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ATP-sensitive K^+ channel channel/enzyme multimer: Metabolic gating in the heart

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Abstract

Cardiac ATP-sensitive K^+ (K_{ATP}) channels, gated by cellular metabolism, are formed by association of the inwardly rectifying potassium channel Kir6.2, the potassium conducting subunit, and SUR2A, the ATP-binding cassette protein that serves as the regulatory subunit. Kir6.2 is the principal site of ATP-induced channel inhibition, while SUR2A regulates K^+ flux through adenine nucleotide binding and catalysis. The ATPase-driven conformations within the regulatory SUR2A subunit of the K_{ATP} channel complex have determinate linkage with the states of the channel's pore. The probability and life-time of ATPase-induced SUR2A intermediates, rather than competitive nucleotide binding alone, defines nucleotide-dependent K_{ATP} channel gating. Cooperative interaction, instead of independent contribution of individual nucleotide binding domains within the SUR2A subunit, serves a decisive role in defining K_{ATP} channel behavior. Integration of K_{ATP} channels with the cellular energetic network renders these channel/enzyme heteromultimers high-fidelity metabolic sensors. This vital function is facilitated through phosphotransfer enzyme-mediated transmission of controllable energetic signals. By virtue of coupling with cellular energetic networks and the ability to decode metabolic signals, K_{ATP} channels set membrane excitability to match demand for homeostatic maintenance. This new paradigm in the operation of an ion channel multimer is essential in providing the basis for K_{ATP} channel function in the cardiac cell, and for understanding genetic defects associated with life-threatening diseases that result from the inability of the channel complex to optimally fulfill its physiological role.

Keywords

K_{ATP} channel; Kir6.2; SUR2A; Eneregetics; Creatine kinase; Adenylate kinase; Glycolysis; Sulfonylurea receptor; Phosphotransfer; Nucleotide; ATP-binding cassette; Action potential; Heart failure; Potassium channel opener; Knock-out

1. Introduction

ATP-sensitive K^+ (K_{ATP}) channels are molecular combinations of an inwardly rectifying K^+ channel, Kir6.x, and a regulatory module, the sulfonylurea receptor SUR [1–3]. Biogenesis of the heteromultimeric K_{ATP} channel occurs through combinations of subunit isoforms that define the intrinsic properties and tissue specificity of the channel complex [3,4]. Physical association of the Kir6.2 and SUR2A isoforms generates cardiac K_{ATP} channels that are

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expressed in high density at the sarcolemma [5,6]. By virtue of their integration with cellular energetic networks and their ability to decode metabolic signals, K_{ATP} channels set membrane excitability to match demand for homeostatic maintenance [7–11]. Recent progress in the understanding of K_{ATP} channel structure and function has been founded on the dissection of channel subunit properties, mapping of channel coupling with cellular energetics and definition of the metabolic sensing role in both healthy and diseased cells.

Under conditions of metabolic surplus, the cardiac K_{ATP} channel responds by closure while metabolic challenge provokes channel opening with consequent K^+ efflux, action potential shortening, and limitation of potentially damaging intracellular Ca^{2+} loading [10,12,13]. The basic gating of the K_{ATP} channel that underlies the channel's metabolic response occurs in reaction to the balance at the channel site of inhibitory and stimulatory nucleotides, ATP and ADP, respectively [1,14]. The way in which the cellular metabolic state is "read" incorporates generation and delivery of nucleotide signals to the K_{ATP} channel subunits, and nucleotide interactions with specialized channel domains that ultimately secure signal processing and translation into pore gating [15,16].

In this way, K_{ATP} channels mediate a homeostatic membrane response to the metabolic insults of ischemia or hypoxia contributing to a cardioprotective outcome [17,18]. Recent studies indicate an even broader function for cardiac K_{ATP} channels in the tolerance of cardiomyocytes to numerous acute and chronic metabolic challenges, including sympathetic surge, and physical training [10,19]. Furthermore, the concept of K_{ATP} channel-mediated myocardial protection has been expanded to include balancing increased performance to meet augmented demands of stress while avoiding an excessive response that could result in cellular injury and/or arrhythmia [10,19–21]. The homeostatic role of K_{ATP} channels is underscored by studies of altered channel behavior in heart disease. Channel gene mutations that disrupt K_{ATP} channel function [14] and/or defects in signaling pathways proximal to the channel site [20] compromise the channel's ability to optimally respond to metabolic challenge. Thus, proper metabolic gating of K_{ATP} channels is vital in limiting acute adverse myocardial outcomes under stress, and in evading injury that precipitates the development or progression of chronic heart disease [10,11,14,19,20].

2. Kir6.2 pore-forming subunit: site of K_{ATP} channel ATP inhibition

Tetramers of Kir6.2 subunits comprise the pore of K_{ATP} channel complexes [3,22]. The pore-forming Kir6.2 subunits cannot readily traffic to the plasma membrane alone, without the regulatory SUR module, due to a C-terminal RKR endoplasmic reticulum retention signal [23,24]. When engineered to be expressed independently of SUR through truncation of the C-terminal amino acids, the Kir6.2 subunit was identified as critical for K_{ATP} channel inhibition by intracellular ATP [25,26]. Although a conventional nucleotide binding motif has not been identified within the Kir6.2 sequence, photoaffinity labeling and scanning with sulfhydryl group reagents accomplished by mutagenesis identified that both N- and C-termini may contribute to recognition of ATP [27,28,29]. The most recently developed model implicates two Kir6.2 subunits in coordination of one ATP molecule [30]. While K185 and R201 in the C-terminus of one subunit and R50 in the N-terminus of another Kir6.2 have been directly implicated in the interaction with the γ- and β-phosphate of ATP, respectively, the adenine ring of ATP interacts with E179 and R301 of the second subunit. Mutations that impact the ATP-responsiveness of Kir6.2 alter cardiac mechanical properties at rest and produce a poor myocardial response to ischemic and metabolic challenge [31], and in non-cardiac tissues are associated with disease due to deficient metabolic coupling [32,33].

Assembly of Kir6.2 with SUR enhances ATP-induced inhibition of the channel pore [25], and defines the tissue-specific burst kinetics of K_{ATP} channel behavior [5,34,35]. Furthermore,

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fundamental K_{ATP} channel properties, including stimulation by MgADP and potassium channel openers as well as inhibition by sulfonylurea drugs that are absent in truncated Kir6.2 channels, are rescued by co-expression of Kir6.2 with SUR [25,36].

3. SUR regulatory module: nucleotide binding and catalysis

SUR, the regulatory subunit of the K_{ATP} channel, incorporates two bundles of six hydrophobic transmembrane-spanning domains (TMD) that are fused to hydrophilic nucleotide binding domains (NBD) also known as the ATP-binding cassettes (ABC). By virtue of structure and sequence homology, SUR belongs to the ABCC subfamily of ABC proteins (http://www.gene.ucl.ac.uk/nomenclature/genefamily/abc.html), that includes the multidrug resistance-associated protein (MRP1 or ABCC1) and the cystic fibrosis transmembrane regulator (CFTR or ABCC7), that generally exist as dimers with two ABC modules and two TMDs (TMD-ABC-TMD-ABC [37]). SUR (TMD_0-TMD-ABC-TMD-ABC), in addition to its unique property of association with a distinct protein subunit, Kir6.2, also contains an additional bundle of five TMDs (TMD_0), proposed to anchor SUR to the Kir6.2 channel pore [38]. Typical for ABCs, two highly conserved motifs, Walker A (GXXXXGKS/T) and Walker B (four hydrophobic residues followed by aspartate) frame a signature LSGGQ linker motif [39,40]. The crystal structure of homologous proteins indicates that the ε-amino acid and/or the main chain nitrogen of the invariant lysine of the Walker A motif participates in the binding of the β- and γ-phosphate of ATP whereas the Walker B aspartate coordinates Mg^{2+} through hydrogen bonding to a water molecule [41]. Together with a histidine switch region, postulated to polarize the attached water molecule for hydrolysis, as well as the Q-loop (between the Walker A and the signature motifs) that interacts with the γ-phosphate of ATP through a water bond, Walker motifs form sites for nucleotide binding and hydrolysis within SUR module (Fig. 1).

Testing of nucleotide binding and hydrolytic properties of SUR with photoaffinity labeling has revealed that NBD1 can be labeled with 8-azido-ATP even in the absence of Mg^{2+} and γ-phosphate analogs (e.g. orthovanadate) which is usually applied in order to prolong the life-time of nucleotide-NBD complexes securing the probability for covalent bonding of azido-nucleotides in a binding pocket [42,43]. Thus in contrast to certain ABC proteins NBD1 of SUR apparently does not hydrolyze ATP but possesses relatively stable nucleotide binding. Indeed, in the presence of Mg^{2+}, NBD1 but not NBD2 of SUR could be labeled with 8-azido [γ-^{32}P]ATP suggesting that at least one hydrolytic cycle occurred at the second domain prior to labeling leaving the possibility only for tagging of NBD2 with of 8-azido-[α-^{32}P]ATP [44, 45]. Asymmetrical properties of nucleotide interactions with NBDs correlate with the different structural homology between NBDs. Specifically, in the cardiac K_{ATP} channel, the two NBDs of SUR2A share a 28% sequence homology, such that they are more similar to their counterparts in CFTR than they are to each other (i.e. ~40% sequence homology of SUR2A and CFTR NBD1s, as well as ~40% between the NBD2s of these two ABC proteins). In the SUR close relatives MRP1 and CFTR NBD2 also typically more catalytically active than NBD1 [44–46].

The rate of the catalytic reaction of SUR was directly probed in immunoprecipitates of SUR2A and in purified constructs containing either NBD1 or NBD2 fused to maltose binding protein MBD [47]. In fact, [^{32}P]P_i generation from γ-labeled [^{32}P]ATP was catalyzed by purified MBD-NBD2 and to a lesser extent if at all by MBD-NBD1, confirming primary catalytic activity at NBD2 [47]. Magnesium-dependent NBD2 ATPase activity was within the range reported for other ABC proteins [9,47]. Site-directed mutagenesis of the lysine residue in the Walker A motif of NBD2 or mutation in the Walker B aspartate suppressed this ATPase activity, and produced K_{ATP} channels with a higher sensitivity to ATP compared to wild-type Kir6.2/SUR2A [47,48] indicating that the hydrolytically-driven transition from the MgATP-

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to the MgADP-liganded conformations of SUR influences the gating properties of K_{ATP} channels.

4. SUR catalysis-mediated Kir6,2 channel gating

While SUR2A shares the major property of ATP interaction and hydrolysis with other ABCC proteins, no transport function coupled to catalysis, typical for MRP1 protein, has so far been identified. Rather, in the SUR/Kir6.2 complex, similarly to CFTR that also functions as a channel [45,49–51], an intrinsic catalysis is not required for passive ion permeation down the elecrtochemical gradient but could be involved in allosteric regulation of pore gating. Specifically, coupling of discrete conformations in the SUR catalytic cycle with channel gating was solved using nucleotide trapping procedures in conjunction with on-line channel recording when intrinsic enzymatic activity was enhanced by elevating temperature [9]. Capture of ATPase intermediates was achieved using γ-phosphate analogs, orthovanadate (V_i) and beryllium-fluoride (BeF_x), which stabilize the catalytic cycle in distinct conformations [49]. Orthovanadate forms a pentavalent pyramidal structure, like γ-phosphate in ATP undergoing hydrolysis, and stabilizes post-hydrolytic states whereas pre-hydrolytic intermediates can be distinguished using beryllium-fluoride which forms a tetrahedral structure, and mimics γ-phosphate in ATP prior to hydrolysis. Although both V_i and BeF_x in the presence of nucleotides arrest NBD2ATPase activity, the outcome of the distinct catalytic intermediates induced by these γ-phosphate analogs on the K_{ATP} channel regulation is opposite [9]. Trapping the prehydrolytic MgATP-bound state at the SUR ATPase with beryllium-fluoride (*SUR*MgADP·BeF*$_x$) translates into pore closure or negative channel gating. Recruitment of a posthydrolytic MgADP-bound state by orthovanadate (*SUR**MgADP·V*$_i$), promotes opening of ATP-inhibited K_{ATP} channels or positive channel gating. Trapping the SUR ATPase by beryllium-fluoride and orthovanadate requires divalent cations, hydrolyzable ATP or externally applied ADP, and the structural intactness of NBD2, underscoring the involvement of operational ATP hydrolysis in channel regulation.

Elevated levels of intracellular MgADP, as it occurs under metabolic stress [15,52–55] effectively decelerate ATPase cycling increasing the probability of the post-hydrolytic conformation associated with channel opening [9,14]. In this way, nucleotide exchange between the SUR ATPase and intracellular metabolic pathways could couple K_{ATP} channel function with cellular energetics. In the cell, the high-rate of the major phosphotransfer reaction, catalyzed by creatine kinase, scavenges the ATPase product and facilitates disengagement of posthydrolytic conformations promoting SUR ATPase cycling. Indeed, the rate of ATP hydrolysis at NBD2 can be doubled by creatine kinase, reversing MgADP-induced K_{ATP} channel opening highlighting a critical role for phosphotransfer reactions in setting the balance between the lifetime/probability of MgADP versus MgATP-bound conformational intermediates in the SUR ATPase cycle [9,14]. As a consequence of metabolic stress, a drop of creatine kinase flux compromises ADP removal from ATPase sites and suspends the ATPase cycle in a MgADP-bound state producing channel opening [12,15,16].

Expanding on the conventional nucleotide competition model of K_{ATP} channel gating, such mechanism underscores a critical role for the regulation of substrate/product-dependent engagement of SUR into ATP/ADP-bound intermediates during the ATPase cycle. Stopped-flow spectroscopy, in conjunction with steady-state characteristics of NBD2 catalysis, has allowed determination of the reaction kinetic constants defining the ATPase cycle of SUR2A [14]. The derived values indicated that the rate-limiting step of the NBD2 ATPase cycle is the actual dissociation of ADP. A normally progressing MBP-NBD2 ATPase catalysis is characterized by a relatively long lifetime of the *NBD2·ADP* intermediate state in comparison with shorter lifetimes in other intermediates *NBD2·ATP* and *NBD2·ADP·P*$_i$. Thus, the probability that NBD2 will adopt the ADP-bound state through the ATPase cycle will be higher

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than the probability that this state will form through simple competitive binding in the absence of ATPase activity [14]. Hence, the SUR catalytic activity provides conformational changes that would otherwise occur only rarely, as has also been suggested for gating of CFTR channels [51] with analogy to GTP hydrolysis by G-proteins [56].

Structural characteristics of SUR2A suggest specialized domains that particularly influence the ATPase cycle and associated nucleotide-dependent K_{ATP} channel gating [57]. A high resolution three-dimensional model of SUR2A NBD2, obtained with 1 fs time step molecular dynamics simulation, identifies a β-strand, within the SUR tissue-specific carboxy-terminal tail, that may interact with the $Walker_A$ motif due to the spatial geometry of the folded molecule [14]. Mutations flanking the carboxy-terminal β-strand do not affect ATP-binding but dramatically change the rate and kinetics of the ATPase reaction resulting in altered nucleotide-dependent K_{ATP} channel regulation [14]. This finding underscores that SUR-mediated gating of the channel pore depends not only upon nucleotide binding to NBDs as classically proposed, but also on the predominant conformational intermediate adopted by the intrinsic ATPase cycle. Furthermore, differences in the pattern of probabilities and lifetimes of individual SUR conformations, in addition to distinct ATPase rates, may contribute to the tissue-specificity of nucleotide-dependent K_{ATP} channel regulation [57,58].

While activation of ATP-inhibited K_{ATP} channels following stabilization of the post-hydrolytic state is the well-established mechanism of MgADP-dependent positive channel gating, the outcome of the pre-hydrolytic MgATP-bound SUR conformation represents a previously unrecognized mode of negative nucleotide-dependent channel gating. Although K_{ATP} channel inhibition is usually attributed exclusively to interaction of ATP with the channel pore, negative channel gating apparently could also be induced following binding of sulfonylurea drugs to SUR [48]. Whereas traditionally the effect of sulfonylurea is believed to be nucleotide-independent, binding of glyburide to SUR has been shown to modulate the nucleotide interaction with SUR [59], suggesting the involvement of ATPase-related intermediates in sulfonylurea-induced K_{ATP} channel inhibition [60,61].

The regulation of K_{ATP} channels by potassium channel openers (KCOs) also relies on the NBD2 ATPase-driven conformations within SUR2A. Arrest of the ATPase in a prehydrolytic, but not posthydrolytic, state prevents potassium channel openers from activating K_{ATP} channels [9]. The effect of potassium channel openers could be abolished by non-hydrolyzable ATP analogs, and rescued in the absence of active hydrolysis by recruitment of a posthydrolytic conformation with MgADP [9]. Disengagement of the posthydrolytic conformation by ADP scavenging through the creatine kinase system reverses the effect of potassium channel openers [9,47]. Thus, entry of SUR into a posthydrolytic conformation plays a permissive role in mediating the action of potassium channel openers. By affecting the ATPase activity [47], potassium channel openers stabilize the posthydrolytic MgADP-bound conformation [9]. This is in accord with the finding that ATP-inhibited K_{ATP} channels, when activated by potassium channel openers, exhibit single-channel kinetic behavior indistinguishable from that measured in the presence of MgADP [35,47]. Further, in other SUR isoforms, potassium channel openers maintain MgADP binding, thereby prolonging channel activity following removal of the nucleoside diphosphate [62]. Conversely, intermediates in the ATPase cycle may define the affinity of bound ligands to SUR, as proposed for ATP hydrolysis in other ABC proteins [63, 64]. Specifically, the slow off-rate of potassium channel openers in the presence of magnesium nucleotides [65] suggests that engagement in the posthydrolytic conformation may in turn stabilize potassium channel opener binding. Taken together these data support the hypothesis that K_{ATP} channel regulation by Mg^{2+}-nucleotides and drugs recruits a common allosteric transduction pathway that originates from conformational inter-conversion of NBDs in SUR and culminates with structural “adaptation” of the channel pore.

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Although such allosteric coupling implicates a determinate linkage between the states of the channel pore and the set of NBD2 ATPase conformations, an individual intermediate of NBD2 ATPase cycle may not directly translate into regulation of the channel pore. Indeed, during a normally operating ATPase, NBD2 during each single cycle unavoidably adopts the ADP-bound conformation that potentially could activate K_{ATP} channels. Paradoxically, ATP-regenerating systems that facilitate ATPase cycling and therefore promote transit through the MgADP-bound intermediate state, do not activate K_{ATP} channels [9,15,16,45]. It is only through arrest of the SUR ATPase in a pre- or post-hydrolytic conformation that specific channel outcome, pore closure or antagonism of ATP-induced pore inhibition, can be achieved [9]. In other words, antagonism of ATP-induced channel inhibition can only be secured through prolongation of the lifetime or increase in the probability of posthydrolytic intermediates [9, 14]. In turn, this would indicate that conformational transitions of NBD2 in SUR are not directly translated into a specific state of the channel pore, but rather mediated by an intervening structural rearrangement with the time characteristics slower than the life-time of the post-hydrolytic state. An additional slow-rate conformational rearrangement that transmits the information provided by catalytic intermediates of SUR to the channel pore may rely on dimerization of the two NBDs as has recently been established for a number of ABC proteins [29,66].

5. NBD dimerization and K_{ATP} channel gating

In the structure of the NBD2 monomer of SUR2A [14] as well as other ABC members, the ATP-binding site is exposed, suggesting that the catalytic site can be completed by interaction with another domain [51,66,67]. Evidence of physical proximity of NBDs [68] has hinted that one NBD monomer could complete the binding pocket of the adjacent one.

In the K_{ATP} channel complex cooperative interaction, rather than separate contributions of each of the NBD in SUR2A, is critical for coupling NBD2 ATP intermediates and the functional state of the K_{ATP} channel pore [69,70]. A conformational/functional association of the two NBDs of SUR is supported by biochemical studies of cooperative nucleotide binding. Specifically, MgADP, either by direct binding or as a product of ATP hydrolysis at NBD2, facilitates ADP/ATP-binding to NBD1, an effect abolished by mutations in the Walker A and Walker B motifs of NBD2 [42,43,59]. Furthermore, mutation of the Walker A lysine of NBD1 is crucial for K_{ATP} channel activation induced by stabilization of $NBD2{\cdot}ADP{\cdot}V_i$ or inhibition by $NBD2{\cdot}ADP{\cdot}BeF_x$ complexes, indicating a joint action of NBD1 and NBD2 on channel gating [69]. Cooling of membrane patches, which prevents dissociation of pre-bound nucleotides and “freezes” the conformational intermediates of SUR, activates cardiac K_{ATP} channels in the presence of inhibitory ATP following fast reheating [69]. Such an observation indicates a relatively long-lived cooling-induced conformational rearrangement characterized by barricade of nucleotides in the binding pockets presumably following association of NBDs. Pre-incubation of membrane patches either with MgADP alone or with non-hydrolyzable analogs of ATP prevents K_{ATP} channel activation after reheating. Mutation of the Walker A lysine that impairs nucleotide binding to NBD1 also abolishes cooling/reheating-induced K_{ATP} channel activation, as do mutations in the Walker A lysine and Walker B aspartate critical for hydrolysis in NBD2. Thus, ATP-binding to NBD1, cooperatively supported by hydrolysis at NBD2, is a necessary step in securing the proper structural arrangement of SUR that translates into positive gating of K_{ATP} channels. This paradigm finds support where cooperative binding of two ATP molecules has been demonstrated in other ABC proteins, such as histidine permease [21], maltose transporter [71], MRP1 [72] and P-glycoprotein [73], where cooperative nucleotide binding provides a kinetic control for the formation of a closed NBD dimer [74].

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The determination of crystal structures of several bacterial ABC proteins provides direct evidence that the NBD of ABC proteins physically interact, forming a NBD:NBD dimer [41, 75,76]. However, this finding implicates a different topology of signature and Walker motifs within a NBD dimer, making it difficult to generalize a single dimer structure to all ABC proteins [66]. Furthermore, in the presence of nucleotide, the biochemical or structural evidence of dimer formation was obtained only for Rad50 but not for ABC transporters [41, 67]. Implementation of Rad50 architecture as a template for modeling of other ABC structures is limited, since in contrast to conventional ABC transporters Rad50 lacks membrane spanning domains and interacts with nucleotides via regions that are not conserved among ABC proteins [41,67]. The most recent crystal structure of MJ0796 obtained with the highest resolution of any NBD nucleotide-sandwich dimer allowed modeling of the SUR1 NBD dimer on the basis of sequence alignment [77]. In this structure, an ATP molecule is “sandwiched” between the Walker A motif of the first NBD and the linker motif of the second NBD (Fig. 1) [29,41]. Thus, one ATP molecule interacts with portions of both NBD.

Evidence for NBD dimerization along with the hydrolytic activity within NBDs, provides the basis for various mechanisms of substrate transport by ABC proteins. It has been suggested that dimerization of the NBDs, induced by ATP-binding, represents a key conformational change ultimately leading to dislocation of the signature motifs, ATP hydrolysis and substrate transport, providing the rationale for the existence of two NBD components in all ABC transporters. To this end, hydrolysis would administrate a catalytic passage to dissociation of NBDs aimed to reset the molecular transport machinery to an initial state following the liberation of inorganic phosphate and ADP [41,78]. Remarkably, biochemical studies as well as crystal structures of intact ABC transporters did not reveal significant structural modification induced by the transition of NBDs from the ATP- to the ADP-bound state [74,79,80] indicating that individual reorientation of each NBD is not sufficient for altering of the whole protein structure. Therefore, it appears that it is through dimerization of the NBDs that a more generic structural rearrangement—“power stroke” [74]—can be accomplished to allosterically couple intrinsic catalysis with an associated protein function.

6. Allosteric regulation of the K_{ATP} channel complex

In this regard, the allosteric regulation of the K_{ATP} channel complex seems unique among enzymatic systems since it implicates not only structural coupling and cooperativity, induced by nucleotide interaction within the regulatory channel module, but also transmission of such modification to the associated pore-forming subunit aimed at gating otherwise passive ion permeation. Indeed, the classical allosteric model was successfully applied to nucleotide-dependent K_{ATP} channel gating [15] which presumed that four identical binding sites for ATP and ADP co-exist within the octameric stoichiometry of the K_{ATP} channel complex [22]; binding of ATP to the pore-forming Kir6.2 subunit inhibits channel opening [25,26], whereas binding of ADP to the corresponding regulatory SUR subunit antagonizes ATP-binding to Kir6.2 [43,81]. Although this classic allosteric model of channel regulation was in agreement with experimentally defined nucleotide-dependent gating (i.e. ATP concentration-dependence, saturation of MgADP-induced channel activation), it only postulated intersubunit communication and provided neither the molecular mechanism nor the structural determinants underlying SUR-Kir6.2 interaction.

Regulation of the K_{ATP} channel complex seems to require a complex allosteric hierarchy since the effects of non-nucleotide channel regulators (potassium channel openers, sulfonylureas) also rely on the nucleotide-dependent state of both NBDs in the SUR regulatory module. This suggests that conformational rearrangements of NBDs induced by exogenous (openers and sulfonylurea) and endogenous (Mg-nucleotides) ligands are coupled with the open or closed state of the channel pore through a common, integrating allosteric pathway. Such a suggestion

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stems from a number of experimental observations. Specifically, endogenous as well as exogenous channel regulators (sulfonylurea and KCO) produced the same kinetic fingerprint for channel behavior significantly modulating the probability of burst termination with a similar pattern of changes in interburst kinetics [35,47]. Activation of K_{ATP} channels by potassium channel openers apparently involves cooperative interaction of NBDs since their effect/binding is dependent on intracellular adenine nucleotides and Mg^{2+}, requires the intactness of both NBDs, and relies on post-hydrolytic SUR conformations [40,47,65,82]. Biochemical studies have demonstrated that binding of a sulfonylurea to SUR modulates the cooperative interaction between NBDs inducing ATP dissociation from NBD1 [59]. Neutralization of a number of positively charged arginine moieties in Kir6.2 produced a K_{ATP} channel phenotype insensitive to both MgADP-induced activation and sulfonylurea-induced inhibition [83]. Thus, a structural component within SUR2A to be considered as a candidate common transducer of conformational rearrangements from the regulatory sub-unit to the Kir6.2 channel pore may bear a significant negative charge complementary to positively charged residues in Kir6.2. In this regard a 15 amino acid stretch of negatively charged aspartate and glutamate residues located in SUR downstream of NBD1 in cytoplasmic loop 6 (CL6) between TMD1 and TMD2 is strategically suitable to facilitate read-out of cooperative interactions between NBDs.

7. The K_{ATP} channel complex as a component of the cellular energetic network

The established homeostatic role for K_{ATP} channels in providing metabolic sensing and adjusting membrane excitability under physiologic or pathologic stress implies an integration of channel gating with the cellular energetic network. However, channel sensing of bulk nucleotide levels is limited since the effect of MgADP reaching saturation (at > 100 μM) shifts the range for ATP inhibition (IC_{50} from ~30 to ~300 μM) which is still far below intracellular ATP levels (6–10 mM), implying that MgADP-dependent K_{ATP} channel regulation is insufficient for channel gating at the normally high cytosolic concentrations of ATP [15,54, 84,85]. This indicates that the bulk cytosolic nucleotide composition cannot be the sole determinant of K_{ATP} channel function. Rather, K_{ATP} channels could sense local nucleotide concentrations set by ATPases in the submembrane space at a level distinct from that of the “bulk” cytosol [52,54,86], provided that significant diffusional limitations within the cell exist to establish distinct cellular compartments [15,87–89]. However, such cellular compartmentalization would obstruct proper energetic sensing by K_{ATP} channels, as channel gating would be distorted by local fluctuations of nucleotides, remaining weakly dependent on the cellular metabolic status. Therefore, energetic signaling to the channel site must be managed by systems capable of shunting diffusional barriers transmitting nucleotide signals in order to secure beneficial channel activity in accord with cellular energetic demand.

Cells with high and fluctuating energy demands, such as cardiomyocytes, possess well-defined phosphotransfer systems that facilitate energetic signaling between sites of ATP production and ATP utilization or sensing [7,90]. Structural and functional interactions of K_{ATP} channel proteins with the phosphotransfer enzymes, adenylate kinase (AK: 2ADP ↔ ATP + AMP) and creatine kinase (CK: ADP + CrP ↔ ATP + Cr), are assured by the wide distribution of these enzymes in distinct cellular compartments, including membranes, and their physical association with K_{ATP} channel sub-units [8,15,91]. Creatine phosphate, the substrate for creatine kinase, facilitates ATP-induced K_{ATP} channel inhibition, a regulation absent in knockout mice lacking the creatine kinase gene *M-CK* [15]. Analogously, AMP, the substrate for adenylate kinase, antagonizes ATP-induced K_{ATP} channel closure, an effect lost in cardiomyocytes from mice lacking the adenylate kinase *AK1* gene [8]. Thus, phosphotransfer reactions regulate K_{ATP} channels providing a mechanism for coupling channel behavior with the cellular energetic state.

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In principle, K_{ATP} channel function can be regulated by phosphotransfer enzymes based on product/substrate exchange in the channel microenvironment or through phosphotransfer-dependent modification of the channel's catalytic properties. While the first mechanism is intuitive, the second possibility stems from experimental data indicating alterations in the Michaelis–Menten constant and the maximal rate of the ATPase reaction of purified NBD2 in the presence of phosphotransfer systems. These experiments indicate that creatine or pyruvate kinases significantly increase (~50%) the maximal ATPase reaction rate (Selivanov et al., unpublished data) that would not be possible if phosphotransfer enzymes would solely regulate product/substrate exchange.

Theoretical and experimental analysis of cooperative interaction between the two prototypic phosphotransfer enzymes, creatine kinase (CK) and adenylate kinase (AK) systems, in a compartmentalized cellular environment reveals unique properties of these enzymes in transmission of energetic signals to sarcolemmal K_{ATP} channels [8,15,16]. Specifically, signal transmission from the cytosol to the submembrane compartment is limited due to restricted diffusion of nucleotides, creatine (Cr) and creatine phosphate (CrP). However, facilitated diffusion provided by CK and AK phosphotransfer systems that could bridge diffusional barriers essentially dissipates nucleotide gradients imposed by membrane AT-Pases and diffusional restrictions. Under cardiomyocyte stress, phosphotransfer flux through CK is significantly damped [92–94] such that with only a moderate drop in bulk cytosolic ATP, CK could no longer effectively dissipate nucleotide gradients precipitating a significant fall in submembrane CrP, and generation of an amplified nucleotide response at the K_{ATP} channel site (Fig. 2) [16]. According to the CK reaction equilibrium, substitution by creatine of 9.95 out of the total 10.25 mM of creatine phosphate, a 50 μM drop of bulk ATP (from 0.3 to 0.25 mM) and concomitant increase of bulk ADP can be expected. Experimentally it has been demonstrated that under the active submembrane Na/K-ATPase this minor drop in bulk ATP, which by itself is insufficient to induce channel opening, was amplified by the altered creatine kinase flux into significant nucleotide changes in the submembrane space manifested by vigorous activation of K_{ATP} channels [16].

CK-dependent amplification of the nucleotide response can be tuned by the AK system capable of modulating changes in the ATP/ADP ratio in the submembrane compartment, securing transmission of controllable metabolic signals to K_{ATP} channels. Under severe metabolic challenge, provided that a local regenerating system maintains submembrane ATP levels (i.e. glycolysis or an external source of ATP), bulk AK catalysis could elevate AMP flux into the submembrane compartment and promote the generation of submembrane ADP (ATP + AMP $\rightarrow$ 2~ADP) facilitating K_{ATP} channel opening [8,16]. Thus, energetic signals generated in the cytosol are processed by CK and AK systems that are capable of synchronizing K_{ATP} channel function with changes in the cellular energetic state (Fig. 2).

Integration with other cellular systems also provides for potential energetic regulation of the K_{ATP} channel. There are several contributors including physical interactions between the channel and the actin cytoskeleton [95–99], as well as additional metabolic systems such as lactate dehydrogenase (LDH) that, like CK and AK has also been shown to physical associate with K_{ATP} channel proteins [8,91,100]. LDH catalyzes the reaction NADH + pyruvate $\leftrightarrow$ lactate + NAD. Lactate modulates the ATP sensitivity of K_{ATP} channels and would accumulate in the vicinity of LDH, and thus of cardiac K_{ATP} channels, under anaerobic conditions [100]. Other examples include direct channel effects of G protein subunits [101,102], changes in the composition of membrane phospholipids that interact with Kir6.2 to modulate pore function [103,104], activation of the protein kinase systems PKC and PKA with channel effects through phosphorylation/dephosphorylation [105–108], and pH effects through protonation of channel proteins [109].

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8. K_{ATP} channel regulation in cardiac disease

Assigning to the channel catalytic module a role in integrating ion permeation with intracellular metabolic pathways identifies a novel principle in the regulation of cellular excitability. In principle, defects in the function of channel proteins themselves, disruption of intracellular metabolic networks, and/or disturbed communication between K_{ATP} channels and the energetic network can all be envisioned as molecular mechanisms contributing to cardiac disease.

In heart failure, cardiomyocytes undergo extensive remodeling including diminished mitochondrial respiration, suppressed creatine kinase flux, decreased energy storage, and cytoskeletal disruption, although bulk cytosolic ATP levels are preserved [20,92,93]. These changes impact metabolic signal generation, signal trafficking through phosphotransfer and diffusional effects by altered cellular architecture, among other factors, that affect the ability of cellular distress to be properly communicated to K_{ATP} channels. K_{ATP} channels from such hearts display normal basic physical properties and function when the channels are studied in isolation, however channel function within intact cells, when analyzed at both the cellular and organ level, demonstrates a significantly blunted response to stress [20]. Specifically, K_{ATP} channels from failing hearts display an improper response to ATP and CK, a reduced recognition of metabolic distress, and do not provide for appropriate action potential shortening in hearts exposed to hypoxia. Thus the K_{ATP} channels in myopathic hearts are uncoupled from the cellular metabolic state resulting in compromised regulation of membrane excitability. Consequently such hearts are excessively vulnerable to calcium loading and myocyte necrosis under stress that can be largely ameliorated by pharmacologic restoration of K_{ATP} channel opening with K_{ATP} channel opening drugs, such as nicorandil and pinacidil. The observations that K_{ATP} channels from failing hearts function normally in isolation and that protective effects of channel function can be restored by K_{ATP} channel openers indicates that the defect in K_{ATP} channel function in failing hearts is not within the channel itself but within the cellular signaling systems with which the channel is integrated and upon which the channel depends. The resulting inability of the K_{ATP} channel to fulfill its physiologic role under these conditions renders failing hearts more susceptible to injury and dysfunction under stress that could further contribute to the progression of disease by setting up a downward spiral through additional disruption of cellular energetic signaling cascades.

Derangement of metabolic sensing by cardiac K_{ATP} channels can also occur by mutation of channel proteins such as has been discovered in cases of dilated cardiomyopathy in humans [14]. Scanning of genomic DNA identified two mutations in *ABCC9*, which encodes the regulatory SUR2A sub-unit of the cardiac K_{ATP} channel, in individuals with heart failure and rhythm disturbances due to idiopathic dilated cardiomyopathy but not in normal control patients. Three dimensional modeling indicates these missense and frameshift mutations occur in evolutionarily conserved residues that flank the carboxy-terminal β-strand of SUR2A in close proximity to the Walker A motif required for coordination of nucleotides in the catalytic pocket of ABC proteins. Based on this modeling, the mutations are predicted to disrupt protein folding and thus the integrity of NBD2. Indeed, the two mutations do not significantly affect ATP-binding but do reduce the SUR2A ATPase activity and alter the characteristic reaction kinetics, translating into an abnormal distribution of conformations within the NBD2ATPase cycle and an inability of CK to effectively regulate the ATPase activity of the mutant channels. These aberrances in catalysis generate defective K_{ATP} channel phenotypes that are characterized by abnormal responses to both ATP and ADP. Suboptimal tolerance to stress and a propensity towards cardiomyopathy and arrhythmia in mice with genetic disruption of cardiac K_{ATP} channels [10,19,21], supports the implication that the significant metabolic sensing deficit demonstrated in humans with cardiac K_{ATP} channel mutations contributes to development of heart failure and thus further establishes the importance of proper SUR2A

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NBD2 ATPase kinetics in the metabolic signal decoding that permits the cardiac K_{ATP} channel to fulfill its physiologic role.

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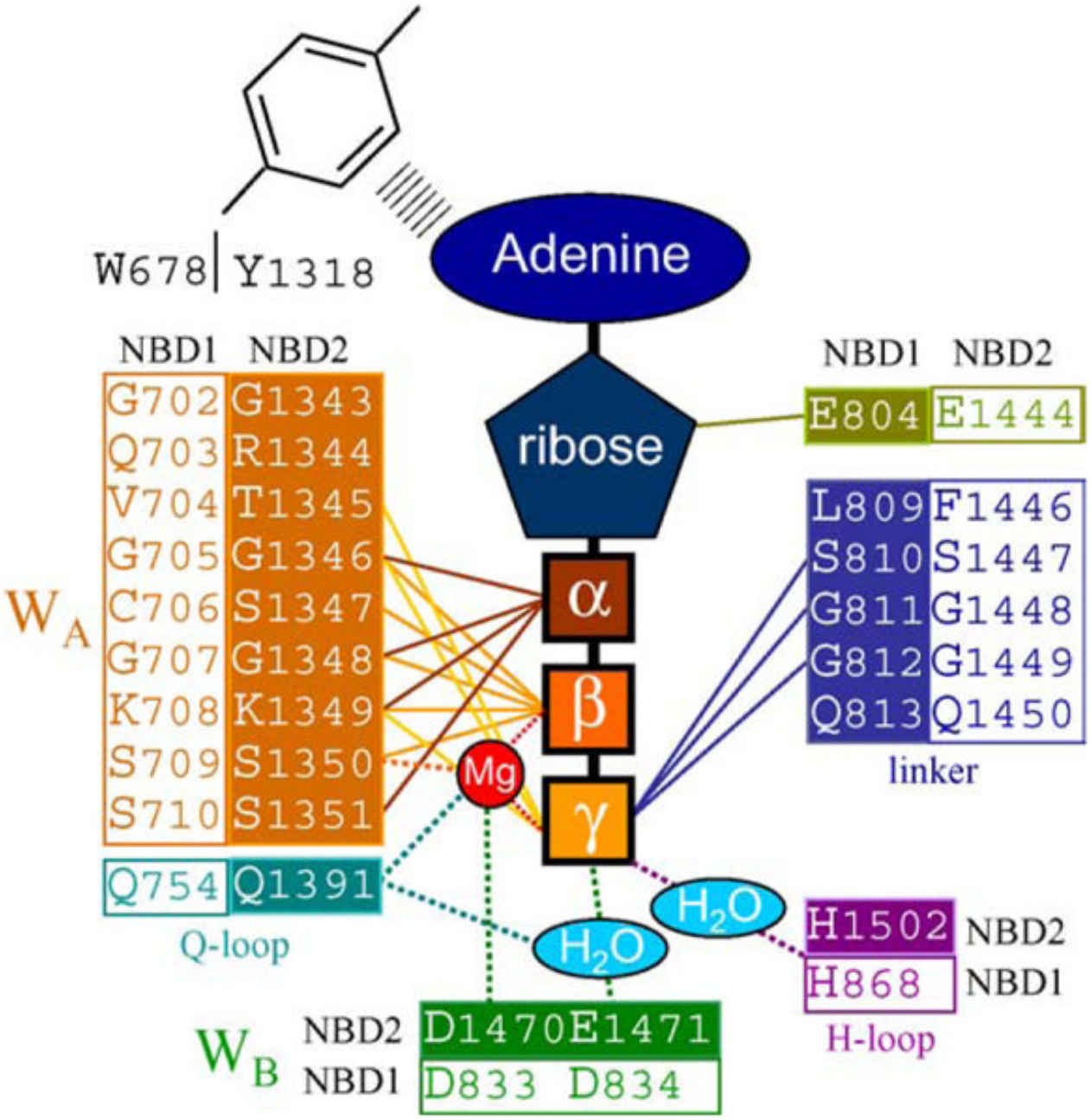

Fig. 1.

Hypothetical coordination of the ATP molecule within SUR. Internal circle (filled elements) symbolizes the first binding pocket formed by the Walker A motif, Q-loop, Walker B motif, H-loop of NBD2 and accomplished with the linker region of NBD1. External circle (clear elements) represents the second binding pocket comprised by the counterparts of NBD1 and NBD2. The scheme is developed based on sequence alignment between NBDs of SUR and MJ0796 protein [67,77].

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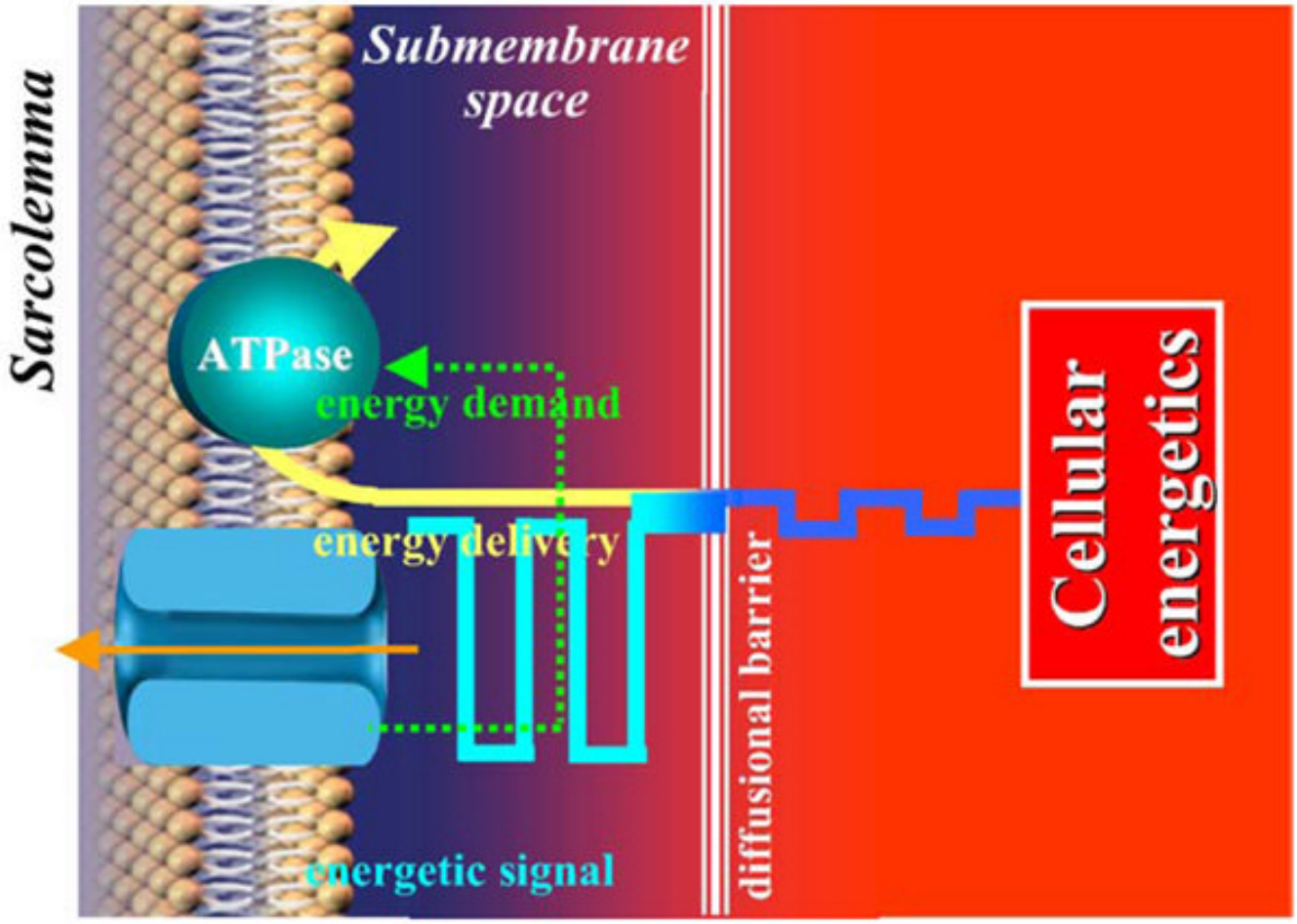

Fig. 2.
In the compartmentalized cellular environment changes in cellular energetics can be transmitted over diffusion barriers, amplified and decoded as a signal by membrane metabolic sensor which triggers adaptive response adjusting energy demand and preserving, thereby, cellular well-being.