Rosalyn Johnson, PhD

Molecular mechanisms of protein phosphatase targeting to ion channels

(Excerpt)

Introduction

The modulation of ion channels by protein phosphorylation is a dynamic process that is controlled by the opposing actions of protein kinases and phosphatases. While the mechanisms by which kinases modulate ion channel activity have been examined extensively, the role of phosphatases in ion channel regulation has received less attention. Because both kinases and phosphatases have broad substrate specificities *in vitro*, and can phosphorylate and dephosphorylate numerous targets, targeting of these enzymes to distinct intracellular locations is critical to achieve signal transduction specificity *in vivo*. The association of kinases and phosphatases with targeting proteins, which specify subcellular localization and mediate unique protein-protein interactions through their targeting domains, compartmentalizes signal transduction. While in some cases these macromolecular complexes may be static, simply serving as a scaffold to bring kinases and phosphatases in close proximity to their substrates, emerging evidence indicates that the dynamic reorganization of signaling complexes is also important. This review will discuss the current state of knowledge regarding the mechanisms by which phosphatases are targeted to ion channels.

Structure and nomenclature of serine/threonine protein phosphatases

Classification of serine/threonine protein phosphatases

Protein phosphatases can be divided into three distinct families: (1) the PPP family, (2) the PPM family, and (3) the PTP family.[1] An emerging body of literature has identified a prominent role

for the PPP family of phosphatases in the regulation of ion channels, and will be the focus of this review. PP1, PP2A and PP2B are the principle members of the PPP family (although more distantly related PP4, PP5, PP6 and PP7 enzymes have been identified[2]), and are differentiated based on their substrate specificity, pharmacological properties and requirement for divalent cations. PP1 prefers the β-subunit of phosphorylase kinase as a substrate and is inhibited by nanomolar concentrations of two small peptide inhibitors, inhibitor-1 (I-1) and inhibitor-2 (I-2). PP2 phosphatases preferentially dephosphorylate the α-subunit of phosphorylase kinase and are insensitive to I-1 and I-2. PP2 phosphatases can be further divided based on their dependence on divalent cations. PP2A does not require divalent cations for its activity, whereas Ca^{2+} and Mg^{2+} regulate the activity of PP2B and PP2C, respectively. Microcystin, okadaic acid and calyculin A potently inhibit the activity of PP1 and PP2A, but have no effect on PP2B and PP2C activity.[1]

Molecular composition of the PPP family of serine/threonine protein phosphatases

PP1, PP2A and PP2B are multimeric enzyme complexes that share a high degree of sequence similarity in their catalytic domain, but differ in their association with regulatory proteins.

i) Protein phosphatase-1 (PP1)

The diverse functions of PP1 are regulated by different holoenzymes, in which the same catalytic subunit (PP1c) is associated with distinct regulatory subunits. Each regulatory subunit contains specific targeting domains that target PP1c to distinct subcellular locations and substrates.[3,4] In addition to their role in PP1c targeting, PP1c regulatory subunits may also alter the substrate specificity or phosphatase activity of PP1c.[3]

ii) Protein phosphatase-2A (PP2A)

PP2A is a heterotrimeric enzyme composed of a catalytic subunit (PP2Ac), and two regulatory subunits (A and B). The diverse functions and subcellular targeting of PP2A are

attributed to the presence of a large number of regulatory B-subunits that individually assemble with a core heterodimer consisting of PP2Ac and an A-subunit.[5] At least 20 B regulatory subunits have been identified, and are divided into four families: the PR44/B family, the PR56/61/B′ family, the PR48/59/72/130/B′′ family and the PR93/PR110/B′′′ family.[5]

iii) Protein phosphatase 2B (PP2B)

PP2B (or calcineurin), is a Ca^{2+}/calmodulin-dependent protein phosphatase.[6] The PP2B holoenzyme is a heterodimer consisting of catalytic A-subunits and regulatory B-subunits. PP2B has a much higher *in vitro* substrate specificity compared with PP1 and PP2A.[6] Besides the core catalytic region, the A-subunit contains several unique structural domains, including a B-interaction domain, a calmodulin (CaM)-binding domain, and an autoinhibitory domain. The autoinhibitory domain inhibits phosphatase activity in the absence of CaM binding. The B-subunit is a Ca^{2+}-sensor protein that contains four EF-hand motifs. When cytosolic Ca^{2+} reaches micromolar levels, Ca^{2+}-CaM promotes the formation of an activated A-B heterodimer.[6]

Protein phosphatase targeting subunits – key players in signaling specificity

Phosphatase targeting subunits have emerged as key players in signal transduction. Each targeting subunit contains a number of domains that dictate: (1) its phosphatase interacting partner; (2) its interaction with other kinases and scaffolding proteins; and (3) its targeting to a specific subcellular locations (e.g. plasma membrane versus nuclear membrane).[3]

Protein sequences that determine interactions with distinct protein phosphatase subtypes

i) PP1

The “RVXF” motif has been identified as a primary PP1c binding motif. It conforms to the consensus sequence (R/K)X_{0-1}(V/I)(P)(F/W), where X can be any residue and P refers to any residue but proline.[7] This sequence is necessary and sufficient to mediate interactions between PP1c and

its regulatory subunits[3]. The RVXF binding channel on PP1c is a hydrophobic groove remote from the catalytic core, and is formed by the top rear edges of two central β-sheets. This channel is flanked by a negatively charged region, which can accommodate several basic residues commonly found N-terminal to the RVXF sequence in many targeting subunits.[8] The requirement for this short degenerate sequence accounts for the lack of structural similarity between known PP1c targeting subunits. The available evidence suggests that the RVXF motif serves as an anchor for the initial binding of regulatory subunits to PP1c. This increases the local concentration of interacting proteins, and thereby promotes binding at secondary, lower affinity sites.[8] It is these secondary interaction sites that are believed to impart altered activity and substrate specificity on PP1c.[7,9]

ii) PP2A

Little is known about the molecular basis for the interaction of B subunits with the PP2Ac-A heterodimer, although a Huntington/elongation/A-subunit/TOR motif (HEAT) motif has been identified as important.[10] The A-subunit of PP2A is an elongated molecule that consists of 15 tandem repeats of 39 amino acids, termed HEAT motifs. Each repeat is composed of two superimposed α-helices. The stacking of these repeats within the A-subunit gives rise to a stable protein with an overall asymmetrical and elongated structure that resembles a hook.[10] Exposed hydrophobic surfaces are localized to intra-repeat turns that connect two helices of each HEAT motif, and form the sites of interaction with the PP2Ac and B-subunits. The first ten hydrophobic surfaces are important for interactions with regulatory B-subunits, while the last five hydrophobic surfaces mediate interactions with PP2Ac.[5]

iii) PP2B

Protein sequences that dictate PP2B binding to its interacting partners are less clear. In some cases, loosely defined binding determinants have been identified. For example, a binding

site for PP2B has been mapped to the C-terminal region of AKAP79/150 between residues 318 and 357.[11] This sequence has some similarity to the binding sites of other PP2B-interaction proteins, including a loosely conserved "PIXIXIT" motif (where X represents any amino acid).[11] Additional sequences are likely to contribute to PP2B interactions.[11]

Protein phosphatase targeting by A-Kinase Anchoring Proteins (AKAPs)

A kinase anchoring proteins (AKAPs) have emerged as key players in the targeting of phosphatases to ion channels, and are a group of functionally, rather than structurally, related proteins. AKAPs have the following properties in common: (1) they interact with RI or RII regulatory subunits of protein kinase A (PKA) via a conserved amphipathic helix; (2) they have unique localization signals and targeting domains that direct them to distinct subcellular locations; and (3) they possess a subset of protein-interaction domains that allow them to scaffold certain combinations of kinases and phosphatases.[12] The AKAPs identified as important for targeting phosphatases to ion channels will be discussed in later sections of this review.

Subcellular localization signals as important determinants of phosphatase targeting

The regulation of ion channels by phosphatases requires their targeting to specific subcellular locations, such as the plasma membrane or the sarcoplasmic reticular membrane. This is accomplished by a subset of membrane-specific targeting signals. AKAP15/18 is targeted to the plasma membrane via lipid modification involving both myristoylation of an N-terminal glycine residue and palmitoylation of cysteine residues[13,14] AKAP79/150 localizes to the neuronal postsynaptic membrane via three polybasic targeting regions that participate in electrostatic interactions with membrane phospholipids.[15] mAKAP contains a targeting domain consisting of three spectrin-like repeats; this domain targets mAKAP preferentially to the perinuclear membrane or the sarcoplasmic reticular membrane.[16] Subtle differences in membrane localization

signals on phosphatase targeting subunits, combined with the expression of a unique subset of protein-protein interactions motifs, are likely to affect the targeting of phosphatases to distinct membrane locations, and therefore influence signaling specificity.

Leucine zipper motifs – key players in protein phosphatase targeting to ion channels

Recently, leucine zipper (LZ) motifs have been identified as key players in kinase and phosphatase targeting to several ion channels, including ryanodine receptors (RyR2), L-type Ca^{2+} channels, and the KCNQ1/KCNE1 K^+ channel.[17-20] Because LZ motifs are present in several other ion channels, including the *Shaker* K^+ channel[21], the Ca^{2+}-activated K^+ channel SK4[22], cyclic nucleotide-gated channels[23] and inositol triphosphate receptors[24], the identification and characterization of binding sequences for kinases and phosphatases will likely have broad implications.

Leucine zipper structure

The LZ motif is a α-helical structure that forms coiled-coils. Coiled-coils are comprised of heptad repeats $(abcdefg)_n$ in which hydrophobic residues occur at positions "*a*" and "*d*" to form the hydrophobic face of the helix; "*b, c, e, f , g*" are hydrophilic residues that form the solvent-exposed part of the coiled-coil.[25] In canonical LZs, a leucine residue occupies every seventh position within four heptad repeats, allowing two LZs to come together to form the coiled-coil structure. LZs classically contain a leucine residue at position "*d*" because of its flexible side chain, although the canonical leucine residue can be replaced by an isoleucine or valine.[25] Residues occupying the "*e*" and "*g*" positions exhibit a restricted range of substitutions, and electrostatic interactions between the "*e*" and "*g*" side-chains from neighboring helices are believed to help specify binding partners.[26] Discontinuities in coiled-coils have been described, such as non-helical discontinuities, skip residues (e.g. *abcdeffg*), omissions of three residues from the heptad pattern (stutters), or omissions of four residues from the heptad pattern.[25] Such variations are also likely

to influence binding specificity. Substitution of an alanine for one or more of the "*d*" leucines or isoleucines in a LZ is sufficient to diminish the ability of the LZ to mediate protein-protein interactions.[27] Mutation at this position has been used experimentally to study the role of LZs in mediating protein-ion channel interactions.

Protein phosphatase targeting to ion channels via leucine zipper motifs

The targeting of phosphatases to ion channels via LZ motifs has been studied primarily in ion channels that participate in control of cardiac function. Three ion channels have received particular attention: RyR2, KCNQ1/KCNE1, and the L-type Ca^{2+} channel – key players in cardiac excitation-contraction (E-C) coupling.[28] Stimulation of the sympathetic nervous system (SNS) in response to exercise or stress leads to activation of β-adrenoceptors (β-ARs) in cardiac myocytes and PKA-dependent phosphorylation of numerous protein targets, including ion channels. This culminates in an increase in heart rate (associated with a decrease in action potential duration) and contractility (due to elevations in contractile Ca^{2+}). These events allow cardiac output to meet stress-induced increases in metabolic demand. The precise control of the phosphorylation state of ion channels, and therefore ion channel activity, is critical in order maintain appropriate Ca^{2+} homeostasis in the face of elevated SNS activity.[29] In this context, the coordinated action of kinases and phosphatases, compartmentalized by key adapter proteins such as AKAPs, is essential. The following sections describe a few examples of the coordinated control of ion channel activity by macromolecular complexes containing ion channels, kinases, phosphatases and targeting proteins.

i) Ryanodine receptors

Ryanodine receptors (RyRs) are found in both excitable and non-excitable cells, functioning as Ca^{2+} release channels in the sarcoplasmic reticulum (SR) or endoplasmic reticulum (ER),

respectively. RyR1 is the predominant isoform in skeletal muscle, RyR2 is predominant in the heart, and RyR3 is found primarily in smooth muscle.[30] RyR2 is a tetramer composed of four RyR2 polypeptides and four FK506-binding proteins (FKBP12.6) that bind to the cytosolic domain of RyR2. FKBP12.6, as a regulatory protein, stabilizes RyR2 channel function and facilitates coupled gating between neighboring RyRs during Ca^{2+}-induced Ca^{2+} release (CICR).[30] In the heart, Ca^{2+} release from intracellular stores represents the major source of contractile Ca^{2+}.[31]

RyRs contain a large cytosolic domain that participates in the regulation of channel gating, and also serves as a scaffold for regulatory proteins.[31] Marx et al. (2001) identified three LZ motifs in the cytosolic domain of RyR2, and subsequently determined that these domains were critical for targeting PP1, PP2A and PKA to the channel via three distinct targeting proteins. Spinophilin was found to bind the N-terminal-most LZ (LZ1) and target PP1 to RyR2, while PR130 targeted PP2A via its interaction with the second LZ (LZ2); mAKAP targeted PKA to the third LZ (LZ3) motif.[17] Single channel experiments in lipid bilayers demonstrated that cAMP-dependent regulation of RyR2 required anchoring of mAKAP/PKA to RyRs via LZ interactions; peptides containing LZ motifs that competitively disrupted mAKAP-RyR2 interactions prevented PKA-mediated phosphorylation of the channel.[17] To establish a functional role for LZ-mediated association of RyR2 with phosphatases, the authors showed that protamine-induced activation of RyR2-bound phosphatases dephosphorylated RyR2.[17] These experiments provide compelling evidence for the role of kinase and phosphatase anchoring in the regulation of RyR2 *in vitro*; future studies should examine the functional significance of this macromolecular complex in intact myocytes.

The study by Marx et al. (2001) illustrated the specificity of LZ interactions - each LZ motif on RyR interacted with a distinct targeting protein. This unique targeting is likely imparted by subtle differences in the amino acid sequence, and therefore structure, of each LZ motif. For

example, LZ3, which interacts with mAKAP, includes one "skip" residue (eight residues instead of seven between "*d*" amino acids). In contrast, a proline residue is located in the "*a*" position within the LZ motif on spinophilin.[17,32,33]

Although controversial[34], some reports have found evidence of RyR2 hyperphosphorylation in human failing hearts that is associated with defective RyR2 function.[35] This hyperphosphorylation was found to be co-incident with reduced association of PP1 and PP2A with RyR2, suggesting that dephosphorylation might be impaired in heart failure patients.[35] If correct, these data provide an important example of how phosphatase targeting to ion channels can have important clinical implications.

ii) KCNQ1-KCNE1

SNS stimulation of the heart leads to a cAMP-dependent increase in the activity of the slowly activating delayed rectifier K^+ channel (I_{Ks}).[36] This enhances the magnitude of the repolarizing current during the cardiac action potential, and provides an important counteracting force to the stimulatory effects of PKA on L-type Ca^{2+} channels.[29] Two gene products form I_{Ks}: the pore-forming α-subunit, KCNQ1, and an auxillary β-subunit, KCNE1.

The I_{Ks} channel in cardiac myocytes forms a macromolecular complex with PP1 and PKA. These interactions are coordinated by the binding of a targeting protein, Yotiao, to a LZ motif in the KCNQ1 C-terminus (residues 588-616 in human KCNQ1).[36] Both PKA and PP1 regulate the phosphorylation of an N-terminal serine residue (Ser27) in the KCNQ1 subunit.[36] Reconstitution of PKA- and PP1-mediated regulation of the KCNQ1/KCNE1 current in CHO cells requires co-expression of KCNQ1/KCNE1 and Yotiao. Furthermore, mutation of the KCNQ1 LZ motif that mediates KCNQ1's interaction with Yotiao eliminates the functional regulation of I_{Ks} currents by PKA, as well as phosphorylation at Ser27.[36]

Interestingly, an inherited long QT (LQT) syndrome mutation has been identified in Finnish families that has been mapped to the first "*e*" position (G589D) in the C-terminal LZ of KCNQ1.[37,38] In the presence of this mutation, PP1 and PKA fail to co-immunoprecipitate with KCNQ1. Therefore, abnormal phosphatase targeting may be an underlying mechanism of LQT syndrome.[36]

iii) L-type Ca^{2+} channels

PKA-dependent phosphorylation of the α_{1c}- and β_{2a}-subunits of L-type Ca^{2+} channels enhances channel open probability.[39,40] In the heart, this leads to increased Ca^{2+} influx and enhanced cardiac contractility.[29] AKAP15/18 has been identified as the anchoring protein responsible for the targeting of PKA to L-type Ca^{2+} channels in cardiac muscle ($Ca_v1.2$)[19] and skeletal muscle ($Ca_v1.1$)[20] through direct interactions with a LZ motif on the distal C-terminus of the pore-forming α_1-subunit. In intact cardiac myocytes, disruption of the LZ-mediated interaction between AKAP15 and $Ca_v1.2$ with LZ-containing peptides prevented β-AR-dependent regulation of channel activity. Therefore, there appears to be a functional significance to this interaction.[19]

Neuronal $Ca_v1.2$ channels form a macromolecular complex that contains β-ARs, adenylyl cyclase, PKA, PP2A, and an unknown molecular scaffold.[41] The site of PP2A's interaction with $Ca_v1.2$ has been mapped to the distal C-terminus of the channel, downstream of its principle phosphorylation site (Ser1928).[42] While truncation of $Ca_v1.2$'s α_1-subunit downstream of Ser1928 did not affect its phosphorylation by PKA in intact cells, dephosphorylation of this site was inhibited, indicating that PKA and PP2A are targeted to $Ca_v1.2$ via different targeting subunits.[43]

(end of excerpt)

References

1. Luan, S. Protein phosphatases in plants. *Annu Rev Plant Biol* **54**, 63-92 (2003).

2. Cohen, P.T. Novel protein serine/threonine phosphatases: variety is the spice of life. *Trends Biochem Sci* **22**, 245-51 (1997).
3. Cohen, P.T. Protein phosphatase 1--targeted in many directions. *J Cell Sci* **115**, 241-56 (2002).
4. Zolnierowicz, S. Type 2A protein phosphatase, the complex regulator of numerous signaling pathways. *Biochem Pharmacol* **60**, 1225-35 (2000).
5. Lechward, K., Awotunde, O.S., Swiatek, W. & Muszynska, G. Protein phosphatase 2A: variety of forms and diversity of functions. *Acta Biochim Pol* **48**, 921-33 (2001).
6. Klee, C.B., Ren, H. & Wang, X. Regulation of the calmodulin-stimulated protein phosphatase, calcineurin. *J Biol Chem* **273**, 13367-70 (1998).
7. Wakula, P., Beullens, M., Ceulemans, H., Stalmans, W. & Bollen, M. Degeneracy and function of the ubiquitous RVXF motif that mediates binding to protein phosphatase-1. *J Biol Chem* **278**, 18817-23 (2003).
8. Egloff, M.P. et al. Structural basis for the recognition of regulatory subunits by the catalytic subunit of protein phosphatase 1. *Embo J* **16**, 1876-87 (1997).
9. Terrak, M., Kerff, F., Langsetmo, K., Tao, T. & Dominguez, R. Structural basis of protein phosphatase 1 regulation. *Nature* **429**, 780-4 (2004).
10. Janssens, V. & Goris, J. Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. *Biochem J* **353**, 417-39 (2001).
11. Dell'Acqua, M.L., Dodge, K.L., Tavalin, S.J. & Scott, J.D. Mapping the protein phosphatase-2B anchoring site on AKAP79. Binding and inhibition of phosphatase activity are mediated by residues 315-360. *J Biol Chem* **277**, 48796-802 (2002).
12. Feliciello, A., Gottesman, M.E. & Avvedimento, E.V. The biological functions of A-kinase anchor proteins. *J Mol Biol* **308**, 99-114 (2001).
13. Trotter, K.W. et al. Alternative splicing regulates the subcellular localization of A-kinase anchoring protein 18 isoforms. *J Cell Biol* **147**, 1481-92 (1999).
14. Gray, P.C. et al. Primary structure and function of an A kinase anchoring protein associated with calcium channels. *Neuron* **20**, 1017-26 (1998).
15. Dell'Acqua, M.L., Faux, M.C., Thorburn, J., Thorburn, A. & Scott, J.D. Membrane-targeting sequences on AKAP79 bind phosphatidylinositol-4, 5-bisphosphate. *Embo J* **17**, 2246-60 (1998).
16. Kapiloff, M.S., Schillace, R.V., Westphal, A.M. & Scott, J.D. mAKAP: an A-kinase anchoring protein targeted to the nuclear membrane of differentiated myocytes. *J Cell Sci* **112 (Pt 16)**, 2725-36 (1999).
17. Marx, S.O. et al. Phosphorylation-dependent regulation of ryanodine receptors: a novel role for leucine/isoleucine zippers. *J Cell Biol* **153**, 699-708 (2001).
18. Marks, A.R., Marx, S.O. & Reiken, S. Regulation of ryanodine receptors via macromolecular complexes: a novel role for leucine/isoleucine zippers. *Trends Cardiovasc Med* **12**, 166-70 (2002).
19. Hulme, J.T., Lin, T.W., Westenbroek, R.E., Scheuer, T. & Catterall, W.A. Beta-adrenergic regulation requires direct anchoring of PKA to cardiac CaV1.2 channels via a leucine zipper interaction with A kinase-anchoring protein 15. *Proc Natl Acad Sci U S A* **100**, 13093-8 (2003).

20. Hulme, J.T., Ahn, M., Hauschka, S.D., Scheuer, T. & Catterall, W.A. A novel leucine zipper targets AKAP15 and cyclic AMP-dependent protein kinase to the C terminus of the skeletal muscle Ca2+ channel and modulates its function. *J Biol Chem* **277**, 4079-87 (2002).
21. McCormack, K. et al. A role for hydrophobic residues in the voltage-dependent gating of Shaker K+ channels. *Proc Natl Acad Sci U S A* **88**, 2931-5 (1991).
22. Joiner, W.J., Wang, L.Y., Tang, M.D. & Kaczmarek, L.K. hSK4, a member of a novel subfamily of calcium-activated potassium channels. *Proc Natl Acad Sci U S A* **94**, 11013-8 (1997).
23. Zhong, H., Molday, L.L., Molday, R.S. & Yau, K.W. The heteromeric cyclic nucleotide-gated channel adopts a 3A:1B stoichiometry. *Nature* **420**, 193-8 (2002).
24. Galvan, D.L., Borrego-Diaz, E., Perez, P.J. & Mignery, G.A. Subunit oligomerization, and topology of the inositol 1,4, 5-trisphosphate receptor. *J Biol Chem* **274**, 29483-92 (1999).
25. Lupas, A. Coiled coils: new structures and new functions. *Trends Biochem Sci* **21**, 375-82 (1996).
26. Walshaw, J. & Woolfson, D.N. Open-and-shut cases in coiled-coil assembly: alpha-sheets and alpha-cylinders. *Protein Sci* **10**, 668-73 (2001).
27. Simmerman, H.K., Kobayashi, Y.M., Autry, J.M. & Jones, L.R. A leucine zipper stabilizes the pentameric membrane domain of phospholamban and forms a coiled-coil pore structure. *J Biol Chem* **271**, 5941-6 (1996).
28. Hulme, J.T., Scheuer, T. & Catterall, W.A. Regulation of cardiac ion channels by signaling complexes: role of modified leucine zipper motifs. *J Mol Cell Cardiol* **37**, 625-31 (2004).
29. Bers, D.M. & Guo, T. Calcium signaling in cardiac ventricular myocytes. *Ann N Y Acad Sci* **1047**, 86-98 (2005).
30. Bers, D.M. Macromolecular complexes regulating cardiac ryanodine receptor function. *J Mol Cell Cardiol* **37**, 417-29 (2004).
31. Fill, M. & Copello, J.A. Ryanodine receptor calcium release channels. *Physiol Rev* **82**, 893-922 (2002).
32. Moitra, J., Szilak, L., Krylov, D. & Vinson, C. Leucine is the most stabilizing aliphatic amino acid in the d position of a dimeric leucine zipper coiled coil. *Biochemistry* **36**, 12567-73 (1997).
33. Sarrouilhe, D., di Tommaso, A., Metaye, T. & Ladeveze, V. Spinophilin: from partners to functions. *Biochimie* (2006).
34. Xiao, B. et al. Characterization of a novel PKA phosphorylation site, serine-2030, reveals no PKA hyperphosphorylation of the cardiac ryanodine receptor in canine heart failure. *Circ Res* **96**, 847-55 (2005).
35. Marx, S.O. et al. PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* **101**, 365-76 (2000).
36. Marx, S.O. et al. Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. *Science* **295**, 496-9 (2002).
37. Piippo, K. et al. A founder mutation of the potassium channel KCNQ1 in long QT syndrome: implications for estimation of disease prevalence and molecular diagnostics. *J Am Coll Cardiol* **37**, 562-8 (2001).
38. Kurokawa, J., Chen, L. & Kass, R.S. Requirement of subunit expression for cAMP-mediated regulation of a heart potassium channel. *Proc Natl Acad Sci U S A* **100**, 2122-7 (2003).

39. De Jongh, K.S. et al. Specific phosphorylation of a site in the full-length form of the alpha 1 subunit of the cardiac L-type calcium channel by adenosine 3',5'-cyclic monophosphate-dependent protein kinase. *Biochemistry* **35**, 10392-402 (1996).
40. Gerhardstein, B.L., Puri, T.S., Chien, A.J. & Hosey, M.M. Identification of the sites phosphorylated by cyclic AMP-dependent protein kinase on the beta 2 subunit of L-type voltage-dependent calcium channels. *Biochemistry* **38**, 10361-70 (1999).
41. Davare, M.A. et al. A beta2 adrenergic receptor signaling complex assembled with the Ca2+ channel Cav1.2. *Science* **293**, 98-101 (2001).
42. Gao, T. et al. cAMP-dependent regulation of cardiac L-type Ca2+ channels requires membrane targeting of PKA and phosphorylation of channel subunits. *Neuron* **19**, 185-96 (1997).
43. Hall, D.D. et al. Binding of protein phosphatase 2A to the L-type calcium channel Cav1.2 next to Ser1928, its main PKA site, is critical for Ser1928 dephosphorylation. *Biochemistry* **45**, 3448-59 (2006).