The Sulfonylurea Receptor, an Atypical ATP-Binding Cassette Protein, and Its Regulation of the $K_{\text{ATP}}$ Channel

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Abstract—ATP-binding cassette (ABC) proteins are highly conserved and widely expressed throughout nature and found in all organisms, both prokaryotic and eukaryotic. They mediate myriad critical cellular processes, from nutrient import to toxin efflux using the energy derived from ATP hydrolysis. Most ABC proteins mediate transport of substances across lipid membranes. However, there are atypical ABC proteins that mediate other processes. These include, but are not limited to, DNA repair (bacterial MutS), ion transport (cystic fibrosis transmembrane receptor), and mRNA trafficking (yeast Elf1p). The sulfonylurea receptor (SUR) is another atypical ABC protein that regulates activity of the potassium ATP channel ($K_{\text{ATP}}$). $K_{\text{ATP}}$ is widely expressed in nearly all tissues of higher organisms and couples cellular energy status to membrane potential. $K_{\text{ATP}}$ is particularly important in the regulation of insulin secretion from pancreatic $\beta$-cells and in regulating action potential duration in muscle cells. SUR is indispensable for normal channel function, and mutations in genes encoding SURs increase the susceptibility to diabetes, myocardial infarction, and heart failure. Here, we review the structure and function of ABC proteins and discuss SUR, its regulation of the $K_{\text{ATP}}$ channel, and its role in cardiovascular disease. (Circ Res. 2008;102:164-176.)

Key Words: sulfonylurea receptor ■ SUR2 ■ ATP-binding cassette (ABC) protein ■ $K_{\text{ATP}}$ ■ myocardium

ATP-binding cassette (ABC) proteins constitute one of the largest and most diverse families of proteins. ABC proteins use the energy derived from ATP hydrolysis to mediate their actions, usually transport of molecules across lipid bilayers. Presently, there are ~6000 known ABC proteins; they are found in all cells, from bacteria to humans, and are highly conserved.1 Expression is highly variable across species, with humans having only 48 known ABC genes2 compared with E coli, which has 79 ABC genes, making up a remarkable 5% of the genome.3 ABC proteins serve 3 major functions within cells: import of molecules, export of molecules, and regulation of intracellular processes. ABC proteins, which mediate the first 2 of these functions, are sometimes referred to as traffic ABC proteins. ABC importers are only found in prokaryotes, where they play a critical role in bringing essential nutrients into cells. Eukaryotes have evolved other forms of nutrient acquisition and, as a result, do not contain any known ABC importers. ABC exporters are found in both prokaryotes and eukaryotes and serve many functions. In prokaryotes, the most clinically significant function of exporters is to mediate the efflux of toxins such as antibiotics via the prokaryotic transporter MsbA and other associated ABC proteins.4 This function underlies the development of antimicrobial resistance in bacteria and represents one of the greatest challenges to modern medicine. Similarly, ABC transporters mediate antimicrobial resistance in both fungi5 and yeast.6 In plants, ABC proteins play a role in developmental processes,7 regulation of growth and function via vacuolar transport of molecules within the cell,8 removal of toxins such as herbicides,9 and transport of molecules that...
regulate plant responses to environmental stressors such as microbial pathogens, herbivores, and UV radiation. In animals, ABC transporters play a role in many important physiological processes. In humans, these include, but are not limited to, cholesterol transport, antigen presentation to T cells, cellular iron trafficking, bile acid transport, and maintenance of the blood–brain barrier.

In prokaryotes, nearly all ABC transporters are found in the plasma membrane. Interestingly, eukaryotic ABC transporters are located in the plasma membrane as well as in the membranes of peroxisomes, lysosomes, vacuoles, endosomes, the Golgi apparatus, the endoplasmic reticulum (ER), and the mitochondria. For instance, ABCA5 localizes to the lysosomal membrane and, although the normal physiological function remains unknown, ABCA5 knockout (KO) mice develop a dilated cardiomyopathy with associated congestive heart failure, a phenotype similar to other forms of lysosomal diseases. The mitochondrial ABC transporter mABC1 has been identified recently as a component of a mitochondrial structure with KATP channel activity and has been shown to be cytoprotective in the face of oxidative stress in neonatal rat cardiomyocytes. Another mitochondrial protein, MTABC3, a product of the ABCB6 gene, plays a key role in heme biosynthesis via transport of mitochondrial porphyrins. Adrenoleukodystrophy results from abnormal accumulation of very long chain fatty acids, resulting from impaired β-oxidation in peroxisomes. This disease has been linked to mutations in the protein ALD, a product of the ABCD1 gene; however, its pathophysiological role in adrenoleukodystrophy is not yet known.

Here, we review the structure and function of SUR as a model of ABC function and review its role in cardiovascular disease. An additional article in this review series focuses on the role of ABC transporters in the processing and trafficking of cholesterol in the cardiovascular system.

**ABC Proteins and Human Disease**

Given the diverse and important roles of ABC proteins, and their divergent subcellular locations, it is no surprise that mutations in ABC genes lead to a number of clinically important disease states. The prototypical human disease caused by a mutant ABC protein is cystic fibrosis. Cystic fibrosis is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene (also known as ABCC7). CFTR is unique in that it is the only ion channel expressed in the ABC family. Decreased function of CFTR makes the affected membranes essentially impermeable to chloride ions, resulting in the diseased phenotype. Recently, Tangier disease, a rare disorder characterized by abnormal, diffuse cholesterol deposition and accelerated atherosclerosis, has been shown to be attributable to a mutation in the ABCA1 gene. This gene is critical for the normal production of mature high-density lipoprotein (HDL) particles. Mutations in ABCA1 also cause familial HDL deficiency (reviewed in detail elsewhere). Other disorders associated with mutations in ABC proteins include Dubin–Johnson syndrome and other inherited diseases of bile transport, adrenoleukodystrophy, surfactant deficiency and other pulmonary disorders, lamellar ichthyosis, immunodeficiency, DEND (developmental delay, epilepsy, and neonatal diabetes) syndrome, and X-linked sideroblastic anemia with ataxia (Table 1). P-Glycoprotein (PGY1), also known as the human multidrug resistance protein (MDR1), is a product of the ABCB1 gene that is widely distributed and mediates toxin efflux out of mammalian cells. PGY1 plays a major role in the resistance to chemotherapeutic agents and also likely plays a role in the efflux of immunosuppressants in posttransplantation patients, contributing to clinically observed variations in organ rejection.

There are many ABC transporters expressed in the heart, and they are becoming more appreciated for their role in cardiac physiology and disease. As mentioned previously, ABCA5 KO mice develop dilated cardiomyopathy. ABCG2 is expressed in cardiac vascular endothelium and is upregulated in ischemic and nonischemic dilated cardiomyopathy, although its function is not known. PGY1 is not normally expressed in the myocardium but has been shown to be expressed in myocytes exposed to both acute ischemia/reperfusion and chronic ischemia. MRPI, a product of the ABCCI gene, is upregulated in mouse hearts after treatment with the cardiotoxic chemotherapeutic agent doxorubicin, suggesting a possible protective role in the myocardium. Interestingly, MRPI is also upregulated in the myocardium in response to acute and chronic exercise in rats and may play a role in cardioprotection against acute and chronic oxidative stress. Dystrophic cardiac calcification, an uncommon disorder that is occasionally a long-term complication of myocardial infarction, is caused by mutations in the ABCG6 gene. Perhaps the most widely studied ABC proteins in myocardial disease are those that regulate cholesterol transport (ABCA1, ABCG1, ABCG5, and ABCG8) and the sulfonfonylurea receptor, which is an atypical ABC protein that is part of the sarcolemmal potassium ATP (KATP) channel.

**Structure of ABC Proteins**

**Typical ABC Proteins**

A typical ABC protein contains 4 domains, 2 nucleotide-binding domains (NBDs) (also referred to as ABC domains), and 2 transmembrane domains (TMDs). Domains can be encoded as separate polypeptides (most common in prokaryotes), dimers, trimers, or complete proteins. The TMDs are highly hydrophobic, and each contains 6 membrane-spanning segments. These domains form the pore through which substrates cross and are also believed to determine substrate specificity (Figure 1). This is illustrated by the crystal structure of the bacterial FlhuA protein, which forms a transmembrane pore that is covered by a hydrophilic plug; this plug moves upon binding of ferrichrome, the ligand transported by this protein. Further crystal structures of other bacterial ABC proteins show varying pore conformations and additional gating mechanisms based on substrate specificity.

The ABC domains are hydrophilic and are present at the cytoplasmic aspect of the membrane. These domains consist of ≥215 amino acids (aa), are highly conserved throughout evolution, and can bind to and hydrolyze ATP. The ABC domains contain 3 major conserved motifs: the Walker A and Walker B domains, which are necessary for binding of ATP,
and the signature, or linker motif (also known as the C region), LSGGQ.50 The ABC domain is the defining feature of the ABC family of proteins, and its presence is used to identify new members of this class of molecules.55 Although most ABC proteins contain 2 ABC and 2 TMD, there are half-ABC proteins containing only 1 of each domain. These half-proteins can exist as either homo- or heterodimers. Numerous crystal structures have shown that the 2 ABC domains dimerize such that they are antiparallel in organization with the conserved Walker A site of 1 NBD aligning with the conserved linker motif of the opposite NBD.53,54,56 The configuration of the NBDs is critical for normal function, and crystal structures have shown the location of many mutations in the NBDs that cause cystic fibrosis, suggesting the possible mechanistic flaw associated with particular CFTR mutations.56,57

In addition to these core domains, there is a wide variety of accessory components that play key roles in the function of specific ABC transporters.54 Many accessory components are proteins that transport the substrates to the ABC transporters. These accessory proteins are highly specific and are structurally altered by binding with the ABC transporter.54 Not surprisingly, these accessory components also induce structural changes in the associated ABC transporter that facilitate transport.59 These accessory proteins add yet another layer of diversity to the family of ABC transporters.

### Table 1. ABC Genes Associated With Human Diseases

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alias</th>
<th>Disease</th>
<th>Proposed Pathophysiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA1</td>
<td>ABC1, CERP</td>
<td>Tangier disease25–27; familial HDL deficiency23</td>
<td>Abnormal transport of cholesterol to apolipoproteins</td>
</tr>
<tr>
<td>ABCA3</td>
<td>ABC3</td>
<td>Surfactant deficiency23 and other pulmonary syndromes of newborns</td>
<td>Abnormal transport of phosphatidylcholine and phosphatidylglycerol23</td>
</tr>
<tr>
<td>ABCA4</td>
<td>ACR</td>
<td>Stargardt macular degeneration; retinitis pigmentosa; age-related macular degeneration22</td>
<td>Abnormal transport of retinol/phospholipids derivatives</td>
</tr>
<tr>
<td>ABCA12</td>
<td>Lamellar ichthyosis type 234</td>
<td></td>
<td>Unclear, possibly attributable to altered lipid transport in keratinocytes</td>
</tr>
<tr>
<td>ABCB2</td>
<td>TAP1</td>
<td>Immune deficiency25</td>
<td>Unstable HLA class I peptide presentation</td>
</tr>
<tr>
<td>ABCB3</td>
<td>TAP2</td>
<td>Immune deficiency22</td>
<td>Altered peptide presentation on HLA class I</td>
</tr>
<tr>
<td>ABCB4</td>
<td>PGY3</td>
<td>Progressive familial intrahepatic cholestasis type 322</td>
<td>Impaired secretion of phosphatidylcholine into bile</td>
</tr>
<tr>
<td>ABCB7</td>
<td>ABC7</td>
<td>X-linked sideroblastic anemia with ataxia27, 38</td>
<td>Defective transport of iron/sulfur complexes out of mitochondria</td>
</tr>
<tr>
<td>ABCB11/BSEP</td>
<td>SPGP</td>
<td>Progressive familial intrahepatic cholestasis type 229</td>
<td>Impaired transport of a number of bile salts into bile</td>
</tr>
<tr>
<td>ABCC2</td>
<td>MRP2</td>
<td>Dubin-Johnson syndrome20</td>
<td>Impaired secretion of bilirubin glucuronosides into bile</td>
</tr>
<tr>
<td>ABCC6</td>
<td>MRP6</td>
<td>Pseudoxanthoma elasticum</td>
<td>Unknown</td>
</tr>
<tr>
<td>ABCC7</td>
<td>CFTR</td>
<td>Cystic fibrosis24</td>
<td>Loss of Cl− ion transport</td>
</tr>
<tr>
<td>ABCC8</td>
<td>SUR1</td>
<td>Transient neonatal diabetes mellitus (TNDM)23; persistent hyperinsulinemic hypoglycemia of infancy (PHHI)23,29 ; DEND syndrome36</td>
<td>Altered SUR1-regulated KATP channel gating</td>
</tr>
<tr>
<td>ABCC9</td>
<td>SUR2</td>
<td>Dilated cardiomyopathy</td>
<td>Impaired KATP channel activity</td>
</tr>
<tr>
<td>ABCD1</td>
<td>ALD</td>
<td>X-linked adrenoleukodystrophy21, 31</td>
<td>Presumed defective transport of very long chain fatty acids</td>
</tr>
<tr>
<td>ABCG5</td>
<td>White 3</td>
<td>Sitosterolemia (mostly Asian patients)</td>
<td>Impaired transport of plant and fish sterols</td>
</tr>
<tr>
<td>ABCG8</td>
<td>Sitosterolemia (mostly white patients)</td>
<td>Impaired transport of plant and fish sterols</td>
<td></td>
</tr>
</tbody>
</table>

For a comprehensive review, see Dean and colleagues2,22 and Singaraja et al.28

**Figure 1.** Schematic of a typical ABC protein. Typical ABC proteins have 2 pore-forming TMDs and 2 NBDs. These highly conserved regions give the entire class of proteins their name. The orange stars denote the ATP-binding sites on the NBDs.

**Atypical ABC Proteins**

Although the majority of ABC proteins directly transport molecules across membranes, there are several ABC proteins that are not involved in substrate transport and are referred to as “atypical” ABC proteins (Table 2).50 These proteins, which clearly do not mediate transport, are classified as ABC proteins because of the presence of the highly conserved ABC domain. Interestingly, many of these atypical ABC proteins have been modified to bind nucleic acids. A classic example of an atypical ABC protein is the bacterial UvrA, which functions in UV-induced DNA repair.60 MutS is another bacterial ABC protein, which plays a critical role in DNA mismatch repair.61 MutS contains the highly conserved ABC motif and forms homo- or heterodimers for function, whereas the transmembrane domain has essentially been replaced by structural motifs involved in the recognition and binding of DNA.61 Similarly, Rad50 contains conserved ABC domains and regulates DNA double-strand break repair. Rad50 also requires dimerization with a total of 4 domains, 2
DNA-binding domains and 2 ABC domains, for normal function. The SMC (structural maintenance of chromosomes) proteins are another class of molecules that contain the classic ABC motif and also bind DNA. SMC proteins are highly conserved from bacteria to humans and play a critical role in the organization of chromosomes; their role as dynamic ATPases is only beginning to be appreciated. The yeast protein Elf1p is also an ABC protein and plays a direct role in the transport of mRNA from the nucleus to the cytoplasm, although whether or not Elf1p binds RNA is still unclear.

In addition to DNA binding, atypical ABC proteins perform other functions. The eukaryotic elongation factor 3 (eEF3) is an ABC protein that plays a key regulatory role in translation. As mentioned, SUR is another atypical ABC protein. SUR associates with one of the Kir6.x potassium channel subunits to form ATP-sensitive potassium channels (K_{ATP}). This represents a unique function among the ABC family of proteins. Unlike other atypical ABC proteins, SUR has maintained its transmembrane domain but is not thought to be directly involved in the transport of molecules across the membrane. In recent years, K_{ATP} channels have become increasingly recognized for their importance in both diabetes and cardiovascular diseases. These channels are an active area of research and are the targets of several different drugs.

The K_{ATP} Channel: Structure and Function

The K_{ATP} channel serves as a sensor of the cellular metabolic state and couples the metabolism of the cell with the membrane potential, primarily by sensing intracellular ATP levels. It is an octamer composed of 4 Kir6 subunits and 4 SUR subunits (Figure 2A). The Kir6 subunits regulate transport of potassium, whereas the SUR subunits play a regulatory role, modulating channel activity depending on cellular ATP levels. Kir6 is a member of the inwardly-rectifying family of K⁺ channels. Two Kir6 isoforms exist, Kir6.1 and Kir6.2, both of which are expressed in the heart, with Kir6.2 being the predominant form in the sarclemma. SUR is an atypical ABC protein that binds to Kir6 subunits to form functional K_{ATP} channels. There are two SUR isoforms, SUR1 and SUR2, the latter of which has two major splice variants, SUR2A and SUR2B. In mice, there have been 2 additional splice variants described. The first encodes a transcript without exon 14 and is only found in the heart. The second is characterized by splicing of exon 17, which encodes a region next to the Walker A site of NBD1 and alters channel gating. SUR1 and −2 are the products of the ABCC8 and ABCC9 genes, respectively. SUR2A is highly expressed in cardiac tissue, whereas SUR1 is most abundantly expressed in the brain and pancreas (Table 3). SUR2B is expressed in vascular smooth muscle cells. SUR1 and SUR2 are full ABC proteins, containing 2 TMD and 2 ABC domains. Similar to other ABC proteins including PGY1 and CFTR, SUR also has an additional 5-helix transmembrane domain termed TMD0 that is connected to the remainder of the SUR subunit by a linker region, L0 (Figure 2). The TMD0 and L0 regions are able to regulate K_{ATP} channel opening, thus suggesting a close functional and physical interaction. Coimmunoprecipitation studies have demonstrated a tight association between TMD0 and Kir6. A 3D model of SUR1/Kir6.2 obtained by single-particle electron microscopy confirms this finding, showing that TMD0 is likely in close association with the outer transmembrane helix of Kir6.2 and L0 is likely in close association with the N terminus of Kir6. The current working model suggests that the Kir6.x subunits, which constitute the pore of K_{ATP}, are located centrally, whereas the SUR subunits are located peripherally, with the important TMD0 domain situated adjacent to the associated Kir6.2 subunit (Figure 2B).

A crystal structure of the K_{ATP} channel is needed for better understanding of the structure and function of the K_{ATP} channel.

The binding of SUR to Kir subunits serves a dual purpose. First, this binding allows translocation of the channel to the plasma membrane. The K_{ATP} channel has a remarkably intricate network of internal targeting signals. These targeting signals regulate proper ER trafficking of the K_{ATP} channel subunits. There are retrograde signals that block ER export and are present in both the Kir and SUR subunits. These signals are blocked on proper dimerization with the respective subunits. Lack of interaction between Kir and SUR results in retention within the ER, and 1:1 subunit stoichiometry is required for cell surface expression. In addition, there is also an antegrade-targeting signal in the C terminus of SUR that is required for release from the ER/Golgi and resultant plasma membrane expression. Second, the interaction between SUR and Kir subunits contributes to the regulation of the channel itself. Specifically, the binding and hydrolysis of ATP at the canonical NBDs of SUR regulates potassium channel activity. Based on data garnered from bacterial ABC
proteins, the NBDs dimerize when bound to nucleotides, facilitating hydrolysis in a cooperative fashion. Binding of ADP induces a conformational change in the structure of the ABC proteins that likely regulates protein function. Direct evidence for this process is lacking for SUR, but circumstantial data, the SUR1/Kir 3D model, and mutational studies indicate that the NBDs of SUR likely dimerize as well. ATP can also inhibit KATP channel activity by binding to the Kir subunits. This tonic inhibition at the Kir subunit is counteracted by Mg2+-bound nucleotide binding at the SUR NBDs.

Although the exact mechanism by which SUR regulates Kir6 is not known, it is generally felt to be attributable to a conformational change in SUR induced by cellular ATP turnover. In other ABC proteins, this conformational change is directly linked to transport across the membrane, whereas in SUR, it is thought to regulate Kir6. SUR, as previously mentioned, interacts with Kir6 via the TMD0 domain and L0, and recent evidence suggests that binding of ATP to the NBDs mediates a conformational change in the TMD0/L0 region. A potential model for this interaction is shown in Figure 2B and 2C. By coupling membrane excitation to the metabolic state of the cell, these channels play key roles in various tissues.

The KATP Channel: Physiological and Pathophysiological Functions of SUR1

The KATP channel plays distinct and important physiological roles in several metabolically active tissues, including the pancreas, brain, and muscle. The KATP channel is best characterized in the pancreas, where it is composed of SUR1 and Kir6.2 subunits and plays a prominent role in regulating insulin secretion in the β-cells. The KATP channel is also expressed in other cells of the endocrine pancreas, including the glucagon-secreting α-cells and the somatostatin-secreting δ-cells. In pancreatic β-cells, high levels of ATP, corresponding to increased plasma glucose levels, result in KATP channel closure. This closure leads to membrane depolarization, Ca2+ influx, and subsequent insulin release (Figure 3A). Binding of ATP to Kir6.2 subunits mediates this inhibition, and SUR1 markedly potentiates this effect. Sulfonylureas, which bind to SURs, are a class of antidiabetic drugs widely used in the treatment of type 2 diabetes mellitus. These drugs bind to the SUR subunit in multiple locations and inhibit KATP channel activity, thus increasing insulin release. In the brain, KATP channels are expressed in various regions and seem to play a role in seizure prevention. They also function as centrally acting glucose sensors. Further, these channels may also play a role in neuronal ischemic preconditioning, similar to what has been observed in cardiac muscle, as described in more detail below.

Mutations affecting the KATP channel result in several disease states that alter pancreatic β-cell function. Many of these mutations are found in the SUR1 subunits of pancreatic KATP channels. Persistent hyperinsulinemic hypoglycemia of infancy (PHHI) is a disorder caused by mutations in several genes, including ABCC8, the gene for SUR1. PHHI is characterized by inappropriately high levels of insulin secretion despite severe hypoglycemia.
disequilibrium between the SUR1 to diabetes. However, there is significant linkage and a common polymorphism in the KATP channel to the plasma membrane, likely because fall into two groups. Some mutations prevent trafficking of these disorders characterized by onset of mild to severe hyperglycemia in the first few months after birth. These disorders are continuous activation of the channel. Transient neonatal diabetes (TND) and permanent neonatal diabetes (PND) are permanent activation of the KATP channel, resulting in membrane hyperpolarization, reduced calcium influx, and reduced insulin secretion.

There are also mutations of the SUR1 subunit that result in continuous activation of the channel. Transient neonatal diabetes (TND) and permanent neonatal diabetes (PND) are disorders characterized by onset of mild to severe hyperglycemia in the first few months after birth. These disorders are at different ends on the same spectrum of disease and, depending on the mutation and the corresponding increase in function, can be transient or persistent, requiring lifelong treatment. Despite the early transient nature of TND, this disease can relapse by the time of adolescence in ≈50% of patients with certain types of mutations. There are several genes associated with this disorder, but recent work has shown numerous activating mutations within the ABCC8 gene, encoding SUR1, that result in either phenotype. All of these mutations have the common pathophysiology of permanent activation of the KATP channel, resulting in membrane hyperpolarization, reduced calcium influx, and reduced insulin secretion.

The recent spate of genome-wide association studies has shown a strong link between the E23K polymorphism of KCNJ11, the gene encoding Kir6.2, and diabetes. Presently, there are no genome-wide association studies linking ABCC8/SUR1 to diabetes. However, there is significant linkage disequilibrium between the KCNJ11 E23K polymorphism and a common polymorphism in ABCC8, A1369S. In fact, these 2 polymorphisms are almost always found together and seem to be protective of progression to diabetes in patients enrolled in the Diabetes Prevention Program. These patients all had impaired glucose tolerance at baseline, and the authors suggest that KCNJ11 E23K polymorphism may function at an earlier stage, namely in the development of impaired glucose tolerance.

Mild cases are readily treatable, although severe forms can be lethal and may require subtotal pancreatectomy. There are numerous ABCC8/SUR1 mutations, and they generally fall into two groups. Some mutations prevent trafficking of the KATP channel to the plasma membrane, likely because of inappropriate exposure of the previously mentioned trafficking signals. Other mutations result in a permanently closed channel that is insensitive to the normal effects of nucleotide binding. Both types of mutations lead to a lack of KATP channel activity, which results in constant calcium influx and hence continuous, inappropriate insulin secretion.

See the text for more details.

### Table 3. The SUR Isoforms

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Gene</th>
<th>Kir6 Subunit†</th>
<th>Tissue Specificity‡</th>
<th>Nucleotide Affinity</th>
<th>Primary Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUR1</td>
<td>ABCC8</td>
<td>Kir6.2</td>
<td>Brain; pancreas</td>
<td>High</td>
<td>Pancreatic β-cells: regulating insulin release</td>
</tr>
<tr>
<td>SUR2A</td>
<td>ABCC9</td>
<td>Kir6.2</td>
<td>Heart</td>
<td>Low</td>
<td>Myocardial cells: regulating action potential duration</td>
</tr>
<tr>
<td>SUR2B</td>
<td>ABCC9</td>
<td>Kir6.2 and Kir6.1</td>
<td>Heart; eye; brain; cerebellum; colon</td>
<td>Intermediate to high</td>
<td>Smooth muscle cells, including vascular: regulating action potential duration and vasodilation</td>
</tr>
</tbody>
</table>

Further details have been published previously. Note: listed is the predominant Kir6.x subunit associated with the SUR isoform. These SUR isoforms associate with the other Kir6.x subunit under certain conditions and in some tissues. †Tissues listed are those in which SUR isoforms are highly expressed. Each isoform is expressed at lower levels in other tissues.

The K_ATP Channel: Physiological and Pathophysiological Functions of SUR2

K_ATP channels in the heart play important roles in cardiac homeostasis. SUR2A is predominantly expressed in the myocardium, whereas SUR2B is predominantly found in the vascular smooth muscle. This tissue-specific distribution has important functional consequences, and the regulation of K_ATP channels by SUR2x is dependent on subcellular concentrations of high-energy phosphates, as well as structural differences between the two SUR2 subtypes.

**SUR2A Regulates K_ATP by Nucleotide Catalysis and Is a Key Sensor of Cellular Energy Status**

As previously mentioned, SUR contains 2 NBDs, which likely work in concert to regulate K_ATP channel activity. K_ATP channels in the pancreas are usually in the open conformation, whereas K_ATP channels in the myocardium are in the closed state under normal conditions. This crucial physiological difference is regulated by the different subtypes of SUR in each tissue. In all SUR subtypes, both NBDs are responsible for nucleotide binding, whereas NBD2 carries out ATP hydrolysis. However, SUR1 and SUR2A have markedly different binding affinities for ATP and ADP, which is believed to result, at least in part, in their opposite effects on K_ATP channel gating in these 2 tissues.

For SUR1, the binding affinities of NBD1 for ATP and ADP, expressed as K_i values, are ≈4.4 and 26 µmol/L, respectively. For the NBD2 of SUR1, the K_i values for ATP and ADP are 60 and 100 µmol/L, respectively. SUR2A has much lower nucleotide affinity, as shown by higher K_i values: at NBD1, the K_i values for ATP and ADP are 110 and 86 µmol/L, respectively, whereas at NBD2, they are 120 and 170 µmol/L, respectively. The NBDs of SUR2B have nucleotide affinities similar to those of SUR1 (K_i values at NBD1 for ATP and ADP of 51 and 66 µmol/L, and at NBD2, of 38 and 67 µmol/L, respectively). These data show that the binding affinities of NBD1 are much lower for SUR2 isoforms than SUR1 and that the SUR2A isoform has a much lower nucleotide-binding affinity than either SUR1 or SUR2B. Additional evidence suggests that the binding of Mg^2+ to
these nucleotides is critical for nucleotide hydrolysis at NBD2 and for proper channel gating.103,104

The high-affinity SUR1 readily responds to increased intracellular ATP secondary to a rise in glucose. This results in hydrolysis of MgATP to MgADP at NBD2. It is this binding of MgADP to NBD2 that induces the proposed conformational change in SUR, leading to increased channel activity. Because of the high levels of ATP and the high affinity for ATP at NBD2 of SUR1, there is continuous cycling, consequent release of ADP, and K_{ATP} channel closure, leading to release of insulin as described previously. In the myocardium, however, the K_{ATP} channel is closed under normal conditions, partly because of the typically high ATP content of this tissue. However, under states of stress, cellular ADP levels rise. As ADP levels rise, the NBD2 of SUR2A becomes fixed in the post-hydrolytic state and is bound to MgADP, resulting in decreased ATP cycling at NBD2 and consequent channel opening (Figure 3B).105 The lower nucleotide affinity at NBD2 of SUR2A likely decreases the propensity for ATP to displace ADP from NBD2, thus allowing NBD2 to remain bound to ADP and facilitate channel opening. Normally, ADP levels are as much as 50-fold lower than ATP levels, even under states of significant metabolic stress.67 As such, it is possible that the reduced affinity of SUR2A for ATP plays a significant role in the response of myocardial K_{ATP} channels to small but significant changes in ADP levels. In addition to this, SUR1 is a more efficient ATPase than is SUR2A,102 although it is unclear how much the variable nucleotide affinities for the 2 NBDs influence enzymatic activity. The common denominator of channel gating by SUR appears to be MgADP binding to and stimulating channel activity, despite much higher levels of cellular ATP.

How can such minor changes in ADP be capable of regulating channel activity in the presence of such overwhelming amounts of ATP? The energy-sensing system of cardiac cells is found focally throughout the cell and, at the sarcolemma, is composed of K_{ATP}, along with several other enzymes of the phosphotransfer system.106 This energy “compartmentalization” allows the energy-sensing system, which includes K_{ATP}, to sense minor fluctuations in cellular energy status even when there are not significant total changes in cellular ATP levels.106 This microenvironment allows K_{ATP} to be in close association with enzymes such as creatine kinase (CK), adenylyl kinase (AK), the muscle form of lactate dehydrogenase (m-LDH) and pyruvate kinase (PK).106 AK is an essential part of intracellular metabolic networks, and it directly regulates the K_{ATP} channel response to dynamic stress by transmitting signals from the mitochondria to the surface K_{ATP} channel.107 Treatment of cells with oligomycin, a mitochondrial F1/F0 ATP synthase inhibitor, prevents K_{ATP} channel opening, providing evidence for this link.107 AK physically associates with K_{ATP}, having been detected by both immunoblotting and by measurement of AK activity in K_{ATP} immunoprecipitates with a Kir6.2 antibody.108 The m-LDH is also physically associated with K_{ATP} and is necessary for the K_{ATP}-mediated cytoprotection from ischemia.108 CK, a major scavenger of free ATPase products, also transduces signals to this subsarcolemmal compartment, where it is found in high concentration.109 Like AK, CK has been shown to physically associate with K_{ATP} in cardiomyocytes, and it has been shown to specifically associate with the SUR2A subunit but not with the Kir subunits.110 In intact hearts, hypoxia has been shown to reduce CK flux.109 This results in a reduction of the normal transfer of ATP to the subsarcolemmal compartment by CK, leading to locally higher ADP concentrations. This reduced flux therefore compromises removal of ADP from SUR2A NBD2 and hence reduces nucleotide cycling, leading to opening of the channel.109 Both AK isoform 1 KO mice and CK KO mice demonstrate markedly reduced K_{ATP} channel gating in response to changes in cellular energy status.107,109

Thus, phosphotransfer reactions are another important means
of regulating $K_{\text{ATP}}$ channel activity and transducing the changes in cellular energy status to $K_{\text{ATP}}$ channels.

The cycling of nucleotides, in particular the presence of MgADP at NBD2, likely results in a conformational change that causes channel gating. As mentioned previously, it is felt that this conformational change acts through the TMD0/L0 domain, whereby binding of MgADP at NBD2 overrides the inhibition of ATP on Kir6.2; however, this has not been proven directly. There is also limited evidence that TMD0/L0 plays a role in the differential gating patterns between SUR2A and SUR1. More evidence is needed to better elucidate the role of the SUR2A TMD0/L0 domain on $K_{\text{ATP}}$ channel function.

In addition to this regulation by SUR2A, $K_{\text{ATP}}$ channel activity is also influenced by the higher ATP content of myocardial tissue and the direct, noncatalytic inhibition of Kir6.2 by ATP. Thus gating of the $K_{\text{ATP}}$ channel is attributable to a complex interplay among SUR subtype, SUR NBD nucleotide affinity and hydrolysis, Kir6.2 nucleotide binding, underlying cellular metabolism, and subcellular energy compartmentalization.

### SUR2A and SUR2B Have a Variable C Terminus

SUR2B is a splice variant of the $ABCC9$ gene. The only difference between SUR2A and SUR2B is the use of a different exon at the C terminus such that the proteins are identical except for the C-terminal 42 aa. Despite this minimal difference, there is a significant difference in channel activity between these 2 subtypes. The C-terminal 42 aa of SUR2B are similar to SUR1. SUR2B mRNA is expressed ubiquitously. SUR2B mRNA is the predominant isoform expressed in vascular smooth muscle, where its protein product interacts with Kir6.1, whereas in other forms of smooth muscle, SUR2B associates with Kir6.2. SUR2B has a nucleotide affinity that is much greater than SUR2A and shows channel gating activity similar to that of SUR1. Structural analysis suggests that the SUR2B C terminus actually interacts with the NBDs, thus regulating the conformational change associated with nucleotide binding. When SUR is coexpressed with Kir6.2 in HEK cells, the C terminus of SUR2A appears to have an inhibitory effect on NBD2 MgADP-induced channel activation. Replacing the C-terminal 42 aa of SUR1 or SUR2B with that of SUR2A results in reduced MgADP-dependent channel activity, similar to what is seen with functional SUR2A. Further research has narrowed the critical regulatory region to a 7-aa sequence within this C terminus. More research is needed to fully define the mechanistic differences caused by this altered C terminus.

### $K_{\text{ATP}}$ Channels and Cardioprotection

As mentioned, myocardial $K_{\text{ATP}}$ channels are closed under normal physiological conditions. However, these channels open at the time of a metabolic insult, reducing cardiac excitability and protecting the myocardium against damage. This occurs by a reduction in action potential duration, which leads to decreased $Ca^{2+}$ flux. This decreases contraction and therefore reduces the consumption of cellular ATP stores. The sarcolemmal $K_{\text{ATP}}$ channel may play a significant role in the process of ischemic preconditioning (IPC), the phenomenon whereby a previous, brief ischemic event results in subsequent protection against a later, more severe ischemic event. IPC induces alterations in cellular bioenergetics that preserve myocardial function; this effect is lost in Kir6.2 KO mice, indicating that $K_{\text{ATP}}$ channels play a role in myocardial protection mediated by IPC. Furthermore, $K_{\text{ATP}}$ channels are vital in the myocardial response to stress, and in either Kir6 KO models or models overexpressing dominant-negative Kir6 subunits, there is a blunted adaptive response to stress, resulting in heart failure and other cardiac defects. Altered calcium handling, resulting from excessive calcium influx that is not properly balanced to the cellular energy supply, is a central feature of this blunted response to IPC and the resulting cardiac defects in response to stress.

Interestingly, recent data show that SUR2 KO mice still exhibit cardioprotection in response to adrenergic stress. In fact, SUR2 KO mice actually have been shown to have significantly reduced infarct size and better left ventricular developed pressure compared with wild-type controls in response to ischemia/reperfusion. These data indicate that the complete, canonical Kir6.2-SUR2A sarcolemmal $K_{\text{ATP}}$ channels are not required for cardioprotection as suggested by the Kir6 KO data. An additional finding was that the calcium channel blocker nifedipine blunts this protective response in SUR2 KO mice. Therefore, it seems that altered calcium handling is a common underlying feature of these models; however, the increased calcium in this case would seem to be having beneficial effects. These data make it clear that there is much yet to be elucidated regarding the underlying pathways of IPC.

Indeed, although sarcolemmal $K_{\text{ATP}}$ channels clearly regulate myocardial response to stress and likely play a role in IPC, there are many other mediators of this process. Recent evidence shows that $K_{\text{ATP}}$ channel recruitment and activity is regulated by the AMP activated protein kinase (AMPK), itself a key mediator of cellular metabolic status. Furthermore, sarcolemmal $K_{\text{ATP}}$ is not the only potassium channel to regulate ischemic preconditioning. Recently, the mitochondrial $K_{\text{ATP}}$ (mito$K_{\text{ATP}}$) channel has been shown to play a role in ischemic preconditioning, independent of sarcolemmal $K_{\text{ATP}}$. This channel is reviewed elsewhere, but it is interesting to note that mito$K_{\text{ATP}}$ is believed to be composed of several proteins, 1 of which is mABC1, an ABC protein whose role in this complex is not known but whose overexpression is protective against oxidant stress in neonatal rat cardiomyocytes. Another layer of complexity regarding the role of $K_{\text{ATP}}$ in IPC has been added by the recent finding that SUR2A is expressed not only in the sarcolemma but also in the mitochondria of cardiomyocytes. It remains to be seen whether functional SUR-containing $K_{\text{ATP}}$ channels will be found in the mitochondria and, if so, what role they play in IPC or general cardiac cellular physiology.

### SUR Subunits Respond Differently to Pharmacotherapeutic Agents That Regulate $K_{\text{ATP}}$ Channels

$K_{\text{ATP}}$ channels are targets of many different pharmacological compounds, including both inhibitors of channel activity and
activators of channel activity. Many of these drugs act through the SUR subunit. The best-known drugs that regulate K\textsubscript{ATP} channels are the sulfonylureas. Sulfonylureas are widely used to treat type 2 diabetes mellitus, acting as insulin secretagogues by inducing β-cell K\textsubscript{ATP} channel closure on binding to SUR1.\textsuperscript{109} Different molecules within this class have different binding affinities for SUR subtypes, with some being very specific for SUR1 (eg, nateglinide, mitiglinide), some being moderately specific for SUR1 (eg, glyburide, glimepiride), and others being essentially not selective (eg, repaglinide).\textsuperscript{128} Given this spectrum of specificity, it would be expected that some sulfonylureas have adverse cardiac effects by preventing the protective effects of IPC because of their ability to close K\textsubscript{ATP} channels, and at least one of these, repaglinide, lists “myocardial ischemia” as a possible adverse reaction. Experimentally, glibenclamide (moderately SUR1-selective) eliminated the beneficial effects of IPC, whereas mitiglinide (highly SUR1-selective) did not, suggesting that their different binding affinities may play a role.\textsuperscript{129} Another study showed that glimepiride did not block IPC, unlike glibenclamide,\textsuperscript{130} both of which are moderately SUR1-selective.\textsuperscript{128} This was attributable to the inability of glimepiride to block mitoK\textsubscript{ATP}, whereas glibenclamide did, again illustrating the multiple pathways that underlie IPC.\textsuperscript{130} As reviewed elsewhere, concern over a possible link between sulfonylureas and adverse myocardial events extends back almost 40 years; however, recent clinical evidence seems more reassuring, but data are still mixed.\textsuperscript{131}

K\textsubscript{ATP} channel openers in theory could be beneficial in ischemic heart disease by inducing channel opening and thereby reducing Ca\textsuperscript{2+} influx, mimicking IPC. One drug in particular, diazoxide, is interesting because, in vitro, it is able to promote IPC; however, it is believed to have higher affinity for the mitochondrial than the sarcolemmal K\textsubscript{ATP} channel. Despite this theoretical benefit, no K\textsubscript{ATP} channel openers are regularly used clinically to treat disease. These channel openers are reviewed elsewhere.\textsuperscript{132}

**K\textsubscript{ATP} Channels in the Endothelium**

In the endothelium, K\textsubscript{ATP} channels play an important role in the regulation of vascular tone. Vascular K\textsubscript{ATP} channels are composed mainly of the SUR2B isoform and Kir6.2, although Kir6.1 may also associate with SUR2B in certain vascular tissue, including the coronary arteries.\textsuperscript{133} Vascular K\textsubscript{ATP} channels are activated by vasodilators such as β-adrenergic agonists and adenosine via the protein kinase A pathway and are inhibited by vasoconstrictors such as angiotensin II via protein kinase C.\textsuperscript{134} Vasodilators produced during an ischemic insult are thought to diffuse from the myocardium and activate these channels. This results in hyperpolarization, reduced calcium flux, and vasodilation (Figure 3C).\textsuperscript{135}

**SUR Subunits Cause K\textsubscript{ATP} Channel Dysfunction in Cardiac Disease**

Evidence of a potential role for SUR in cardiovascular disease comes from muscle-specific SUR2 KO studies. SUR1 KO mice also have been generated, but these studies are beyond the scope of this discussion.\textsuperscript{136} A SUR2 KO was generated by deleting NBD1; therefore, this KO does not distinguish between SUR2A and SUR2B isoforms.\textsuperscript{137} SUR2 KO mice exhibited hypertension, sudden death, and increased coronary vasospasm.\textsuperscript{138} The presence of vasospasm and hypertensive clearly implicate vascular smooth muscle K\textsubscript{ATP} channels and the SUR2 isoform in the regulation of vascular tone. Interestingly, though, transgenic expression of normal SUR2B, which reconstitutes normal sarcolemmal K\textsubscript{ATP} channels, does not abrogate the incidence of vasospasm in these SUR2-null mice, suggesting additional unknown effects of SUR2 on muscle cell biology.\textsuperscript{139} The pattern of ECG changes seen in these mice is the same as that observed in humans with Prinzmetal’s, or variant, angina,\textsuperscript{138} although no known human mutations in ABCC9 have yet been associated with spasm. However, a rare mutation found in an Italian population encoding for a Val734Ile mutation in ABCC9 has been associated with a 6.4-fold increased risk of early myocardial infarction (defined as myocardial infarction before 60 years of age) compared with control patients.\textsuperscript{140} At present, the pathophysiological mechanism behind this increased risk is not known, but given the excessive vasospasm noted in the SUR2 KO, the authors posit that this mutation may increase the incidence of vasospasm, resulting in myocardial infarction.

Another ABCC9 mutation in SUR2A has been identified recently in a patient with adrenergic-inducible atrial fibrillation.\textsuperscript{141} This mutation, T1547I, occurs at a highly conserved residue in the unique C-terminal 42-aa domain of SUR2A. As previously stated, this domain constitutes the only difference between SUR2A and SUR2B and is thought to play a major role in the differential gating exhibited by these 2 isoforms.\textsuperscript{113} This residue is predicted to be in close proximity to the Walker A motif of NBD2, and the T1547I mutation alters the normal hydrogen bonding that stabilizes this motif.\textsuperscript{141} This mutant SUR2A, coexpressed with Kir6.2, showed reduced responsiveness to ADP, while retaining the normal inhibitory effects of ATP, thus resulting in decreased channel gating.\textsuperscript{141}

Experimental evidence also supports a possible role for SUR2A in ischemic heart disease. Using the rat-derived cardiac cell line, H9c2, exposure to 24 hours of mild hypoxia increased expression of SUR2A but not Kir6.2; this upregulation also led to a corresponding increase in plasma membrane K\textsubscript{ATP} channel density.\textsuperscript{142} Of note, Kir6 is expressed at much higher levels than SUR2A in both adult cardiomyocytes\textsuperscript{143} and H9c2 cells.\textsuperscript{144} When cells pretreated with mild hypoxia (P\textsubscript{O2} = 100 mm Hg), but not normoxia (P\textsubscript{O2} = 140 mm Hg), were exposed to more severe hypoxia followed by reoxygenation, they were protected against cell death.\textsuperscript{142} Transgenic mice overexpressing SUR2A show much improved cell survival and markedly reduced infarct size in response to ischemia, further supporting the role that increased SUR2A expression has on ischemic heart disease.\textsuperscript{144}

In light of the data discussed above, it is interesting to note that there is no significant change in the structure of the myocardium of SUR2 KO mice despite the fact that these KO mice had no detectable myocardial SUR2 expression.\textsuperscript{123,137} Despite this, mutations in ABCC9 have been identified in idiopathic dilated cardiomyopathy patients.\textsuperscript{146} The 2 identified mutations disrupt the variable C terminus of SUR2A,
again pointing to the importance of this structure in regulating K$_{\text{ATP}}$ channels. These mutations both disrupt the tertiary structure of NBDD2, specifically reducing the ATPase catalytic activity without affecting nucleotide binding. This reduced ATPase activity causes aberrant sensing of cellular energy status and prevents the normal regulation of channel activity by phosphotransfer systems such as CK. Because an estimated half of all cases of dilated cardiomyopathy are deemed to be idiopathic, it seems likely that further mutations affecting the ABCC9 gene will be found in the future.

**Conclusions**

ABC proteins play critical roles in all organisms throughout nature. They regulate many processes critical to life. In the heart, the atypical ABC protein SUR plays a critical role in the regulation of K$_{\text{ATP}}$ channels; this regulatory role is unique among ABC transporters described to date. SUR plays a key part in the intricate regulation between cellular energy status and membrane potential. Experimental models suggest that the cardiac and vascular isoform, SUR2, plays an important role in IPC and ischemic heart disease, coronary vasomotor tone, the development of hypertension, and heart failure. Despite this, much has yet to be learned about SUR and its role in cardiovascular disease. For instance, in SUR2 KO mice, coronary vasospasm is related to ST elevation and atrioventricular heart block on the ECG, yet the role of SUR in the cardiac electrical conduction system has yet to be elucidated to any significant extent. Furthermore, SUR2A levels have been shown to decline in an animal model of aging, but a role for SUR2A levels in human aging or an association between the development of cardiac disease and aging has yet to be established. Nonetheless, research on ABC proteins in the cardiovascular system will continue to progress, hopefully leading to future therapies targeted at cardiac disease.

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None.

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Sulfonylurea Receptor and Regulation of $K_{\text{ATP}}$

Burke et al


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