Potassium Channel Phosphorylation in Excitable Cells: Providing Dynamic Functional Variability to a Diverse Family of Ion Channels

Phosphorylation of potassium channels affects their function and plays a major role in regulating cell physiology. Here, we review previous studies of potassium channel phosphorylation, focusing first on studies employing site-directed mutagenesis of recombinant channels expressed in heterologous cells. We then discuss recent mass spectrometric-based approaches to identify and quantify phosphorylation at specific sites on native and recombinant potassium channels, and newly developed mass spectrometric-based techniques that may prove beneficial to future studies of potassium channel phosphorylation, its regulation, and its mechanism of channel modulation.

Potassium channels (KChs) are important and diverse determinants of membrane potential and regulate a wide variety of cellular processes. There are over 100 genes encoding the pore-forming α subunits of KChs in the mammalian genome (19), making them the most diverse subset of ion channels and one of the largest families of cell signaling proteins. Many KCh types are broadly expressed and are crucial in establishing the resting potential and ionic driving force across membranes of virtually every cell type. Other KChs are expressed only in excitable tissue (neurons, skeletal, and cardiac muscle) where they can assume very limited cellular and subcellular patterns of expression and regulate specific and local aspects of electrical signaling. Still others are devoted to regulating salt and water balance in epithelial cells and hormone secretion in endocrine cells. KChs are complicated multi-subunit membrane proteins, comprising four pore-forming α subunits that assemble into homo- or hetero-tetramers, often associated with auxiliary subunits (74). Diversity of KCh structure and function arises from a large number of different KCh genes and from combinatorial assembly of the encoded subunits. Subunit assembly occurs during biosynthesis and generates protein complexes of fixed subunit composition. Dynamic and reversible changes in KCh structure and function come about through post-translational modification of these channel complexes, with the predominant form being cycles of phosphorylation and dephosphorylation events carried out by a wide variety of protein kinases and protein phosphatases, respectively.

Protein kinases are another diverse group of signaling proteins, with individual kinases exhibiting specificity for distinct target sequences, complex patterns of cellular and subcellular expression, and regulation by diverse signaling pathways and metabolic conditions (38). The intersection of these two diverse sets of signaling functionalities via KCh phosphorylation allows a cell to dynamically respond to changes in extracellular and intracellular environment with changes in membrane electrical properties. KCh modulation by reversible changes in channel phosphorylation state has been the object of intense study for over three decades. However, only recently, through application of mass spectrometry (MS)-based proteomic techniques, has the rapid and reliable identification of chemical phosphate present on KCh proteins purified from intact cells and tissues been possible. These studies have begun to reveal a remarkable extent of in vivo KCh phosphorylation.

That phosphorylation is an important physiological regulator of KCh function began with the seminal work of Kandel, Greengard, and colleagues, who showed that serotonergic neurotransmission alters neuronal excitability and underlies acquisition of short-term memory in the marine invertebrate Aplysia californica (7, 8, 30). Subsequent studies revealed that this response was mediated entirely through cAMP-dependent protein kinase (PKA)-dependent inhibition of a specific KCh, termed the S channel (65, 66). Subsequent studies in mammalian neurons, and in a variety of nonneuronal cells, revealed diverse signaling pathways regulating KCh phosphorylation state, as indicated by phosphorylation-dependent changes in the biophysical properties of the target channels and diverse aspects of cellular physiology were dynamically controlled by KCh phosphorylation. However, identification of specific KCh proteins that were the substrates for the phosphorylation events, and specific sites modified, remained an elusive research objective, whereas similar studies on the primary pore-forming
and voltage-sensing α subunits of voltage-gated sodium and calcium channels yielded definitive results (39). In general, KChs exhibit molecular complexity that exceeds other channel types, confounding biochemical purification of individual KChs using classical biochemical approaches (e.g., ion exchange or size exclusion chromatography). KChs are tetramers of the primary pore-forming α subunits (sodium and calcium channels have only one pseudotetrameric α subunit), and different α subunits can co-assemble into a wide variety of biochemically similar but distinct KChs. Moreover, most cells express a variety of KChs such that a rich source of a single channel type is not found.

The problems associated with purification and chemical analysis of phosphorylation on specific members of a highly related gene family present in low amounts in native tissues have been recently overcome with two technical advances. The first is the availability of subtype-specific antibodies for individual KCh α subunits, which has allowed for immuno-affinity isolation of specific channel complexes from native tissue. The second major advance has been in mass spectrometric-based approaches to chemically identify phosphate on small amounts of purified channel proteins and to unambiguously identify and quantify specific sites of covalent modification. Recent application of these techniques to purify native and recombinant KChs and analyze their phosphorylation state has led to identification and quantitation of a number of in vivo phosphorylation sites on KChs as well as insights into their role in regulating channel function.

**Phosphorylation-Dependent Modulation of KCh Gating**

The large number of KCh subtypes, and the lack of sharp biophysical and pharmacological differences between them, can confound identification of specific KCh subtypes underlying specific physiological processes in native cells. As such, the bulk of studies addressing the modulation of KChs by phosphorylation have been performed in heterologous systems expressing homo-tetrameric recombinant channels, using pharmacological activation of endogenous or exogenous protein kinases and phosphatases to modulate channel function. This is typically followed by mutagenesis of candidate phosphorylation sites to identify specific residues necessary for the observed response. Although in practical terms it is relatively safe to assume that any residues identified are likely to act as phosphorylation sites, it is important to remember that this approach identifies residues necessary for functional modulation and not the phosphorylation sites themselves; this requires chemical analysis of covalently bound phosphate.

Whole cell channel activity, expressed as the total ionic current ($I$), is the product of three factors: the number of channels in the plasma membrane ($n$), the probability that the channel will be open under a given set of conditions (the open probability or $P_o$), and the single channel or unitary conductance ($i$), such that $I = nP_o i$. Studies on the effects of phosphorylation on KCh function have identified pronounced effects on $n$ and $P_o$ as detailed below (effects on $i$ have not been extensively investigated). Effects on $n$ or the number of channels in the plasma membrane can be due to effects on biosynthetic trafficking that will alter the proportion of synthesized channels that reach the plasma membrane, to the stability of channels in the plasma membrane, and to targeting of channels to and/or clustering in specific membrane compartments. Effects on $P_o$ can come from altering the sensitivity of the channel to activating stimuli (e.g., the voltage-dependence of activation of voltage-gated or Kv channels, ATP for KATP channels, etc.), the microscopic kinetics of channel activation, the mean open time, and the rate of deactivation. Certain KChs also exhibit time-dependent inactivation. Although each of these parameters is intrinsically encoded in the primary structure of an individual KCh isoform, each can also serve as a potential target for modulation to affect channel $P_o$ and examples of each type of modulation are found in the literature (28).

A number of recent studies have provided clear examples of phosphorylation-dependent modulation of $P_o$. The voltage-dependence of activation of Kv2.1 channels and the kinetics of channel activation are dramatically affected by phosphorylation state (53). Kv2.1 is maintained at a highly phosphorylated state in many cell types, and regulation is primarily achieved through signaling events that induce dephosphorylation, with subsequent effects on increased $P_o$ due to hyperpolarizing shifts in voltage-dependent activation and an acceleration of activation kinetics (53). Kv4.2 channels, which underlay transient Kv currents in neurons and cardiac muscle, are suppressed by phosphorylation due to depolarizing shifts in voltage-dependent activation (26). Studies utilizing in vitro phosphorylation and microsequencing of recombinant Kv4.2 fragments led to chemical identification of PKA sites on Kv4.2 by PKA (3) and ERK (1). Activity of Kir6.2, the pore-forming subunit of KATP channels, which link metabolism to membrane excitability and regulate insulin secretion from pancreatic β-cells, is upregulated by PKC-mediated phosphorylation to affect $P_o$ by stabilizing the open state of the channel (35). Large-conductance calcium-activated BK channels in native (10, 62, 64) and heterologous cells exhibit phosphorylation-dependent shifts in voltage dependence and/or apparent calcium affinity. A complex interplay exists between modification of the BK COOH terminus by splicing and phosphorylation, with specific proteins kinases conferring activation in one splice variant background and inhibition in another (73). Moreover, the impact of phosphorylation...
A number of studies have revealed potent modulation of KCh inactivation by phosphorylation. Treatment of *Xenopus* oocytes expressing Kv3.4 with a PKC activator eliminates N-type inactivation, and mutation of two individual serine residues dampened this response [the effects were amplified in the double mutation of two individual serine residues dampened inactivation (72)]. Combined with the large number (~150) of potential phosphorylation sites on the BK COOH terminus, these characteristics can confer tremendous range of function to BK channel phosphorylation.

A number of studies have revealed potent modulation of KCh inactivation by phosphorylation. Treatment of *Xenopus* oocytes expressing Kv3.4 with a PKC activator eliminates N-type inactivation, and mutation of two individual serine residues dampened this response [the effects were amplified in the double mutant (14)]. Structural studies of synthetic peptides corresponding to amino acids 1–30 of Kv3.4 in the unphosphorylated and doubly phosphorylated forms revealed that, although the unphosphorylated peptide has a compact structure that serves as a suitable pore blocker, the phosphopeptide structure was looser and less effective at blocking the pore (4). A similar effect of phosphorylation in slowing N-type inactivation of Kv1.4 channels has also been observed (57), although in this case the functional modulation is sensitive to mutation of a serine residue that lies well outside of the inactivation ball. Increased phosphorylation of both Kv3.4 and Kv1.4 enhances *I* by removing inactivation and increasing *P*~o~.

**Phosphorylation-Dependent Modulation of KCh Trafficking**

Phosphorylation also affects *I* via mechanisms that impact channel number *n* through acute effects on channel appearance at, or removal from, the plasma membrane. As for KCh gating, mechanisms that impact channel number are intrinsically encoded in primary structure but can be further modulated by phosphorylation. Kv1.2 currents in *Xenopus* oocytes are increased on PKA activation, a response suppressed by mutation of a consensus PKA site in the Kv1.2 NH~2~ terminus (22), although structural studies of this domain have raised questions as to whether this can be the true PKA phosphorylation site since it is not surface exposed (41). Kv1.2 currents are decreased on tyrosine kinase activation (23) through increased Kv1.2 endocytosis dependent on an NH~2~ terminal tyrosine (49). Kv1.2 expression is increased by tyrosine phosphatase activation (75). Tyrosine kinase activation also destabilizes Kv1.2 in the membrane by inhibiting its association with the actin-binding protein cortactin, a response dependent on a COOH-terminal tyrosine (20). Thus phosphorylation at two distinct tyrosine residues on Kv1.2 exerts distinct effects on endocytosis and channel number *n*. Tyrosine phosphorylation of other ion channels [e.g., Kv1.1 (24), Kv1.5 (21)] also leads to suppression of ionic currents, presumably through similar effects, and PKC activation yields reduced hERG currents with little or no effect on channel activation or inactivation (11).

Kv7.2/Kv7.3 (KCNQ2/KCNQ3) channels, which form the molecular correlate of the neuronal M current, are also modulated by phosphorylation (61). PKA activation enhances Kv7.2/Kv7.3 currents, an effect that is abrogated by mutation of an NH~2~ terminal consensus PKA site (55). Tyrosine kinase activation yields slow inhibition of Kv7.2/Kv7.3 expression through a mechanism that involves NH~2~ and COOH-terminal tyrosines on Kv7.3 (27). The slowly activating potassium current in mammalian heart, arising from Kv7.1 channels, is increased on sympathetic stimulation to increase heart rate. The increased currents are due to PKA-mediated phosphorylation of Kv7.1 requiring an NH~2~-terminal serine and countered by the effects of protein phosphatase 1 (PP1). Interestingly, modulation of Kv7.1 requires a macromolecular signaling complex of the auxiliary subunit MinK/KCNE1, PKA, PP1, and the scaffolding protein Yotiao (39). PKA-mediated modulation of Kv4.2 channels also involves the formation of a macromolecular complex, in this case requiring auxiliary KChIP3 subunits (60). It is likely that phosphorylation-dependent modulation of other KChs involves similar macromolecular complexes located at discrete membrane sites.

Kv3.1b currents are suppressed upon PKC activation in heterologous systems (15, 31). Analysis of putative phosphorylation sites using metabolic labeling with [32P]orthophosphate, PKC activation, and site-directed mutagenesis, in parallel with detailed electrophysiological analyses, led to identification of a critical COOH-terminal serine (36). Experiments with a phosphospecific antibody confirmed phosphorylation at this site, and its dramatic downregulation in auditory neurons in response to auditory stimulation. This correlates with increased Kv3.1b current that facilitates high-frequency spiking (67). This serves as a prominent example, whereby a physiologically significant modulation of a KCh mediates a neurophysiological response in vivo, akin to that originally observed in *Aplysia*, but now with the added identification of a specific phosphorylation site on a molecularly identified KCh protein. More recent studies revealed a similar activity-dependent downregulation of phosphorylation at specific sites on Kv2.1 (53).

A number of studies have focused on phosphorylation-dependent effects on biosynthetic trafficking of KChs to the plasma membrane. Activation of PKA or glucocorticoid-inducible kinase-1 increases Kir1.1 surface expression by suppressing an endoplasmic reticulum retention signal on the Kir1.1 COOH terminus (50, 77). Trafficking of two-pore Task-1 (KCNN3) KChs is also modulated by phosphorylation, in this case mediated by the phosphorylation-dependent binding of I4-3-3 proteins to suppress the function of a dibasic ER retention motif (51). Phosphorylation of...
KChs can also affect their clustering at specific plasma membrane sites. Kir2.3, like many other KCh α subunits, has a consensus PDZ binding motif at its very COOH terminus. Kinase activation prevents binding of Kir2.3 to PDZ-containing proteins like PSD-95, a response mediated through a serine within the PDZ binding motif (12). Phosphorylation of Kv2.1 also affects its clustering (44), although the precise mechanism is not known. In this regard, KCh phosphorylation serves as a potential mechanism for dynamically regulating the density of KChs at discrete plasma membrane sites.

Mass Spectrometric Approaches for the Identification of KCh Phosphorylation

A single KCh subunit can potentially be phosphorylated by multiple protein kinases on the same or different sites. These sites are further subjected to dephosphorylation by multiple protein phosphatases. As such, a comprehensive identification and quantitation of KCh phosphorylation sites is needed to fully understand physiological regulation of KCh function by phosphorylation. Conventional phosphoprotein biochemistry and mutagenesis approaches have not been adequate to provide comprehensive information as to the specific sites phosphorylated, especially when there exist multiple sites on a given subunit. These approaches also do not allow for easy quantification of changes in phosphorylation state at specific sites. Sites identified solely through mutagenesis experiments as playing a role in mediating protein kinase- or phosphatase-dependent channel regulation also have the caveat that they may act in a secondary regulatory capacity and not be the true site of covalent phosphate modification. As such, sensitive and reliable techniques for analysis of chemical phosphate on KCh proteins are critical to understanding the molecular mechanisms underlying regulation of channel function by phosphorylation.

Proteomic techniques utilizing MS have emerged as the preeminent methods to analyze complex protein samples (2, 76), including identification of phosphorylation sites. Analyses of phosphoproteins can be difficult, since, although 30% of all proteins are thought to be regulated through phosphorylation (38), the phosphorylated pool is generally of relatively low abundance in cells and is present as a mixed population with varying stoichiometries of phosphorylation at individual sites. Other proteins present in complex samples can interfere with the analysis of phosphorylation on the target protein of interest. MS is a very powerful technique for the identification of phosphorylation sites on proteins, but it has some limitations.

**FIGURE 1.** Mass spectrometric techniques for the relative quantification of protein phosphorylation states

A: strategy for SILAC analysis of regulated KCh phosphorylation sites. Cells expressing a native or recombinant KCh are grown in normal or stable isotope-labeling media for at least five cell doublings. One dish is stimulated, after which the cells are lysed, and equal amounts of lysates from unstimulated and stimulated cells are mixed. KCh protein is then purified and subjected to SDS-PAGE, and the KCh protein band is excised and digested with trypsin. The resultant tryptic peptide mixture is analyzed by LC-MS/MS.

B: strategy for quantification of KCh phosphorylation sites using iTRAQ. KCh proteins purified from different cell or tissue samples are reduced, alkylated, and digested with trypsin. Each sample is then separately labeled with one of the four iTRAQ reagents (termed 114, 115, 116, and 117). After labeling, the samples are mixed and analyzed by LC-MS/MS.

C: strategy for quantification of KCh phosphorylation sites using proteolytic 18O-labeling method. KCh proteins purified from two different cell or tissue samples are digested with trypsin, either in natural H216O or H218O, to obtain differential mass labeling. Equal amounts of cell lysates are mixed and analyzed by LC-MS/MS.
Because phosphorylation is labile and preferentially lost over peptide backbone fragmentation, the resulting spectra may not allow for unambiguous sequence assignment. Ion signals in MS corresponding to phosphopeptides are significantly suppressed in the presence of nonphosphorylated peptides, and phosphopeptides can be poorly fragmented in MS. KChs are hydrophobic membrane proteins, and their analysis by MS techniques is more difficult than the analysis of hydrophilic proteins. Although many of the difficulties arise from their hydrophobicity, the basic residues associated with transmembrane segments also prevent random protonation in MS and therefore often inhibit production of fragment ions necessary for the sequence analysis. Therefore, the transmembrane segments of KChs seldom appear in MS.

Even with these limitations, phosphorylation sites with potentially biological functions can be elucidated through MS techniques. The successful identification of phosphorylation sites usually requires prior isolation or enrichment of the target KChs before MS analysis. The most successful strategies integrate target-protein and/or phosphorylation-specific enrichment steps, or the use of various combinations of protein digestion and peptide-separation methods. The enrichment of target proteins is most readily achieved using immunopurification techniques employing KCh-specific antibodies (53, 71). A powerful approach to enrich for phosphopeptides in general is immobilized metal-affinity chromatography (IMAC), which relies on the affinity of phosphate group for metal ions (e.g., Fe$^{3+}$ or Ga$^{3+}$) bound to solid-phase supports (17, 34, 56). Titanium oxide columns have also been used for the enrichment of phosphopeptides (33, 55). Recently, the selectivity of strong cation exchange (SCX) chromatography has also been used for the isolation of phosphopeptides, as phosphate groups reduce the isoelectric point of phosphopeptides (5, 34).

Several MS techniques have been applied specifically for the detection of phosphopeptides in complex mixtures (25, 68–70). Precursor ion and neutral loss scanning are currently the methods of choice for sequencing phosphopeptides. In many cases of phosphorylation at serine or threonine residues, a neutral loss of phosphoric acid (H$_3$PO$_4$, –98 Da) or phosphate (HPO$_3$, –80 Da) by a gas-phase $\beta$-elimination reaction is observed on collision-induced dissociation (CID). Analyses of spectra for neutral loss of phosphate on CID were used in identification and quantitation of phosphorylation sites on the Kv2.1 KCh (53, 54). Phosphotyrosine residues do not typically undergo neutral loss of phosphate due to gas-phase $\beta$-elimination, and precursor ion scans targeting the immonium ion of phosphotyrosine (m/z 216.043) can instead be used (68, 69). Other techniques that have not yet been applied to KCh phosphorylation studies include tandem MS using electron capture dissociation (ECD), where only the peptide backbone is cleaved, resulting in typical c and z ions, whereas amino acid modifications, like phosphorylation, remain intact, allowing for unambiguous assignment in the phosphopeptide sequence (13, 32, 63). Electron transfer dissociation (ETD) fragments peptides by transferring an electron from a radical anion to a protonated peptide, inducing fragmentation of the peptide backbone and identification of phosphorylation sites as with ECD (9, 47).

**Quantification of KCh Phosphorylation Using Mass Spectrometry**

Recently, MS has been employed to obtain quantitative information on KCh phosphorylation at specific sites critical to understanding both their regulation and the mechanistic basis of functional modulation. MS approaches to quantify phosphorylation generally use stable isotopes to differentially label two samples with mass tags such that the samples can be mixed and analyzed simultaneously. One method that has recently been applied to studies of KCh phosphorylation (53, 54) is stable isotope labeling with amino acids in cell culture (SILAC) (Figure 1A). SILAC involves metabolic labeling of one sample with a “light” amino acid and another with a “heavy” amino acid (i.e., containing $^2$H instead of $^1$H, $^{13}$C instead of $^{12}$C, or $^{15}$N instead of $^{14}$N). Incorporation of the heavy amino acid and its mass tag into a peptide generates an obvious and predictable mass shift (e.g., 6 Da for $^{13}$C$_6$-Arg or 8 Da for $^{13}$C$_6$,$^{15}$N$_2$-Lys). The advantage of

![FIGURE 2. Schematic structure of Kv2.1](http://physiologyonline.physiology.org)
using $^{13}$C$_6$-Arg or $^{13}$C$_6$,$^{15}$N$_2$-Lys labeling, as in studies of Kv2.1 (53, 54), is that every tryptic peptide will exhibit a uniform mass difference (6 Da or 8 Da). A limitation of SILAC is the requirement for metabolic labeling for several cell divisions to completely saturate the cellular amino acid pool with label. Therefore, this method is only applicable to analysis of samples in long-term cell or organotypic culture.

Alternative methods that have not yet been applied to studies of KChs allow for stable isotopic labeling in vitro and quantification of protein phosphorylation in samples of any origin. These include isobaric tags for relative and absolute quantitation (iTRAQ) (FIGURE 1B), which consists of the covalent labeling (through an NHS ester that reacts with free NH$_2$-termini and lysine side chains) of up to four samples with isobaric (same nominal mass) tags that appear in the tandem mass spectrum at $m/z$ 114, 115, 116, and 117 but, through the presence of a carbonyl balance group, add up to the same mass and still allow for peptide identification (52, 58, 79). iTRAQ has successfully been applied in the quantification of protein phosphorylation (16, 29, 78). Proteolytic $^{18}$O-labeling (6, 45) (FIGURE 1C) involves oxygen incorporation ($^{18}$O if the digestion is performed in H$_2^{18}$O water) at the newly created COOH terminus during both trypsin-catalyzed peptide bond hydrolysis and subsequent oxygen exchange reactions. However, since either one or two carboxyl oxygens can be exchanged, variability in mass differences between labeled and unlabeled peptide pairs for multiple charged species can create problems with quantification. Unlike SILAC, iTRAQ, and proteolytic $^{18}$O-labeling techniques, which provide relative quantitation, AQUA is an absolute quantification method that involves introduction of a known amount of stable isotope-labeled synthetic peptide during proteolytic digestion (18, 40). AQUA has been used to quantitatively study phosphorylation (40), although it has not yet been applied to studies of KChs.

**Application of Mass Spectrometry to the Identification and Characterization of Phosphorylation Sites on KChs**

Recently, MS has been used in studies of phosphorylation of the Kv7.2/Kv7.3 (71) and Kv2.1 (53, 54) KChs. Recombinant Kv7.2/Kv7.3 channels purified from HEK293 cells were found by matrix-assisted laser desorption/ionization (MALDI)-MS to be phosphorylated on peptides from the S4-S5 loop, and the COOH terminus (71). Kv2.1, which has 76 serine, 36 threonine, and 13 tyrosine residues in large cytoplasmic NH$_2$- and COOH-terminal domains, is extensively phosphorylated in mammalian brain (43, 48). Kv2.1 is subject to calcineurin-mediated dephosphorylation in response to increased excitatory synaptic activity (44) and hypoxia/ischemia (43). Recombinant Kv2.1 in HEK293 cells is phosphorylated at multiple residues in the S4-S5 loop, and the COOH terminus.
also dephosphorylated after calcineurin activation in response to Ca\(^{2+}\) ionophore or muscarinic stimulation (46), affecting both Kv2.1 gating and localization.

Immunopurification of Kv2.1 from heterologous cells and from rat brain followed by liquid chromatography-tandem MS (LC-MS/MS) led to the unambiguous identification of 15 serine and 1 threonine residues as being chemically modified with phosphate (53) (FIGURE 2). SILAC was also used to quantify changes in Kv2.1 phosphorylation in response to calcineurin-dependent dephosphorylation and revealed that 7 of the 16 phosphorylation sites in Kv2.1 were dephosphorylated upon calcineurin activation (53) (FIGURE 3). SILAC was also used to analyze Kv2.1 phosphorylation in HEK293 and COS-1 cells, between which Kv2.1 exhibits significant phosphorylation-dependent differences in voltage-dependent gating and clustering (46, 48). These analyses revealed that different levels of phosphorylation at the same sites, and not the use of different sites, underlies the differences in Kv2.1 gating and clustering in the different cell backgrounds. Subsequent studies using phosphospecific antibodies targeting individual Kv2.1 phosphorylation sites provided additional quantitative information as to spatial and temporal changes in Kv2.1 phosphorylation at specific sites in response to changes in neuronal activity (42). Together, these studies emphasize the remarkable extent of KCh phosphorylation and its impact on KCh function.

In summary, although modulation of phosphorylation is a well known mechanism regulating diverse aspects of function of most types of KChs, the identification of the specific phosphorylation sites and their regulation has remained problematic. Overall, the majority of phosphorylation sites remain uncharacterized, with only a few in vivo sites being reported. Moreover, those sites identified in mutagenesis studies as being necessary for a specific functional response may reflect only a small subset of those present on native KCh proteins. Application of the MS-based approaches described above to the identification and analysis of KCh phosphorylation sites will allow for rapid advances in our understanding of their extent, nature, and regulation, and role in KCh function.

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References


