The Na-K-ATPase, or sodium pump, is the molecular machine for ATP-dependent and coupled transport of Na⁺ and K⁺ across the plasma membrane. The Na⁺ gradient produced by the Na-K-ATPase is the energy source for cellular uptake of many nutrients. It is also the major force for maintaining the balance of electrolytes and fluids at the whole body level (28). A functional Na-K-ATPase consists of two noncovalently linked α and β subunits (40, 75). An additional γ subunit also exists in some tissues as a regulator of Na-K-ATPase (76). Studies over the past decade have made several new advances in understanding the physiological functions of the Na-K-ATPase. These include 1) identification of the molecular mechanism by which the Na-K-ATPase transduces ouabain binding to the activation of protein kinase cascades and the generation of second messengers such as inositol 1,4,5-trisphosphate (IP₃) and calcium transients; 2) the demonstration of endogenous Na-K-ATPase ligands and their role in the development of hypertension and cardiac remodeling; 3) the involvement of Na-K-ATPase in control of cancer cell growth. These findings have been detailed in some recent reviews (5, 8, 68, 85). A noticeable fact in these studies is that involvement of protein-protein interactions rather than the pumping activity alone contributes to the regulatory effects of the Na-K-ATPase on cellular functions. It is known that the plasma membrane is compartmentalized into structurally and functionally different microdomains. These microdomains may sequester specific proteins and lipids, while excluding others, to form a dynamic center for regulating cellular processes such as signal transduction, vesicular transport, and cargo delivery (50, 63, 71, 73). Although special lipid composition and protein markers have been used to classify different microdomains such as lipid rafts and caveolae (65), the formation of microdomains may be more functionally oriented. To this end, it is important to note that the calcium-signaling microdomains have been the focus of investigation for many laboratories for decades. Interestingly, recent studies have identified several important protein interactions of the Na-K-ATPase and revealed that these protein interactions play a pivotal role in the formation of functionally distinct calcium signaling microdomains, which will be the focus of this mini-review.

Interaction Between the Na-K-ATPase and the Na/Ca Exchanger

Calcium is a highly versatile intracellular signal and is responsible for regulating numerous cellular processes such as contraction, secretion, fertilization, proliferation, apoptosis. Consistently, cells have developed a series of mechanisms that exert an exquisite control of calcium signaling. This is achieved, at least partially, by the formation of different calcium-signaling microdomains. For example, in cardiac muscle, T-tubular and sarcoplasmic reticulum (SR) membranes can form “diad junctions” that allow calcium to flow through the plasma membrane calcium channel, to bind and open the ryanodine receptor, and to trigger the subsequent larger calcium release from the SR and muscle contraction (27, 65). In neuronal cells, a high concentration of calcium was visualized in a size of ~0.15 μm² area at the presynaptic active zone (60). The sites in the cytoplasm for these compartmentalized high calcium concentration profiles are defined as “calcium concentration microdomains” (47). Biochemical studies reveal that the plasma membrane calcium channels are targeted to presynaptic sites by specific protein-protein interactions that involve both the intracellular and extracellular channel domains (12, 74). Disruption of the interaction between the synaptic protein interaction sites in the calcium channels with SNARE proteins reduces effectiveness of calcium release, providing evidence for the importance in
the proximity of proteins involved in calcium movement in cells (12).

The Na-K-ATPase is known to be important in regulation of intracellular calcium. For example, binding of ouabain to the Na-K-ATPase raises intracellular calcium in cardiac myocytes, resulting in increases in myocardial contraction. This serves as a basis for using digitalis drugs to treat congestive heart failure. Mechanistically, the Na-K-ATPase was originally hypothesized to regulate calcium entry through the Na/Ca exchanger (NCX) by altering intracellular Na concentration in cardiac myocytes (2, 10, 42). Physical coupling among the Na-K-ATPase, NCX, and SR calcium store was first demonstrated in smooth muscle cells (50). Blaustein and colleagues (39, 72) have recently provided further support to the notion that the Na-K-ATPase interacts with NCX to form a specific calcium-signaling microdomain in many different cell types, including smooth muscle cells and astrocytes. Specifically, they have proposed that the interaction between the NCX with either α2 or α3, but not α1, isoform of the Na-K-ATPase forms a signaling microdomain that is responsible for the ouabain-induced calcium increases in astrocytes and smooth muscle cells (39, 72). Moreover, they have identified that the NH2 termini of α2 and α3 isoforms contain a common structural motif that allows these isoforms to be targeted to the plasma membrane microdomains, overlapping the "functional" SR/endoplasmic reticulum (ER) (72). The NCX activity in these microdomains is regulated by the local Na+ or Ca2+ concentrations, but the ion binding sites for the regulation are not the sites participating in the ion transport. Differential regulatory mechanisms were also found between the cardiac/neuronal-specific NCX isoform and the kidney isoform (7, 22, 23).

The cardiac/neuronal-specific NCX type 1 (NCX1) isoform consists of nine transmembrane (TM) helices, with an extracellular NH2 terminus and a cytosolic COOH terminus (49). An amphoteric sequence, located close by the intracellular end of TM6, is considered an important site in regulation by Na+ and acidic phospholipids (19, 52). When ouabain binds to the Na-K-ATPase, an increase in Na+ near the calcium oscillations has been observed in renal epithelial cells (58). Blaustein and colleagues (39, 72) have hypothesized that the interaction between Na-K-ATPase and the NCX1 in cardiac myocytes (21). Moreover, it has been found that the NH2 termini of α2 and α3 isoforms contain a common structural motif that allows these isoforms to be targeted to the plasma membrane microdomains, overlapping the "functional" SR/endoplasmic reticulum (ER) (72). The NCX activity in these microdomains is regulated by the local Na+ or Ca2+ concentrations, but the ion binding sites for the regulation are not the sites participating in the ion transport. Differential regulatory mechanisms were also found between the cardiac/neuronal-specific NCX isoform and the kidney isoform (7, 22, 23).

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ouabain-induced changes in the interaction between Na-K-ATPase and IP3R complex. These findings suggest that the Na-K-ATPase may be able to tether PLC and IP3Rs to a calcium-regulatory microdomain to facilitate calcium release in renal epithelial cells (54). These findings led the authors to propose that ouabain-induced changes in the interaction between Na-K-ATPase and IP3Rs may be sufficient to stimulate calcium release from the calcium store (54). Alternatively, these interactions may alter the gating properties of IP3Rs. It is important to mention that ouabain-induced calcium oscillations have also been found in cells other than renal epithelial cells (46, 87).

The formation and regulation of this microdomain may also involve another important component of Na-K-ATPase signaling pathway, the Na-K-ATPase/Src complex (44, 78). The Na-K-ATPase and Src are enriched in caveolae of renal epithelial cells (83), and they directly interact with each other (78). Moreover, the Na-K-ATPase/Src complex serves as a receptor for ouabain to increase protein tyrosine phosphorylation and consequently stimulate protein kinase cascades and phospholipase (78). For example, activation of the Na-K-ATPase/Src receptor complex by ouabain transactivates EGF receptor and subsequent results in the activation Ras/ERK cascade. Moreover, activation of this receptor has been found to stimulate tyrosine phosphorylation of PLC-γ and the generation of IP3 (80). Because activation of this pathway plays an important role in regulation of IP3Rs, we examined whether the Na-K-ATPase/Src complex is involved in the formation of a calcium-signaling microdomain. GST pull-down assays have revealed that the central loop of the Na-K-ATPase α1 subunit actually interacts with PLC-γ, whereas the NH2 terminus binds IP3Rs. These findings suggest that the Na-K-ATPase may be able to tether PLC and IP3Rs into a calcium-regulatory microdomain to facilitate ouabain-activated signal transduction. In accordance, both PLC-γ and IP3Rs co-immunoprecipitated with the Na-K-ATPase/Src complex, and ouabain increased formation of this signaling microdomain in a Src-dependent manner. Moreover, we found that activation of the Na-K-ATPase/Src receptor complex by ouabain stimulated tyrosine phosphorylation of IP3Rs. Functionally, activation of this Na-K-ATPase/Src/PLC-γ complex by ouabain led to the opening of IP3Rs and a rise in intracellular calcium. Inhibition of either Src or PLC was sufficient to block ouabain-induced calcium transients. Taken together, these findings indicate that the Na-K-ATPase functions as an important scaffold capable of bringing IP3Rs to their effector PLC-γ to facilitate calcium release in response to ouabain-induced activation of receptor Na-K-ATPase/Src complex.

Receptor

Although recent studies of the Na-K-ATPase and other cardiac and smooth muscle Na+/K+ channels have described this channel as an effector for cell signaling pathways, these ion channels also have properties of a receptor for endogenous and exogenous substances (4). In this regard, the Na-K-ATPase functions as a regulator of intracellular 

"In addition to its effect on calcium entry through NCX, early studies suggested that ouabain and other cardiotonic steroids might activate L-type Ca2+ channels and stimulate Ca2+ release from SR and/or ER."
IP3Rs complex. This complex plays an important role ER-associated protein junctate is also found to bind finally, the effects of ATP on PKC activation and ER calcium release were determined, we found that the α1 knockdown desensitized the ATP-induced Ca2+ release but not PKC activation. Moreover, expression of the NH2 terminus of the Na-K-ATPase α1 subunit, as expected, disrupted formation of the Na-K-ATPase/IP3R complex and attenuated ER Ca2+ release provoked by ATP. Finally, the α1 knockdown also reduced both angiotensin II and EGF-induced ER Ca2+ release. Taken together, these findings support the notion that interaction between the Na-K-ATPase and IP3Rs is important for ER calcium signaling emanated from activation of the receptor Na-K-ATPase/Src complex as well as several other PLC-coupled receptors.

Structural Feature of the Na-K-ATPase-Mediated Protein Interaction

The recently resolved crystal structure showed that the pig Na-K-ATPase α1 subunit, similar to the SERCA calcium ATPase, contains three major domains [60]. The A domain consists of the NH2 terminus and the second cytoplasmic domain (CD2) connected to transmembrane helices M2 and M3. The enzyme also has a highly conserved phosphorylation (P) domain that is close to the membrane and a nucleotide binding (N) domain. Among them, A and N domains are extruded and more exposed, which is consistent with the fact that most of the binding motifs found in the α1 subunit reside in these two domains. The N domain of the Na-K-ATPase α1 subunit interacts with many proteins, including Src and PLC-γ. These interactions are important for the receptor function of the Na-K-ATPase. Moreover, they provide spatial proximity between the generation of IP3 and opening of IP3R by ATP. In addition, the A domain also interacts with arrestin 2 and spinophilin as well as structural proteins such as ankyrin [18, 41]. The interaction with arrestin and spinophilin is important for intracellular trafficking of the Na-K-ATPase [41].

The A domain of the Na-K-ATPase is another site of the molecule that is involved in protein-protein interaction. For example, a three-amino acid sequence (GGVGDVLRK) at the NH2 terminus of the Na-K-ATPase α1 subunit is essential for binding to IP3R [90]. Interestingly, Blausin and colleagues have identified that L and A domain flanking the LKK sequence, are important for targeting the α2 and the α3 to the NCX signaling microdomains in astrocytes [72]. In addition, the A domain also interacts with the SH2 domain of Src, caveolin-1, PTEN kinase, and ankyrin. These interactions are important for the signaling function of Na-K-ATPase. It is interesting to note that the Na-K-ATPase α1 subunit often contains two binding sites for the same partner proteins such as ankyrin, Src, and caveolin-1 [18, 83].

Although interaction with membrane transporters, channels, receptors, protein kinases, and phosphates constitutes formation of the aforementioned calcium signaling microdomains, interaction with structural proteins such as ankyrin, adducin, collagen, and 14-3-3 protein may play an important role in stabilizing the microdomain structure [26, 29, 51, 86]. For instance, the actin cytoskeleton is involved in coordinating interactions among IP3Rs, the Na-K-ATPase (54), and other signaling partners. 

Recent studies have shown that the Na-K-ATPase plays an important role in the regulation of IP3Rs localization and function in different types of cells. A recent study demonstrated that the Na-K-ATPase co-localizes with IP3Rs in astrocytes and this could be an important mechanism of sortilin-mediated IP3R internalization and recycling [84]. Besides proteins, the Na-K-ATPase can interact with lipids and lipoproteins. For example, the Na-K-ATPase has been shown to interact with cholesterol and phospholipids and this interaction can affect its activity [35]. In addition, the Na-K-ATPase is also involved in the regulation of ionic channels and the regulation of cell volume [46]. The most recent study demonstrated that the Na-K-ATPase can interact with cholesterol and this interaction can affect the expression of cholesterol transporters [40].
The notion that Na-K-ATPase is contained in cholesterol-enriched microdomains also has the domain that is critical for binding N (Na) and Pi/Src complex.

Interestingly, the ER IP3R complex also has the domain that is critical for binding N (Na). The second protein-protein interaction with caveolin-1, caveolin-2) causes dominantly inherited type 4 long-QT cardiac arrhythmia in humans (55, 57). Finally, a similar protein complex has also been detected in cardiac myocytes other than cardiac myocytes (72).

In addition to ankyrin, caveolin-1 appears to be another important structural protein. It plays a role in targeting the Na-K-ATPase into caveolae (83). It is known that caveolae serve as an important calcium signaling microdomain in cardiac myocytes (81). Significantly, a loss-of-function (E1425C) mutation in caveolin-2 (also known as caveolin 2) causes dominantly inherited type 4 long-QT cardiac arrhythmia in humans (55, 57). Finally, a similar protein complex has also been detected in cells other than cardiac myocytes (72).

Besides protein-protein interaction, the lipids composition and their specific binding with membrane proteins are important for the formation of microdomains. Indeed, the Na-K-ATPase, as an ion pump, functions only when it resides in the membrane with proper composition of lipids. Removal of the phospholipids from the plasma membrane of cardiac cells led to almost complete inhibition of Na-K-ATPase activity (32). In addition to phospholipids, cholesterol was also found to regulate Na-K-ATPase activity. Na-K-ATPase activity in lens fiber cells was much lower than that in lens epithelial cells, which was closely correlated with cholesterol levels in these cells (15). Depletion of cholesterol from the cell membrane was found to induce biphasic response in Na-K-ATPase activity (30). The mechanism of such regulation was considered to be a direct and high-affinity interaction between IP3R and ankyrin-2 (18, 78, 83). The 11-amino acid sequence of the Na-K-ATPase resides in a cholesterol-enriched caveolae structure. Cholesterol depletion could disrupt the caveolae structure and diminish the signaling function of the Na-K-ATPase (45, 83).

**Perspectives**

Studies of the past 10 years have identified many important protein interactions of the Na-K-ATPase. The interactions among the Na-K-ATPase, protein kinase, membrane transporters/channels, and structural proteins ensure formation of dynamic and cell-specific calcium-signaling microdomains. It is important to recognize that the aforementioned investigations only mark the beginning of a fascinating new field. Besides identification and functional characterization of new partners such as TRPCs, poly-cystin-1, and protein phosphatases (31, 62, 89), studies have to be conducted to understand the dynamics, regulation, and isoform- and cell-specific aspects of these interactions among the Na-K-ATPase and its partners. Further efforts of many laboratories are clearly required. It is also important to recognize that we know little about how the ion-transporting function of Na-K-ATPase is related with its signaling function in the regulation of these cell functions. However, the continual efforts will eventually provide insights into the newly appreciated functions of the Na-K-ATPase and their role in cell biology and animal physiology.

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