Heteromeric Assembly of Human Ether-à-go-go-related Gene (hERG) 1a/1b Channels Occurs Cotranslationally via N-terminal Interactions*†§

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Alternate transcripts of the human ether-à-go-go-related gene (hERG1) encode two subunits, hERG1a and 1b, which form potassium channels regulating cardiac repolarization, neuronal firing frequency, and neoplastic cell growth. The 1a and 1b subunits are identical except for their unique, cytoplasmic N termini, and they readily co-assemble in heterologous and native systems. We tested the hypothesis that interactions of nascent N termini promote heteromeric assembly of 1a and 1b subunits. We found that 1a and 1b N-terminal fragments bind in a direct and dose-dependent manner. hERG1 hetero-oligomerization occurs in the endoplasmic reticulum where co-expression of N-terminal fragments with hERG1 subunits disrupted oligomerization and core glycosylation. The disruption of core glycosylation, a cotranslational event, allows us to pinpoint these N-terminal interactions to the earliest steps in biogenesis. Thus, N-terminal interactions mediate hERG 1a/1b assembly, a process whose perturbation may represent a new mechanism for disease.

Potassium channels encoded by the human ether-à-go-go-related gene (hERG1) are important in controlling cardiac excitability, neuronal firing frequency, tumor cell proliferation, and smooth muscle function (1–7). Clinically, they are considered the primary target for acquired long QT syndrome (LQTS) (4, 5, 8) caused when drugs intended for other therapeutic targets block hERG1 channels and trigger catastrophic ventricular arrhythmias and sudden death. Mutations in hERG1 give rise to congenital LQTS (9). Although only a fraction of the more than 200 potential disease-causing mutations in hERG have been analyzed in heterologous expression systems, most reduce surface membrane expression of channels because mutant subunits fail to exit the endoplasmic reticulum (ER) (10–15). Little is known about why such LQTS-2 mutants fail to mature. In theory, maturation defects could result from misfolding, failed oligomeric assembly, or trafficking defects during translocation of channel complexes to the plasma membrane (16, 17). Drugs can rescue a subset of mutants, as if by stabilizing the internal vestibule of the pore and thus compensating for folding or oligomerization defects (18).

We are only beginning to understand how ion channels assemble (19). Studies of certain voltage-gated potassium (Kv) channels and neurotransmitter receptors indicate that assembly of related subunits is specified by N-terminal interactions (20–22). In homomeric Kv1.3 channels, N termini interact as they emerge from the translocon, well before subunit synthesis is complete (23). In contrast to Kv or ligand-gated channels, where homologous N termini mediate association, hERG subunits present an unusual challenge: they exist as two isoforms that are identical except for structurally divergent N termini. The hERG 1a N terminus comprises ~396 residues, whereas the 1b N terminus is a mere 56 residues, the first 36 of which are unique (Fig. 1A). The two subunits assemble in native tissues and in heterologous systems (24) where, in the absence of hERG 1a, 1b subunits fail to form robust homomeric currents (25, 26). If the N termini are involved in assembly for hERG channels as for other potassium channels, the underlying mechanisms may differ substantially from those regulating association of homologous domains. Understanding these mechanisms is an important first step to uncovering new determinants of LQTS that occur because of defects in biogenesis.

In this study we tested the hypothesis that cotranslational interactions between the 1a and 1b N termini promote heteromeric subunit association. We show that hERG 1a and 1b N termini interact in cellular and in vitro assays. We utilized a truncated 1b subunit, which is retained in the ER, as a reporter of early biogenic events. Homo-oligomers of this subunit are core-glycosylated, but glycosylation can be disrupted by the introduction of heteromerizing 1a N-terminal fragments. Thus, heteromeric interactions occur before the addition of the glycan group in the ER. Such non-homotypic interactions between structurally dissimilar N termini occur cotranslationally and likely function to facilitate the heteromerization of hERG1 channel subunits.

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§ The abbreviations used are: hERG1, human ether-à-go-go related gene; ER, endoplasmic reticulum; aa, amino acids; TM, transmembrane; GFP, green fluorescent protein; GST, glutathione S-transferase; LQTS, long QT syndrome; HEK, human embryonic kidney; IP, immunoprecipitate; Endo H, endoglycosidase H.
Early Heteromeric hERG1 Assembly

EXPERIMENTAL PROCEDURES

Plasmids—For mammalian expression, hERG 1a (aa 1–1159) and 1b (aa 1–819) cDNA were subcloned into pcDNA3.1 vector (Invitrogen). C-terminal-truncated clones, lacking 461 aa of the distal region, 1aACT (aa 1–698) and 1bACT (aa 1–358), were subcloned into pcDNA3.1/Myc-His vector (Invitrogen). hERG1 N-terminal fragments with the first two transmembrane (TM) domains were subcloned using PCR with a 3′ oligo that introduced a FLAG tag sequence at the end of the second TM (aa 476 for 1a and 136 for 1b). Amplified fragments were cloned into pcDNA3.1 vector and sequenced.

For bacterial expression, 1a N terminus (1aNT, aa 1–67) was cloned into pET28a (+) vector (Novagen) resulting in an N-terminal His tag. The 1b N terminus (1bNT, aa 1–67) was cloned into pGex4T-1 vector (Amersham Biosciences) and bears a GST tag on the N-terminal end. Both pGex4T-1 and pET28a clones were transformed into Rosetta(DE3)pLysS cells (Novagen).

Antibodies—The generation of pan-ERG1 rabbit antibody against the C terminus of hERG1, and 1a and 1b isoform-specific rabbit antibodies has been described previously (24). For Western blotting, the antibodies were used at 1:5000, 1:150, and 1:1000 dilutions, respectively. Mouse anti-c-Myc (Santa Cruz Biotechnology) was used at a dilution of 1:250. Mouse anti-c-Myc (Clontech) was used at 1:200 dilution. Mouse pan-hERG1 antisera were produced in collaboration with Neoclone (Madison, WI) and used at 1:500.

Cell Culture—Human embryonic kidney (HEK)-293 cells were cultured in Dulbecco’s modified Eagle’s medium at 37 °C. A cell line stably expressing 1bACT protein was generated by transfecting HEK-293 cells with Myc-tagged 1bACT DNA and growing in medium containing 1 mg/ml neomycin for selection. Separate cell colonies were selected, and lysate from them was probed with 1b-specific antibody to confirm expression. Cell lines expressing 1bACT were then maintained in 500 µg/ml neomycin.

Protein Expression and Purification from HEK-293 Cells—Cells were transfected with appropriate quantity of DNA at 70–80% confluence using TransIT-LT1 kit (Mirus). Membrane preparations were made, 48 h post-transfection, by solubilizing cells in 150 mM NaCl, 25 mM Tris-HCl, pH 7.4, 20 mM NaEDTA, 10 mM NaEGTA, 5 mM glucose, and 0.5–1% (v/v) Triton X-100 followed by sonication and incubation for 15 min. Lysates were cleared of debris by centrifugation at 10,000 × g for 15 min, and the supernatant quantified using the Bradford assay. Immune complexes were eluted in 5 ml of 250 mM LSB (225 mM Tris-HCl, pH 6.8, 5% SDS, 50% glycerol, 200 mM dithiothreitol) at 65 °C for 5 min. Eluted protein complexes, alongside their input lysate, were size-separated by SDS-PAGE and Western-blotted using standard methods.

Co-immunoprecipitation was used to isolate complexes of the 1aNT<sub>im</sub> fragment with the truncated 1bACT construct in stably expressing 1bACT cells to observe the effects of association on 1bACT core glycosylation. In contrast, in transient co-transfections the effects could be seen in the lysate without co-immunoprecipitation. We inferred that the inability to observe an effect in stable cell lysates without co-immunoprecipitation is because of the reduced efficiency of co-expression of the two constructs compared with transient transfections in which both constructs are simultaneously introduced and coordinately translated.

Protein Expression and Purification in Escherichia coli—Recombinant proteins were purified as per manufacturer’s protocol (Amersham Biosciences and Novagen). Purified 1aNT-His<sub>6</sub> protein was eluted from Ni<sup>2+</sup> beads (Qiagen) with 250 mM imidazole and desalted and exchanged in 20 mM HEPES, pH 7.4, 500 mM NaCl by Amicon 10K column (Millipore). Purified proteins were size-separated by SDS-PAGE, stained by Coo massie Blue, and quantified by comparison with bovine serum albumin protein standards.

GST Pull-down Assays—Binding assays were carried out by incubating the indicated amounts of soluble 1aNT-His<sub>6</sub> with 2 µM immobilized recombinant fusion proteins, 1bNT or GST. Reactions were incubated with agitation for 2 h at 4 °C in a total volume of 150 µl of TBS buffer containing 0.5% Triton X-100 (binding buffer). Bound proteins were washed three times with 1 ml of binding buffer, and eluted by boiling in LSB. The entire binding reaction was subjected to SDS-PAGE and Coo massie Blue staining.

Endoglycosidase Analysis—Deglycosylation was performed according to the manufacturer’s protocol. 8 µg of denatured lysate was treated with either endoglycosidase H (Roche Applied Science) or PNGase F (New England Biolabs) overnight at 37 °C. In control reactions, the enzymes were replaced with buffer.

Pulse Chase—HEK-293 cells were grown to 70% confluence and transfected with 10 µg of 1bACT DNA with either 10 µg of 1aNT<sub>im</sub> or 10 µg of pcDNA3.1 using Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> (27). Two days post-transfection, cells were starved for 15 min, pulsed with 250 µCi of <sup>35</sup>S Promix (Amersham Biosciences) per dish for 5 min. Cells were chased with unlabeled medium for the indicated times. Cells were suspended in lysis buffer and incubated for 30 min at 4 °C. Radiolabeled lysates were clarified by centrifugation, quantified, and immunoprecipitated with either 1b-specific (for pcDNA3.1-transfected cells) or 1a-specific (1aNT<sub>im</sub>-transfected cells) antibodies for 3 h, and immune complexes were isolated with protein A beads for 12–16 h at 4 °C. Proteins were eluted with LSB, heated at 65 °C for 5 min, and size-separated by SDS-PAGE. Gels were fixed in a 25% isopropyl alcohol and 10% acetic acid solution, dried at 80 °C for 2 h, and exposed to a phosphorscreen. Pulse chase for 1bACT stable cells was set up similarly with indicated pulse and chase times.

Fractional Centrifugation—HEK-293 1bACT cells were transfected with DNA for either pcDNA3.1 vector or 1aNT<sub>im</sub>. Cells were homogenized in 750 µl of protease inhibitor (Roche

JOURNAL OF BIOLOGICAL CHEMISTRY 9875
Early Heteromeric hERG1 Assembly

FIGURE 1. hERG1 subunits assemble in the ER. A, schematic illustrating different cytoplasmic N-terminal domains of hERG 1a and 1b. Labels indicate the N-linked glycosylation site and protein domains ether-α-o-glycosylation (eog). Per-Arnt-Sim (PAS), putative cyclic nucleotide binding domain (CNBD), and tetramerization coiled-coil (TCC). B, lysate from HEK-293 cells transiently expressing 1a and 1b was immunoprecipitated with 1b-specific rabbit antibody, and the Western blot was probed with mouse antibody against the common C terminus of hERG1 isoforms. The immature species typically dominates in transient transfections. The mature and immature 1a glycoforms migrate at 155 and 135 kDa, respectively. Immature and mature 1b proteins migrate at 85 and 95 kDa, respectively.

micitab)-supplemented 10 mm Tris-HCl, pH 7.4, 250 mm sucrose, 2 days post-transfection. Cells were lysed manually using a 25-gauge needle. Lysate was centrifuged at 1,000 x g to remove crude cellular debris (1,000 x g pellet). Supernatant was collected and centrifuged at 10,000 x g for 30 min to separate large membranes (e.g. ER, Golgi, plasma membrane; 10,000 x g pellet). The supernatant was subjected to a final centrifugation at 100,000 x g for 90 min to separate small organelles (e.g. endosomes, lysosomes; 100,000 x g g pellet) and the cytosol (100,000 x g supernatant). Each pellet was dissolved in 300 μl of lysis buffer (protease-inhibitor supplemented 50 mm Tris-HCl, pH 7.2, 1 mm EDTA, 150 mm sodium chloride, 1% Triton X-100). Up to 1% Triton X-100 was added to lyse the cytosolic fraction.

Densitometry and Statistical Analysis—Coomassie Blue-stained gels and x-ray films of Western blots were quantified by measuring optical density using LabWorks Image and Acquisition Analysis Software (Upland, CA). Data were analyzed using PRISM 2.0 software (GraphPad, San Diego).

For in vitro binding assays (Fig. 3), data from three separate experiments were normalized, plotted, and fitted with variable slope sigmoidal dose-response Equation 1.

\[
Y = \frac{Y_{\text{Max}} - Y_{\text{Min}}}{1 + 10^{\log EC_{50} - X}} \times \text{Hill Slope}
\]

where X is the logarithm of concentration and Y is the response.

For assessing the degree of 1bΔCT homo-oligomerization in the absence or presence of the disrupting 1aNT\text{tm} fragment (Fig. 5), we used the formula in Equation 2.

Level of homo-oligomerization

\[
= \frac{(1b\Delta CT-\text{GFP} / 1b\Delta CT-\text{Myc})_{\text{input}}}{(1b\Delta CT-\text{GFP} / 1b\Delta CT-\text{Myc})_{\text{ip}}}
\]

This formula allowed us to take in account the variability in 1bΔCT-GFP and 1bΔCT-Myc protein expression in different sets of lysates. Data were analyzed by running column statistics for one-sample Student’s t test.

For disruption experiments (Fig. 6), optical density (OD) values for core glycosylated/total 1bΔCT from lysates of cells co-transfected with 1bΔCT and each of the disruption fragments were individually compared with the fractional OD value from 1bΔCT + pcDNA3.1 co-transfected lysate. Data from three experiments were analyzed using a Student’s unpaired t test and a two-tailed p value and are presented as mean ± S.E.; n indicates the number of experiments.

RESULTS

hERG 1a and 1b N Termini Interact in Mammalian Cells—To identify the soluble domains required for channel association, we first tested whether heteromeric interactions were preserved in the absence of the C terminus, which might mediate heteromerization. We found that 1aΔCT and 1bΔCT proteins co-expressed in HEK-293 cells could be co-immunoprecipitated using 1a-specific antibodies (Fig. 2B, lanes 1 and 2). This association requires interaction of the truncated proteins in vivo, because 1aΔCT and 1bΔCT did not associate in mixed lysates independently expressing the two constructs (Fig. 2B, lanes 3 and 4). We conclude that the C terminus, which includes a tetramerization coiled-coil (TCC) domain (34), is not required for hERG 1a/1b heteromeric association.

To determine whether an intact hydrophobic core is required to mediate association, we made more extensive C-terminal truncations leaving only the N terminus and the first two TM domains (1aNT\text{tm} and 1bNT\text{tm}, Fig. 2C). Including the first two TMs allows the short N terminus of the 1b subunit to fully emerge from the translocon and attain proper tertiary structure (19). We co-expressed 1aNT\text{tm} and 1bNT\text{tm} in HEK-293 cells, and immunoprecipitated the cell lysate with 1a-spe-
Early Heteromeric hERG1 Assembly

1aNT from 0.3 to 10 μM in separate reactions, eluted bound complex from the beads, and resolved the eluate using SDS-PAGE. Binding of 1aNT to GST-1bNT was sufficiently robust to be visualized by Coomassie Blue staining (Fig. 3A, lanes 1–6). The low efficiency of 1aNT binding is attributable to the large degree of degradation of 1bNT (visible on gel, under the indicated GST-1bNT polypeptide) and to competing homotypic association of 1aNT fragments (supplemental Fig. S1). Immobilized GST used as a negative control did not show binding to the 1a N terminus at any concentration used (Fig. 3A, lanes 7–12). A dose-response curve plotting the OD of soluble 1aNT bound to GST-1bNT as a function of 1aNT concentration shows binding occurs with an EC_{50} of 0.96 ± 0.2 μM and a Hill coefficient of 1.52 (Fig. 3B). These results indicate that the 1a and 1b N termini bind each other directly and thus potentially mediate association of heteromeric subunits during assembly.

**Truncated hERG 1b Serves as a Reporter for Early ER Events**—To determine whether hERG 1a and 1b N termini interactions play a role in biogenesis, we next sought an approach to perturb their interaction and measure the effect on early events in assembly. We discovered that the C-truncated construct 1bΔCT exists not in core and maturely glycosylated forms, as is the case for full-length 1b (24), but rather in core and unglycosylated forms (Fig. 4). We identified the core glycoform based on its sensitivity to both PNGase F and Endoglycosidase H (Endo H), which collapsed the 38-kDa 1bΔCT to the same size as the lower, unglycosylated band migrating at ~35 kDa (Fig. 4A). Fig. 4B shows mature, full-length hERG 1b is insensitive to Endo H, thus ensuring the specificity of the enzyme treatment. The presence of the core and unglycosylated species presented an opportunity to identify, via perturbation, early events leading up to core glycosylation (see below).

**1a N-terminal Fragments Disrupt hERG1bΔCT Oligomerization**—We exploited ER glycosylation of 1bΔCT to identify the role of heteromeric N-terminal interactions in early assembly of hERG channels. We hypothesized that core glycosylation might report oligomerization of 1bΔCT, and that disruption of these events by 1aNT fragments would provide evidence of heteromeric N-terminal interactions. We first tested...
Early Heteromeric hERG1 Assembly

FIGURE 3. hERG 1a and 1b N termini interact in vitro. A, left panel, Coomassie Blue-stained gel showing direct interaction between 1a and 1b N termini. Lanes 1–6, co-sedimentation of 1aNT with immobilized GST-1bNT (arrows). Each reaction contained indicated amounts of soluble 1aNT-His6. The amount of 1aNT bound to 1bNT was roughly 50–500 ng, falling within the sensitivity and linear range of the Coomassie Blue stain (64). 100 percent of bead-bound material was loaded. The two species do not interact in mixed lysates from cells co-transfected with 1aNT and 1bNT. B, optical density of the bound 1aNT (upper arrow in A) at each concentration (lanes 1–6) was divided by the corresponding GST-1bNT input (lower arrow) to control for loading. Each fractional OD for 1aNT was then normalized to the maximum (lane 6) and plotted against the concentration of soluble 1aNT.

FIGURE 4. C-terminal-truncated 1b reports core glycosylation. A, Western blot probed with 1b-specific antibody shows 1bCT is reduced by both PNGase F (lane 1 and Endo H (lane 3) treatment. B, in contrast, full-length hERG 1b has a mature, Endo H insensitive band as well as an Endo H-sensitive band. PNGase F removes all glycan groups from 1b. Lane 2 in each case is the untreated lysate. M, mature; CG, core-glycosylated; UG, unglycosylated.

whether 1bCT subunits oligomerize by co-expressing 1bCT-Myc with 1bCT-GFP in HEK-293 cells. Using Myc antibodies, we found the larger 1bCT-GFP fusion protein co-immunoprecipitated with the 1bCT-Myc (Fig. 5A, lanes 1 and 2). The two species do not interact in mixed lysates from cells independently expressing the two constructs (Fig. 5A, lanes 4 and 5). Reciprocal immunoprecipitates using GFP antibodies gave similar results (data not shown).

If 1a and 1b N termini efficiently interact, we expect 1aNT\textsubscript{tm} fragments to disrupt hERG1\textsubscript{1a}1bCT homo-oligomerization. Indeed, as is evident from Western blot results, co-transfection with comparable amounts of 1aNT\textsubscript{tm} (see supplemental Fig. S2) caused a significant reduction in homo-oligomerization (Fig. 5A, lanes 6 and 7). Association of 1bCT-Myc and 1bCT-GFP oligomers was dramatically reduced by 80 ± 13% (p = 0.0011, N = 4; Fig. 5B). Thus, the 1aNT fragment disrupts 1bCT oligomerization. These experiments demonstrate that avid interaction between 1a and 1b N termini mediate heteromeric assembly.

1a N-terminal Fragments Cause Accumulation of Unglycosylated 1b\textsubscript{CT}—The perturbation of 1bCT oligomerization could occur by one of two mechanisms: the 1aNT\textsubscript{tm} fragment prevents 1b N-terminal interactions during biogenesis, or it causes dissociation of extant 1b oligomers. Preventing early assembly is expected to disrupt core glycosylation, whereas any disruption of preformed 1bCT oligomers is not. By measuring core glycosylation, we can pinpoint the timing of the 1aNT\textsubscript{tm} and 1bCT interaction.

We cotransfected 1bCT into HEK-293 cells with one of the following constructs: empty vector (control), 1aNT\textsubscript{tm}, or Ndel (lacking N-terminal residues 1–354; cf. Fig. 1A). We assayed the effects of the disrupting fragments in 1bCT lysates by measuring the core glycoform as a fraction of the total (core-plus unglycosylated) signal. As compared with the control, cotransfection of 1bCT with 1aNT\textsubscript{tm} resulted in significant reduction in core-glycosylated 1bCT, suggesting that the heteromeric N termini interacted prior to the glycosylation step (p < 0.005, n = 3; Fig. 6, A and B). Ndel also reduced core glycosylation but to a lesser extent (p < 0.05, n = 3), and we could not rule out whether this reduction was because of its competition for the glycosylation machinery. In contrast, the greater enrichment of unglycosylated 1bCT by 1aNT\textsubscript{tm} could not have resulted from a direct exhaustion of the glycosylation machinery, because 1aNT\textsubscript{tm} lacks the hERG1 N-linked glycosylation site. The 1aNT\textsubscript{tm} fragment associated primarily with the unglycosylated 1bCT in co-immunoprecipitation experiments, indicating the increase in unglycosylated 1bCT was because of its direct physical association with the disrupting fragments (Fig. 6C). Together, the results above indicate that N-terminal interactions disrupt oligomerization and core glycosylation, reflecting early biogenic events.

Interaction with hERG 1a N Terminus Prevents Maturation of 1bCT—As an alternative explanation for the increase in abundance of unglycosylated 1bCT just described, we considered whether 1a N-terminal fragments might promote degradation of 1bCT rather than prevent core glycosylation. If so, we would expect to see an accumulation of unglycosylated 1bCT species in the cytosol en route to degradation as part of the unfolded protein response (35, 36). We used pulse-chase labeling to test this possibility. We co-transfected 1bCT with vector (as a control) or with 1aNT\textsubscript{tm} fragment in HEK-293 cells. We radiolabeled cells for 5 min, and immunoprecipitated
termini associate early, prior to the core glycosylation of the S5-P linker as it emerges from the translocon.

**DISCUSSION**

In the current study, we investigated the biochemical basis for heteromeric assembly of hERG1 channels during biogenesis. We showed that hERG 1a and 1b subunits assemble in the ER. Their N termini interact in vitro in a direct and dose-dependent manner. As illustrated in the model in Fig. 8, the 1a N-terminal fragment interacts with the 1b N terminus and prevents oligomerization of a truncated 1b construct (1bΔCT). Cotranslational glycosylation of 1bΔCT is concomitantly reduced, suggesting heteromeric N termini interact prior to glycosylation and therefore cotranslationally. Heteromeric interaction and inhibition of glycosylation occurred within 5 min of the onset of protein synthesis as measured with pulse chase. Thus, N-terminal interactions are crucial to early assembly in the biogenesis of hERG1 channels.

Mechanisms controlling homomultimeric and hetero-multimeric assembly of membrane proteins have been explored in several other systems. In the distantly related Shaker Kv channels, homo-oligomerization is facilitated by the association of homologous tetramerizing (T1) interaction domains (22, 37, 38). Nascent N-terminal T1 domains interact cotranslationally, associating as they are produced on the ribosome and well before the pore-forming domains have been synthesized or properly folded (23, 39–41). The efficiency of this process depends on the presence of the ER membrane, which is thought to concentrate the interacting domains that would otherwise be diluted in the cytosol (20, 41). Moreover, the transmembrane anchors facilitate the appropriate folding of the contiguous N terminus (42–46). The interaction of the T1 domains is also thought to regulate heteromeric assembly: channels from divergent Kv subfamilies (e.g. Kv1.1, Kv2.1) can assemble, albeit inefficiently, only if their T1 domains have been removed (47); if the T1 domains are present, only heteromers from within a subfamily (e.g. Kv1.1, Kv1.3) will be produced (48). In contrast to Kv and ligand-gated channels, where oligomerization occurs via the interactions of homologous N termini (19, 21, 49, 50), our results indicate the assembly of hERG 1a and 1b proceeds by the interaction of structurally dissimilar domains.
Whether hERG 1a channels have an N-terminal oligomerization domain similar to T1 remains controversial. A hERG 1a N-terminal fragment termed NAB associates as tetramers in solution (51), whereas in crystal structures the same protein fragment exists strictly as monomers (52). Although crystallography also revealed a PAS domain predicted to be involved in protein-protein interactions (53), the specific 1a N-terminal domains mediating heteromeric association remain to be determined.

The perturbation of 1bCT oligomerization by the 1aNTtm fragment indicates the 1b N termini interact cotranslationally during homo-oligomerization. This result was not necessarily predicted from analysis of the short, 36-amino acid 1b N terminus, which exhibits an excess of 8 positively charged residues. Our results indicate these charges do not present an electrostatic barrier to 1b N-terminal homo-oligomerization.

Why glycosylation is disrupted in the presence of heteromerizing N termini is not known. The hERG subunit has a single glycosylation site, and the core glycosylation machinery may require a double glycan moiety not present in the 1bCT-1aNTtm complex. Alternatively, failure to fully oligomerize or global folding disturbances may reduce accessibility to the core glycosylation machinery. The heteromerizing fragment may also prevent topological changes that allow exposure of the glycosylation site to the lumen.

A tangential but important result from this experiment is that truncation of the C terminus results in accumulation of the core glycoform and reveals a requirement for the C terminus in Golgi-mediated hERG 1b glycosylation. Expression of 1aCT gave similar results (supplemental Fig. S3), indicating that the hERG1 C terminus is of general importance in Golgi-mediated glycosylation. We speculate that the absence of the C terminus deprives truncated hERG1 proteins of interactions with forward trafficking partners that ensure their arrival at the Golgi. Alternatively, C-terminal truncation may prevent interaction with proteins normally required to retain hERG1 in the Golgi long enough to ensure appropriate glycosylation (54, 55). Golgi-mediated glycosylation of hERG1 channels is not a requirement for function or trafficking but it does enhance channel stability (28). Therefore,
The presence of 1b might regulate 1a N-terminal interactions with the β1 integrin and in turn determine the localization of hERG 1a/β1 integrin complexes in caveolae-containing lipid rafts.

hERG1 channels have been intensively studied because of their role as a target for LQTS. Trafficking defects have been identified as a major mechanism underlying congenital LQTS (10, 63). Unraveling the assembly process may provide clues to the varied mechanisms by which hERG1 mutations, many of which are unique to the 1a N terminus, can result in LQTS. Mutations could reduce the efficiency of heteromeric hERG1 channel assembly, or mediate dominant-negative interactions between 1a and 1b subunits, resulting in ER retention, enhanced degradation, reduced surface expression and lower current magnitudes, all processes capable of reducing repolarizing current and causing LQTS. Additionally, C-terminal mutations could interfere with complex glycosylation thereby destabilizing channels at the cell surface and potentially reducing hERG1 current.

In summary, our results demonstrate that the N termini interact in a direct, dose-dependent manner and mediate heteromeric association of hERG1 subunits during channel biogenesis. This interaction, which takes place between structurally divergent N-terminal domains of the respective 1a and 1b subunits, is a critical step in the assembly of this unique channel.

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