Channel Triage

Emerging Insights into the processing and quality control of hERG potassium channels by DnaJA proteins 1, 2 and 4

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Molecular chaperones have long been known to be critical for protein homeostasis as well as oligomeric complex assembly. The ubiquitous Hsp70 family (heat shock protein of 70 kDa) of chaperones is a key player in this arena and interacts with a variety of client proteins. A comprehensive mechanistic understanding of Hsp70 action remains elusive, however the common mechanism underlying the diverse functions of Hsp70s is the apparent ability of these chaperones to bind and sequester unfolded regions of a wide array of substrate proteins, thereby maintaining these clients in functionally competent states. The client binding and release kinetics of Hsp70s are governed by an intrinsic ATPase activity. Not surprisingly, the chaperone activity of Hsp70s is shaped by a large number of associated co-chaperones, one group of which is known as the DnaJ/Hsp40 co-chaperone family. Shrier, Young and colleagues have now added to our understanding of how a subset of DnaJ co-chaperones (i.e. DnaJA1, DnaJA2 and DnaJA4) act in concert with Hsc70, (the constitutive heat shock cognate protein of 70 kDa), to regulate the maturation and trafficking of hERG (human ether-a-go-go-related gene or KCNH2) potassium channels.

In the heart, the rapidly activating delayed rectifier K+ current (I_{Kr}) that is encoded by hERG is critical for ventricular repolarization. Loss of hERG channel function causes type 2 long QT syndrome (LQT2) that is clinically characterized by an increased risk of ventricular arrhythmia and sudden death. Given hERG’s involvement in LQT2, cellular mechanisms that regulate hERG expression are the subject of considerable interest as potential avenues for therapeutic intervention. In this study, the authors reasoned that chaperone-mediated processes would be responsible for helping newly synthesized hERG channels adopt their biologically active conformation(s) at the cell surface as well as for targeting non-viable channels for degradation. More than 200 naturally occurring, loss-of-function mutations have been identified in hERG. Many mutant channels never reach the plasma membrane, but rather are retained in the endoplasmic reticulum and destroyed by proteasomal ER-associated degradation (ERAD). From an electrical perspective, a breakdown in the coordinated folding, trafficking, assembly and degradation of hERG would reduce the number of functional hERG channels and lead to inadequate action potential repolarization. This realization fueled the search for the cellular chaperone machinery responsible for the delivery and maintenance of functional hERG channels at the plasma membrane. Now, in their latest chapter to this story, the authors have used a proteomic screen of immunoprecipitated HA-tagged hERG channels transfected into cell lines to identify hERG-associated folding machinery. Their data reveal that the closely related DnaJA1, DnaJA2 and DnaJA4 co-chaperones act as key modulators of the degradation pathway for wild-type and mutant forms of hERG.

Experimentally, the authors utilized cDNA constructs encoding the full-length HA-tagged hERG channel to transiently express channels in HEK 293 cells and stably express tagged channels in HeLa...
Since DnaJ’s appear to guide nascent hERG channels toward endoplasmic reticulum-mediated degradation rather than export, the authors reasoned that binding of DnaJ to hERG folding intermediates would recruit Hsc70, which would subsequently recruit the E3 ubiquitin ligase CHIP (C-terminus of Hsc70-interacting protein). This latter enzyme would ubiquitylate the chaperone-bound hERG channel, thereby targeting it for proteosomal degradation. These predictions were tested in pulse-chase experiments, which showed that DnaJAs and CHIP reduce both the immature and mature forms of hERG in a time dependent manner. Furthermore, the authors’ evaluation of the established hERG mutant G601S, which displays temperature sensitive trafficking, demonstrated that DnaJA1 and DnaJA2 reduced the trafficking efficiency of this mutant hERG channel, even at the permissive temperature (26 °C). Shrier and colleagues then tested the channel specificity of DnaJA1, DnaJA2, and DnaJA4 by examining the maturation of the related HCN2 potassium channel and the unrelated CFTR chloride channel. While all three DnaJAs promoted degradation of HCN2, only DnaJA2 over expression reduced CFTR maturation. Collectively these results provide compelling evidence that DnaJA activation of Hsc70 is important for maintaining normal levels of hERG channels and may have broader implications for the dynamic control of various types of channels.

In summary, the cell biological characterization presented in this study provides us with a more accurate physical picture of the protein machinery responsible for early ‘triage’ decisions regarding the folding, maturation and/or degradation of hERG channels. The conceptual picture emerging from this and related studies is that identity of chaperones responsible for monitoring the “quality control” of hERG folding intermediates is complex, and that the DnaJ’s play a decisive role in recruiting chaperone machinery that either facilitates endoplasmic reticulum degradation or export.