This Review is part of a thematic series on the Biology of Cardiac Arrhythmias, which includes the following articles:

- Antiarrhythmic Drug Target Choices and Screening
- Inherited Arrhythmogenic Diseases: The Complexity Beyond Monogenic Disorders
- Genomics in Sudden Cardiac Death
- Regulation of Ion Channel Expression
- Biology of Cardiac Arrhythmias: Ion Channel Protein Trafficking
- Computational Insights: Chaos and Wave Theory
- Gene Therapy and Cell Therapy of Cardiac Arrhythmias

Eduardo Marbán and Gordon Tomaselli, Editors

This series is in honor of Harry A. Fozzard, 8th Editor of Circulation Research.

Biology of Cardiac Arrhythmias
Ion Channel Protein Trafficking

Brian P. Delisle,* Blake D. Anson,* Sridharan Rajamani, Craig T. January

Abstract—The mechanisms underlying normal and abnormal cardiac rhythms are complex and incompletely understood. Through the study of uncommon inheritable arrhythmia syndromes, including the long QT and Brugada syndromes, new insights are emerging. At the cellular and tissue levels, we now recognize that ion channel current is the sum of biophysical (gating, permeation), biochemical (phosphorylation, etc), and biogenic (biosynthesis, processing, trafficking, and degradation) properties. This review focuses on how heart cells process ion channel proteins and how this protein trafficking may be altered in some cardiac arrhythmia diseases. In this review, we honor Dr Harry A. Fozzard, a modern pioneer in cardiac arrhythmias, cell biology, and molecular electrophysiology. As a scientist and physician, his writings and mentorship have served to foster a generation of investigators who continue to bring this complex field toward greater scientific understanding and impact on humankind. (Circ Res. 2004;94:1418-1428.)

Key Words: arrhythmia ■ ion channels ■ protein trafficking ■ cell biology

It is well understood that cardiac arrhythmias are a common and sometimes lethal manifestation of many acquired and inherited diseases affecting the cardiovascular system. Electrophysiologists have long sought to understand and treat the arrhythmogenic mechanisms of these diseases to sustain life and improve its quality. Beginning in 1949 with the first reports of cardiac action potential recordings, including normal and diseased human heart, extraordinary progress has been achieved in understanding mechanisms of normal and abnormal cell electrophysiology and heart rhythm generation. The reductionist approach has resulted in the identification of multiple families of ionic currents, their associated channel and transporter proteins, and the genes encoding their multiple subunit structures. Similarly, evolution of knowledge about the complex cellular processes that modulate ion channel function, govern cell-to-cell communication, and generate different mechanisms of arrhythmias have added to our understanding of normal and abnormal electrophysiological properties. From these findings emerged the concept that abnormalities in surface membrane ion channel function are central to arrhythmogenesis. More recent observations that mutated ion channels identified in patients and studied in experimental models undergo altered expression or are incorrectly processed within cells have broadened our view of the

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TABLE 1. Posttranslational Processing of Proteins

A protein, once synthesized on a ribosome, has several outcomes:

- It must fold into a proper tertiary structure
- It may assemble with other proteins (similar or not) to form a larger mature molecule (eg, K⁺ channel oligomerization, accessory subunit addition, etc)
- It may be cleaved into one or more smaller mature protein molecules (eg, Ca²⁺ channel α₁β subunits)
- It may be transported (traffic) to another intracellular location(s) and be sorted for delivery to its final destination
- It may be targeted at any stage for degradation (eg, ubiquitin–proteosomes, lysosomes)

Potential mechanisms underlying changes in ion channel currents. Thus, it is now recognized that the macroscopic current in heart and other excitable tissues is the sum of 2 processes: (1) biophysical (gating, ion permeation) and biochemical (phosphorylation, etc) modulation of surface membrane ion channels and (2) biogenic modulation (biosynthesis, processing, trafficking, and degradation) of channel protein. Incorrect processing of mutant proteins within cells, in fact, has become an increasingly important mechanism causing many diseases, and the potential for correcting defective protein processing offers novel therapeutic approaches.

Protein Processing in Cells

The 1972 Nobel Prize for Chemistry was awarded to Dr Christian Anfinsen for work suggesting that the primary amino acid sequence of a protein is sufficient for proper folding in vitro. Since that time, it has become apparent that protein biogenesis in vivo is more complex, requiring additional proteins that assist with peptide processing and trafficking, energy input, posttranslational modification, protein quality control, and degradation. These processes are outlined in Table 1. It is important to recognize that the bulk of our information has been derived from lower organisms and expression studies in heterologous systems.

Transcription and Translation

Regulating the expression of ion channels at the cell surface begins at the level of gene transcription and mRNA stability. Genomic DNA contains binding sites for transcriptional proteins and their regulatory elements (transcriptional activators and repressors). During transcription, RNA polymerases generate long strands of heteronuclear RNA that contain an untranslated 5’ region, multiple exons (amino acid encoding RNA sequences) and introns (noncoding RNA sequences of incompletely understood function) interspersed among the exons, and an untranslated 3’ region. Removal of the intronic sequences (splicing) occurs in the nucleus, creating mRNA consisting of exons to define the coding region as well as 5’ and 3’ noncoding regions that promote mRNA translation and stability. Normal mRNA splicing can vary for a single gene product, thereby generating multiple transcripts with different coding regions and/or differing translation efficiencies. Similarly, pathogenic nucleotide substitutions, deletions, and insertions can affect mRNA synthesis and stability, thereby altering the amount of mRNA available for subsequent peptide generation.

Messenger RNA is exported from the nucleus to the cytoplasm where ribosomal translates coding regions into polypeptide chains. Ribosomal translation begins with the codon AUG, which encodes the amino acid methionine. This “start” codon is usually preceded by a 6-nucleotide consensus motif (GCCACC, the Kozak sequence). In eukaryotic cells, translocation of the nascent polypeptide from the ribosome into the endoplasmic reticulum (ER) is thought to occur cotranslationally and is initiated when specific signal sequences (amino acid motifs) in the elongating polypeptide bind a cytosolic signal recognition particle that then targets the complex to receptors within the ER membrane. For cytosolic proteins, the targeting signal sequence is almost invariably found within the amino terminal. Ion channels, however, have the added complexity of possessing several transmembrane spanning domains. Consequently, these proteins contain numerous signal sequences to direct ER (and ultimately plasma) membrane crossings that determine the cytoplasmic and extracellular domains, and these signaling sequences are not restricted to the amino terminal. For example, in voltage-gated K⁺ channels (Kv1.3), as well as in cystic fibrosis transmembrane conductance regulator (CFTR) Cl⁻ channels, the first ER targeting translocation signal sequence is contained within the second transmembrane spanning domain, nearly 200 amino acids downstream from the amino terminal. Additional transmembrane domains also interact with the ER membrane to initiate and terminate translocation to allow for complex transmembrane protein spanning structures. Although unknown, the mechanisms driving translocation of cardiac ion channels are presumed to be similar.

Posttranslational Modification of Proteins

As a peptide is inserted into the ER, it is almost immediately bound by protein chaperones (so termed because they monitor immature proteins) that are proposed to facilitate maturation and the formation of proper tertiary structure required for protein function. Many chaperone proteins are ATPases and may have other enzymatic activities as well. Most newly synthesized proteins rapidly undergo coreglycosylation, the site-specific (an amino acid motif usually containing an asparagine) addition and trimming of mannose-rich sugar moieties, which may assist in subsequent protein folding, maturation, and stability.

Ion channels exist as large protein complexes with a centrally located pore that allows for ion flow across membranes. For most Na⁺ and Ca²⁺ channels, the α (pore-forming) subunit is synthesized as a monomeric protein derived from a single gene product that has 4 repeating domains of similar structure. For K⁺ channels, however, the α subunit is a multimeric protein formed by the coassembly of multiple peptides either from the same or from related genes. Most voltage-gated cardiac K⁺ channels (Kv) are assumed to require the coassembly of 4 subunit peptides to form a tetrameric protein channel structure. For at least some K⁺ channels, assembly involves the formation of dimers, which then dimerize again to form the tetrameric channel.
unmasking, of ER retention and export signal sequences, thus protein folding and assembly results in the masking, or units that serve multiple regulatory roles. Thus, not only must eases. Furthermore, most ion channels have accessory subunits that serve multiple regulatory roles. Thus, not only must the \( \alpha \) subunit proteins interact and assume a proper 3-dimensional structure but also the accessory subunit proteins must assemble correctly to form multimeric macromolecular complexes, and for most proteins this is thought to occur in the ER.\(^{18,19} \)

After folding and assembly, the protein is transported out of the ER through vesicle budding where it travels along cytoskeletal elements to the ER–Golgi intermediate complex (ERGIC).\(^{20} \) Work on \( \kappa_{5,5} \) ion channels has shown that proper protein folding and assembly results in the masking, or unmasking, of ER retention and export signal sequences, thus providing a mechanism governing export from the ER.\(^{21} \) From the ERGIC, the peptide travels through the Golgi complex where residues core-glycosylated in the ER undergo further processing (“complex glycosylation”). The extent of glycosylation varies as sugar moieties are added and trimmed repeatedly during maturation.\(^{11} \) On reaching the trans-Golgi, proteins are sorted based on specific sequences and earmarked for their final destination, be it other intracellular compartments, the plasma membrane, or extracellular space.

### Quality Control and Protein Degradation

A protein must attain a correct tertiary or native structure to function properly. Cells have evolved a complex set of quality-control monitoring processes that prevent many non-native or misfolded proteins from reaching their intended destinations by tagging such proteins as abnormal and targeting them for premature degradation. These quality-control mechanisms, however, remain poorly understood. In mammalian cells, the majority of the quality control is thought to occur in the ER.\(^{22} \)

Protein degradation is essential for homeostasis because everything a cell synthesizes must ultimately be degraded, secreted, or shed; accumulation of metabolic products usually is lethal to a cell. The principal pathways for degradation depend on the type of protein (membrane, cytosolic, etc) and the reason for degradation. Once identified by quality-control mechanisms, most misfolded proteins are retrotranslocated out of the ER to undergo ATP-dependent ubiquitination, which is followed by degradation within proteasomes.\(^{23,24} \) Proteins and other cellular components can also be degraded through autophagic destruction by lysosomes.\(^{25,26} \)

### Ion Channels in Human Heart Disease

Cardiac action potentials reflect changes in membrane conductance governed by dozens of different ion channels and electorgenic exchangers. Inherited long QT syndrome (LQTS) was recognized 40 years ago as 2 distinct clinical phenotypes, the Romano-Ward and the Jervell and Lange-Nielsen syndromes.\(^{27–29} \) Elucidating the causes of LQTS has been central to progress in identifying several human ion channel genes and in understanding genetic and molecular mechanisms of cardiac ion channel diseases. The manifestations of LQTS arise from polygenic causes, with 7 forms of inherited LQTS described to date (LQT1–7; Table 2). The phenotypes are often attributed to “loss of function” or “gain of function” mutations in the affected ion channels. LQT1, 2, 5, and 6 are thought to prolong the plateau of cardiac action potentials by reducing \( K^+ \) channel currents (loss of function) activated during depolarization. LQT1 and 5 are characterized by mutations in \( KCNQ1 \) (\( KvLQT1 \)) and \( KCNE1 \) (\( MinK \)), which encode the \( \alpha \) and \( \beta \) subunits, respectively, for the slowly activating delayed rectifier \( K^+ \) current (\( I_{Ks} \)). LQT2 and 6 are characterized by mutations in \( KCNH2 \) (\( human-ether-a-go-go-related gene; HERG \)) and \( KCNE2 \) (\( MiRP1 \)), which encode the \( \alpha \) and putative \( \beta \) subunits, respectively, for the rapidly activating delayed rectifier \( K^+ \) current (\( I_{Kr} \)). LQT7 is caused by mutations in \( KCNJ2 \) (\( Kir2.1 \)), which reduces the inward rectifier \( K^+ \) channel current (\( I_{K1} \)) to slow the return of the membrane to the resting potential. LQT4 was recently associated with abnormalities in ankyrin-B protein, which promotes the formation of macromolecular signaling complexes to reduce repolarizing current. In contrast, LQT3 arises from mutations in \( hNaV1.5 \) (\( SCN5a \)), which encodes the cardiac Na\(^+ \) channel. These cause failure of normal inactivation to increase late Na\(^+ \) current (gain of function) and prolong action potentials. More than 200 mutations in the genes encoding for LQT1–7 are known, with LQT1 and 2 patients accounting for most of LQTS. Additional congenital arrhythmia syndromes continue to be described, and these are summarized in Table 2. Unlike LQTS, some of these involve gain of function mutations in \( K^+ \) channels (short QT syndrome) and loss of function mutations in \( Na^+ \) channels (Brugada syndrome, cardiac conduction disease, etc).

The functional effects of gene mutations are often studied by expressing appropriately engineered cDNA in heterologous systems. For ion channels, the cellular or macroscopic current (\( I \)) is the product of the number of functional channels in the cell surface membrane (\( N \)), the probability that the channel is open (\( Po \)), and the single channel conductance (\( i \)). This paradigm is illustrated in Figure 1 for HERG current (\( I_{HERG} \)). Changes in \( Po \) and \( i \) can be detected by measuring macroscopic and/or single channel currents, whereas understanding changes in \( N \) requires a multifaceted approach of biochemical, molecular, immunocytochemical, and electrophysiological techniques.

### Protein Trafficking: Lessons From LQT2

Nearly 100 LQT2-linked mutations have been identified in HERG channels. The majority of mutations are single nucleotide changes causing single amino acid substitutions in the channel protein (missense mutations). Only a small number of these mutations have been expressed and functionally characterized, with the consistent finding that LQT2 mutations cause reduced \( I_{HERG} \). A somewhat surprising finding was that this occurs by several different cellular mechanisms, as illustrated in Figure 1.\(^{30–32} \) (1) Reduced \( N \) arises from mutations that generate a trafficking-defective protein with the immature channel retained in the ER. A reduced \( N \) can
also arise from nonsense mutations that cause codon deletions or frame shifts to generate a truncated protein, and recent evidence also suggests that some mutations reduce $N$ by decreasing gene transcription efficiency. 33 (2) Altered $P_o$ can arise through mutations affecting a channel’s gating and kinetic properties. (3) Altered $i$ can arise through mutations affecting channel permeability/selectivity (typically pore mutations). (4) In channels that form as multimeric proteins (ie, $K^+$ channels), dominant-negative interactions between co-assembled mutant and wild-type (WT) channel subunits can potentially affect $N$, $P_o$, or $i$. Furthermore, a mutation can reduce $I_{HERG}$ by combinations of these cellular mechanisms. 32,34–38

Protein trafficking defects reduce the delivery of channels to the cell surface membrane and have emerged as a common LQT2 disease mechanism. These mutations are not restricted

TABLE 2. Cardiac Ion Channel Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Current</th>
<th>Chromosome</th>
<th>Defective Gene</th>
<th>Key Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long QT: Romano-Ward (LQT1–6) and Andersen Syndrome (LQT-7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LQT1</td>
<td>$I_{Ks}$, $+$ amplitude</td>
<td>11p15.5</td>
<td>$KvLQT1$ (KCNQ1)</td>
<td>Wang, et al, 1996[36]</td>
</tr>
<tr>
<td>LQT2</td>
<td>$I_{Ks}$, $+$ amplitude</td>
<td>7q35–36</td>
<td>$HERG$ (KCNH2)</td>
<td>Curran et al, 1995[54]</td>
</tr>
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<td>LQT3</td>
<td>$I_{Na}$, $+$ late current</td>
<td>3p21–24</td>
<td>$SCN5a$ (hNaV1.5)</td>
<td>Wang et al, 1995[57]</td>
</tr>
<tr>
<td>LQT4</td>
<td>altered cell $Ca^{2+}$</td>
<td>4q25–27</td>
<td>$ANKB$</td>
<td>Schott et al, 1995[58]</td>
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<td></td>
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<td></td>
<td></td>
<td>Mohier et al, 2003[39]</td>
</tr>
<tr>
<td>LQT5</td>
<td>$I_{Ks}$, $+$ amplitude</td>
<td>21q22.1–22.2</td>
<td>$MinK$ (KCNE1)</td>
<td>Splawski et al, 1997[100]</td>
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<td>LQT6</td>
<td>$I_{Ks}$, $+$ activation</td>
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<td>$MIRP1$ (KCNE2)</td>
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</tr>
<tr>
<td>LQT7</td>
<td>$I_{Ks}$, $+$ amplitude</td>
<td>17q23</td>
<td>$Kir2.1$ (KCNJ2)</td>
<td>Tristani-Firouzi et al, 2002[102]</td>
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<tr>
<td>Long QT: Jervell and Lange-Nielsen</td>
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<tr>
<td>JLN1</td>
<td>$I_{Ks}$, $+$ amplitude</td>
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<td>$KvLQT1$ (KCNQ1)</td>
<td>Neyroud et al, 1997[103]</td>
</tr>
<tr>
<td>JLN2</td>
<td>$I_{Ks}$, $+$ amplitude</td>
<td>21q22.1–22.2</td>
<td>$MinK$ (KCNE1)</td>
<td>Schultz-Bahr et al, 1997[104]</td>
</tr>
<tr>
<td>Sudden infant death syndrome (LQTS in neonates)</td>
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<tr>
<td></td>
<td>$I_{Ks}$, $+$ amplitude</td>
<td>11p15.5</td>
<td>$KvLQT1$ (KCNQ1)</td>
<td>Schwartz et al, 2000[105]</td>
</tr>
<tr>
<td></td>
<td>$I_{Na}$, $+$ late current</td>
<td>3p21–24</td>
<td>$SCN5a$ (hNaV1.5)</td>
<td>Ackerman et al, 2001[106]</td>
</tr>
<tr>
<td></td>
<td>$I_{Ks}$, $+$ amplitude</td>
<td>7q35–36</td>
<td>$HERG$ (KCNH2)</td>
<td>Lupoglazoff et al, 2004[107]</td>
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<tr>
<td>Brugada syndrome</td>
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<tr>
<td>BS1</td>
<td>$I_{Na}$, $+$ amplitude</td>
<td>3p21–24</td>
<td>$SCN5a$ (hNaV1.5)</td>
<td>Chen et al, 1998[108]</td>
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<td>3p22–25</td>
<td>$SCN5a$ (hNaV1.5)</td>
<td>Weiss et al, 2002[109]</td>
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<td>Idiopathic ventricular fibrillation</td>
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<tr>
<td>IVF</td>
<td>$I_{Ks}$, $+$ amplitude</td>
<td>3p21–24</td>
<td>$SCN5a$ (hNaV1.5)</td>
<td>Akai et al, 2000[110]</td>
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<td>Catecholaminergic (Biventricular) ventricular tachycardia</td>
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<tr>
<td>CPVT1</td>
<td>$+$ cell $Ca^{2+}$</td>
<td>1q42–43</td>
<td>hRyR2</td>
<td>Priori et al, 2001[111]</td>
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<tr>
<td>CPVT2</td>
<td>$+$ cell $Ca^{2+}$</td>
<td>1p13–21</td>
<td>CASQ2</td>
<td>Postma et al, 2002[112]</td>
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<tr>
<td>Cardiac conduction disease</td>
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</tr>
<tr>
<td>CCD</td>
<td>$I_{Na}$, $+$ amplitude</td>
<td>3p21–24</td>
<td>$SCN5a$ (hNaV1.5)</td>
<td>Schott et al, 1999[114]</td>
</tr>
<tr>
<td>Sick sinus syndrome</td>
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<tr>
<td></td>
<td>$I_{Na}$, $+$ amplitude</td>
<td>3p21–24</td>
<td>$SCN5a$ (hNaV1.5)</td>
<td>Benson et al, 2003[115]</td>
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<td>15q24–25</td>
<td>$HCN4$</td>
<td>Schulze-Bahr et al, 2003[116]</td>
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<td>Atrial fibrillation</td>
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</tr>
<tr>
<td></td>
<td>$I_{Na}$, $+$ amplitude</td>
<td>11p15.5</td>
<td>$KvLQT1$ (KCNQ1)</td>
<td>Chen et al, 2003[117]</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>$KvLQT1$ (KCNQ1)</td>
<td>Brugada et al, 1997[118]</td>
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<td></td>
<td></td>
<td>6q14–16</td>
<td>$KvLQT1$ (KCNQ1)</td>
<td>Ellinor et al, 2003[119]</td>
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<td>Short QT syndrome</td>
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<tr>
<td>SQT1</td>
<td>$I_{Ks}$, $+$ amplitude</td>
<td>7q35–36</td>
<td>$HERG$ (KCNH2)</td>
<td>Brugada et al, 2003[120]</td>
</tr>
<tr>
<td>SQT2</td>
<td>$I_{Ks}$, $+$ amplitude</td>
<td>11p15.5</td>
<td>$KvLQT1$ (KCNQ1)</td>
<td>Bellocq et al, 2004[121]</td>
</tr>
</tbody>
</table>
to a single region of the HERG channel (Figure 2); rather, trafficking-defective mutations have been found in several regions of the protein, including the N-terminus (T65P, threonine to proline at amino acid position 65), transmembrane regions (N470D, A561V), pore region (G601S, Y611H, V612L, T613M, L615V), and the C-terminus (R752W, N861I) including the cyclic nucleotide-binding domain (F805C, V822M, R823W). These mutations can be distinguished from WT and normally trafficking mutant channels because they are minimally present in the cell membrane. Electrophysiologically, there is little or no detectable current, and on Western blot analysis, immature (135-kD) core-glycosylated protein is present, but the mature (155-kD) complexly glycosylated protein is absent, as discussed. Using immunocytochemistry, several investigators have shown directly that trafficking-defective LQT2 channel protein (for the T65P, A561V, G601S, Y611H, R752W, V822M, N861I mutations) is located predominantly intracellular with a perinuclear pattern, and 2 mutations (T65P and Y611H) have been shown directly to colocalize with resident ER proteins. These data suggest that trafficking-defective mutant channels are retained in the ER as core-glycosylated protein.

LQT2 mutant subunits differ in their ability to coassemble with WT channel subunit proteins. Coexpression of the trafficking-defective N470D, A561V, V612L, T613M, and L615M mutations with WT protein results in a variable reduction of $I_{HERG}$ because of a dominant-negative effect of the mutant. Ficker et al showed that A561V coassembled with WT subunits, trapping most of the HERG protein in the ER. In contrast, the trafficking-defective LQT2 mutants G601S and R752W did not cause dominant-negative suppression of $I_{HERG}$. Thus, at least some trafficking-defective LQT2 mutant subunits have dominant-negative suppression of $I_{HERG}$. Thus, at least some trafficking-defective LQT2 mutant subunits have dominant-negative suppression of $I_{HERG}$.

**Figure 1.** Macroscopic $I_{HERG}$ depends on the number of functional channels inserted in the plasma membrane ($N$), the open probability of the channel ($P_o$), and its unitary conductance ($i$). WT channel protein is transcribed in the nucleus, synthesized in the ER, exported to the Golgi apparatus, and inserts into the plasma membrane. LQT2 channels may reduce $I_{HERG}$ by altering gating properties ($\Delta P_o$), altering unitary conductance ($\Delta i$), by reducing the number of channels inserted into the plasma membrane ($\downarrow N$), or by combinations of these effects.

**Figure 2.** Trafficking-defective LQT2 mutations in the HERG channel. A schematic cartoon representing a single HERG channel $\alpha$-subunit shows the structural domains including 6 transmembrane helices (S1–S6). The cylinders represent putative $\alpha$ helices. The outer pore region between the S5–S6 helices contains complex glycosylation (N) and selectivity filter (G–F–G) sites. The C-terminus contains a cyclic nucleotide-binding domain (black) and a putative ER retention signal (R–G–R) that can regulate the surface expression of HERG channel protein. Reported trafficking-defective LQT2 mutations (amino acids identified in red) occur throughout much of the HERG channel protein.
negative interactions that suppress the surface expression of WT subunits. Because little is known about channel multimerization in myocytes, care must be taken in translating these in vitro findings.

**HERG Maturation Through the Secretory Pathway**

After synthesis and core-glycosylation in the ER, HERG protein is exported to the Golgi apparatus for complex glycosylation, sorting, and eventual insertion into the surface membrane. As with most large proteins, molecular chaperones are assumed to promote the proper folding of newly synthesized protein and facilitate degradation of misfolded protein. Ficker et al identified 2 cytosolic molecular chaperones, heat conjugate/stress-activated protein 70 (Hc/sp 70) and heat shock protein 90 (Hsp 90), that immuno-noprecipitated with the core-glycosylated, immature HERG protein.47 The Hc/sp 70 family of proteins binds to the hydrophobic regions of newly synthesized proteins in the cytosol to stabilize intermediate steps in protein folding. Hsp 90 proteins prevent the aggregation of misfolded proteins in vitro, but whether it is involved in the normal folding of proteins remains unclear.9 Inhibition of Hsp 90 with geldanamycin prevented the maturation of WT HERG channels, reduced the surface expression of $I_{\text{HERG}}$, and promoted the ubiquination of the channel protein. These data suggest that Hc/sp 70 and Hsp 90 proteins associate with the cytosolic domains of immature HERG proteins in the ER membrane and that Hsp 90 promotes HERG channel maturation.

ER resident proteins may avoid forward transport through the secretory pathway because they contain amino acid sequences in the lumen (eg, K-D-E-L) or cytosol (K-K-X-X and R-X-R) that result in their retrograde transport back to the ER. Similar sequences have been identified in ion channel sequences in the lumen (eg, K-D-E-L) or cytosol (K-K-X-X and R-X-R) that result in their retrograde transport back to the ER. In HERG channels, Kupershmidt et al identified a leucines (R-G-R to L-G-L) increased $I_{\text{HERG}}$. Coexpressing GM130 with WT HERG channels reduced functional $I_{\text{HERG}}$. Roti Roti et al2also showed that V822M and R823W, which are trafficking-defective LQT2 mutations in the nucleotide-binding domain, disrupted the interaction of GM130 with the HERG C-terminal fragment. Given this result, it is tempting to speculate that disruption of the HERG and GM130 interaction may contribute to the phenotype of some trafficking-defective LQT2 mutations.

**Reversing the Trafficking-Defective LQT2 Phenotype: New Therapeutic Strategies**

In mammalian cells, reducing the culture temperature can restore the oligomerization and export of mutant proteins that are retained in the ER.53,54 Reducing cell culture temperature also increases surface expression of several trafficking-defective LQT2 channels. Additional methods that increase the surface expression of LQT2 trafficking-defective channels have been identified, including incubating cells in: (1) molar concentrations of glycerol; (2) drugs that bind to and cause HERG channel block or their analogues (E4031, cisapride, astemizole, quinidine, fexofenadine); or (3) the sarcoplasmic/endoplasmic reticulum Ca-ATPase (SERCA) inhibitor thapsigargin.31,37–39,43,44,55 The finding that some mutant channels can be “pharmacologically rescued” to function in the cell membrane has obvious therapeutic implications.

Rescue, however, is selective for specific trafficking-defective LQT2 mutations. The T65P, N470D, G601S, R752W, F805C, V822M and R823W mutations are rescued by lowering incubation temperature, which is thought to promote native protein folding by stabilizing intermediate steps in the protein-folding pathway. The T65P, N470D, and

multiple consensus sites for N-linked glycosylation (N-X-S/T), it is the asparagine at position 598 (N598) that appears to undergo N-linked core- and complex-glycosylation.12 Several experimental approaches have shown that HERG subunit protein is first synthesized as $\approx132$-kD peptide in the ER, which rapidly undergoes N-linked core-glycosylation to increase the molecular mass to $\approx135$-kD. In the Golgi apparatus, complex glycosylation increases the molecular mass of each channel subunit to $\approx155$-kD.12,32,44,50 Several biological functions have been attributed to core- and complex glycosylation, including promoting proper protein folding, ER export, altering protein function, and regulating protein stability. Initially disruption of N-linked glycosylation was thought to prevent the efficient surface expression of HERG channels.51 More recent studies have shown that glycosylation-deficient channels are expressed in the cell surface membrane and can generate $I_{\text{HERG}}$. However, pulse-chase labeling experiments suggest that the glycosylation deficient channel protein has a shorter half-life compared with WT HERG channels.12

In a yeast 2-hybrid screen of human heart library, a Golgi-resident protein, GM130, interacted with a HERG C-terminal fragment encoding amino acids 667 to 1159.52 However, unlike the Hc/sp 70 and Hsp 90, GM130 immuno-noprecipitates with both immature core-glycosylated and mature complexly glycosylated HERG protein. Coexpressing GM130 with WT HERG channels reduced functional $I_{\text{HERG}}$ suggesting that GM130 can regulate $I_{\text{HERG}}$. Roti Roti et al2also showed that V822M and R823W, which are trafficking-defective LQT2 mutations in the nucleotide-binding domain, disrupted the interaction of GM130 with the HERG C-terminal fragment. Given this result, it is tempting to speculate that disruption of the HERG and GM130 interaction may contribute to the phenotype of some trafficking-defective LQT2 mutations.
G601S (but not R752W, F805C, or R823W) are rescued by incubating in HERG channel blocking drugs. Amino acid residues on the inner portion of the sixth transmembrane segment (S6) of HERG channel protein constitute the drug-binding domain for HERG channel-blocking drugs.56,56 Mutating the drug-binding domain minimizes drug block of HERG channels and prevents the pharmacological rescue of the LQT2 trafficking-defective mutant G601S, suggesting that the inner pore domain may underlie both pharmacological rescue as well as drug-block of \( I_{\text{HERG}} \). Although the mechanism of pharmacological rescue is incompletely understood, binding of HERG channel-blocking drugs may help to stabilize intermediate states of the mutant channel protein that promote export of the channel out of the ER. At concentrations required for pharmacological rescue most drugs block \( I_{\text{HERG}} \), thereby limiting their therapeutic value.38,43 However, fexofenadine (terfenadine carboxylate) was shown to increase current in the N470D and G601S mutations at drug concentrations lower than \( I_{\text{HERG}} \) blocking concentrations, thus uncoupling channel block from pharmacological rescue.55 Thapsigargin, an inhibitor of SERCA channels, can also rescue the trafficking-defective LQT2 mutations G601S and F805C (but not N470D). The mechanism of thapsigargin-mediated rescue is distinct from that of rescue by HERG channel blockers for 2 reasons. Thapsigargin does not block \( I_{\text{HERG}} \) at concentrations that cause pharmacological rescue, and thapsigargin rescues LQT2 mutations that HERG channel-blocking drugs do not.44 The mechanism underlying thapsigargin rescue is speculative, although it appears to involve alterations in luminal ER \([\text{Ca}^{2+}]\) that directly affect protein folding or \( \text{Ca}^{2+} \)-dependent molecular chaperones. Interestingly, thapsigargin has also been shown restore surface expression of mutant channels in other diseases that prevent ER export, such as in CFTR.58

The proposed mechanism for trafficking-defective channels is that the protein is incorrectly folded and therefore earmarked for intracellular retention by cellular quality-control mechanisms. Rescue interrupts this. This postulate is supported by the observation that G601S and R752W have increased interactions with Hsc70 and Hsp90 compared with WT channels. Rescue reduced these chaperone interactions for G601S and R752W.47 It is also important to note that different methods of rescue do not increase the surface expression of WT channels. Taken together, the data suggest that rescue by several methods increases the probability that a mutant protein will reach its native conformation. The observation that different methods of rescue do not affect similarly all LQT2 trafficking-defective channels suggests that these mutations differentially affect the protein folding and oligomerization steps.

Beyond LQT2: Other Proarrhythmic Syndromes Linked to Trafficking-Defective Channels

Trafficking-defective mutations have been described for the cardiac \( \text{Na}^{+} \) channel and the inward rectifier \( \text{K}^{+} \) channel identified from patients with inherited arrhythmia syndromes. Baroudi et al showed that the Brugada syndrome mutation R1432G and the double mutation R1232W/T1620M reduced \( I_{\text{Na}} \), and that mutant protein colocalized with the ER resident protein calnexin, suggesting ER trapping.59,60 Another trafficking-defective \( \text{Na}^{+} \) channel mutation, M1766L, was identified in an infant with ventricular tachycardia.61,62 Surface expression of M1766L increased in mammalian cells by reducing culture temperature, coexpressing the \( \text{Na}^{+} \) channel \( \beta 1 \) subunit, or incubating cells in the \( \text{Na}^{+} \) channel blocker mexiletine.61 Surprisingly, the trafficking-defective phenotype of the mutation differed depending on the clone of the cardiac \( \text{Na}^{+} \) channel (hH1, hH1a, or hH1b). The M1776L mutation prevented the ER export of channel protein in the hH1 and hH1a clones, but not the hH1b clone, which contains a common polymorphism.62 These data suggest that polymorphisms may modify mutant phenotype expression through unknown intragenic actions.

Multiple mutations in the \( \text{KCNJ2} \) gene have been described in patients with Andersen syndrome. In heterologous expression systems, these mutations cause marked reductions in \( \text{K}^{+} \) current. At least some mutations (\( \Delta S95 \) 98 and \( \Delta S314 \) 315) are reported to be trafficking-defective, with the channels failing to reach the surface membrane.63 Both of these mutations had a dominant-negative effect on surface expression of WT channel protein.

These findings with LQT2, Brugada syndrome, and Andersen syndrome mutations show that defects in protein trafficking are a relatively common consequence of gene mutations and provide one mechanism for loss of ion channel current in inherited arrhythmia syndromes. Given that loss of function occurs in many of the inherited arrhythmia syndromes (Table 2), it is reasonable to speculate that defective protein trafficking will be a common mechanism identified in many other inherited arrhythmia syndromes and disease models.

A scheme for categorizing alterations in cardiac ion channels in inherited and possibly some acquired arrhythmia diseases is shown in Table 3 and is in keeping with the nomenclature developed for other inheritable diseases (see later). Class 1 mutations include abnormalities at the level of gene transcription or in protein translation that result in no, truncated, nonsense, or altered protein levels. For example, the nucleotide deletion HERG 1261delA leads to an in-frame reading abnormality that is thought to result in a truncated, nonfunctional HERG protein.64 Similarly, mutation/polymorphism in an \( \text{hNaV} 1.5 \) promoter results in altered expression of \( \text{Na}^{+} \) channels by altering transcriptional activity to produce loss of functional current.33 Class 2 mutations produce abnormal posttranslational protein processing (protein folding, oligomerization, etc) and lead to defective protein trafficking. As discussed, these mutations appear to be common, have been identified in \( \text{KCNH2}, \text{KCNJ2}, \) and \( \text{hNaV}1.5 \) genes, and span the length and organization of the channel proteins (for HERG, see Figure 2). Multiple intracellular mechanisms

<table>
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<th>TABLE 3. Classification Scheme for Cardiac Ion Channel Mutations</th>
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appear to contribute to protein trafficking defects; thus, ultimately, subdivisions of class 2 mutation effects may emerge. Class 3 mutations result in ion channels that have altered biophysical properties because of changes in channel gating and/or kinetics. Mutations in this class also appear to be common in LQTS and other arrhythmia syndromes and are found in different regions and domains of channel proteins. An example is the T474I mutation in HERG channels, where voltage-dependent gating is markedly shifted resulting in I_{HERG} activation at more negative voltages, but with decreased I_{HERG} at more positive voltages. A recently described HERG mutation, N588K, which results in failure of the channel to inactivate normally to cause an increase in I_{HERG} during the action potential plateau, and was identified in 2 families with short QT syndrome. Most LQT3 mutations result in altered gating of the cardiac Na^+ channel with marked slowing of inactivation to increase late I_{Na}. Class 4 mutations result in altered or absent channel selectivity or permeability. These gene mutations are located near the ion channel pore region and have been associated with severe LQTS disease phenotypes. For example, the G628S mutation in HERG channels results in a normally trafficking protein that inserts correctly into the cell surface membrane, yet the mutation causes loss of channel function. One amino acid downstream is the N629D mutation, which causes altered channel inactivation and a reduction in selectivity for K^+ over Na^+. The classification scheme shown in Table 3 provides insight into understanding the impact of ion channel gene mutations on the control of macroscopic current (I); class 1 and 2 mutations alter I_{Na}, class 3 mutations alter I_{K}, and class 4 mutations alter I. Clearly, some mutations result in changes in channel properties that can be attributed to multiple classes.

**Defective Protein Trafficking in Other Inheritable Diseases**

Defective protein trafficking has emerged as an important mechanism in many diseases.

**Familial Hypercholesterolemia and the Low-Density Lipoprotein Receptor**

Cholesterol, although a small molecule, plays a vital role in biology. Familial hypercholesterolemia is one of several genetic causes of an elevated low-density lipoprotein (LDL) concentration in plasma. In pioneering work, Brown and Goldstein identified mutations in the gene encoding the LDL receptor that caused defective protein trafficking, as well as affecting LDL receptor internalization, ligand-binding, and appropriate targeting of the LDL receptor in hepatocytes. More than 700 mutations have been identified in the gene encoding the LDL receptor protein (http://www.ucl.ac.uk/fh/). These mutations usually are grouped functionally according to the pattern of disruption of the structure and function of LDL receptor. Class I mutations have no receptors synthesized. Class II mutations have LDL receptor protein synthesized, but it is trafficking-defective and is retained in the ER. Class III mutations result in receptor protein that traffics to reach the cell surface but fails to bind LDL normally. Class IV mutations result in receptors that reach the cell surface and bind LDL, but fail to cluster in coated pits for subsequent internalization. It is interesting that ~50% of LDL receptor mutations act to disrupt receptor protein trafficking from the ER to the Golgi complex.

**Cystic Fibrosis and CFTR**

Cystic fibrosis is a monogenic recessive disease caused by mutations in the gene encoding for the CFTR protein. It is an important disease in protein trafficking. CFTR is a cAMP-dependent Cl^- channel that regulates fluid composition across the epithelial cell membranes. More than 1000 mutations in the gene have been described (http://www.genet.sickkids.on.ca/cgi-bin/WebObjects/MUTATION). Similar to the LDL receptor mutations, CFTR mutations are usually grouped according to the pattern of disruption of the CFTR protein. Class I mutations result in failed synthesis of CFTR protein. Class II mutations cause defective protein trafficking with the CFTR protein retained in the ER. Class III mutations cause defective regulation of normally trafficking CFTR protein. Class IV mutations cause an abnormal channel conductance. The most common mutation in cystic fibrosis is the deletion of the codon for a phenylalanine at position 508 (ΔF508) of CFTR protein and is found in 70% of affected patients. This mutation is thought to cause the CFTR protein to fold incorrectly and be retained in the ER, thus failing to traffic to the plasma membrane. It is then retrotranslocated from the ER to undergo ubiquitination and proteosomal degragation. The ΔF508 CFTR protein is particularly interesting because it is capable of forming functional channels under experimental conditions. In both heterologous expression and epithelial tissue systems, the protein trafficking defect can be “corrected” by reducing incubation temperature (<30°C) and by incubation with a variety of chemical agents such as glycerol, trimethylamine N-oxide, sodium 4-phenylbutyrate, and deuterated water, which are proposed to act as pharmacological chaperones to cause misfolded CFTR protein to assume a configuration that permits trafficking to the surface membrane. These findings have driven the search for new pharmacological strategies for the treatment of patients with cystic fibrosis.

**Charcot-Marie-Tooth Disease and Connexins**

Charcot-Marie-Tooth disease (CMTX) is a complex polygenic neuropathic disorder that constitutes the most common form of inheritable disease in the peripheral nervous system. Connexins are a family of homologous integral gap junction membrane proteins that form channels for the transmission of electrical signals and diffusion of small ions and molecules between coupled cells. One form of CMTX results from mutations in the gap junction protein β1 gene (GJB1), which encodes connexin 32 (Cx32). More than 200 mutations throughout GJB1 have been identified. Several molecular mechanisms have been proposed for Cx32 mutations to cause CMTX, including: (1) mRNA for Cx32 is not transcribed; (2) mRNA is normally transcribed but little protein is produced; (3) Cx32 protein is synthesized but not properly transported to the plasma membrane; (4) the Cx32 protein is transported normally to the plasma membrane but does not form functional channels; and (5) the Cx32 protein inserts in the surface membrane and forms gap junction channels that function abnormally.
Nephrogenic Diabetes Insipidus and Aquaporin Channels

Nephrogenic diabetes insipidus (NDI) is characterized by the inability to concentrate the urine resulting in excessive urine production and thirst. The genetic basis of NDI is multifactorial and includes mutations in the aquaporin-2 channel (AQP2) gene. This channel is vasopressin-regulated and is found in the kidney at distinct sites along nephrons and collecting ducts. More than 20 mutations in AQP2 have been identified in patients with autosomal-recessive NDI. Functional expressions of several AQP2 mutations have shown that some result in nonfunctional channel proteins, whereas others produce a small increase in osmotic water permeability. Several AQP2 mutant proteins show impaired transport from the ER. Interestingly, Tamarappoo and Verkman found that the trafficking defect of several AQP2 mutations was corrected by incubation in glycerol to rescue functional channels, and immunostaining confirmed their redistribution from ER to the cell surface.

The diseases described are but a few examples of pathological conditions caused by gene mutations that may result in failure of proteins to traffic normally through the ER to reach their intended destinations. Presently, nearly 100 diseases have been reported that involve abnormal protein trafficking or “traffic jams” within cells. Furthermore, it is clear that while some mutations interfere with the ability of a protein to undergo normal processing and intracellular trafficking, the same mutation may have minimal or no effect on the protein’s function provided it reaches its intended target. Thus, therapeutic strategies to correct or restore protein trafficking, even partially, may have important clinical benefits.

Summary: Genomics and Proteomics

The study of inherited arrhythmia syndromes is one area in which human genomics has led to new information and insight into the polygenic nature of some cardiac arrhythmias. Undoubtedly, more genes and mutations for cardiac arrhythmia diseases await discovery. Concomitantly, proteomics is resulting in the identification of multiple molecular and cellular mechanisms that underlie the phenotypic expression of gene mutations. These discoveries potentially may lead to new proteomic-based, as well as gene-based, therapies in many diseases. Presently, molecular models do not exist that accurately predict the functional consequences of most ion channel gene mutations on biogenic or biophysical function. Whereas the identification of new genes and gene mutations remains an essential component for progress in understanding disease mechanisms, this must be accompanied by functional expression studies to identify the mechanisms for molecular and cellular dysfunction of the encoded proteins.

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