Frontiers: Skeletal muscle sodium pump regulation: a translocation paradigm

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Benziane B, Chibalin AV. Skeletal muscle sodium pump regulation: a translocation paradigm. Am J Physiol Endocrinol Metab 295: E553–E558, 2008. First published April 22, 2008; doi:10.1152/ajpendo.90261.2008.—The skeletal muscle sodium pump plays a major role in the removal of K+ ions from the circulation postprandial, or after a physical activity bout, thereby preventing the development of hyperkalemia and fatigue. Insulin and muscle contractions stimulate Na+-K+-ATPase activity in skeletal muscle, at least partially via translocation of sodium pump units to the plasma membrane from intracellular stores. The molecular mechanism of this phenomenon is poorly understood. Due to the contradictory reports in the literature, the very existence of the translocation of Na+-K+-ATPase to the skeletal muscle cell surface is questionable. This review summarizes more than 30 years work on the skeletal muscle sodium pump translocation paradigm. Furthermore, the methodological caveats of major approaches to study the sodium pump translocation in skeletal muscle are discussed. An understanding of the molecular regulation of Na+-K+-ATPase in skeletal muscle will have important clinical implications for the understanding of the development of complications associated with the metabolic syndrome, such as cardiovascular diseases or increased muscle fatigue in diabetic patients.

Na+-K+-ATPase; translocation; insulin; contraction; membrane traffic; ouabain

Na+-K+-ATPase is a membrane cation pump that is critically involved in the maintenance of intracellular sodium and potassium concentrations and participates in the maintenance of cell volume and electrochemical gradients. In addition to these general functions, Na+-K+-ATPase promotes membrane re polarization and reuptake of extracellular potassium in excitable cells, including skeletal muscle (8). Hydrolysis of ATP by Na+-K+-ATPase fuels the coupled transport of K+ into the cell and Na+ out of the cell. Thus the maintenance of the intracellular K+ concentration is a dynamic and fundamental process involving Na+-K+-ATPase (8). Skeletal muscle is a primary storage site for dietary K+, and the sodium pump plays a major role in the removal of this ion from the circulation under physiological conditions (i.e., after a meal or exercise bout), thereby preventing the development of hyperkalemia and muscle fatigue (28).

Skeletal muscle contains one of the largest pools of Na+-K+-ATPase in the body and expresses α (α1 and α2)- and β (β1 and β2)-subunits. Small amounts of the α3-subunit are also detected in skeletal muscle (8). The functional unit of the sodium pump is an α/β-heterodimer. The phospholemman (PLM, FXYD1) forms a complex with α/β-dimers in heart and skeletal muscle. Interaction of PLM with sodium pump units modulates activity of Na+-K+-ATPase (5).

In view of the fundamental importance of Na+-K+-ATPase, its regulation can be achieved by multiple mechanisms, including changes in the intrinsic activity, subcellular distribution, and cellular abundance. In skeletal muscle, on a long-term basis, thyroid hormone and exercise training lead to an increase in the Na+-K+-ATPase subunit expression and pump activity (8). The short-term sodium pump regulation includes elevation in the intracellular Na+ concentration, increased sensitivity to intracellular Na+ concentration, and covalent modification of the Na+-K+-ATPase subunits. The catalytic α-subunit of Na+-K+-ATPase and PLM are substrates for protein kinases (5, 8), and the pump phosphorylation is an important molecular mechanism for the short-term control of its activity in response to hormonal stimulation.

Phenomenon and Methodology

One mechanism to control the skeletal muscle sodium pump activity is through regulation of Na+-K+-ATPase cell surface abundance. There are contradictory reports in the literature; the possibility that sodium pump units are translocated to the plasma membrane has been highlighted by some workers studying the skeletal muscle Na+-K+-ATPase regulation (1, 2, 15–18, 22, 26, 27, 31, 34, 36) but not observed by others (8, 9, 29).

Early researchers proposed a “translocation hypothesis” to describe the mechanism by which sodium pump units were recruited to the plasma membrane from an intracellular compartment to explain the effect of insulin treatment on [3H]ouabain binding to intact frog muscles (15). The mechanism was further confirmed in experiments with subcellular fractionation of insulin-stimulated frog muscle and considers that an “unmasking of inactive pumping sites” (12) may account for the increase in sodium pump activity. These studies are based on the evidence that the Na+-K+-ATPase activity and [3H]ouabain binding were increased in the plasma membranes isolated from insulin-stimulated muscles, with parallel decreases in these parameters in intracellular membrane fractions (19, 31).

A translocation of the α/β-Na+-K+-ATPase dimer to the plasma membrane from intracellular stores has been observed in response to in vivo insulin stimulation in rat skeletal muscle (16, 26). Similar results were obtained in experiments with in vitro rat soleus muscle incubated in the presence of insulin (6). Importantly, an acute bout of exercise (36) and in situ electrically stimulated muscle contraction (34) led to translocation of both α1- and α2-subunits to the skeletal muscle plasma membrane. These studies were performed on rat muscle using gradient centrifugation-based subcellular fractionation.

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Perspectives

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atom protocols that have been successfully developed to monitor GLUT4 translocation (11, 13, 20). Using a completely different membrane isolation technique, namely a giant sarcotubule vesicles isolation, Juel et al. (17, 18) demonstrated that acute exercise promotes translocation of the sodium pump α2- and β-subunits to the plasma membrane in human and rat skeletal muscle. Thus the skeletal muscle sodium pump translocation phenomenon appears to be conserved between species.

In contrast to muscle subcellular fractionation studies, different results have been observed using [3H]ouabain binding to intact rat muscles. This method has previously been utilized in experiments performed using amphibian muscle. The technique developed by Clausen and Kohn (9) did not detect translocation of the α2-subunit in either electrically stimulated or insulin-treated muscle (29). This stimulation protocol did not lead to an increase in saturated [3H]ouabain binding capacity in intact rat soleus muscle. Instead, the investigators concluded that the effect observed in the early experiments with frog muscles was due to the short incubation and inadequate saturation, since the ouabain binding rate is dependent on pump activity, which can be increased by insulin and contractions independently from translocation. The authors also observed that in subcellular fractionation experiments the yield of plasma membrane/sarcotubule recovery was very low, making it difficult to establish whether this fraction was representative of the entire pool of the sodium pump in skeletal muscle. These authors therefore suggested that the early reported increase in Na\(^{+}\)-K\(^{+}\)-ATPase subunit plasma membrane abundance (6, 16–18, 26, 31) was an artifact arising from the different membrane purification methods and did not reflect translocation in intact tissue.

However, conclusions based on only [3H]ouabain binding data are equivocal. There are a number of possibilities to reconcile the discordant views regarding the translocation of Na\(^{+}\)-K\(^{+}\)-ATPase. For one, differences in either the membrane turnover rate between frog and rat skeletal muscle, temperatures used during incubation, or incubation time with [3H]ouabain could explain the different results obtained in ex vivo [3H]ouabain binding experiments. Secondly, it is also important to take the rate of pump translocation and speed of sodium pumps turnover at the muscle cell surface into account. Ouabain binding itself leads to a rather rapid internalization of the pump unit/ouabain complexes into cells (21, 23, 30). Conversely, the effect of muscle contraction on translocation of the Na\(^{+}\)-K\(^{+}\)-ATPase subunits to the sarcolemma is already reversed after 30 min after exercise (17). Thus ex vivo ouabain binding to intact skeletal muscle after longer time points will reflect total and not cell surface sodium pump content (in the case of rodent skeletal muscle, it will be a measure of ouabain-sensitive α2-subunit content). Indeed, data from Clausen’s laboratory (29) demonstrated increased [3H]ouabain binding to intact skeletal muscle in response to stimuli after shorter incubation times (15–30 min), which could be interpreted as increased plasma membrane content. After longer incubation times, the effect disappears due to reinternalization of the pump units and [3H]ouabain-Na\(^{+}\)-K\(^{+}\)-ATPase complex uptake. Notably, stimulation with insulin increased [3H]ouabain binding measured after 15 min to intact primary human skeletal muscle differentiated myotubes, and this effect was abolished by inhibitors of phosphatidylinositol 3-kinase (PI 3-kinase), PKC, and ERK1/2 (1). Thus the [3H]ouabain binding assay could be used as a tool to assess changes in the sodium pump cell surface abundance, if binding times are short enough or membrane traffic events are blocked, while saturated [3H]ouabain binding capacity is a “golden standard” for quantitative assessment of total sodium pump content in skeletal muscle and other tissues.

One way to prevent the pump reinternalization is by decreasing the temperature at the end of the stimulation period and during the [3H]ouabain binding step to “freeze” cellular membranes and slow all trafficking events. Indeed, [3H]ouabain binding performed at 16–18°C with isolated rat soleus muscle after either insulin stimulation or in vitro electrically stimulated muscle contraction at 30°C reveals a significant increase in ouabain binding in stimulated rodent skeletal muscle (Fig. 1). However, if the [3H]ouabain binding is performed at 30°C, there is no difference in the binding between control and stimulated muscle. Importantly, even in the control group, [3H]ouabain binding reaches maximal values. These data provide evidence that [3H]ouabain binding performed at 30°C reflects total, rather than cell surface sodium pump content, in isolated rat skeletal muscle. Thus the long-term [3H]ouabain binding performed at a temperature that allows constitutive exo- or endocytosis cannot be used as a tool to assess the cell surface abundance of Na\(^{+}\)-K\(^{+}\)-ATPase in skeletal muscle.

The structural complexity of skeletal muscle tissue, with inherent difficulties in applications of optical techniques, has

![Graph](http://ajpendo.physiology.org/)

**Fig. 1.** Effect of temperature on [3H]ouabain binding to insulin- or contraction-stimulated rat soleus muscle. The soleus muscles from male Wistar rats (80–100 g body wt) were subjected to insulin stimulation or isometric contractions essentially as described previously (3) in a standard Krebs-Ringer bicarbonate buffer at 30°C. After stimulation, the muscles were transferred in K\(^{+}\)-free Krebs-Ringer buffer containing [3H]ouabain (2 × 10\(^{-6}\) M) and then incubated for 210 min at 16–18°C or 30°C, followed by four 30-min washouts in ice-cold K\(^{+}\)-free Krebs-Ringer buffer. [3H]ouabain binding site content was determined directly and expressed as per gram wet weight. Values are means ± SE; n = 4–6 muscles. *P < 0.05 vs. basal.

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made subcellular fractionation the preferred and almost irreplaceable method to isolate membrane compartments for the study of membrane proteins trafficking in skeletal muscle. These techniques were successfully used to study insulin- and contraction-stimulated GLUT4 translocation in skeletal muscle (11, 20). Importantly, all the GLUT4 translocation data obtained by subcellular fractionation methods have been confirmed using real-time optical experiments with different cell models utilizing labeled GLUT4 molecules and, most recently, using indirect in vivo measurements in green fluorescent protein-GLUT4 fusion protein transgenic mice (24).

Subcellular fractionation experiments provide evidence that Na\(^{+}\)-K\(^{+}\)-ATPase, together with the glucose transporter GLUT4, is insulin- and contraction-responsive proteins in skeletal muscle. In vivo cellular imaging of tagged sodium pump molecules will help to resolve the issue of the Na\(^{+}\)-K\(^{+}\)-ATPase translocation mechanism further.

The increase in Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha_2\)-subunit plasma membrane abundance in response to an insulin injection in rat skeletal muscle has been confirmed by immunoelectron microscopy. The location of the sodium pump contained in intracellular vesicles has been reported to be in the subsarcolemmal and triadic region (26, 27). These results provide morphological evidence that the sodium pump is present both in the plasma membrane and in the intracellular compartments in skeletal muscle and that the pump cell surface content increases in response to insulin.

Cell surface biotinylation has been developed as robust method to label membrane proteins that expose lysine residues to extracellular space in cell culture (14). Subcellular fractionation experiments provide evidence that the Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha_2\)-subunit plasma membrane abundance increases in response to insulin in skeletal muscle (6, 16, 26), while \(\alpha_1\)-subunit translocation has been shown to be regulated only by muscle contraction (34, 36). Using a sensitive biotinylation technique that has not been previously applied to muscle tissue, Al-Khallil et al. (2) have demonstrated that insulin induces not only Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha_2\)-subunit but also \(\alpha_1\)-subunit translocation to the cell surface in isolated rat epitrochlearis muscle and cultured human skeletal muscle cells. In a parallel subcellular fractionation experiment, an insulin-mediated increase in the \(\alpha_1\)-subunit abundance in the plasma membrane fraction was undetected. However, an intracellular membrane fraction obtained from insulin-stimulated muscles was depleted of \(\alpha_1\)-subunit. Thus even without a noticeable increase in the \(\alpha_1\)-subunit abundance in the plasma membrane fraction, a reduction of the \(\alpha_1\)-subunit abundance in the intracellular membrane fraction can be taken as evidence for an insulin-sensitive \(\alpha_1\)-subunit pool. Most likely, a subfraction containing the target plasma membrane for the insulin-sensitive pool of the \(\alpha_1\)-subunit was unrecovered on sucrose gradient, whereas the increase in the \(\alpha_1\)-isoform abundance in response to insulin was robust and measurable by labeling of cell surface Na\(^{+}\)-K\(^{+}\)-ATPase, although the magnitude of the response was greater in the case of the \(\alpha_2\)-subunit. Thus both of the Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha\)-subunit isoforms that are predominantly expressed can translocate to the cell surface in response to insulin or contractions. Importantly, subunit translocation has been observed in oxidative (6, 26, 34; soleus and red gastrocnemius) and glycolytic (2) (epitrochlearis) skeletal muscle fiber types.

The effect of either an acute exercise bout or in vitro electrically stimulated muscle contraction on sodium pump plasma membrane abundance has recently been studied by Juel’s laboratory (22). Cell surface biotinylation, outer-membrane-enriched fractions, and sarcolemma giant vesicles isolation have been used to assess cell surface proteins abundance. All three techniques reveal a similar increase (36–41%) in Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha_2\)-subunit cell surface content. The Na\(^{+}\)-K\(^{+}\)-ATPase activity, measured at \(V_{\text{max}}\) as K\(^{+}\)-stimulated 3-O-methylfluorescein phosphatase, was increased in giant sarcolemma vesicles but not in total muscle homogenates obtained from muscles of previously exercise rats. These findings provide evidence to suggest that the contraction-induced increase in the sodium pump activity in skeletal muscle is mostly due to an increase in cell surface pump content, rather than changes in the intrinsic activity of the pump units. Interestingly, caveolin-3 abundance is also increased at the cell surface of contracted muscles, and the \(\alpha_1\)- and \(\alpha_2\)-subunits are coimmunoprecipitated with caveolin-3. However, the magnitude of the increase in caveolin-3 abundance is 2.2-fold lower than the increase in \(\alpha_2\)-subunit abundance (22). Thus Na\(^{+}\)-K\(^{+}\)-ATPase translocation from caveolae can partly explain the mechanism by which muscle contraction increases the cell surface abundance of Na\(^{+}\)-K\(^{+}\)-ATPase. The remaining pool of the \(\alpha_2\)-subunit could be translocated via caveolin-3-independent vesicular trafficking. The possible role of caveolae as a compartment for intracellular localization of the sodium pump in skeletal muscle has been suggested earlier based on immunoelectron microscopy observations (27).

Surface labeling of plasma membrane proteins in cell culture and in isolated skeletal muscles ex vivo may be an important approach for studying the sodium pump and sodium pump-associated proteins traffic, as it permits the use of inhibitors of different signaling molecules before biotinylation. This technique could also be used to visualize possible Na\(^{+}\)-K\(^{+}\)-ATPase trafficking defects in skeletal muscle from genetically modified animal models. However, subcellular fractionation and gradient centrifugation techniques will remain important methods to study membrane trafficking in skeletal muscle if sufficient quantities of tissue are available. The mild conditions of the method allow for an assessment of enzymatic activity and interaction of the proteins of interest in membrane fractions.

**Signaling Pathways Regulating the Sodium Pump Translocation**

The sodium pump translocation to the cell surface concomitant with an increase in the pump activity in skeletal muscle has been observed in response to insulin, acute exercise, or in vitro muscle contraction. A striking similarity between the sodium pump and GLUT4 translocation in response to insulin stimulation and muscle contraction has provoked speculation that traffic of the sodium pump in skeletal muscle is regulated in a manner similar to that of GLUT4 trafficking, and similar signaling pathways are activated. Indeed, like GLUT4 translocation, the sodium pump translocation is sensitive to the inhibition of PI 3-kinase and atypical PKCs (1, 2). However, data from immunoelectron microscopy studies and immunosialation of specific vesicles indicate that the GLUT4 glucose transporter and Na\(^{+}\)-K\(^{+}\)-ATPase do not localize to the same intracellular vesicles in skeletal muscle (25). In contrast...
to GLUT4, phosphorylation of the sodium pump subunits plays the crucial role in the regulation of its trafficking. Rat α1-subunit translocation to the cell surface in response to insulin correlates with dephosphorylation of Ser18 (35), a PKC phosphorylation site that is critically involved in rat α1-subunit endocytosis (7). However, human α1- and α2-subunits and rat α2-subunit lack Ser18, although they exhibit a similar translocation response to insulin and inhibitors of PI 3-kinase and PKC (1, 2). Thus despite these species-specific and isoform-specific differences, a common mechanism for triggering sodium pump translocation is likely to exist (Fig. 2).

Na\(^{+}\)-K\(^{-}\)-ATPase in rat skeletal muscle and human differentiated skeletal muscle cells is phosphorylated on multiple sites in response to insulin (1, 6). Moreover, this phosphorylation acts as a triggering signal to promote Na\(^{+}\) transporters at the plasma membrane or blocks the Na\(^{+}\)/H\(^{+}\) antiporter (7). However, human α1- and α2-subunits and rat α2-subunit lack Ser18, although they exhibit a similar translocation response to insulin and inhibitors of PI 3-kinase and PKC (1, 2). Thus despite these species-specific and isoform-specific differences, a common mechanism for triggering sodium pump translocation is likely to exist (Fig. 2).

\[\text{Na}^{+}-\text{K}^{-}\text{-ATPase} + \text{Insulin} \rightarrow \text{Na}^{+}\text{-K}^{-}\text{-ATPase}_{\text{cell surface}}\]

Fig. 2. Na\(^{+}\)-K\(^{-}\)-ATPase regulation and its traffic in skeletal muscle. Insulin or muscle contraction causes phosphorylation of Na\(^{+}\)-K\(^{-}\)-ATPase subunits and promotes translocation of Na\(^{+}\)-K\(^{-}\)-ATPase from an intracellular storage site to the plasma membrane or blocks the Na\(^{+}\)-K\(^{-}\)-ATPase recycling from the plasma membrane to an intracellular pool. Increased Na\(^{+}\)-K\(^{-}\)-ATPase abundance at the muscle cell surface leads to an increase in K\(^{+}\) uptake and Na\(^{+}\) excretion. PLM, phospholemman; PI 3-kinase, phosphatidylinositol 3-kinase; IRS, insulin receptor substrate; AP2, adaptor protein-2; AICAR, 5-aminoimidazole-4-carboxamide-β-riboside.

Notably, exercise and muscle contraction are stronger activators of the ERK1/2 signaling pathway than insulin. Thus ERK1/2 signaling could be important for contraction-induced sodium pump translocation. The ex vivo contraction-mediated increase in cell surface Na\(^{+}\)-K\(^{-}\)-ATPase content was abolished by PD-98059, a MEK1 inhibitor, in rat epitrochlearis muscle (A. Chibalin and O. Kotova, unpublished observations). However, the role of ERK1/2 in phosphorylation and activation of the sodium pump in skeletal muscle in response to contractions remains to be elucidated. Interestingly, muscle contractions induce a transient increase in the α2-subunit tyrosine phosphorylation in rat soleus muscle, while phosphorylation of the α1-isofrom was unchanged (34). In rat soleus muscle, insulin stimulation also leads to a transient increase in tyrosine phosphorylation of the α2-subunit but not the α1-subunit (6). Taken together, these results with contraction-mediated translocation of the α2 subunit to the cell surface suggest an insulin-like response.

Exercise and ex vivo muscle contraction are potent activators of AMP-activated protein kinase (AMPK), a metabolic master switch that regulates cellular energy homeostasis. In rats, a three h infusion of 5-aminoimidazole-4-carboxamide-β-β-riboside (AICAR), a pharmacological AMPK activator, leads to an acute fall in the plasma K\(^{+}\) concentration ([K\(^{+}\)], without an increase in K\(^{+}\) urinary excretion, concomitant with an increase in AMPK phosphorylation (40). The effect of AICAR on plasma [K\(^{+}\)] was blunted in mice overexpressing a kinase dead form of AMPK (40). Thus AMPK activation may be a potential mechanism by which exercise activates the sodium pump in skeletal muscle, a tissue that contains >70% of body K\(^{+}\) and regulates plasma [K\(^{+}\)] (28). However, AICAR infusion is without effect on Na\(^{+}\)-K\(^{-}\)-ATPase activity, as measured in skeletal muscle homogenates (40). Moreover, plasma membrane abundance of the α2-subunit was also unchanged (40). An independent study (22)
indicates that 1 h after a single AICAR injection, the abundance of the α2-subunit in skeletal muscle was decreased by 20%. This observation is compatible with recent report (37) indicating that CO2-induced AMPK activation in lung cells promotes Na\(^{+}\)-K\(^{+}\)-ATPase endocytosis. These findings support the hypothesis that once AMPK has been activated, most of the energy-consuming processes in the cell are switched off. However, the mechanism of whole body AICAR-induced potassium clearance and how AMPK activation affects K\(^{+}\) fluxes and Na\(^{+}\)-K\(^{+}\)-ATPase activity in cell remains to be determined.

The role of PLM as an important player in the physiological regulation of Na\(^{+}\)-K\(^{+}\)-ATPase in skeletal muscles is just beginning to be uncovered. PLM is expressed mostly in heart and skeletal muscle, with a predominant expression noted in the sarcolemma. Unphosphorylated PLM binds to the Na\(^{+}\)-K\(^{+}\)-ATPase α-subunits and inhibits the pump activity by decreasing Na\(^{+}\) affinity or \(V_{\text{max}}\) (5, 10). PLM phosphorylation disrupts the PLM-α-subunit interaction and stimulates the pump. Phosphorylation by PKA at Ser68 only increases the apparent affinity for Na\(^{+}\) of the α1/β- and α2/β-isoforms, while phosphorylation by PKC at Ser63 and Ser68 increases \(V_{\text{max}}\) for α2/β-isozyme and not for the α1/β-isozyme (4). In rat skeletal muscle, PLM associates with the α1- and the α2-isoforms (10, 33). Insulin induces phosphorylation of PLM (38). The level of PLM expression is also increased by exercise training (33). In rats, acute exercise translocates PLM to the muscle outer membrane fraction and sarcolemma giant vesicles, with a 2- to 3.5-fold increase compared with sedentary control, together with a concomitant 67% increase in Na\(^{+}\)-K\(^{+}\)-ATPase \(V_{\text{max}}\) (32). The same exercise protocol produces an increase in the cell surface sodium pump abundance only by 36–41% (22). These data provide evidence to suggest that the translocation of PLM to plasma membrane may further increase the \(V_{\text{max}}\) of Na\(^{+}\)-K\(^{+}\)-ATPase. Whether muscle contraction leads to an increase in PLM phosphorylation or whether kinases different from PKA and PKC phosphorylate PLM in skeletal muscle remains to be determined.

**Future Horizons**

After more than 30 years of study, the mechanism governing Na\(^{+}\)-K\(^{+}\)-ATPase translocation in skeletal muscle is still incompletely understood. Several major questions still need to be answered. The nature and origin of the compartments where the sodium pump is stored during basal conditions are unclear. Specific Na\(^{+}\)-K\(^{+}\)-ATPase-containing intracellular vesicles need to be isolated and its composition needs to be determined. Similarities and differences between insulin- or contraction-induced mechanisms for the Na\(^{+}\)-K\(^{+}\)-ATPase translocation need to be studied in further detail. A kinetic analysis of the Na\(^{+}\)-K\(^{+}\)-ATPase redistribution between plasma membrane and intracellular compartments needs to be performed to establish whether either an increase in exocytosis, a decrease in endocytosis, or both of these processes acting in parallel lead to an accumulation of the sodium pump molecules at the cell surface in response to insulin or contractile stimuli. The role of small GTPases and GTPase-activating proteins in insulin- or contraction-induced translocation of the sodium pump remains to be determined. Phosphorylation of the Na\(^{+}\)-K\(^{+}\)-ATPase subunits and associated proteins, especially the human isoforms, needs to be studied in detail. In vivo phosphorylation sites should be mapped, and the nature of specific protein kinases, phosphorylating the sodium pump in response to either insulin or contraction, and their regulation should be uncovered. The role of phospholemman in skeletal muscle ion and nutrient transport should be determined. A whole array of techniques developed in studies of GLUT4 translocation and membrane traffic in neurons, including online monitoring of the trafficking events in living cell, can now be applied to study the mechanism governing sodium pump translocation. An increased understanding of the precise molecular processes involved in the regulation of Na\(^{+}\)-K\(^{+}\)-ATPase by either insulin or contraction-mediated signaling pathways will facilitate the development of novel therapeutic strategies for patients with cardiovascular diseases, metabolic syndrome and complications of diabetes mellitus.

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