channels that exhibit defective trafficking (37, 38). Although the basis of transport block of this class of mutant proteins is unknown, it is possible that these mutations cause a major collapse in protein struc-

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ture such that they are not amenable to stabilization. Indeed, mutant proteins that are rescued by glycerol show unperturbed biological activity indicating a subtle change in structure (39, 40). It is conceivable that for mutant proteins with major structural defects the critical free energy barrier, which is a rate-limiting step in folding, cannot be overcome in the presence of chemical chaperons or by cooling (41, 42).

Our observations suggest that the structural defect of HERG<sub>340–899</sub> is recognized very early in biogenesis, possibly during translocation-assisted folding and integration into the ER membrane (43–45). Consequentially, the rapid decrease in the levels of HERG<sub>340–899</sub> could be the result of co-translational proteolysis with the outcome of rapid degradation of nascent chain during synthesis (46). Indeed, in the presence of 26 S proteasome inhibitors the steady state expression levels of HERG<sub>340–899</sub> peal that of the wild type protein. Therefore, regardless of the pathways involved and the basis of transport block, degradation is at least partly responsible for reduced levels and high turnover rate of HERG<sub>340–899</sub>.

Relevance to LQTS—Consistent with our findings, previous reports indicate that HERG-C is required for the generation of functional HERG currents (47, 48). Within the COOH-terminal cytoplasmic domain we have now identified a fragment comprising residues 860–899 as being essential for the production of functional HERG channels. By controlling exit from the ER, this fragment regulates HERG trafficking to the cell surface. In addition, our data suggests that naturally occurring mutations resulting in truncations or amino acid substitution in HERG-C have similar consequences. These findings provide a unifying mechanism for LQTS associated with a subset of mutations in HERG-C. Although we have shown that residues 860–899 are pivotal for the intracellular trafficking of HERG, other signals in the HERG-C may contribute to the quality control/structure of the ion channel. This is suggested by mutations upstream of segment 860–899 that lead to mislocalization of HERG (9, 12). Multiple and distinct signals of HERG-C could play a role in proper trafficking and folding of the ion channel. Alternatively, residues 860–899 may be a part of a larger domain within the COOH terminus that is involved in maturation of the ion channel. We are currently exploring these two possibilities.

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