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Subcellular targeting of phosphatases: a novel function of ankyrins

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IN THE PAST DECADE, mutations in over a dozen genes have been linked to a predisposition for cardiac arrhythmias and sudden death (15). Most of these genetic alterations involve genes coding for cardiac ion channels, transporters, and exchangers. In 2003, Mohler et al. (14) demonstrated that mutations in ankyrin-B, a protein that anchors ion-conducting proteins, could also cause a congenital arrhythmia syndrome. Single nucleotide changes in the gene encoding ankyrin-B (ANK2) cause congenital long QT syndrome type 4 (LQT-4), a condition characterized by prolonged ventricular repolarization, ventricular tachyarrhythmias, syncope, and sudden death (20). To date, approximately nine loss-of-function mutations in ankyrin-B have been linked to a wide spectrum of electrophysiological phenotypes that not only include features reminiscent of the long QT syndrome but also bradycardia, sinus arrhythmia, junctional escape rhythm, conduction block, and catecholamine-induced ventricular tachycardia (5, 11, 13).

The cellular manifestations of ankyrin-B mutations attest to the role of ankyrins as targeting proteins that ensure proper insertion of ion channels and transporters into the appropriate cellular membranes and microdomains (13). The ankyrin family consists of three genes (ANK1, ANK2, and ANK3) that produce ankyrin-R ("restricted" expression), ankyrin-B ("broad" expression), and ankyrin-G ("giant" size, or "general" expression), respectively. Several alternatively spliced variants have been identified for each ankyrin gene, which contributes to individual differences with regard to tissue specificity and subcellular localization (5). The primary protein structure of each ankyrin isoform, however, consists of three functional domains: an amino-terminal membrane-binding domain, a spectrin-binding domain, and a carboxyl-terminal death domain. The membrane-binding domain contains 24 ANK repeats, which form four independently folded subdomains of six repeats each (2, 5). This membrane-binding domain does not, as suggested by its name, bind to cell membranes but rather mediates recognition of and binding to subsets of membrane-associated proteins, including ion channels, transporters, and cell adhesion molecules (12). The multivalent character of the membrane-binding domain of ankyrin allows a simultaneous binding to multiple membrane proteins and thereby the formation of macromolecular complexes. The COOH-terminal domain, which displays great divergence between the different ankyrin isoforms, is believed to contribute to intermolecular interactions in conjunction with the membrane-binding domain (1). Finally, the spectrin-binding domain, which interacts with β-spectrin, is thought to mediate a linkage of membrane proteins to the cytoskeleton.

Previous studies have provided evidence for an important physiological function of the spectrin-binding domain of ankyrin-B (14). The LQT4-associated ankyrin-B mutation E1425G results in aberrant subcellular localization of the Na^+ pump (Na^+/K^+-ATPase), the Na^+/Ca^{2+} exchanger (NCX), and the inositol-1,4,5-trisphosphate receptor (14). The elegant study by Bhasin et al. (3) in this issue of *American Journal of Physiology-Heart and Circulatory Physiology* demonstrates a novel role of ankyrin-B, and the spectrin-binding domain in particular, in targeting protein phosphatases to cellular microdomains in cardiac myocytes. This work suggests that ankyrins play a previously unrecognized role in the phosphorylation-dependent regulation of ion-conducting proteins in the heart. Moreover, these findings lead to new questions: whether other enzymes are associated with ankyrins, whether there are differences in phosphatase binding to ankyrin in heart disease, and whether there are naturally occurring mutations in ankyrins that disrupt the phosphatase-binding site.

Defects in the phosphorylation-dependent regulation of ion channel macromolecular complexes have been previously linked to cardiac arrhythmias and heart failure (21). It has become apparent that the proper subcellular organization of kinases and protein phosphatases with anchoring proteins, or by direct binding to ion channels and transporters, is critically important for cardiac homeostasis. For example, the slowly inactivating K^+ channel, which is strongly regulated by β-adrenergic stimulation (4), forms a macromolecular complex consisting of α (KCNQ1) and β (KCNE1) channel subunits, as well as the A kinase-anchoring protein called yotiao. The anchoring protein yotiao targets key enzymes involved in phosphorylation (PKA) and dephosphorylation [protein phosphatase 1 (PP1)], which ensures high efficiency and specificity of channel modulation. The long QT syndrome-associated mutation G589D in KCNQ1 disrupts yotiao binding to the K^+ channel and results in defective phosphorylation-dependent regulation of the inactivating K^+ channel complex (9). Another example is the cardiac ryanodine receptor (RyR) channel complex, which consists of four pore-forming RyR subunits, each associated with multiple regulatory subunits (e.g., calmodulin and FKBP12.6), kinases (protein kinase A and calmodulin-dependent kinase II), and PP1 and PP2A (21). It has been shown that PP2A is targeted to the RyR channel complex via anchoring protein PR130 (10). Decreased binding of PP2A to RyR has been demonstrated in failing human hearts, suggesting that abnormal targeting of protein phosphatases may contribute to enhanced RyR phosphorylation and RyR-mediated Ca^{2+} release in heart failure (17).

A clear future direction of the study by Bhasin and colleagues (3) is to determine whether PP2A plays a role in the phosphorylation-dependent regulation of ankyrin-B activity and the ability of ankyrin to target protein complexes to the proper subcellular locations. In addition, it would be interesting to assess whether ankyrin serves solely as a scaffolding protein to recruit PP2A to targets or whether ankyrin actively participates in the phosphorylation/dephosphorylation-dependent allosteric regulation of target proteins. Recent studies have indeed shown that ankyrin-binding proteins Na^+/K^+-ATPase, Na^+/Ca^{2+} exchanger, and inositol-1,4,5-trisphosphate receptor

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are actively regulated by PP2A (6, 16, 18), although the role of ankyrin in these signaling events remains to be studied. Since arrhythmias in patients with mutations in ankyrin-B are frequently evoked by stress or exercise (13), it is tantalizing to speculate that some mutations might disrupt PP2A-mediated dephosphorylation, which could lead to increased phosphorylation of ankyrin-bound ion-conducting proteins. Recent studies have revealed that abnormal phosphorylation of ion channel subunits may indeed contribute to lethal arrhythmias in patients with congenital long QT syndrome (9), atrial fibrillation (7, 19), and heart failure (8).

During the past few years, it has been clearly demonstrated that ankyrin-B has an important role in cardiac myocytes by enabling intermolecular interactions and targeting key regulatory proteins to subcellular domains. Since the roles of ankyrins for cellular physiology continue to be elucidated, ankyrin-B may emerge as a promising new target for the treatment of cardiac arrhythmias.

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