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N- and *O*-linked glycosylation coordinate cell-surface localization of a cardiac potassium channel

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The biogenesis and processing of membrane and secretory proteins takes place at the endoplasmic reticulum (ER) where they are integrated into or translocated across the ER membrane. A remarkable element implicated in biogenesis of this class of protein is that they are almost always processed by various cotranslational and posttranslational modifications which ultimately determine protein stability, localization and function. Defaults in biogenesis, processing or trafficking of membrane proteins are often the direct cause of various human diseases. In particular, some forms of cardiac arrhythmias, such as the long QT syndrome (LQTS), are caused by aberrant processing and defective trafficking of ion channels to the plasma membrane (Anderson *et al.* 2006).

The LQTS is a congenital heart disease characterized by an abnormally long QT interval measured with a body surface electrocardiogram and increase risk of torsade de pointes, a fatal cardiac arrhythmia. Several forms of LQTS arise from mutations in genes encoding main or auxiliary subunits of potassium channels implicated in the repolarization phase of cardiac action potential. Some of these mutations generate dysfunctional ion channels, thus delaying the repolarization phase of the cardiac action potential and prolonging the QT interval. In contrast, other mutations have no or little effect on the function of potassium channels but instead result in misprocessing and defective trafficking of the channel protein to the plasma membrane where they normally function (Anderson *et al.* 2006).

In human heart, KCNE1 and KCNQ1 co-assemble to form the slow outward current (I_{Ks}) of the repolarization phase of the action potential. Homozygous mutations in *KCNE1* or *KCNQ1* genes gives rise to the Jervell and Lange–Nielsen syndrome (JLNS), a form of LQTS associated with congenital deafness (Splawski *et al.* 1997). In a recent issue of *The Journal of Physiology*, Chandrasekhar *et al.* (2011) investigated the molecular mechanisms by which one mutation in *KCNE1* diminish the contribution of I_{Ks} to the repolarization phase of the cardiac action potential, giving rise to JLNS. They found a novel posttranslational modification in the KCNE1 subunit and they suggest that abolishing this modification by a natural mutation is associated with defective trafficking of the channel protein to the plasma membrane.

One common type of modification of ion channels involves addition of oligosaccharide chains to asparagine (N) residues, a process referred to as N-linked glycosylation. Several cardiac ion channels undergo N-linked glycosylation and mutations in consensus N-linked glycosylation sites have been identified in potassium channels implicated in LQTS (Anderson *et al.* 2006). In contrast, much less if known about processing of cardiac ion channels by *O*-linked glycosylation. In eukaryotic cells, several types of *O*-linked glycosylation exist but they all involve addition of carbohydrate moieties to serine (S) or threonine (T) amino acids. In their study, Chandrasekhar *et al.* (2011) pointed to multiple consensus sites for *O*-linked glycosylation in KCNE1 and experimentally identified the actual T residue that undergoes glycosylation. They showed KCNE1 undergoes both *N*- and *O*-linked glycosylation when heterologously expressed with KCNQ1 in cells. By exploring a transgenic mouse model stably expressing concatenated KCNE1–KCNQ1 fusion protein, they found that the cardiac I_{Ks} complex acquires *O*-linked glycosylation *in vivo*. Mutational analysis identified a T at position 7 (T7) as the sole site for *O*-linked glycosylation. Interestingly, this same site is critical for *N*-linked glycosylation at N5, allowing abrogation of both modifications with a single mutation. Indeed, one such mutation exists in patients with LQTS and it severely compromises the glycosylation and trafficking of KCNE1–KCNQ1 channel complexes (Bas *et al.* 2001). In contrast, mutations that eliminate either *N*- or *O*-linked glycans separately have minimal effect on the functional activity, biogenesis and trafficking of the channel proteins. Notably, the latter conclusions were based on investigations of glycosylation sites that were created by mutagenesis. The authors argue that the consensus *N*- and *O*-linked glycosylation sites overlap in native KCNE1 sequence, thus making it difficult to investigate these glycosylation types separately by mutational analysis. However, in the absence of additional information, it is unclear as to why it is not possible to create mutants that would allow separate analysis of native *N*- and *O*-linked glycosylation sites. Theoretically, mutation of N5 should abrogate *N*-linked glycosylation without affecting *O*-linked glycosylation, thereby enabling investigations aimed at identifying the consequence of *N*-linked glycosylation on biogenesis and function of the KCNE1–KCNQ1 complex. Another alternative to separately investigate the consequence of *N*- and *O*-linked glycosylation in biogenesis and trafficking of KCNE1 involves pharmacological treatments which inhibit distinct glycosylation types. For example, treatment of live cells with tunicamycine specifically abolishes *N*-linked glycosylation, thereby allowing investigation of this defect without interference from defects associated with *O*-linked glycans.

One important novelty of the studies reported by Chandrasekhar *et al.* (2011) is the discovery that the cardiac I_{Ks} complex is modified by *O*-linked glycans. However, the precise consequence of this modification for the function and biogenesis of the ion channel remains unknown. The authors suggest that this modification is potentially important for subcellular localization of the I_{Ks} complex in cardiac cells. This hypothesis can be tested by investigating the localization of mutant channels in cardiac cell lines (such as the HL-1 cell line) or in a transgenic mouse model similar to the one described by Chandrasekhar *et al.* (2011). An alternative function for this modification is to allow regulation of I_{Ks} based on physiological demand. It is well known that the I_{Ks} channel is modulated by phosphorylation as a result of adrenergic stimuli. Thus, specific protein

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modifications can allow interaction with molecular machinery involved in regulation of I_{Ks} or the specific targeting of the ion channel to the proximity of adrenergic receptors (Marx *et al.* 2002). Undoubtedly, a better understanding of the implication of *O*-linked glycosylation for I_{Ks} function requires investigations in native cardiac cells and under physiological conditions.

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