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A-Kinase Anchoring Protein 9 and I_{Ks} Channel Regulation

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Abstract

A-kinase anchoring proteins (AKAPs) create compartmentalized environment inside the cell to bring various signaling molecules to their targets. In the heart, a slowly activating potassium channel (I_{Ks}) important for cardiac repolarization is tightly regulated by the sympathetic nervous system in an AKAP-dependent manner. I_{Ks} channel forms a macromolecular complex with AKAP9 and other enzymes, such as PKA, phosphatase, adenylyl cyclase and phosphodiesterase, all of which are responsible to control the phosphorylation state of the channel. Such a complex thus ensures the I_{Ks} channel to be regulated properly to maintain the normal cardiac rhythm. Disruptions of various elements of the complex have been found to cause severe pathological consequences, including the Long QT Syndrome.

A-kinase anchoring proteins (AKAPs) are scaffolding proteins that provide spatiotemporal control of enzymatic activities by serving as the structural and functional links between the associating enzyme molecules and their end targets, such as ion channels[1]. In the heart, where the excitation and contraction of cardiac muscles need to be tightly regulated to provide adequate and continuous blood supply to body organs, a growing list of AKAPs that includes mAKAP, AKAP15/18, Gravin and AKAP9 *etc*, has been shown to play critical roles in cardiac physiology and pathology[2-4]. In this review, we focus on AKAP9, also known as Yotiao, which was initially found to bind NMDA receptor subunit NR1[5, 6] and belongs to a multispliced AKAP family that includes the longer transcript AKAP350/AKAP450/CG-NAP [7-9] localized in the golgi and centrosomal regions. We will discuss the role of AKAP9 in the regulation of a slowly activating cardiac potassium channel (I_{Ks}) as well as its relevance to cardiac arrhythmic disorders, such as the Long QT Syndromes (LQTs).

I. Role of AKAP9 in I_{Ks} channel regulation and LQTs

The I_{Ks} channels are comprised of the pore-forming α- subunit KCNQ1 that conducts ionic current, as well as the auxiliary β-subunit KCNE1 that renders the channel its characteristic biophysical properties, including the slow kinetics [10, 11]. The voltage-gated I_{Ks} channels open in response to depolarization to repolarize the cardiac cells. Mutations in both KCNQ1 and KCNE1 disrupt channel function, prolong action potential duration (APD) and cause LQT1 [12] and 5[13], respectively. I_{Ks} channels are regulated by the sympathetic nervous system (SNS) via the β-adrenergic receptor/Gs/cAMP/PKA pathway. Walsh and Kass first demonstrated in the Guinea pig ventricular cells that cAMP analogues caused a large increase in the I_{Ks} amplitude as well as a slowing in the current decay during deactivation [14]. Later, Kurokawa et al reconstituted such effects in a recombinant expression system by coexpressing AKAP9 (Yotiao) and demonstrated that AKAP9 forms a

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macromolecular complex with KCNQ1, Type II regulatory subunit of PKA (RII), and protein phosphatase 1 (PP1) and brings PKA to the vicinity of the channel to phosphorylate a serine residue (S27) on KCNQ1 amino terminus[15]. AKAP9 binds KCNQ1 carboxy terminus via a leucine zipper (LZ) motif. A previously known LQT mutation (G589D) near the LZ motif disrupted the interaction between Yotiao and KCNQ1 as well as the functional regulation of I_{Ks} by PKA phosphorylation [15]. These results suggest for the first time that (1) AKAP9 plays an indispensable role in I_{Ks} channel regulation by SNS; (2) Dysfunctional SNS regulation of I_{Ks} channel may lead to pathological consequences such as LQT.

The role of AKAP9 in I_{Ks} channel regulation and LQTs was further demonstrated in a subsequent paper [16]. AKAP9 was shown to possess two binding sites for KCNQ1, one located on its N-terminus (residues 29-46), the other on its C-terminus (residues 1574-1643). By performing sequence analysis targeted at these two regions in a large LQT cohort with negative genotype, Chen et al identified a putative AKAP9 mutation (S1570L) in a LQT patient and her family. The mutation is close to the C-terminal binding site on AKAP9 and was shown to reduce KCNQ1 binding and phosphorylation. More importantly it diminished cAMP-induced enhancement of I_{Ks} channel activities and was predicted by computer simulation to prolong APD, especially when cells were stimulated by isoproterenol. These results provided direct evidence that a defective AKAP9 may cause LQT.

II. Molecular components of the AKAP9/I_{Ks} complex

AKAP9 provides a structural entity to integrate various signaling molecules and the targeted I_{Ks} channel. Two pairs of enzymes have been shown to associate with AKAP9. The first pair involves PKA and PP1, which phosphorylates or dephosphorylates the channel, respectively. The second pair involves adenylyl cyclase (AC) and phosphodiesterase (PDE), which increases or decreases cAMP inside the cell, respectively, to control PKA activities. Together the two pairs of opposing enzymes act to balance and fine-tune the phosphorlation state of I_{Ks} channel (Figure 1).

1. PKA. PKA holoenzyme consists of two regulatory subunits and two catalytic subunits. The regulatory subunits associate with their catalytic counterparts under low cAMP level. Upon receptor stimulation, cAMP concentration rises. The catalytic subunits are released from the regulatory subunits. One of the essential functions of AKAPs is to harbor the RII subunits. All AKAPs have a signature RII binding motif, which features an amphipathic helix of 14-18 residues that are able to dock to RII with high affinity[17]. Early works identified a region on AKAP9 (residues 1440-1457: LEEEVAKVIVSMSIAFAQ) as the primary binding site for PKA RII [6, 18]. Recently crystal structures of AKAP binding motif bound to RII have been solved. The structures showed that the RII binding motif of AKAP binds to a hydrophobic interface formed by the two N-termini (D/D domain) of the RII dimer[19, 20]. It remains to be seen whether the RII binding site on AKAP9 for RII follows the same pattern. The details in the crystal structures will allow us to design peptides to disrupt interaction between AKAP and RII, thus providing valuable tools to study AKAP function[21, 22]. The primary function of the AKAP9-bound PKA is to phosphorylate the S27 residue on KCNQ1. But it is also noteworthy that AKAP9 itself is a PKA substrate and that phosphorylation of AKAP9 participates in the process of I_{Ks} channel regulation [23, 24]. Whether AKAP9 is self-phosphorylated by the associating PKA is an interesting possibility that remains to be tested.
2. PP1. PP1 is a serine/threonine phosphatase that dephosphorylates its substrate. Scaffolding proteins such as AKAPs provide the non-specific PP1 a structural platform to act on a specific group of substrates [25-27]. The role of PP1 in the I_{Ks}

regulation is evidenced not only by its detection in the I_{Ks}/AKAP9 macromolecular complex, but also by the functional experimental data. Addition of okadaic acid, a PP1 inhibitor, enhanced the effect of cAMP-dependent I_{Ks} regulation [15]. Previous works have shown that PP1 bound to residues 1171-1229 of AKAP9. A hallmark PP1 binding motif (KVxF) is present in AKAP9, but was found to be not essential for PP1 interaction [6]. Lack of finer mapping for PP1 binding site hinders further studies of the physiological role of PP1 in I_{Ks} regulation.

3. AC. Membrane bound ACs are activated by Gs-coupled receptors, including the β-adrenergic receptors, and are responsible for cAMP synthesis, a prelude to PKA activation. Recent studies showed that ACs associated with various AKAPs, including AKAP79/150, mAKAP and AKAP9 [28-31]. AKAP9 associates with AC1, 2, 3, and 9, but not 4, 5, and 6. Residues 808-957 of AKAP9 bind directly to AC2 N-terminus. Interestingly, expression of AKAP9 inhibited the activity of AC2 and 3, but not AC1 or 9 [32]. Disrupting the interaction between AKAP9 and AC enhanced AC activities. However, it is unclear which AC subtype is present in the AKAP9/I_{Ks} complex and what role it may play in the regulation of I_{Ks} activity.

4. PDE. PDEs hydrolize cyclic nucleotides in the cells. Some isoforms of PDE specifically degrade cAMP, a key second messenger, thus providing negative controls over the downstream effectors, including PKA [33]. More importantly, PDEs create localized cAMP gradients/compartments to fine-tune various cellular functions [34, 35]. PDEs have been shown to associate with AKAPs. For example, PDE4D3 was shown to interact with mAKAP [36] and AKAP450 [37]. Terrnoire et al demonstrated that PDE4D3, but not PDE4D5, is a member of the I_{Ks}/AKAP9 complex and interacts with AKAP9 [38]. Addition of PDE4D3 to the expression system diminished the cAMP-induced I_{Ks} channel regulation. These results point to an interesting possibility: if PDE4D3 activity were to be down-regulated in disease, or if a patient were to carry a mutation that renders I_{Ks} channel unable to bind PDE4D3, these would create a situation where I_{Ks} channel gains in function. Gain in function of I_{Ks} channel is known to associate with atrial fibrillation[39]. This intriguing hypothesis remains to be tested.

In summary, the AKAP9/I_{Ks} macromolecular complex integrates various elements in the Gs/cAMP/PKA pathway. AKAP9 is pivotal in creating a compartmentalized environment that ensures I_{Ks} channels to function properly. Many questions remain to be answered. For example, it is unclear how AKAP9 coordinates various signaling molecules, which have seemingly opposing functions, to act in concert to control the phosphorylation state of I_{Ks} channels. It is of great interest to test or to visualize the changes in the local cAMP gradient as a result of the altered enzyme activities. Also intriguing is the question that how phosphorylation of a single serine residue (S27 in KCNQ1) may cause such a significant change in channel biophysical properties. With newly developed experimental techniques and tools, these questions will be addressed to provide insights into the molecular mechanism of how I_{Ks} channels are regulated by the SNS.

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Abbreviations footnote

AKAP	A kinase-anchoring protein
PKA	protein kinase A
AC	adenylyl cyclase
PP1	protein phosphatase 1
PDE	phosphodiesterase
I_{Ks}	the slow outward potassium current
SNS	sympathetic nervous system
LZ	leucine zipper

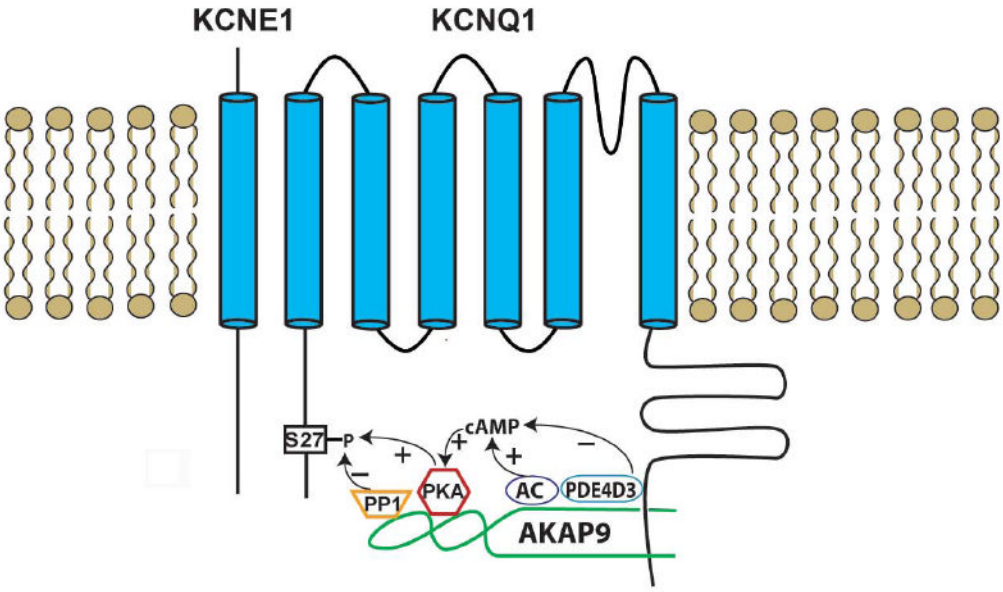

Figure 1.

A schematic diagram of the I_{Ks} /AKAP9 (Yotiao) macromolecular complex. I_{Ks} channels are comprised of α-subunits (KCNQ1) and β-subunits (KCNE1). S27 is a PKA phosphorylation site on KCNQ1 N-terminus. AKAP9 interacts with KCNQ1 C-terminus and harbors two pairs of enzymes that control the phosphorylation state of the channel. PKA phosphorylates I_{Ks} channel, while PP1 dephosphorylate it. AC and PDE control cAMP level, which in turn activates PKA.