Published in final edited form as:
Curr Pharm Biotechnol. 2011 April 1; 12(4): 621–635.

Regulation of ABC Transporter Function Via Phosphorylation by Protein Kinases

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Abstract

ATP-binding cassette (ABC) transporters are multispanning membrane proteins that utilize ATP to move a broad range of substrates across cellular membranes. ABC transporters are involved in a number of human disorders and diseases [1]. Overexpression of a subset of the transporters has been closely linked to multidrug resistance in both bacteria and viruses and in cancer. A poorly understood and important aspect of ABC transporter biology is the role of phosphorylation as a mechanism to regulate transporter function. In this review, we summarize the current literature addressing the role of phosphorylation in regulating ABC transporter function. A comprehensive list of all the phosphorylation sites that have been identified for the human ABC transporters is presented, and we discuss the role of individual kinases in regulating transporter function. We address the potential pitfalls and difficulties associated with identifying phosphorylation sites and the corresponding kinase(s), and we discuss novel techniques that may circumvent these problems. We conclude by providing a brief perspective on studying ABC transporter phosphorylation.

Keywords

ABC transporter; CK2; kinase; LC/MS; PKA; PKC; phosphorylation; regulation

1. INTRODUCTION

ATP-Binding Cassette (ABC) transporters are found in species ranging from bacteria to man [2]. ABC transporters are involved in a variety of human health related problems and diseases (Table 1). The transporters are found in a number of cellular organelles, including the mitochondria, vacuole/lysosome, and plasma membrane, and are responsible for the transport of chemical compounds across cellular membranes in a nucleotide dependent manner [1–4]. The ABC transporters have been divided into 5 subfamilies according to sequence similarity of their nucleotide binding domains (NBDs): ABCA-ABCD and ABCG (ABCE and ABCF are not transporters, have no membrane spans, and are localized to the cytosol) [1,3]. In general, the ABC transporters structurally consist of an “ABC Core” which contains two membrane spanning domains (MSDs or TMDs) and two NBDs that are connected by intracellular loops (Fig. 1) [1,2]. However, transporters can exist as heterodimers of “Half” transporters or “Long” ABC transporters, which contain an additional N-terminal extension (NTE) (Fig. 1) [1,4–6].

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CONFLICT OF INTEREST
There are no conflicts of interest for the authors of this paper.

Although much is known about the function of the ABC transporters, post-translational regulation of transporter function has remained relatively uncharacterized until recently. A significant problem associated with identifying proteins that regulate ABC transporter function is the detection of interacting proteins. For example, identifying an interacting kinase(s) and the corresponding post-translational modification(s) is difficult [4,7]. The difficulty arises from the fact that ABC transporters are tethered to the membrane but site-specific or truncation mutants often misfold or un- fold and as a result traffic incorrectly [4]. The development and improvement of a number of techniques including the membrane yeast-two hybrid (MYTH) assay and proteomic methods, such as co-immunoprecipitation-affinity chromatography followed by liquid chromatography-mass spectrometry (LC/MS) proteomic analysis, over the last decade has greatly reduced this barrier [4]. Many phosphorylation sites within ABC transporters have been identified as a result of these improvements. In many cases, the corresponding kinase(s) has also been identified [4,7]. This review will focus on regulation of ABC transporter function via phosphorylation, the kinases that are involved, and the current and future technologies that are and will be available to identify new kinases that phosphorylate the ABC transporters as well as their corresponding phosphorylation sites.

2. PHOSPHORYLATION AS A MECHANISM OF ABC TRANSPORTER REGULATION

Phosphorylation is one of the most common mechanisms of post-translational protein regulation in the cell [8]. A broad range of cellular proteins are regulated via kinase-mediated phosphorylation. Phosphorylation occurs under greatly varying conditions in response to multiple stimuli [9,10]. Phosphorylation is critically important for the regulation of a number of cellular processes including transcription, ubiquitination, protein degradation, protein subcellular localization, and, most notably, protein function [10]. The availability of ATP as a donor and the ease of reversibility of phosphorylation are likely the reasons why the cell has so extensively adopted phosphorylation as a mechanism to regulate so many cellular processes [8]. Therefore, it is not surprising that abnormal phosphorylation is associated with many human diseases and conditions (misregulation of a number of kinases is strongly associated with multidrug resistance (MDR) in cancer) [9,10]. Many diseases occur as the result of mutated phosphorylation sites [8]. It is important to note that many toxins and pathogens exert their effects by altering cellular phosphorylation [8]. One such example of a phosphorylation-altering toxin is microcystin, which is effluxed by the green algae that produces it via the mycH ABC transporter. This toxin inhibits Type 1 and 2A protein phosphatases. Importantly, microcystin is a substrate for P-gp (ABCB1) in fish and is believed to regulate the expression and possibly the function of fish P-gp via inhibition of protein phosphatases [11–13].

A critical gap in our understanding of ABC transporter biology is the mechanism by which the transporters are regulated at the post-translational level [4]. A number of studies have identified phosphorylation as a mechanism of transporter regulation. Phosphorylation sites have been identified in members of almost every ABC transporter subfamily from yeast to man. It is reasonable to believe that all the transporters are regulated by phosphorylation and a variety of other post-translational modifications to some extent. Regulation of ABC transporter function by phosphorylation provides cells with a simple, low energy, fast, and efficient way to change transporter function. Other modifications that may regulate ABC transporter function or expression, such as sumoylation or ubiquitination, require more energy and are less efficient. A comprehensive list of phosphorylation sites identified by LC/MS for the human ABC transporters is provided in Table 2. Below we will discuss the role of phosphorylation as a mechanism to regulate the function of each ABC subfamily. We

will conclude this review with a prospectus for how modern proteomics will aid in identifying the post-translational regulators and post-translational modifications of the ABC transporter family.

3. ABC TRANSPORTER SUBFAMILIES; CONSEQUENCES OF THEIR PHOSPHORYLATION

3.1. ABCA

The ABCA subfamilies of proteins are involved in a variety of cellular functions and are associated with a number of human diseases [1,2]. Most notable is the role of ABCA1 in cholesterol efflux and Tangier disease. ABCA4 is critically important for the efflux of the retinol-AC conjugate from retinal pigment epithelium cells. Mutations in ABCA4 result in Stargardt disease, which is similar to macular degeneration [1,2]. Of these two transporters, ABCA1 has been the most extensively studied. For many years protein kinase A (PKA) was known to play an important role in regulating cholesterol efflux; however, the mechanism for this regulation was unknown. In 2002 PKA-regulated cholesterol efflux was finally shown to be the result of direct phosphorylation of ABCA1[14]. The study identified two PKA phosphorylation sites at serine (Ser)1042 within NBD1 and the homologous site, Ser2054, within NBD2 (it is thought that the NBDs share significant homology due to gene duplication) [14]. Although both sites are PKA phosphorylation sites *in vitro*, mutation analysis *in vivo* strongly suggests that Ser2054 is the dominant phosphorylation site [14]. Phosphorylation of Ser2054 is constitutive and required for full transporter activity. Mutation of Ser2054 to alanine (Ala) results in a 50% decrease in ABCA1-mediated cholesterol efflux *in vitro* [14]. In 2004, Roosbeek *et. al.* published biochemical evidence for phosphorylation of the CK2 consensus sites at threonine (Thr)1242, Thr1243, and Ser1255 within the “R-like” domain (Fig. 1), a shorter version of the ABCC7 R domain found between NBD1 to MSD2 [15]. Mutation of these sites individually to alanine (Ala) results in a partial loss of protein function [15]. Further, treatment of cells with a CK2 inhibitor resulted in decreased phosphorylation of recombinant NBD1-R1 (from ABCA1), which supports the initial finding that Thr1242, Thr1243, and Ser1255 are phosphorylated directly by CK2 [15].

Importantly, phosphorylation of ABCA1 plays a role in protein stability [16,17]. Martinez *et. al.* showed that phosphorylation of ABCA1 at Thr1286 and Thr1305, within the “R-like” domain, promotes calpain-mediated ABCA1 degradation [16]. Mutation of both sites to Ala resulted in a 3.1-fold increase in cell surface expression and a 2.3-fold increase in cholesterol efflux as compared to wild type (WT) [16]. Further, Yamauchi *et al.* provided evidence suggesting that ABCA1 is stabilized through a protein kinase Cα(PKCα)-dependent phosphorylation mechanism [17]. Together, these studies suggest a role for phosphorylation in the regulation of ABCA1 protein activity and stabilization/degradation.

3.2. ABCB

The ABCB subfamily of ABC transporters is a structurally and functionally diverse group of proteins that is conserved in all mammals (reviewed elsewhere) [1,2,18]. Members of the ABCB subfamily have been directly linked to multiple diseases including cholestasis, immune deficiency, and sideroblastic anemia, and MDR in cancer [19]. The most recognized of the ABCB subfamily is probably ABCB1, which is more commonly referred to as MDR1 or p-glycoprotein, followed by ABCB2 and ABCB3, the transporter-associated with antigen presenting proteins (TAPs) [1,3,19].

3.2.1. ABCB1—Overexpression of ABCB1 is strongly associated with the multidrug resistance (MDR) phenotype in a broad range of cancers [1,20]. Although the role of

ABCB1 in cancer has been extensively studied, very little is known about the role of ABCB1 in normal cellular metabolism and cell protection from environmental stress. In addition, post-translational regulation of ABCB1 function is poorly understood. Interestingly, a large number of studies have suggested that ABCB1 is phosphorylated *in vivo*; however, it is still unclear what role phosphorylation plays in regulating ABCB1 function [8,9,20–43]. In 1987, Mellado and Horwitz published the first evidence suggesting that ABCB1 was phosphorylated [38]. Their work showed that phosphorylation of ABCB1 increased when cells were treated with cAMP. Treatment of partially purified ABCB1 with recombinant PKA (i.e., the catalytic subunit) results in increased phosphorylation of ABCB1 *in vitro* [38]. It is now apparent that ABCB1 is likely phosphorylated by a number of kinases, including PKC and PKA. A number of conflicting studies have been published as to the role of phosphorylation in regulating ABCB1 function and yet no clear consensus has been reached [21–25,29,31–32,36,41,42].

Interestingly, it is important to note that a large number of studies have shown that many of the known inhibitors of PKA, PKC, and many other kinases are both substrates and/or inhibitors of ABCB1 function [44–48]. There is increasing evidence that the same is true for some of the ABCC and ABCG subfamilies of transporters. The role of the kinase inhibitors as substrates and inhibitors of the ABC transporters is extremely important and critical to evaluating the effectiveness of kinase inhibitor use in the clinic [44–48]. Although of extreme importance, the role of kinase inhibitors as substrates and inhibitors of the ABC transporters is not covered in this review. Excellent reviews on this subject can be found elsewhere [44–48].

A number of studies support a role for phosphorylation in the regulation of ABCB1 function [21,22,25,30,31,34,36,39–42]. Overwhelming evidence suggests that PKC is a major player in ABCB1 phosphorylation and regulation [22–25,30–32,37,39,40,42,43]. *Ex vivo* purification of ABCB1 followed by tryptic digestion and peptide sequencing via Edman degradation identified that human ABCB1 is phosphorylated at putative PKC phosphorylation sites: serine 661, 667, and 671 [24,39]. Supporting these findings, *in vitro* kinase assays performed on small peptides derived from the “R-like” domain of ABCB1 identified serine 661, 667, and 671 as potential *in vivo* PKC phosphorylation sites [23,27,28]. PKC-dependent phosphorylation of ABCB1 is stimulated by the PKC activator 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and okadaic acid [24] and inhibited by sphingosine stereoisomers [40]. Work by Idriss *et. al.* examining ABCB1 function in purified vesicles from sf9 cells suggests that phosphorylation within the “R-like” domain is specific for PKCα and not PKCε [31]. Further phosphorylation within the “R-like” domain regulates ABCB1 ATPase activity [31,42]. PKCα-mediated phosphorylation appears to regulate ABCB1-dependent efflux of anions, which suggests a role for ABCB1 in Cl^- channel regulation [30–32,42–43]. Interestingly, mutation of all the possible PKC phosphorylation sites within the “R-like” domain of ABCB1 does not alter ABCB1 function [29]. However, the results of this study must be taken with caution as the function of the mutants was assessed in yeast and not in mammalian cells [29]. It is not uncommon to find that studies performed in yeast with mammalian homologues differ in their results as compared with the homologous studies carried out in human/mammalian cells [49–51].

In addition to PKC, evidence suggests that PKA and CK2 phosphorylate ABCB1 and regulate ABCB1 function [24,27–28,39]. PKA phosphorylates serine 667, 671, and 683 in human ABCB1 *in vitro* and *in vivo* [24,28,39]. These studies suggest that serine 667 and 671 are substrates for both PKC and PKA. CK2 phosphorylates up to five sites of ABCB1 within a small tryptic fragment (631–658) within the “R-like” domain similar to PKC and PKA [27]. Interestingly, GTP can stimulate ABCB1 phosphorylation and the intracellular ATP/GTP ratio has a clear effect on ABCB1 phosphorylation status [33,35]. In conclusion,

ABCB1 function is regulated by phosphorylation and this regulation is quite dynamic due to the involvement of multiple kinases and multiple phosphorylation sites [34].

3.2.2. ABCB2 and ABCB3 (TAP1 and TAP2)—ABCB2 and ABCB3 are commonly referred to as the ATP-binding cassette transporters associated with antigen processing or TAPs [1,2]. ABCB2 and ABCB3 are required for the transport of antigenic peptides from the cytosol into the lumen of the ER for the assembly of histocompatibility antigen complex class I [1,2]. Heterodimerization is required for ABCB2 and ABCB3 to carry-out their cellular function [1,2]. Li *et al.* showed that a 43-kDa kinase can be found associated with the ABCB2/3 complex [52]. ABCB2/3 transport activity is inhibited by phosphorylation [52]. However, with such limited number of studies analyzing the role of phosphorylation on ABCB2/3 function, no significant conclusions can be made.

3.3. ABCC

The ABCC transporters are distinct from other ABC transporters by two unique features: 1) many transport glutathione conjugates (GS-X) or cotransport glutathione (GSH) and a substrate; and 2) the subfamily has an additional N-terminal extension beyond the “ABC Core” (Fig. 1) [2,53–55]. The N-terminal extension comes in two forms, a long form (comprising 5 additional membrane spans and a cytosolic loop) and a short form (comprising a very large N-terminal cytosolic domain of about 300 amino acids) (Fig. 1) [2,53,54]. The ABCC subfamily is one of the largest ABC transporter subfamilies. The ABCCs are involved in a variety of human diseases including pseudoxanthoma elasticum, cystic fibrosis, and MDR. ABCC7, more commonly referred to as the cystic fibrosis conductance regulator (CFTR) [2], is the most characterized of all the ABC transporters with respect to phosphorylation. Phosphorylation of the ABCC subfamily is discussed below.

3.3.1. ABCC2—ABCC2 is the gene that is responsible for Dubin-Johnson Syndrome (DJS) [1,2]. One of the major roles of ABCC2 in humans is thought to be the transport of conjugated bilirubin across the plasma membrane and into the canacular space where it can be removed from the body via hepatobiliary excretion 1,2]. Mutations that render ABCC2 non-functional prohibit the efflux of conjugated bilirubin from the liver. The loss of ABCC2 function or a reduction in transporter function results in the intracellular accumulation of both conjugated bilirubin (glucuronide-bilirubin) and unconjugated bilirubin in the liver [56,57]. Therefore, one mechanism of potential treatment of DJS patients would be to upregulate ABCC2 function, and hence, a critical understanding of ABCC2 regulation is required. To date, only three papers have reported on the posttranslational modification of ABCC2 [58–60]. Of these three reports, only one provides significant evidence to suggest a role for phosphorylation in ABCC2 regulation [60]. Although further investigation is needed, the initial studies described here suggest that patients with Dubin Johnsons Syndrome may benefit from treatment with kinase inhibitors.

3.3.2. ABCC7—ABCC7, or CFTR as it is more commonly known, is the Cl-channel that is responsible for cystic fibrosis when mutated [18,61]. ABCC7 is a short ABCC similar to ABCC4 and 5 (Fig. 1). Mutations in ABCC7, most notably a deletion of phenylalanine (Phe)508, result in misfolding of the protein and retention in the endoplasmic reticulum (ER) [18,61]. Of all the members of the ABC transporter superfamily, ABCC7 regulation via kinase-mediated phosphorylation is the most extensively characterized (see the list of identified phosphorylation sites in Table 2). The first evidence of ABCC7 regulation via phosphorylation was by Tabcharani *et. al.* and Picciotto *et. al.* in 1991 [62–64]. Their studies suggested that ABCC7 is regulated by PKA and PKC in response to cyclic AMP (cAMP) [62–64]. It has now been almost 20 years since this initial discovery and only very recent

work has begun to shed light on the mechanism by which phosphorylation regulates ABCC7 function. ABCC7 phosphorylation and regulation have been extensively reviewed elsewhere [40,65–71], and thus we will briefly summarize the findings and discuss them in the context of the larger ABC transporter superfamily.

A review of the literature suggests that ABCC7 is extensively phosphorylated throughout the R domain, a cytosolic region of the protein that links the NBD1 to MSD2 see Table 2 and Fig. (1). ABCC7 function appears to be regulated by phosphorylation within NBD1 by PKC [72–74], and by extensive PKA- and PKC-mediated phosphorylation within the R domain [64,72,75–82]. In addition, more recent work suggests that ABCC7 function is regulated to a lesser extent by AMPK- and CK2-mediated phosphorylation within the R domain, and dephosphorylation by protein phosphatases [62,83–87].

The R domain of ABCC7 is rich in putative PKA phosphorylation sites [62–64,66,67,71]. In general, individual or combined mutation of PKA sites results in decreased transporter function [62–64,66,67,69,71,72,76,77,80,88–90]. The stimulatory effect of PKA on ABCC7 via phosphorylation is additive with a maximal induction of ABCC7 transporter function around 3-fold over that of the unphosphorylated protein [66,67,71]. Importantly, detailed analysis of the phosphorylation sites relative to each other suggests that phosphorylation at any one site is directly and indirectly affected by phosphorylation at another phosphorylation site(s) within ABCC7 [66,67,71]. PKA has been shown to require AKAP (Ezrin) to physically interact with ABCC7 and inhibition of this interaction blocks PKA phosphorylation of the R domain [82]. This finding suggests that phosphorylation of ABCC7 by PKA is regulated by AKAP.

Similar to PKA, ABCC7 has multiple PKC consensus sites within the R domain [62–64,66,67,71]. Regulation of ABCC7 function via PKC-mediated phosphorylation within the R domain is influenced by PKA- and PKC-mediated phosphorylation at other, non-R domain localized phosphorylation sites [66,67,71]. Therefore, PKA- and PKC-mediated ABCC7 regulation is extremely complex and involves multiple phosphorylation sites [66,67,71]. In the future, it will be very important to analyze the phosphorylation status of ABCC7 under various cellular conditions.

Recent studies shed new light on the mechanism by which phosphorylation regulates ABCC7 function through the R domain [70,78,91,92]. A combination of NMR, computational, and three dimensional cryomicroscopy studies indicate that phosphorylation of the R domain results in conformational changes that alter the function of ABCC7 [68,70,78,92]. The computational studies suggest that the change afforded by phosphorylation pushes the R domain away from the core and NBDs which results in an increase in the apparent size/radius of the protein [68]. This computational model is further supported by three dimensional cryomicroscopy studies which identified that in the absence of phosphorylation of the R domain, the NBDs and the core are more compact in shape and structure [68,70,92]. The culmination of these findings was recently published by Kanelis *et al.* In this study, the authors demonstrate by NMR that the unphosphorylated R domain of ABCC7 folds into the NBDs and the core. Phosphorylation of the R domain inhibits these interaction [92]. Together these studies suggest a model in which phosphorylation of the R domain prevents “compacting” of the ABCC7 protein and opens the core to accept ions for transport [92]. These findings provide important insight into the overall role of phosphorylation in the regulation of all ABC transporters. Although the extensive cytosolic loop in ABCC7 that connects NBD1 to MSD0 is not strictly conserved in other ABC transporters, a similar structure is found in every member of the ABC transporter superfamily [2,19].

3.3.3. ABCC8/9—ABCC8 and ABCC9, more commonly called sulfonyl urea receptors 1 and 2 (SUR1 and SUR2), are "Long" ABCC transporters similar to ABCC1 and ABCC2 [18,19,93]. Unlike other members of the ABCC subfamily, ABCC8 and ABCC9 are part of a multiprotein complex containing multiple subunits of the potassium inward rectifier protein, Kir6. 2. This large protein complex forms classical K_{ATP} channels [94]. Mutations in ABCC8, ABCC9, and Kir6.2 result in familial persistent hyperinsulinemic hypoglycemia (PHIP) [18,19,93,94] and appear to play an important role in cardiomyocyte function [95]. Similar to many other cellular ion channels, the K_{ATP} channels are highly regulated at multiple levels including post-translationally by phosphorylation [96–102].

Multiple studies suggest that ABCC8 is regulated by phosphorylation. One such study reports that ABCC8 (SUR1) is phosphorylated at Ser1571 in a PKA-dependent manner [96]. Phosphorylation of ABCC8 by PKA regulates the basal state and function of the K_{ATP} channels, including burst duration and interburst intervals[96]. Interestingly, the same study identified a PKA phosphorylation site at Ser372 in Kir6.2 and demonstrated that this site induces K_{ATP} channel activity *in vivo* [96]. This work is supported by Lin *et. al.* which identified a second phosphorylation site within Kir6.2 at Thr224 [98]. In addition to PKA regulation, ABCC8 may be regulated by PKC [100]. Work by Ribalet *et. al.* suggests that phosphorylation of both Kir6.2 and ABCC8 is required for proper function of the K_{ATP} channel [100]. To this end, cells expressing functional K_{ATP} channels were inhibited by specific PKC inhibitors [100].

Similar to ABCC8, ABCC9 (SUR2) is phosphorylated by PKA [99,101–103] at Thr633, Ser1387, and Ser1465 [99,103]. Phosphorylation of ABCC9 by PKA induces activation of the K_{ATP} channel [99,103]. This work, together with the studies described above for ABCC8, suggests a role for multisite phosphorylation as a complex mechanism for regulating K_{ATP} channel activity [99]. A challenge to studying the mechanism by which phosphorylation regulates ABCC8 and ABCC9 is determining how their interaction with Kir6.2 is affected. Therefore, it will be interesting to see if recent advances in phosphoproteomics and structural biology will lead to new insights into the role of phosphorylation in regulating K_{ATP} channel activity.

3.4. ABCD

The ABCD subfamilies of ABC transporters are located within the membrane of the peroxisome [1,2]. There are four members of this subfamily, ABCD1–4 [1,2]. Mutations within ABCD1 are responsible for the human disease, adrenoleukodystrophy [1,2]. To date, only one study has reported that members of the ABCD subfamily are regulated by phosphorylation [104]. Tanaka *et. al.* reported that ABCD1 and ABCD3 are phosphorylated *in vivo* and this phosphorylation appears to alter transporter function [104]. Unlike many of the other ABC transporters, which are phosphorylated by Ser/Thr-specific kinases, ABCD1 and ABCD3 appear to be phosphorylated by a tyrosine kinase [104]. In conclusion, it is reasonable to believe that ABCD1 and ABCD3 are regulated via phosphorylation; however, a considerable amount of work remains before any substantial conclusions can be drawn.

3.5. ABCG

The ABCG subfamily of transporters is an extremely diverse subfamily of transporters comprised of five half transporters (ABCG 1,2,4,5, and 8) [2]. The ABCG subfamily includes ABCG2 (BCRP), which is has been strongly associated with drug resistance in cancer, and ABCG5 and ABCG8, which have been shown to be responsible for sitosterolemia [2]. Similar to the ABCD subfamily, only one report to date has identified phosphorylation as a potential regulatory mechanism in this subfamily [105]. Xie *et. al.* reported that phosphorylation of ABCG2 is required for proper function [105]. Their work

suggests that the kinase Pim-1 phosphorylates ABCG2 at Thr362 and that this phosphorylation modulates dimerization of the ABCG2 molecules, which is a requirement for proper function [105]. Mutation of Thr362 to alanine results in cytoplasmic compartmentalization of ABCG2 thereby inhibiting proper localization at the plasma membrane and protein dimerization [105]. Further work is necessary to determine the overall extent to which ABCG2 is phosphorylated.

4. REGULATION OF NON-MAMMALIAN ABC TRANSPORTERS: A BRIEF OVERVIEW

ABC transporters are found in all organisms from bacteria to man [2,18]. The role of ABC transporters in microorganisms has been associated with the efflux of a number of compounds and ions including salt ions, sugars, lipids, and toxins [106–114]. In bacteria and yeast, ABC transporters are associated with multidrug resistance and increased organism virulence [106–114]. Therefore, a better understanding of ABC transporter biology and biochemistry is of critical importance in order to improve treatment of microorganism-associated diseases and infections. In addition, microorganisms, such as the yeast *Saccharomyces cerevisiae,* are excellent models for the study of ABC transporter function and regulation [4,115,116]. In recent years, high throughput genomic and protein interaction studies involving yeast ABC transporters have proven extremely useful in identifying new regulatory pathways including those involving phosphorylation [4,115,116]. Recent advances in phosphoproteomics of the whole yeast proteome have been extremely useful in identifying a number of phosphorylation sites within the ABC transporters [110,117–120]. Together, these approaches have provens the utility and importance of yeast and other microorganisms as model systems to characterize ABC transporter function.

To date a number of studies in yeast and bacteria have identified mechanisms by which ABC transporter function is regulated by phosphorylation [121–129]. In yeast, the ABC transporters that have been suggested to be regulated by phosphorylation include Ste6p, Ycf1p, Pdr5p, and Cdr1p [122–127,129]. Phosphorylation of Ste6p, a protein required for yeast mating, appears to play a critical role in regulating protein localization and recycling [122,123]. The activity of Pdr5p is regulated by Sit4p-mediated phosphorylation [126]. Similarly, Ycf1p function is regulated by the yeast casein kinase 2 alpha protein, Cka1p [124]. Phosphorylation has been suggested to play a dual role for Cdr1p [129] by altering the activity and stability of the transporter [129]. Similar studies can be found for bacterial ABC transporters [121,128]. *Mycobacterium tuberculosis* pathogenicity in mice requires the ABC transporter, Rv1747 [121,128]. Here pathogenicity associated with Rv1747 is regulated, in part, via a Ser/Thr kinase [121,128]. Further insight into the mechanisms regulating ABC transporter phosphorylation in microorganisms may allow for the development of novel kinase inhibitor-based therapeutics for the treatment of microorganismal-associated diseases and infections.

5. PHOSPHOPROTEOMICS: PROSPECTUS WITH RESPECT TO ABC'S

With the increasing development of new phosphopeptide enrichment techniques and the increasing sensitivity of mass spectrometers, the identification of phosphorylation sites on proteins has become quite routine [130]. With the ever increasing number of high quality proteomic cores being established, liquid chromatography-mass spectroscopy (LC/MS) has quickly become the technique of choice [130]. Unlike many of the traditional techniques used to identify phosphorylation of a protein, such as two dimensional gel electrophoresis (2D SDS-PAGE) analysis of [^{32}P]-labeled protein lysates, LC/MS does not require radioactivity, it is more sensitive, and has the ability to identify multiple phosphorylation sites in multiple samples during a single run. This allows for a direct comparison of the

phosphorylation status of a single protein from two separate samples. LC/MS in this manner utilizes a technique called stable isotope labeling of amino acids in tissue culture or SILAC [130].

One of the most important developments in recent years may be the use of immobilized metal affinity chromatography (IMAC) to greatly enrich phosphopeptides from whole-cell trypsinized lysate protein preparations [131]. Currently, ultra high performance liquid chromatography (UHPLC) and nano-HPLC MS/MS analysis in combination with various dissociative MS techniques (ECD and ETD) have increased detection sensitivity well beyond what was capable even a year ago [131]. Improvements in all these areas will increase our ability to detect phosphorylation sites within the ABC transporters, a development that will be essential for understanding the complex regulation of ABC transporter function via multisite phosphorylation.

Although the development of highly sensitive phosphoproteomic detection techniques has vastly improved our ability to identify sites of phosphorylation, it remains extremely difficult to identify phosphorylation sites within membrane bound proteins [7]. As with the study of membrane proteins in general, a great limitation to their biochemical analysis is our ability to enrich them [7]. Therefore, it has become critically important to consider one or multiple enrichment steps [7]. To use LC/MS phosphoproteomics successfully to identify ABC transporter phosphorylation sites, multiple techniques have been used. These techniques include both classical and modern methodologies such as density gradient centrifugation, co-immunoprecipitation (possibly by biotinylation), and purification from cell debris with various detergents [7]. Other techniques that will continue to play an important role in the identification of kinases and kinase-transporter interactions are cross-linking technologies and the modified yeast two hybrid assay (iMYTH). “Cell-shaving” may provide unique insights into the status of the externally oriented loops of the ABC transporters [4,7]. These techniques are extensively reviewed by Cordwell *et. al.* [7].

In conclusion, advanced tools are available to further address how phosphorylation regulates ABC transporter function. More detailed analysis of this type will provide useful insight into not only the cellular function of ABC transporters but also into how these proteins are regulated. Ultimately, the identification of kinases that regulate transporter function may aid in the development of novel kinase inhibitor-based therapies that will more effectively treat ABC transporter-related diseases.

Acknowledgments

CMP is supported by NIH P20RR020171. The authors thank Dr. Mary Vore for her support and her effort in the review of this manuscript. The authors would also like to thank Dr. Zhe-Sheng Chen for the opportunity to submit this manuscript into this review issue of Current Pharmaceutical Biotechnology.

LIST OF ABBREVIATIONS

ABC	ATP-binding Cassette
BCRP	Breast Cancer Related Protein
CFTR	Cystic Fibrosis Transductance Regulator
IMAC	Ion Metal Affinity Chromatography
LC/MS	Liquid Chromatography/Mass Spectrometry
MRP	Multidrug Resistance-associated Protein

P-gp	P-Glycoprotein
TAP	Tandem Affinity Purification
UHPLC	Ultra High Performance Liquid Chromatography
ECD	Electron Capture Dissociation
ETD	Electron Transfer Dissociation

References

1. Gottesman MM, Ambudkar SV. Overview: ABC transporters and human disease. J Bioenerg Biomembr. 2001; 33:453–458. [PubMed: 11804186]
2. Dean M, Allikmets R. Evolution of ATP-binding cassette transporter genes. Curr Opin Genet Dev. 1995; 5:779–785. [PubMed: 8745077]
3. Dean M. ABC transporters, drug resistance, and cancer stem cells. J Mammary Gland Biol Neoplasia. 2009; 14:3–9. [PubMed: 19224345]
4. Paumi CM, Chuk M, Snider J, Stagljar I, Michaelis S. ABC transporters in Saccharomyces cerevisiae and their interactors: new technology advances the biology of the ABCC (MRP) subfamily. Microbiol Mol Biol Rev. 2009; 73:577–593. [PubMed: 19946134]
5. Haimeur A, Conseil G, Deeley RG, Cole SP. The MRP-related and BCRP/ABCG2 multidrug resistance proteins: biology, substrate specificity and regulation. Curr Drug Metab. 2004; 5:21–53. [PubMed: 14965249]
6. Taglicht D, Michaelis S. Saccharomyces cerevisiae ABC proteins and their relevance to human health and disease. Methods Enzymol. 1998; 292:130–162. [PubMed: 9711551]
7. Cordwell SJ, Thingholm TE. Technologies for plasma membrane proteomics. Proteomics. 2009; 10(4):611–627. [PubMed: 19834916]
8. Cohen P. The role of protein phosphorylation in human health and disease. The Sir Hans Krebs Medal Lecture. Eur J Biochem. 2001; 268:5001–5010. [PubMed: 11589691]
9. Johnson LN. The regulation of protein phosphorylation. Biochem Soc Trans. 2009; 37:627–641. [PubMed: 19614568]
10. Salazar C, Hofer T. Multisite protein phosphorylation--from molecular mechanisms to kinetic models. FEBS J. 2009; 276:3177–3198. [PubMed: 19438722]
11. Ame MV, Baroni MV, Galanti LN, Bocco JL, Wunderlin DA. Effects of microcystin-LR on the expression of P-glycoprotein in Jenynsia multidentata. Chemosphere. 2009; 74:1179–1186. [PubMed: 19124144]
12. Contardo-Jara V, Pflugmacher S, Wiegand C. Multi-xenobiotic-resistance a possible explanation for the insensitivity of bivalves towards cyanobacterial toxins. Toxicon. 2008; 52:936–943. [PubMed: 18930753]
13. Pearson LA, Hisbergues M, Borner T, Dittmann E, Neilan BA. Inactivation of an ABC transporter gene, mcyH, results in loss of microcystin production in the cyanobacterium Microcystis aeruginosa PCC 7806. Appl Environ Microbiol. 2004; 70:6370–6378. [PubMed: 15528494]
14. See RH, Caday-Malcolm RA, Singaraja RR, Zhou S, Silverston A, Huber MT, Moran J, James ER, Janoo R, Savill JM, Rigot V, Zhang LH, Wang M, Chimini G, Wellington CL, Tafuri SR, Hayden MR. Protein kinase A site-specific phosphorylation regulates ATP-binding cassette A1 (ABCA1)-mediated phospholipid efflux. J Biol Chem. 2002; 277:41835–41842. [PubMed: 12196520]
15. Roosbeek S, Peelman F, Verhee A, Labeur C, Caster H, Lensink MF, Cirulli C, Grooten J, Cochet C, Vandekerckhove J, Amoresano A, Chimini G, Tavernier J, Rosseneu M. Phosphorylation by protein kinase CK2 modulates the activity of the ATP binding cassette A1 transporter. J Biol Chem. 2004; 279:37779–37788. [PubMed: 15218032]
16. Martinez LO, Agerholm-Larsen B, Wang N, Chen W, Tall AR. Phosphorylation of a pest sequence in ABCA1 promotes calpain degradation and is reversed by ApoA-I. J Biol Chem. 2003; 278:37368–37374. [PubMed: 12869555]

17. Yamauchi Y, Hayashi M, Abe-Dohmae S, Yokoyama S. Apolipoprotein A-I activates protein kinase C alpha signaling to phosphorylate and stabilize ATP binding cassette transporter A1 for the high density lipoprotein assembly. J Biol Chem. 2003; 278:47890–47897. [PubMed: 12952980]

18. Dean M, Annilo T. Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. Annu Rev Genomics Hum Genet. 2005; 6:123–142. [PubMed: 16124856]

19. Dean M. The genetics of ATP-binding cassette transporters. Methods Enzymol. 2005; 400:409–429. [PubMed: 16399363]

20. Ueda K, Cornwell MM, Gottesman MM, Pastan I, Roninson IB, Ling V, Riordan JR. The mdr1 gene, responsible for multidrug-resistance, codes for P-glycoprotein. Biochem Biophys Res Commun. 1986; 141:956–962. [PubMed: 2880583]

21. Chambers TC. Identification of phosphorylation sites in human MDR1 P-glycoprotein. Methods Enzymol. 1998; 292:328–342. [PubMed: 9711565]

22. Chambers TC, McAvoy EM, Jacobs JW, Eilon G. Protein kinase C phosphorylates P-glycoprotein in multidrug resistant human KB carcinoma cells. J Biol Chem. 1990; 265:7679–7686. [PubMed: 1970571]

23. Chambers TC, Pohl J, Glass DB, Kuo JF. Phosphorylation by protein kinase C and cyclic AMP-dependent protein kinase of synthetic peptides derived from the linker region of human P-glycoprotein. Biochem J. 1994; 299(Pt 1):309–315. [PubMed: 7909431]

24. Chambers TC, Pohl J, Raynor RL, Kuo JF. Identification of specific sites in human P-glycoprotein phosphorylated by protein kinase C. J Biol Chem. 1993; 268:4592–4595. [PubMed: 8095261]

25. Chambers TC, Zheng B, Kuo JF. Regulation by phorbol ester and protein kinase C inhibitors, and by a protein phosphatase inhibitor (okadaic acid), of P-glycoprotein phosphorylation and relationship to drug accumulation in multidrug-resistant human KB cells. Mol Pharmacol. 1992; 41:1008–1015. [PubMed: 1377325]

26. Cohen P. The regulation of protein function by multisite phosphorylation--a 25 year update. Trends Biochem Sci. 2000; 25:596–601. [PubMed: 11116185]

27. Glavy JS, Horwitz SB, Orr GA. Identification of the *in vivo* phosphorylation sites for acidic-directed kinases in murine mdr1b P-glycoprotein. J Biol Chem. 1997; 272:5909–5914. [PubMed: 9038209]

28. Glavy JS, Wolfson M, Nieves E, Han EK, Yang CP, Horwitz SB, Orr GA. Identification of *in vivo* phosphorylation sites for basic-directed kinases in murine mdr1b P-glycoprotein by combination of mass spectrometry and site-directed mutagenesis. Methods Enzymol. 1998; 292:342–358. [PubMed: 9711566]

29. Goodfellow HR, Sardini A, Ruetz S, Callaghan R, Gros P, McNaughton PA, Higgins CF. Protein kinase C-mediated phosphorylation does not regulate drug transport by the human multidrug resistance P-glycoprotein. J Biol Chem. 1996; 271:13668–13674. [PubMed: 8662768]

30. Hardy SP, Goodfellow HR, Valverde MA, Gill DR, Sepulveda V, Higgins CF. Protein kinase C-mediated phosphorylation of the human multidrug resistance P-glycoprotein regulates cell volume-activated chloride channels. EMBO J. 1995; 14:68–75. [PubMed: 7828597]

31. Idriss H, Urquidi V, Basavappa S. Selective modulation of P-glycoprotein's ATPase and anion efflux regulation activities with PKC alpha and PKC epsilon in Sf9 cells. Cancer Chemother Pharmacol. 2000; 46:287–292. [PubMed: 11052626]

32. Idriss HT, Hannun YA, Boulpaep E, Basavappa S. Regulation of volume-activated chloride channels by P-glycoprotein: phosphorylation has the final say. J Physiol. 2000; 524(Pt 3):629–636. [PubMed: 10790147]

33. Lelong IH, Cardarelli CO, Gottesman MM, Pastan I. GTP-stimulated phosphorylation of P-glycoprotein in transporting vesicles from KB-V1 multidrug resistant cells. Biochemistry. 1994; 33:8921–8929. [PubMed: 7913830]

34. Lelong-Rebel IH, Cardarelli CO. Differential phosphorylation patterns of P-glycoprotein reconstituted into a proteoliposome system: insight into additional unconventional phosphorylation sites. Anticancer Res. 2005; 25:3925–3935. [PubMed: 16309179]

35. Lelong-Rebel IH, Rebel G, Cardarelli CO, Pastan I, Gottesman MM. Modulation by the ATP/GTP ratio of the phosphorylation level of P-glycoprotein and of various plasma membrane proteins of KB-V1 multidrug resistant cells. Anticancer Res. 2003; 23:2363–2375. [PubMed: 12894516]
36. Ma LD, Marquardt D, Takemoto L, Center MS. Analysis of P-glycoprotein phosphorylation in HL60 cells isolated for resistance to vincristine. J Biol Chem. 1991; 266:5593–5599. [PubMed: 1672314]
37. Masanek U, Stammler G, Volm M. Modulation of multidrug resistance in human ovarian cancer cell lines by inhibition of P-glycoprotein 170 and PKC isoenzymes with antisense oligonucleotides. J Exp Ther Oncol. 2002; 2:37–41. [PubMed: 12415618]
38. Mellado W, Horwitz SB. Phosphorylation of the multidrug resistance associated glycoprotein. Biochemistry. 1987; 26:6900–6904. [PubMed: 3427052]
39. Orr GA, Han EK, Browne PC, Nieves E, O'Connor BM, Yang CP, Horwitz SB. Identification of the major phosphorylation domain of murine mdr1b P-glycoprotein. Analysis of the protein kinase A and protein kinase C phosphorylation sites. J Biol Chem. 1993; 268:25054–25064. [PubMed: 7901220]
40. Sachs CW, Ballas LM, Mascarella SW, Safa AR, Lewin AH, Loomis C, Carroll FI, Bell RM, Fine RL. Effects of sphingosine stereoisomers on P-glycoprotein phosphorylation and vinblastine accumulation in multidrug-resistant MCF-7 cells. Biochem Pharmacol. 1996; 52:603–612. [PubMed: 8759033]
41. Staats J, Marquardt D, Center MS. Characterization of a membrane-associated protein kinase of multidrug-resistant HL60 cells which phosphorylates P-glycoprotein. J Biol Chem. 1990; 265:4084. [PubMed: 1968066]
42. Szabo K, Bakos E, Welker E, Muller M, Goodfellow HR, Higgins CF, Varadi A, Sarkadi B. Phosphorylation site mutations in the human multidrug transporter modulate its drug-stimulated ATPase activity. J Biol Chem. 1997; 272:23165–23171. [PubMed: 9287320]
43. Vanoye CG, Castro AF, Pourcher T, Reuss L, Altenberg GA. Phosphorylation of P-glycoprotein by PKA and PKC modulates swelling-activated Cl- currents. Am J Physiol. 1999; 276:C370. [PubMed: 9950764]
44. Brendel C, Scharenberg C, Dohse M, Robey RW, Bates SE, Shukla S, Ambudkar SV, Wang Y, Wennemuth G, Burchert A, Boudriot U, Neubauer A. Imatinib mesylate and nilotinib (AMN107) exhibit high-affinity interaction with ABCG2 on primitive hematopoietic stem cells. Leukemia. 2007; 21:1267–1275. [PubMed: 17519960]
45. Ozvegy-Laczka C, Cserepes J, Elkind NB, Sarkadi B. Tyrosine kinase inhibitor resistance in cancer: role of ABC multidrug transporters. Drug Resist Updat. 2005; 8:15–26. [PubMed: 15939339]
46. Shi Z, Peng XX, Kim IW, Shukla S, Si QS, Robey RW, Bates SE, Shen T, Ashby CR Jr, Fu LW, Ambudkar SV, Chen ZS. Erlotinib (Tarceva, OSI-774) antagonizes ATP-binding cassette subfamily B member 1 and ATP-binding cassette subfamily G member 2-mediated drug resistance. Cancer Res. 2007; 67:11012–11020. [PubMed: 18006847]
47. Shi Z, Tiwari AK, Shukla S, Robey RW, Kim IW, Parmar S, Bates SE, Si QS, Goldblatt CS, Abraham I, Fu LW, Ambudkar SV, Chen ZS. Inhibiting the function of ABCB1 and ABCG2 by the EGFR tyrosine kinase inhibitor AG1478. Biochem Pharmacol. 2009; 77:781–793. [PubMed: 19059384]
48. Shukla S, Robey RW, Bates SE, Ambudkar SV. Sunitinib (Sutent, SU11248), a small-molecule receptor tyrosine kinase inhibitor, blocks function of the ATP-binding cassette (ABC) transporters P-glycoprotein (ABCB1) and ABCG2. Drug Metab Dispos. 2009; 37:359–365. [PubMed: 18971320]
49. Richter S, Geldner N, Schrader J, Wolters H, Stierhof YD, Rios G, Koncz C, Robinson DG, Jurgens G. Functional diversification of closely related ARF-GEFs in protein secretion and recycling. Nature. 2007; 448:488–492. [PubMed: 17653190]
50. Bowring CE, Llewellyn DH. Differences in HAC1 mRNA processing and translation between yeast and mammalian cells indicate divergence of the eukaryotic ER stress response. Biochem Biophys Res Commun. 2001; 287:789–800. [PubMed: 11563865]

51. Morreale G, Conforti L, Coadwell J, Wilbrey AL, Coleman MP. Evolutionary divergence of valosin-containing protein/cell division cycle protein 48 binding interactions among endoplasmic reticulum-associated degradation proteins. FEBS J. 2009; 276:1208–1220. [PubMed: 19175675]
52. Li Y, Salter-Cid L, Vitiello A, Preckel T, Lee JD, Angulo A, Cai Z, Peterson PA, Yang Y. Regulation of transporter associated with antigen processing by phosphorylation. J Biol Chem. 2000; 275:24130–24135. [PubMed: 10823836]
53. Hipfner DR, Gauldie SD, Deeley RG, Cole SP. Detection of the M(r) 190,000 multidrug resistance protein, MRP, with monoclonal antibodies. Cancer Res. 1994; 54:5788. [PubMed: 7954400]
54. Slovak ML, Ho JP, Bhardwaj G, Kurz EU, Deeley RG, Cole SP. Localization of a novel multidrug resistance-associated gene in the HT1080/DR4 and H69AR human tumor cell lines. Cancer Res. 1993; 53:3221–3225. [PubMed: 8391919]
55. Cole SP, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, Stewart AJ, Kurz EU, Duncan AM, Deeley RG. Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science. 1992; 258:1650–1654. [PubMed: 1360704]
56. Keitel V, Nies AT, Brom M, Hummel-Eisenbeiss J, Spring H, Keppler D. A common Dubin-Johnson syndrome mutation impairs protein maturation and transport activity of MRP2 (ABCC2). Am J Physiol Gastrointest Liver Physiol. 2003; 284:G165–174. [PubMed: 12388192]
57. Keppler D, Konig J. Hepatic secretion of conjugated drugs and endogenous substances. Semin Liver Dis. 2000; 20:265–272. [PubMed: 11076395]
58. Ito K, Wakabayashi T, Horie T. Mrp2/Abcc2 transport activity is stimulated by protein kinase Calpha in a baculo virus co-expression. system. Life Sci. 2005; 77:539–550. [PubMed: 15904671]
59. Minami S, Ito K, Honma M, Ikebuchi Y, Anzai N, Kanai Y, Nishida T, Tsukita S, Sekine S, Horie T, Suzuki H. Posttranslational regulation of Abcc2 expression by SUMOylation system. Am J Physiol Gastrointest Liver Physiol. 2009; 296:G406–413. [PubMed: 19074644]
60. Wimmer R, Hohenester S, Pusl T, Denk GU, Rust C, Beuers U. Tauroursodeoxycholic acid exerts anticholestatic effects by a cooperative cPKC alpha-/PKA-dependent mechanism in rat liver. Gut. 2008; 57:1448–1454. [PubMed: 18583398]
61. Dean M, Hamon Y, Chimini G. The human ATP-binding cassette (ABC) transporter superfamily. J Lipid Res. 2001; 42:1007. [PubMed: 11441126]
62. Tabcharani JA, Chang XB, Riordan JR, Hanrahan JW. Phosphorylation-regulated Cl- channel in CHO cells stably expressing the cystic fibrosis gene. Nature. 1991; 352:628–631. [PubMed: 1714039]
63. Picciotto MR, Cohn JA, Bertuzzi G, Greengard P, Nairn AC. Phosphorylation of the cystic fibrosis transmembrane conductance regulator. J Biol Chem. 1992; 267:12742–12752. [PubMed: 1377674]
64. Cheng SH, Rich DP, Marshall J, Gregory RJ, Welsh MJ, Smith AE. Phosphorylation of the R domain by cAMP-dependent protein kinase regulates the CFTR chloride channel. Cell. 1991; 66:1027–1036. [PubMed: 1716180]
65. Aleksandrov AA, Aleksandrov LA, Riordan JR. CFTR (ABCC7) is a hydrolyzable-ligand-gated channel. Pflugers Arch. 2007; 453:693–702. [PubMed: 17021796]
66. Gadsby DC, Vergani P, Csanady L. The ABC protein turned chloride channel whose failure causes cystic fibrosis. Nature. 2006; 440:477–483. [PubMed: 16554808]
67. Hegedus T, Aleksandrov A, Mengos A, Cui L, Jensen TJ, Riordan JR. Role of individual R domain phosphorylation sites in CFTR regulation by protein kinase A. Biochim Biophys Acta. 2009; 1788:1341–1349. [PubMed: 19328185]
68. Hegedus T, Serohijos AW, Dokholyan NV, He L, Riordan JR. Computational studies reveal phosphorylation-dependent changes in the unstructured R domain of CFTR. J Mol Biol. 2008; 378:1052–1063. [PubMed: 18423665]
69. Seibert FS, Chang XB, Aleksandrov AA, Clarke DM, Hanrahan JW, Riordan JR. Influence of phosphorylation by protein kinase A on CFTR at the cell surface and endoplasmic reticulum. Biochim Biophys Acta. 1999; 1461:275–283. [PubMed: 10581361]
70. Zhang L, Aleksandrov LA, Zhao Z, Birtley JR, Riordan JR, Ford RC. Architecture of the cystic fibrosis transmembrane conductance regulator protein and structural changes associated with phosphorylation and nucleotide binding. J Struct Biol. 2009; 167:242–251. [PubMed: 19524678]

71. Gadsby DC, Nairn AC. Control of CFTR channel gating by phosphorylation and nucleotide hydrolysis. Physiol Rev. 1999; 79:S77. [PubMed: 9922377]
72. Chappe V, Hinkson DA, Zhu T, Chang XB, Riordan JR, Hanrahan JW. Phosphorylation of protein kinase C sites in NBD1 and the R domain control CFTR channel activation by PKA. J Physiol. 2003; 548:39–52. [PubMed: 12588899]
73. Csanady L, Chan KW, Nairn AC, Gadsby DC. Functional roles of nonconserved structural segments in CFTR's NH2-terminal nucleotide binding domain. J Gen Physiol. 2005; 125:43–55. [PubMed: 15596536]
74. Lewis HA, Buchanan SG, Burley SK, Conners K, Dickey M, Dorwart M, Fowler R, Gao X, Guggino WB, Hendrickson WA, Hunt JF, Kearins MC, Lorimer D, Maloney PC, Post KW, Rajashankar KR, Rutter ME, Sauder JM, Shriver S, Thibodeau PH, Thomas PJ, Zhang M, Zhao X, Emtage S. Structure of nucleotide-binding domain 1 of the cystic fibrosis transmembrane conductance regulator. EMBO J. 2004; 23:282–292. [PubMed: 14685259]
75. Becq F, Jensen TJ, Chang XB, Savoia A, Rommens JM, Tsui LC, Buchwald M, Riordan JR, Hanrahan JW. Phosphatase inhibitors activate normal and defective CFTR chloride channels. Proc Natl Acad Sci USA. 1994; 91(19):9160–9164. [PubMed: 7522329]
76. Chang XB, Tabcharani JA, Hou YX, Jensen TJ, Kartner N, Alon N, Hanrahan JW, Riordan JR. Protein kinase A (PKA) still activates CFTR chloride channel after mutagenesis of all 10 PKA consensus phosphorylation sites. J Biol Chem. 1993; 268:11304–11311. [PubMed: 7684377]
77. Chappe V, Hinkson DA, Howell LD, Evagelidis A, Liao J, Chang XB, Riordan JR, Hanrahan JW. Stimulatory and inhibitory protein kinase C consensus sequences regulate the cystic fibrosis transmembrane conductance regulator. Proc Natl Acad Sci USA. 2004; 101:390–395. [PubMed: 14695900]
78. Dulhanty AM, Riordan JR. Phosphorylation by cAMP-dependent protein kinase causes a conformational change in the R domain of the cystic fibrosis transmembrane conductance regulator. Biochemistry. 1994; 33:4072. [PubMed: 7511414]
79. Mathews CJ, Tabcharani JA, Chang XB, Jensen TJ, Riordan JR, Hanrahan JW. Dibasic protein kinase A sites regulate bursting rate and nucleotide sensitivity of the cystic fibrosis transmembrane conductance regulator chloride channel. J Physiol. 1998; 508(Pt 2):365–377. [PubMed: 9508802]
80. Seibert FS, Tabcharani JA, Chang XB, Dulhanty AM, Mathews C, Hanrahan JW, Riordan JR. cAMP-dependent protein kinase-mediated phosphorylation of cystic fibrosis transmembrane conductance regulator residue Ser-753 and its role in channel activation. J Biol Chem. 1995; 270:2158–2162. [PubMed: 7530719]
81. Broughman JR, Sun L, Umar S, Sellin JH, Morris AP. Chronic PKC-beta2 activation in HT-29 Cl. 19a colonocytes prevents cAMP-mediated ion secretion by inhibiting apical membrane CFTR targeting. Am J Physiol Gastrointest Liver Physiol. 2006; 291:G331–334. [PubMed: 16574992]
82. Sun F, Hug MJ, Bradbury NA, Frizzell RA. Protein kinase A associates with cystic fibrosis transmembrane conductance regulator via an interaction with ezrin. J Biol Chem. 2000; 275:14360–14366. [PubMed: 10799517]
83. Thelin WR, Kesimer M, Tarran R, Kreda SM, Grubb BR, Sheehan JK, Stutts MJ, Milgram SL. The cystic fibrosis transmembrane conductance regulator is regulated by a direct interaction with the protein phosphatase 2A. J Biol Chem. 2005; 280:41512–41520. [PubMed: 16239222]
84. Kongsuphol P, Cassidy D, Hieke B, Treharne KJ, Schreiber R, Mehta A, Kunzelmann K. Mechanistic insight into control of CFTR by AMPK. J Biol Chem. 2009; 284:5645–5653. [PubMed: 19095655]
85. Pagano MA, Arrigoni G, Marin O, Sarno S, Meggio F, Treharne KJ, Mehta A, Pinna LA. Modulation of protein kinase CK2 activity by fragments of CFTR encompassing F508 may reflect functional links with cystic fibrosis pathogenesis. Biochemistry. 2008; 47:7925–7936. [PubMed: 18597485]
86. Treharne KJ, Crawford RM, Xu Z, Chen JH, Best OG, Schulte EA, Gruenert DC, Wilson SM, Sheppard DN, Kunzelmann K, Mehta A. Protein kinase CK2, cystic fibrosis transmembrane conductance regulator, and the deltaF508 mutation: F508 deletion disrupts a kinase-binding site. J Biol Chem. 2007; 282:10804–10813. [PubMed: 17289674]

87. King JD Jr, Fitch AC, Lee JK, McCane JE, Mak DO, Foskett JK, Hallows KR. AMP-activated protein kinase phosphorylation of the R domain inhibits PKA stimulation of CFTR. Am J Physiol Cell Physiol. 2009; 297:C94–101. [PubMed: 19419994]

88. Csanady L, Seto-Young D, Chan KW, Cenciarelli C, Angel BB, Qin J, McLachlin DT, Krutchinsky AN, Chait BT, Nairn AC, Gadsby DC. Preferential phosphorylation of R-domain Serine 768 dampens activation of CFTR channels by PKA. J Gen Physiol. 2005; 125:171–186. [PubMed: 15657296]

89. Dahan D, Evagelidis A, Hanrahan JW, Hinkson DA, Jia Y, Luo J, Zhu T. Regulation of the CFTR channel by phosphorylation. Pflugers Arch. 2001; 443(Suppl 1):S92. [PubMed: 11845311]

90. Ostedgaard LS, Baldursson O, Welsh MJ. Regulation of the cystic fibrosis transmembrane conductance regulator Cl- channel by its R domain. J Biol Chem. 2001; 276:7689–7692. [PubMed: 11244086]

91. Baker JM, Hudson RP, Kanelis V, Choy WY, Thibodeau PH, Thomas PJ, Forman-Kay JD. CFTR regulatory region interacts with NBD1 predominantly via multiple transient helices. Nat Struct Mol Biol. 2007; 14:738. [PubMed: 17660831]

92. Kanelis V, Hudson RP, Thibodeau PH, Thomas PJ, Forman-Kay JD. NMR evidence for differential phosphorylation-dependent interactions in WT and DeltaF508 CFTR. EMBO J. 2010; 29:263–277. [PubMed: 19927121]

93. Dean M, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. Genome Res. 2001; 11:1156–1166. [PubMed: 11435397]

94. Babenko AP, Aguilar-Bryan L, Bryan J. A view of sur/KIR6.X, KATP channels. Annu Rev Physiol. 1998; 60:667–687. [PubMed: 9558481]

95. Babenko AP, Gonzalez G, Aguilar-Bryan L, Bryan J. Reconstituted human cardiac KATP channels: functional identity with the native channels from the sarcolemma of human ventricular cells. Circ Res. 1998; 83:1132–1143. [PubMed: 9831708]

96. Beguin P, Nagashima K, Nishimura M, Gonoi T, Seino S. PKA-mediated phosphorylation of the human K(ATP) channel: separate roles of Kir6.2 and SUR1 subunit phosphorylation. EMBO J. 1999; 18:4722. [PubMed: 10469651]

97. Lin YF, Chai Y. Functional modulation of the ATP-sensitive potassium channel by extracellular signal-regulated kinase-mediated phosphorylation. Neuroscience. 2008; 152:371–380. [PubMed: 18280666]

98. Lin YF, Jan YN, Jan LY. Regulation of ATP-sensitive potassium channel function by protein kinase A-mediated phosphorylation in transfected HEK293 cells. EMBO J. 2000; 19:942–955. [PubMed: 10698936]

99. Quinn KV, Giblin JP, Tinker A. Multisite phosphorylation mechanism for protein kinase A activation of the smooth muscle ATP-sensitive K+ channel. Circ Res. 2004; 94:1359–1366. [PubMed: 15087422]

100. Ribalet B, John SA, Weiss JN. Regulation of cloned ATP-sensitive K channels by phosphorylation, MgADP, and phosphatidylinositol bisphosphate (PIP(2)): a study of channel rundown and reactivation. J Gen Physiol. 2000; 116:391–410. [PubMed: 10962016]

101. Shi Y, Chen X, Wu Z, Shi W, Yang Y, Cui N, Jiang C, Harrison RW. cAMP-dependent protein kinase phosphorylation produces interdomain movement in SUR2B leading to activation of the vascular KATP channel. J Biol Chem. 2008; 283:7523–7530. [PubMed: 18198173]

102. Yang Y, Shi Y, Guo S, Zhang S, Cui N, Shi W, Zhu D, Jiang C. PKA-dependent activation of the vascular smooth muscle isoform of KATP channels by vasoactive intestinal polypeptide and its effect on relaxation of the mesenteric resistance artery. Biochim Biophys Acta. 2008; 1778:88–96. [PubMed: 17942071]

103. Shi Y, Wu Z, Cui N, Shi W, Yang Y, Zhang X, Rojas A, Ha BT, Jiang C. PKA phosphorylation of SUR2B subunit underscores vascular KATP channel activation by beta-adrenergic receptors. Am J Physiol. 2007; 293:R1205–1214.

104. Tanaka AR, Tanabe K, Morita M, Kurisu M, Kasiwayama Y, Matsuo M, Kioka N, Amachi T, Imanaka T, Ueda K. ATP binding/hydrolysis by and phosphorylation of peroxisomal ATP-binding cassette proteins PMP70 (ABCD3) and adrenoleukodystrophy protein (ABCD1). J Biol Chem. 2002; 277:40142–40147. [PubMed: 12176987]

105. Xie Y, Xu K, Linn DE, Yang X, Guo Z, Shimelis H, Nakanishi T, Ross DD, Chen H, Fazli L, Gleave ME, Qiu Y. The 44-kDa Pim-1 kinase phosphorylates BCRP/ABCG2 and thereby promotes its multimerization and drug-resistant activity in human prostate cancer cells. J Biol Chem. 2008; 283:3349–3356. [PubMed: 18056989]

106. Kiran MD, Akiyoshi DE, Giacometti A, Cirioni O, Scalise G, Balaban N. OpuC--an ABC transporter that is associated with Staphylococcus aureus pathogenesis. Int J Artif Organs. 2009; 32:600–610. [PubMed: 19856269]

107. Colicchio R, Ricci S, Lamberti F, Pagliarulo C, Pagliuca C, Braione V, Braccini T, Tala A, Montanaro D, Tripodi S, Cintorino M, Troncone G, Bucci C, Pozzi G, Bruni CB, Alifano P, Salvatore P. The meningococcal ABC-Type L-glutamate transporter GltT is necessary for the development of experimental meningitis in mice. Infect Immun. 2009; 77:3578–3587. [PubMed: 19528209]

108. Lamarche MG, Wanner BL, Crepin S, Harel J. The phosphate regulon and bacterial virulence: a regulatory network connecting phosphate homeostasis and pathogenesis. FEMS Microbiol Rev. 2008; 32:461–473. [PubMed: 18248418]

109. Mishra NN, Prasad T, Sharma N, Payasi A, Prasad R, Gupta DK, Singh R. Pathogenicity and drug resistance in Candida albicans and other yeast species. A review. Acta Microbiol Immunol Hung. 2007; 54:201–235. [PubMed: 17896473]

110. Niimi M, Tanabe K, Wada S, Yamazaki A, Uehara Y, Niimi K, Lamping E, Holmes AR, Monk BC, Cannon RD. ABC transporters of pathogenic fungi: recent advances in functional analyses. Nippon Ishinkin Gakkai Zasshi. 2005; 46:249–260. [PubMed: 16282967]

111. Upcroft P. Multiple drug resistance in the pathogenic protozoa. Acta Trop. 1994; 56:195–212. [PubMed: 7911270]

112. Del Sorbo G, Schoonbeek H, De Waard MA. Fungal transporters involved in efflux of natural toxic compounds and fungicides. Fungal Genet Biol. 2000; 30:1–15. [PubMed: 10955904]

113. Davidson AL, Chen J. ATP-binding cassette transporters in bacteria. Annu Rev Biochem. 2004; 73:241–261. [PubMed: 15189142]

114. Raetz CR, Reynolds CM, Trent MS, Bishop RE. Lipid A modification systems in gram-negative bacteria. Annu Rev Biochem. 2007; 76:295–329. [PubMed: 17362200]

115. Cai J, Gros P. Overexpression, purification, and functional characterization of ATP-binding cassette transporters in the yeast, Pichia pastoris. Biochim Biophys Acta. 2003; 1610:63–76. [PubMed: 12586381]

116. Sipos G, Kuchler K. Fungal ATP-binding cassette (ABC) transporters in drug resistance & detoxification. Curr Drug Targets. 2006; 7:471–481. [PubMed: 16611035]

117. Albuquerque CP, Smolka MB, Payne SH, Bafna V, Eng J, Zhou H. A multidimensional chromatography technology for in-depth phosphoproteome analysis. Mol Cell Proteomics. 2008; 7:1389–1396. [PubMed: 18407956]

118. Gruhler A, Olsen JV, Mohammed S, Mortensen P, Faergeman NJ, Mann M, Jensen ON. Quantitative phosphoproteomics applied to the yeast pheromone signaling pathway. Mol Cell Proteomics. 2005; 4:310–327. [PubMed: 15665377]

119. Li X, Gerber SA, Rudner AD, Beausoleil SA, Haas W, Villen J, Elias JE, Gygi SP. Large-scale phosphorylation analysis of alpha-factor-arrested Saccharomyces cerevisiae. J Proteome Res. 2007; 6:1190–1197. [PubMed: 17330950]

120. Smolka MB, Albuquerque CP, Chen SH, Zhou H. Proteome-wide identification of *in vivo* targets of DNA damage checkpoint kinases. Proc Natl Acad Sci USA. 2007; 104:10364–10369. [PubMed: 17563356]

121. Curry JM, Whalan R, Hunt DM, Gohil K, Strom M, Rickman L, Colston MJ, Smerdon SJ, Buxton RS. An ABC transporter containing a forkhead-associated domain interacts with a serine-threonine protein kinase and is required for growth of Mycobacterium tuberculosis in mice. Infect Immun. 2005; 73:4471–4477. [PubMed: 16040957]

122. Kelm KB, Huyer G, Huang JC, Michaelis S. The internalization of yeast Ste6p follows an ordered series of events involving phosphorylation, ubiquitination, recognition and endocytosis. Traffic. 2004; 5:165–180. [PubMed: 15086792]

123. Kolling R. Mutations affecting phosphorylation, ubiquitination and turnover of the ABC-transporter Ste6. FEBS Lett. 2002; 531:548–552. [PubMed: 12435609]

124. Paumi CM, Chuk M, Chevelev I, Stagljar I, Michaelis S. Negative regulation of the yeast ABC transporter Ycf1p by phosphorylation within its N-terminal extension. J Biol Chem. 2008; 283:27079–27088. [PubMed: 18667437]

125. Subba Rao G, Bachhawat AK, Gupta CM. Two-hybrid-based analysis of protein-protein interactions of the yeast multidrug resistance protein, Pdr5p. Funct Integr Genomics. 2002; 1:357–366. [PubMed: 11957110]

126. Chen XJ. Activity of the Kluyveromyces lactis Pdr5 multidrug transporter is modulated by the Sit4 protein phosphatase. J Bacteriol. 2001; 183:3939–3948. [PubMed: 11395457]

127. Eraso P, Martinez-Burgos M, Falcon-Perez JM, Portillo F, Mazon MJ. Ycf1-dependent cadmium detoxification by yeast requires phosphorylation of residues Ser908 and Thr911. FEBS Lett. 2004; 577:322–326. [PubMed: 15556603]

128. Molle V, Soulat D, Jault JM, Grangeasse C, Cozzone AJ, Prost JF. Two FHA domains on an ABC transporter, Rv1747, mediate its phosphorylation by PknF, a Ser/Thr protein kinase from Mycobacterium tuberculosis. FEMS Microbiol Lett. 2004; 234:215–223. [PubMed: 15135525]

129. Wada S, Tanabe K, Yamazaki A, Niimi M, Uehara Y, Niimi K, Lamping E, Cannon RD, Monk BC. Phosphorylation of candida glabrata ATP-binding cassette transporter Cdr1p regulates drug efflux activity and ATPase stability. J Biol Chem. 2005; 280:94–103. [PubMed: 15498768]

130. Rogers LD, Foster LJ. Phosphoproteomics--finally fulfilling the promise? Mol Biosyst. 2009; 5:1122. [PubMed: 19756301]

131. Chen G, Pramanik BN. Application of LC/MS to proteomics studies: current status and future prospects. Drug Discov Today. 2009; 14:465–471. [PubMed: 19429505]

132. Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, Yu L, Brewer C, Collins JA, Molhuizen HO, Loubser O, Ouelette BF, Fichter K, Ashbourne-Excoffon KJ, Sensen CW, Scherer S, Mott S, Denis M, Martindale D, Frohlich J, Morgan K, Koop B, Pimstone S, Kastelein JJ, Genest J Jr, Hayden MR. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet. 1999; 22:336–345. [PubMed: 10431236]

133. Allikmets R, Shroyer NF, Singh N, Seddon JM, Lewis RA, Bernstein PS, Peiffer A, Zabriskie NA, Li Y, Hutchinson A, Dean M, Lupski JR, Leppert M. Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. Science. 1997; 277:1805–1807. [PubMed: 9295268]

134. Powis SJ, Howard JC, Butcher GW. The major histocompatibility complex class II-linked cim locus controls the kinetics of intracellular transport of a classical class I molecule. J Exp Med. 1991; 173:913. [PubMed: 2007857]

135. Momburg F, Roelse J, Neefjes J, Hammerling GJ. Peptide transporters and antigen processing. Behring Inst Mitt. 1994; 94:26–36. [PubMed: 7998911]

136. Strautnieks SS, Bull LN, Knisely AS, Kocoshis SA, Dahl N, Arnell H, Sokal E, Dahan K, Childs S, Ling V, Tanner MS, Kagalwalla AF, Nemeth A, Pawlowska J, Baker A, Mieli-Vergani G, Freimer NB, Gardiner RM, Thompson RJ. A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. Nat Genet. 1998; 20:233–238. [PubMed: 9806540]

137. Allikmets R, Raskind WH, Hutchinson A, Schueck ND, Dean M, Koeller DM. Mutation of a putative mitochondrial iron transporter gene (ABC7) in X-linked sideroblastic anemia and ataxia (XLSA/A). Hum Mol Genet. 1999; 8:743. [PubMed: 10196363]

138. Paulusma CC, Kool M, Bosma PJ, Scheffer GL, ter Borg F, Scheper RJ, Tytgat GN, Borst P, Baas F, Oude Elferink RP. A mutation in the human canalicular multispecific organic anion transporter gene causes the Dubin-Johnson syndrome. Hepatology. 1997; 25:1539–1542. [PubMed: 9185779]

139. Ringpfeil F, Lebwohl MG, Christiano AM, Uitto J. Pseudoxanthoma elasticum: mutations in the MRP6 gene encoding a transmembrane ATP-binding cassette (ABC) transporter. Proc Natl Acad Sci USA. 2000; 97:6001–6006. [PubMed: 10811882]

140. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science. 1989; 245:1066–1073. [PubMed: 2475911]

141. Nestorowicz A, Wilson BA, Schoor KP, Inoue H, Glaser B, Landau H, Stanley CA, Thornton PS, Clement JPt, Bryan J, Aguilar-Bryan L, Permutt MA. Mutations in the sulonylurea receptor gene are associated with familial hyperinsulinism in Ashkenazi Jews. Hum Mol Genet. 1996; 5:1813–1822. [PubMed: 8923011]

142. Mosser J, Lutz Y, Stoeckel ME, Sarde CO, Kretz C, Douar AM, Lopez J, Aubourg P, Mandel JL. The gene responsible for adrenoleukodystrophy encodes a peroxisomal membrane protein. Hum Mol Genet. 1994; 3:265–271. [PubMed: 8004093]

143. Berge KE, Tian H, Graf GA, Yu L, Grishin NV, Schultz J, Kwiterovich P, Shan B, Barnes R, Hobbs HH. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science. 2000; 290:1771–1775. [PubMed: 11099417]

144. Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, Ross DD. A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci USA. 1998; 95:15665. [PubMed: 9861027]

145. Chen CJ, Chin JE, Ueda K, Clark DP, Pastan I, Gottesman MM, Roninson IB. Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoprotein) gene from multidrug-resistant human cells. Cell. 1986; 47:381–389. [PubMed: 2876781]

146. Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu Rev Biochem. 1993; 62:385–427. [PubMed: 8102521]

147. Olsen JV, Blagoev B, Gnad F, Macek B, Kumar C, Mortensen P, Mann M. Global, *in vivo*, and site-specific phosphorylation dynamics in signaling networks. Cell. 2006; 127:635–648. [PubMed: 17081983]

148. Rikova K, Guo A, Zeng Q, Possemato A, Yu J, Haack H, Nardone J, Lee K, Reeves C, Li Y, Hu Y, Tan Z, Stokes M, Sullivan L, Mitchell J, Wetzel R, Macneill J, Ren JM, Yuan J, Bakalarski CE, Villen J, Kornhauser JM, Smith B, Li D, Zhou X, Gygi SP, Gu TL, Polakiewicz RD, Rush J, Comb MJ. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell. 2007; 131:1190–1230. [PubMed: 18083107]

149. Dephoure N, Zhou C, Villen J, Beausoleil SA, Bakalarski CE, Elledge SJ, Gygi SP. A quantitative atlas of mitotic phosphorylation. Proc Natl Acad Sci USA. 2008; 105:10762–10767. [PubMed: 18669648]

150. Hornbeck PV, Chabra I, Kornhauser JM, Skrzypek E, Zhang B. PhosphoSite: A bioinformatics resource dedicated to physiological protein phosphorylation. Proteomics. 2004; 4(6):1551–1561. [PubMed: 15174125]

151. Tao WA, Wollscheid B, O'Brien R, Eng JK, Li XJ, Bodenmiller B, Watts JD, Hood L, Aebersold R. Quantitative phosphoproteome analysis using a dendrimer conjugation chemistry and tandem mass spectrometry. Nat Methods. 2005; 2:591–598. [PubMed: 16094384]

152. Imami K, Sugiyama N, Kyono Y, Tomita M, Ishihama Y. Automated phosphoproteome analysis for cultured cancer cells by two-dimensional nanoLC-MS using a calcined titania/C18 biphasic column. Anal Sci. 2008; 24:161–166. [PubMed: 18187866]

153. Beausoleil SA, Jedrychowski M, Schwartz D, Elias JE, Villen J, Li J, Cohn MA, Cantley LC, Gygi SP. Large-scale characterization of HeLa cell nuclear phosphoproteins. Proc Natl Acad Sci USA. 2004; 101:12130–12135. [PubMed: 15302935]

154. Daub H, Olsen JV, Bairlein M, Gnad F, Oppermann FS, Korner R, Greff Z, Keri G, Stemmann O, Mann M. Kinase-selective enrichment enables quantitative phosphoproteomics of the kinome across the cell cycle. Mol Cell. 2008; 31:438–448. [PubMed: 18691976]

155. Mayya V, Lundgren DH, Hwang SI, Rezaul K, Wu L, Eng JK, Rodionov V, Han DK. Quantitative phosphoproteomic analysis of T cell receptor signaling reveals system-wide modulation of protein-protein interactions. Sci Signal. 2009; 2:ra46. [PubMed: 19690332]

156. Gauci S, Helbig AO, Slijper M, Krijgsveld J, Heck AJ, Mohammed S. Lys-N and trypsin cover complementary parts of the phosphoproteome in a refined SCX-based approach. Anal Chem. 2009; 81:4493–4501. [PubMed: 19413330]

157. Brill LM, Motamedchaboki K, Wu S, Wolf DA. Comprehensive proteomic analysis of Schizosaccharomyces pombe by two-dimensional HPLC-tandem mass spectrometry. Methods. 2009; 48:311–319. [PubMed: 19272449]

158. Brill LM, Xiong W, Lee KB, Ficarro SB, Crain A, Xu Y, Terskikh A, Snyder EY, Ding S. Phosphoproteomic analysis of human embryonic stem cells. Cell Stem Cell. 2009; 5:204–213. [PubMed: 19664994]

159. Jorgensen C, Sherman A, Chen GI, Pasculescu A, Poliakov A, Hsiung M, Larsen B, Wilkinson DG, Linding R, Pawson T. Cell-specific information processing in segregating populations of Eph receptor ephrin-expressing cells. Science. 2009; 326:1502–1509. [PubMed: 20007894]

160. Guo A, Villen J, Kornhauser J, Lee KA, Stokes MP, Rikova K, Possemato A, Nardone J, Innocenti G, Wetzel R, Wang Y, MacNeill J, Mitchell J, Gygi SP, Rush J, Polakiewicz RD, Comb MJ. Signaling networks assembled by oncogenic EGFR and c-Met. Proc Natl Acad Sci USA. 2008; 105:692–697. [PubMed: 18180459]

161. Oppermann FS, Gnad F, Olsen JV, Hornberger R, Greff Z, Keri G, Mann M, Daub H. Large-scale proteomics analysis of the human kinome. Mol Cell Proteomics. 2009; 8:1751–1764. [PubMed: 19369195]

162. Thingholm TE, Larsen MR, Ingrell CR, Kassem M, Jensen ON. TiO(2)-based phosphoproteomic analysis of the plasma membrane and the effects of phosphatase inhibitor treatment. J Proteome Res. 2008; 7:3304–3313. [PubMed: 18578522]

163. Villen J, Beausoleil SA, Gerber SA, Gygi SP. Large-scale phosphorylation analysis of mouse liver. Proc Natl Acad Sci USA. 2007; 104:1488–1493. [PubMed: 17242355]

164. Sui S, Wang J, Yang B, Song L, Zhang J, Chen M, Liu J, Lu Z, Cai Y, Chen S, Bi W, Zhu Y, He F, Qian X. Phosphoproteome analysis of the human Chang liver cells using SCX and a complementary mass spectrometric strategy. Proteomics. 2008; 8:2024–2434. [PubMed: 18491316]

165. Yu LR, Zhu Z, Chan KC, Issaq HJ, Dimitrov DS, Veenstra TD. Improved titanium dioxide enrichment of phosphopeptides from HeLa cells and high confident phosphopeptide identification by cross-validation of MS/MS and MS/MS/MS spectra. J Proteome Res. 2007; 6:4150–4162. [PubMed: 17924679]

166. Townsend RR, Lipniunas PH, Tulk BM, Verkman AS. Identification of protein kinase A phosphorylation sites on NBD1 and R domains of CFTR using electrospray mass spectrometry with selective phosphate ion monitoring. Protein Sci. 1996; 5:1865–1873. [PubMed: 8880910]

167. Wang Y, Du D, Fang L, Yang G, Zhang C, Zeng R, Ullrich A, Lottspeich F, Chen Z. Tyrosine phosphorylated Par3 regulates epithelial tight junction assembly promoted by EGFR signaling. EMBO J. 2006; 25:5058–5070. [PubMed: 17053785]

168. Neville DC, Rozanas CR, Price EM, Gruis DB, Verkman AS, Townsend RR. Evidence for phosphorylation of serine 753 in CFTR using a novel metal-ion affinity resin and matrix-assisted laser desorption mass spectrometry. Protein Sci. 1997; 6:2436–2445. [PubMed: 9385646]

169. Cantin GT, Yi W, Lu B, Park SK, Xu T, Lee JD, Yates JR 3rd. Combining protein-based IMAC, peptide-based IMAC, and MudPIT for efficient phosphoproteomic analysis. J Proteome Res. 2008; 7:1346–1351. [PubMed: 18220336]

170. Wang B, Malik R, Nigg EA, Korner R. Evaluation of the low-specificity protease elastase for large-scale phosphoproteome analysis. Anal Chem. 2008; 80:9526–9533. [PubMed: 19007248]

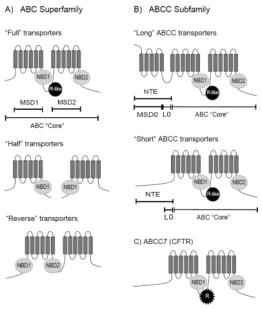

Fig. 1. Predicted topology of ATP-Binding Cassette (ABC) transporters

(A) In general, ABC transporters are structurally characterized as having an ABC "Core" containing two membrane spanning domains (MSDs), also called transmembrane spanning domains (TMDs), and two intracellular nucleotide binding domains (NBDs). A number of ABC transporters exist as "Half" transporters which form homo- and/or heterodimers. **(B)** The ABCC subfamily has N-terminal extension (NTE) in addition to the ABC "Core", The NTE of "Long" ABCC proteins consists of an extra membrane spanning domain (MSD0, TMD0) and an extra linker domain (L0) (also called cytoplasmic loop 3 (CL3)). In "Short" ABCC proteins, the MSD0 is absent. **(C)** The linker region between NBD1 and MSD2 of ABCC7 (CFTR) is extensively phosphorylated and is thus referred to as the regulatory domain (R domain). Similar domains are found in other ABC transporters and are referred to here as the "R-like" domain (annotated in Fig. **1A**).

Table 1

ABC Transporter-Associated Diseases

Disease	Transporter(s)	Reference
Tangier disease	ABCA1	[132]
Stargardt disease	ABCA4	[133]
Immune deficiency	ABCB2 and ABCB3	[134, 135]
Progressive familial intrahepatic cholestasis (PFIC)	ABCB4 and ABCB11	[136]
Sideroblastic anemia and ataxia	ABCB7	[137]
Dubin Johnson Syndrome (DJS)	ABCC2	[138]
Pseudoxanthoma Elasticum (PXE)	ABCC6	[139]
Cystic fibrosis	ABCC7	[140]
Persistent hypoglycemia of infancy	ABCC8 and ABCC9	[141]
Adrenoleukodystrophy	ABCD1	[142]
Sitosterolemia	ABCG5 and ABCG8	[143]
Cancer	ABCB1, ABCC1, and ABCG2	[1, 55, 144–146]

Table 2

Phosphorylation Sites of the ABC Transporters Identified by MS Analysis of Tryptic Derived Peptides

Transporter	Amino acid	Site	Reference
ABCA1	SERINE	1042	[14,15]
	THREONINE	1242	
	THREONINE	1243	
	SERINE	1255	
	SERINE	2054	
ABCA2	SERINE	1327	[147]
	SERINE	1331	
	TYROSINE	1694	[148]
	TYROSINE	2178	[149]
	TYROSINE	2186	[148]
	THREONINE	2412	[149]
ABCA3	TYROSINE	1265	[148]
	TYROSINE	1268	
	TYROSINE	1349	
ABCA4	TYROSINE	400	[148]
ABCA5	TYROSINE	22	[148]
	TYROSINE	58	
	TYROSINE	1299	[150]
ABCA6		NONE IDENTIFIED	
ABCA7		NONE IDENTIFIED	
ABCA8		NONE IDENTIFIED	
ABCA9		NONE IDENTIFIED	
ABCA10	TYROSINE	307	[151]
	SERINE	568	[152]
ABCA11		NONE IDENTIFIED	
ABCA12	TYROSINE	811	[150]
	TYROSINE	814	
ABCA13	TYROSINE	1773	[148]
ABCB1	SERINE	644	[149]
	SERINE	658	
	SERINE	660	
	SERINE	661	[23,24]
	SERINE	667	
	SERINE	671	
	SERINE	683	[149]
	SERINE	686	[23,24]
ABCB2		NONE IDENTIFIED	

Transporter	Amino acid	Site	Reference
ABCB3	THREONINE	458	[149]
	TYROSINE	597	[150]
	TYROSINE	693	
ABCB4		NONE IDENTIFIED	
ABCB5		NONE IDENTIFIED	
ABCB6		NONE IDENTIFIED	
ABCB7	TYROSINE	345	[148]
	TYROSINE	356	[148]
ABCB8		NONE IDENTIFIED	
ABCB9	SERINE	530	[153]
ABCB10		NONE IDENTIFIED	
ABCB11		NONE IDENTIFIED	
ABCC1	TYROSINE	490	[148]
	SERINE	915	[154,155]
	SERINE	917	[156]
	SERINE	918	[150]
	SERINE	919	[157,158]
	TYROSINE	920	[148,159,160]
	SERINE	930	[161,162]
	TYROSINE	1508	[160]
ABCC2	SERINE	283	[149,154]
	TYROSINE	616	[157,158]
	THREONINE	873	[149]
	SERINE	878	
	TYROSINE	885	
	SERINE	900	
ABCC3	SERINE	908	[149,163]
	SERINE	911	
ABCC4	SERINE	313	[152]
	SERINE	314	
	SERINE	601	[164]
	THREONINE	646	[147,149,155]
	TYROSINE	1259	[150]
ABCC5	TYROSINE	10	[160]
	SERINE	505	[149]
	SERINE	509	
	THREONINE	513	
ABCC6	SERINE	681	[156]
	SERINE	902	[165]

Transporter	Amino acid	Site	Reference
	SERINE	1310	[156]
ABCC7	SERINE	422	[166]
	SERINE	489	
	SERINE	511	[86]
	TYROSINE	515	[167]
	SERINE	519	[71]
	SERINE	557	
	THREONINE	582	[77]
	THREONINE	604	
	SERINE	641	[71]
	SERINE	660	[63,166,168]
	SERINE	670	[71]
	THREONINE	682	[77]
	SERINE	686	[63]
	THREONINE	690	[71]
	SERINE	700	[63,166,168]
	SERINE	707	[77]
	SERINE	712	[166,168]
	SERINE	728	[71]
	SERINE	737	[63,166,168]
	SERINE	742	[71]
	SERINE	753	[168]
	SERINE	756	[71]
	THREONINE	757	
	THREONINE	760	
	SERINE	768	[63,166,168]
	THREONINE	787	[71]
	THREONINE	788	
	SERINE	790	[63]
	SERINE	795	[63,166,168]
	SERINE	813	
	THREONINE	1176	[156]
ABCC8	TYROSINE	798	[150]
ABCC9		NONE IDENTIFIED	
ABCC10	THREONINE	463	[169]
	SERINE	467	
ABCC11	TYROSINE	993	[157,158]
ABCC12		NONE IDENTIFIED	
ABCD1	SERINE	733	[149,154,170]

Transporter	Amino acid	Site	Reference
ABCD2		NONE IDENTIFIED	
ABCD3	TYROSINE	143	[152]
ABCD4		NONE IDENTIFIED	
ABCG1		NONE IDENTIFIED	
ABCG2	TYROSINE	362	[105]
ABCG4		NONE IDENTIFIED	
ABCG5		NONE IDENTIFIED	
ABCG8		NONE IDENTIFIED	