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Review

Adenosine kinase and ribokinase – the RK family of proteins

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Abstract. Ribokinase (RK) and adenosine kinase (AK) catalyze the phosphorylation of ribose and adenosine to ribose-5-phosphate and AMP, respectively. Belonging to the RK family of proteins, these enzymes share a number of unique structural and functional elements. Extensive work has been carried out on many aspects of these enzymes in recent years, and we summarize the wealth of information currently available on them. The topics covered include descriptions of the primary and three-dimensional structures of AK and RK, their phylogenetic relationships, biochemical aspects of these enzymes including their reaction mechanisms and ionic requirements, and also work on certain inhibitors of these enzymes. The cellular metabolism and transport of ribose and adenosine are also briefly discussed, as well as the beneficial effects of ribose and adenosine in physiology and how these effects can be harnessed for pharmacological purposes.

Keywords. Adenosine kinase, ribokinase, NXXE motif, gene structure and mutants, enzyme inhibitors, PfkB family of proteins, phosphate dependency, adenosine and ribose metabolism.

Introduction

Ribokinase (RK) and adenosine kinase (AK) are two important enzymes belonging to the RK family of proteins that share a number of unique primary and tertiary structural elements. RK catalyzes the phosphorylation of ribose to ribose-5-phosphate (R-5-P), using ATP as a phosphate donor: Ribose + ATP → R-5-P + ADP. This is the first step in ribose metabolism and functions to trap ribose within the cell after its uptake. The phosphorylated sugar can then enter the pentose phosphate pathway (PPP) for energy production, or be used as a carbon source for the synthesis of nucleotides, histidine, and tryptophan [1, 2]. Adenosine kinase (AK) catalyzes the phosphorylation of adenosine to AMP, also using ATP as a phosphate donor: Adenosine + ATP → AMP + ADP. As one of the most abundant nucleoside kinases in mammalian tissues [3, 4], AK is the first enzyme in the anabolic pathway of adenosine utilization. Hence, it plays a key role in the regulation of intracellular and extracellular levels of adenosine, which exhibits a potent neuromodulating property [5, 6]. Interestingly, both AK and RK are implicated in tissue-protective mechanisms against various ischemic insults.

Although RK and AK were discovered more than 50 years ago [7, 8], and extensive work has been carried out on different aspects of these enzymes, there is no review available that summarizes the wealth of information on them. The purpose of this review is to introduce readers to the excellent work covering different aspects of RK and AK.

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Enzymes	N-terminal sequence	C-terminal sequence
RBSK_ECOLI	41 GGkGaNQAvaAgR-----SganiafiactG	249 DTiAAGDtfnGALI
RBSK_BACSU	38 GGkGaNQAvaAaR-----Lgaqvfmvgkv G	236 DTtGAGDtfnAAFA
RBSK_YEAST	37 GGkGlNQAaaIgKlknpsSRysvrmignvG	277 DTtGAGDtflGGLV
RBSK_HUMAN	52 GGkGaNQCvqSaR-----Lgamtsmvckv G	263 DTtGAGDsfvGALA
ADK_MYCTU	47 GGvGgNMAfaIgV-----Lggevalvgaa G	251 DPtGVGDafrAGFL
ADK_YEAST	63 GGaGqNTArgSaYvl---Gagqvvyfgsv G	287 DTnGAGDafaGGFM
ADK_MOUSE	79 GGsGqNSMkvAqWliqe-Phkaatffgci G	310 DTnGAGDafvGGFL
ADK_HUMAN	80 GGsGqNSIkvAqWmiqq-Phkaatffgci G	311 DTnGAGDafvGGFL
K1PF_BACSU	36 GGkGiNVSrlLkR-----Hhvaskalgfv G	243 NSvGAGDsvvAGFL
K1PF_ECOLI	39 AGkGiNVAkvLkD-----Lgidvtvggfl G	249 STvGAGDsmvGGLI
K1PF_MYCPN	34 GGkGiNMAivMaL-----Fgikptvltfl G	241 STtGAGDtllGVFL
KHK_MOUSE	40 GGnGsNSCtvLsL-----Lgarcafmgsl A	252 DTlGAGDtfnASVI
KHK_HUMAN	40 GGnGsNSCtvLsL-----Lgapcafmgsm A	252 DTlGAGDtfnASVI
KDGK_ECOLI	27 GGdGlNTSvyIaRqvdp-Aaltvhyvtal G	258 DTtAAGDsfsAGYL
LACC_STRPN	36 GGkGlNVTrvLsE-----Fgdsvlatglv G	247 NPvGSGDstvAGIS
FRLD_ECOLI	24 GGnGvNVAvyCtR-----Ygiqpgcitwv G	218 DTmGAGDsfiAGFL

Figure 1. Conserved signature sequences of the RK family enzymes. Conserved amino acid residues are shown capitalized in the alignment. Abbreviations for the enzymes: RBSK, ribokinase; ADK, adenosine kinase; K1PF, 1-phosphofructokinase; KHK, ketohexokinase; KDGK, 2-dehydro-3-deoxygluconokinase; LACC, tagatose-6-phosphate kinase; FRLD, fructoselysine kinase; INGK, inosine kinase; and HLDE, bifunctional enzyme D-hepta-D-heptose-7-phosphate kinase / D-hepta-D-heptose-1-phosphate adenosyltransferase. Abbreviations for the species: ECOLI, *Escherichia coli*; BACSU, *Bacillus subtilis*; MYCTU, *Mycobacterium tuberculosis*; MYCPN, *Mycoplasma pneumoniae*; and STRPN, *Streptococcus pneumoniae.*

Overview of the RK family of proteins

RK genes have been found in both prokaryotes and eukaryotes, and sequence comparisons have shown that they belong to the PfkB family of carbohydrate kinases [9], which phosphorylate the hydroxymethyl groups of a variety of sugar moieties. The PfkB family of enzymes, thus, is also referred to as the RK family, and forms one of the three non-homologous branches of sugar kinases, along with the hexokinase family and the galactokinase family [9]. Initially, the RK family consisted of a small number of evolutionarily related enzymes. Beside RKs from *Escherichia coli* and yeast, other members of this group included fructokinase, 1-phosphofructokinase, 6-phosphofructo-2-kinase (PfkB or PFK2), tagatose-6-phosphate kinase, and inosine kinase (INGK) [9]. Since then, many other enzymes have been added to the RK family, including AK [10, 11] and 2-dehydro-3-deoxygluconokinase [12].

The members of the RK family are identified by the presence of two highly conserved sequence motifs (Fig. 1). The first motif is found in a glycine-rich area located in the N-terminal region of these enzymes. This motif includes two consecutive glycine residues that form part of the hinge between the lid domain and the αβα domain (see next section) [13–16]. The second motif, which is found in the C-terminal region, is involved in ATP binding and the formation of an anion hole [13–16]. This motif also contains a conserved aspartic acid residue which serves as a catalytic base [13–16]. A search of the Swiss-Prot entries (release 54.5 of 13 November, 2007) with the above two sequence motifs (PROSITE [17, 18] accession numbers PS00583 and PS00584, respectively) resulted in 213 overlapping hits. These matches included all of the previously mentioned enzymes of the RK family, as well as new members such as fructoselysine kinase and the bifunctional enzyme D-hepta-D-heptose-7-phosphate kinase / D-hepta-D-heptose-1-phosphate adenosyltransferase from various sources. The list also includes a number of uncharacterized sugar kinases, mostly of bacterial sources.

Although the overall sequence identity between the members of the RK family is less than 30%, their crystal structures are very similar [13–16]. Retaining the overall structure composed of the large αβα sandwich domain and the small lid domain, enzymes in this group differ only to a small extent with respect to the sugar substrate-binding moiety and peripheral structures. On the other hand, the two other families of sugar kinases, hexokinase and galactokinase, have vastly different three-dimensional structures from that of the RK family, showing different frequencies and orders of α-helices and β-strands [19, 20]. At the sequence level, the enzymes of these three families do not show significant sequence similarity [9].

Despite large differences in their primary and tertiary structures, some enzymes in the hexokinase and RK families show similar substrate specificity and carry out identical functions. For instance, fructokinases from *Zymomonas mobilis*, *Streptococcus mutans*, *Lactococcus lactis* and *Bacillus subtilis* [21–24] belong to the hexokinase family, showing all of the characteristic sequence patterns of this family. The other

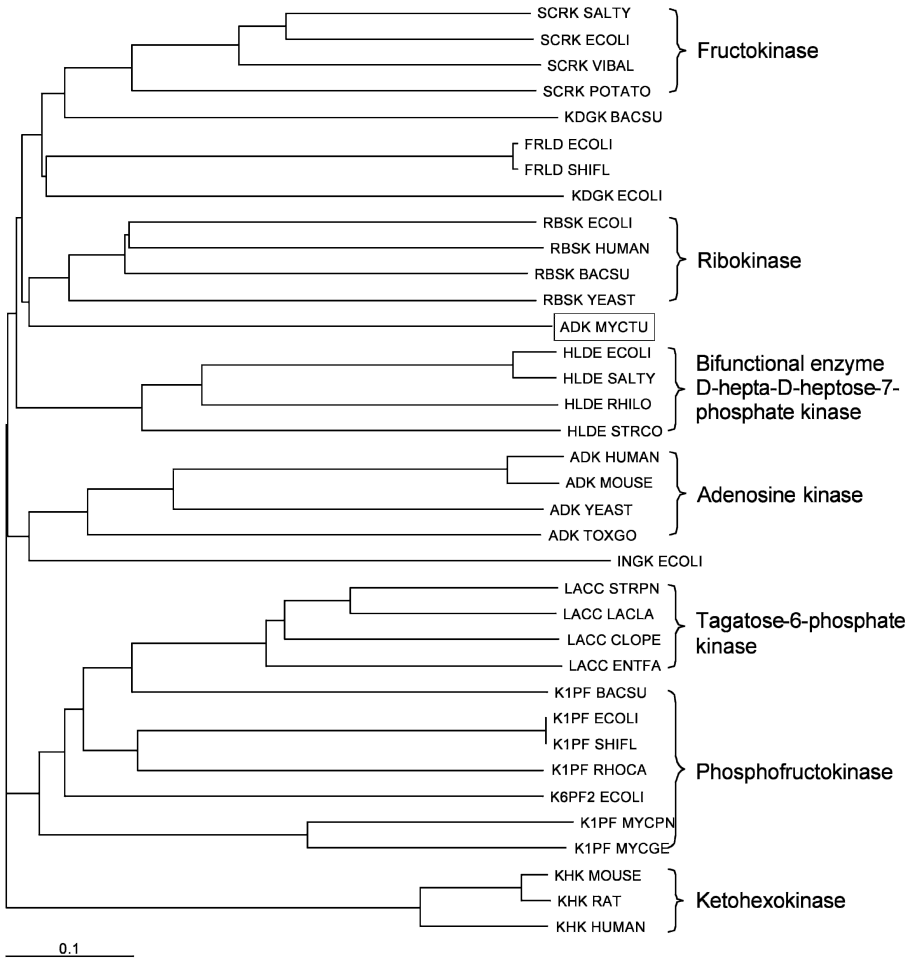


Figure 2. Phylogenetic tree of RK family enzymes. The tree was constructed using the program ClustalX (version 1.83) with full-length sequences as inputs, and the number of bootstrap trials was 1000. Adenosine kinase from *M. tuberculosis* is shown boxed in red. Abbreviations: SCRK, fructokinase; K6PF2, 6-phosphofructo-2-kinase; SALTY, *Salmonella typhimurium*; VIBAL, *Vibrio alginolyticus*; SHIFL, *Shigella flexnery*; RHILO, *Rhizobium loti*; STRCO, *Streptomyces coelicolor*; TOXGO, *Toxoplasma gondii*; LACLA, *Lactococcus lactis*; CLOPE, *Clostridium perfringens*; ENTFA, *Enterococcus faecalis*; RHOCA, *Rhodobacter capsulatus*; and MYCGE, *Mycoplasma genitalium.* Please refer to the legend for Figure 1 for other abbreviations.

fructokinases, such as the ones from *E. coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Vibrio alginolyticus* and potato [25–27], belong to the RK family, and do not show significant sequence similarity to the fructokinases of the hexokinase family. These enzymes have likely resulted from adaptations of different structural frameworks for the same function, i.e. convergent evolution [9].

One can also observe convergent evolution leading to similar substrate specificity within one structural family. For instance, the ability to utilize adenosine as a substrate appears to have arisen at least twice in the RK family. AK activity until recently was only found in eukaryotic organisms. However, a recently identified enzyme from *Mycobacterium tuberculosis* has been denoted as the first, and thus far the only bacterial AK [28]. Sequence alignment indicates that AK from *M. tuberculosis* contains areas which are conserved in other AKs, including residues involved in the binding of the substrates [28]. The *M. tuberculosis* enzyme, however, shows greater overall sequence similarity (representing both identical and conserved substitutions) with RK and fructokinase (35 %) than it does with other AKs (less than 24 %) [28]. AKs from other sources, on the other hand, show more than 50 % sequence similarity with each other. In a phylogenetic tree for the RK family of proteins, *M. tuberculosis* AK branches with the RKs and fructokinases, while other AKs form a separate branch in the tree (Fig. 2) [28]. The substrate specificity of the *M. tuberculosis* enzyme, when measured in terms of catalytic efficiency (k_{cat}/K_m), is also very different from that of other eukaryotic AKs. While human AK favours adenosine approximately 900-fold over ribose as a substrate, *M. tuberculosis* AK does so only about 9-fold. Furthermore, the *M. tuberculosis* enzyme can also phosphorylate fructose as effectively as it can phosphorylate ribose [Park and Gupta, unpublished data]. Thus, the exact role and the preferred substrate(s) of this putative *M. tuberculosis* AK *in vivo* remain to be determined. However, based upon the results of sequence alignment and biochemical studies, as well as the fact that AK is not found in other bacteria, the AK activity exhibited by the *M. tuberculosis* enzyme appears to have resulted from convergent evolution.

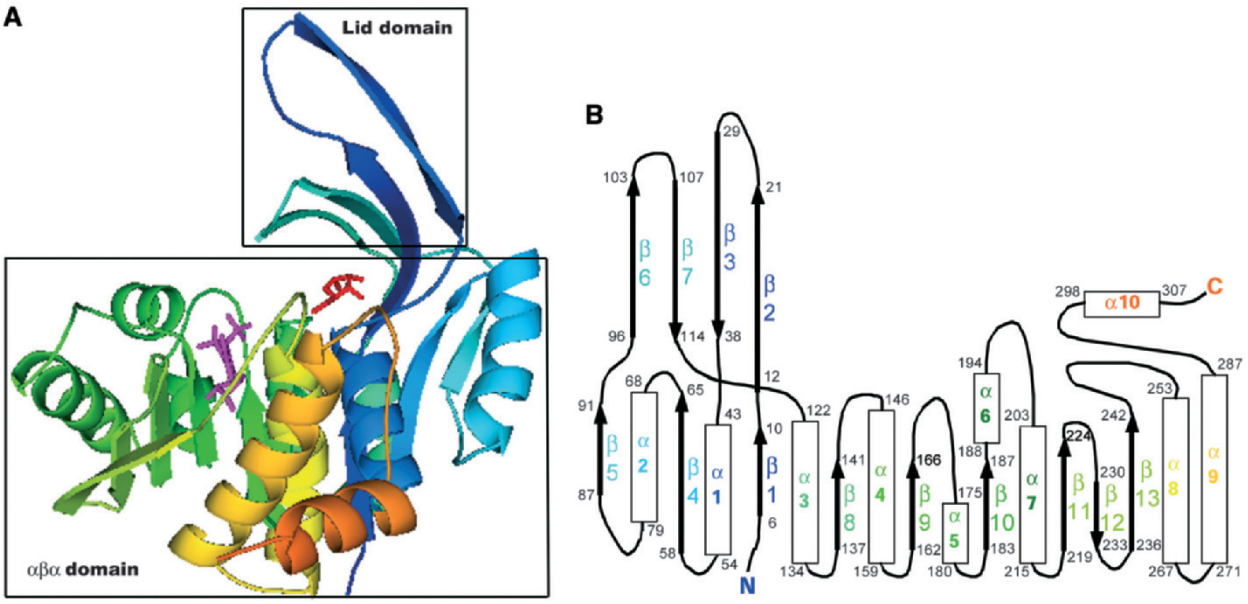


Figure 3. Structure of an *E. coli* ribokinase subunit. (*A*) Ribbon diagram showing the 'closed' form of the enzyme, bound with the substrates, ribose (red) and ADP (magenta). The αβα domain and the lid domain are boxed and labeled. The image was constructed using PyMol (version 0.93) with the PDB file 1RKD, and coloured according to the residue number (orange at the N-terminus and blue at the C-terminus). (*B*) Topology diagram showing the organization and lengths of α-helices and β-strands. The helices and strands are labeled in corresponding colors, as seen in the ribbon diagram. Residue numbers indicate the start and end of each secondary structure.

The RK family of proteins/enzymes has adapted to very broad substrate specificity within a distinct overall structural framework. Low sequence similarity between the family members, as well as their broad distribution throughout all three domains of life, supports the idea that the common ancestor of the RK family enzymes diverged from their ancestor at a fairly early stage of evolution.

Structural aspects of RK and AK

Structure of RK

The first three-dimensional crystal structure of RK was determined in 1998, with the *E. coli* protein [29]. RK exists in solution as a homodimer [30], and each monomer consists of two domains (Fig. 3) [29]. The large domain is formed by a nine-stranded β-sheet, which is flanked by 10 α-helices. This αβα sandwich domain provides most of the specific binding interactions for the substrates, ribose and ATP [29]. The smaller domain, which is composed of four β-strands, acts as a lid over the active site (Fig. 3A). The outer face of this β-sheet provides an interface for the dimer interaction, through which the two β-sheets of the dimer pack orthogonally to each other, forming a flattened β-barrel [29]. The cross-over segment of each subunit also contributes in forming the active site of the dimer partner. The substrate ribose binds in the cleft between the αβα sandwich domain and the smaller β-sheet (lid) domain [29]. The nucleotide binding site is located in a groove in the αβα subunit near the ribose-binding site, but is not entirely covered by the smaller lid-like domain [29]. The anion hole, a feature shared by all members of the RK family [13–15], is created by the main-chain nitrogen atoms of residues 252–255, and is present adjacent to the active site [29].

Comparison of the apo structure of RK to its substrate-bound forms shows a drastic conformational alteration [16, 29]. Although the internal configuration of the αβα sandwich domain and the lid domain remain essentially the same, their spatial relationship is vastly different. This conformational change can be described as an opening and closing of the lid domain, if the αβα domain is taken as a point of reference [16]. In this case, the apo structure takes the 'open' conformation, exposing the ribose-binding site. The substrate-bound structure, on the contrary, takes the 'closed' conformation, in which the bound ribose is almost completely buried under the small lid domain. Measurements of the C^{α}-torsion angles show that lid opening affects the main-chain conformation of residues 39–41, 96 and 113 [16]. These residues form a hinge between the two domains and include the GG switch, another structural feature shared by all members of the RK family [13–16].

Different structures of RK suggest that the large conformational change plays an important role in the function of the enzyme. Based on these structures,

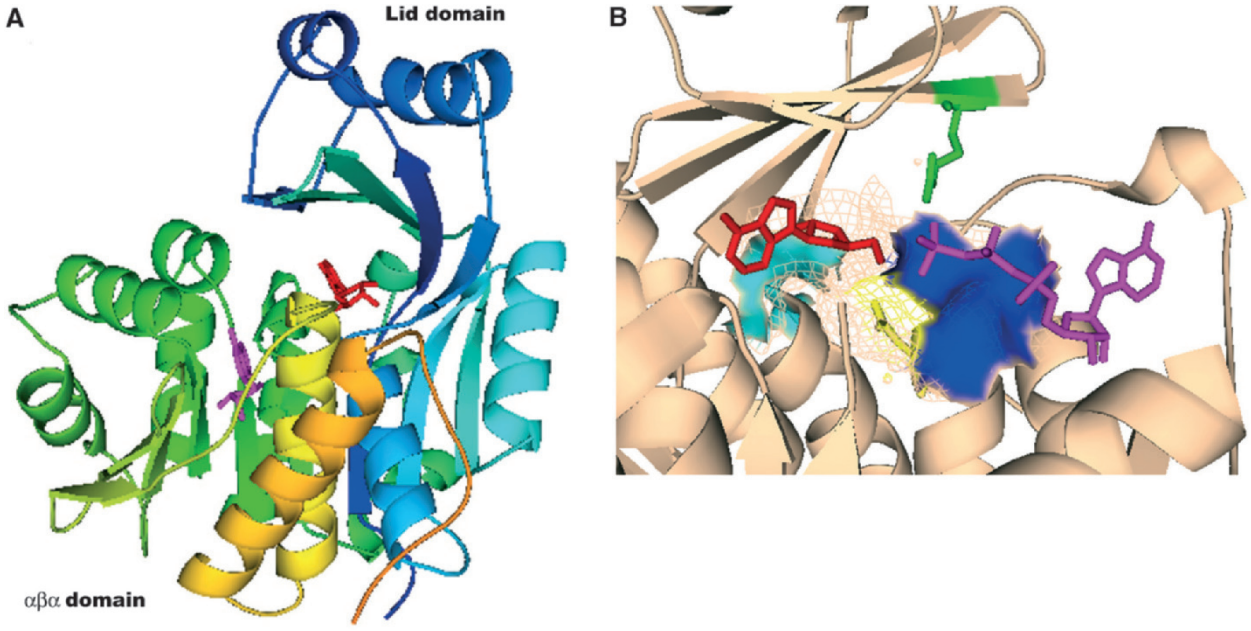


Figure 4. Structure of adenosine kinase. (*A*) Ribbon diagram of human AK showing two bound adenosine molecules (red and magenta), with the enzyme structure coloured in a rainbow scheme (orange at the N-terminus and blue at the C-terminus). (*B*) Close-up view of *Toxoplasma gondii* AK active site. Bound adenosine and AMP-PCP are shown in red and magenta, respectively. A number of other important structural features are also shown in this structure: (i) the surface of the anion hole (in blue); (ii) the surface which the GG switch makes (in cyan); the catalytic base Asp318 (in yellow); and Arg136 of the lid domain which shows a close contact with the bound nucleotide (in green). The diagrams were constructed with the PDB files 1BX4 (human) and 1LII (*T. gondii*), using PyMol (version 0.93).

Sigrell et al. [16, 29] have proposed a mechanism by which the RK reaction proceeds. Initially in the open form, the enzyme allows the entry of ribose, which binds to the active site in the αβα sandwich domain. Ribose binding induces closing of the lid, which is likely to increase the enzyme affinity for the second substrate ATP. The residue Asp255 is believed to function as the catalytic base which abstracts a proton from the O5′-hydroxyl group of ribose. The negatively charged O5′ atom then makes a nucleophilic attack on the γ-phosphate of ATP, resulting in a pentacovalent transition state. This transition state is stabilized by the anion hole in which the γ-phosphate of ATP is positioned. Subsequent movements of the lid domain are supposed to induce the breakdown of the transition state, after which the enzyme takes the open conformation again. The products, R-5-P and ADP are released and the enzyme is ready for another round of catalysis. This sequence of events suggests an ordered Bi Bi reaction mechanism, similar to those proposed for other carbohydrate kinases, which may also show induced fit upon sugar binding [20].

Structure of AK

The human form of AK was characterized by Mathews et al. [14], soon after the RK structure was solved [29]. The active form of AK is a monomer, which is also composed of an αβα sandwich domain and a smaller lid domain (Fig. 4A) [14]. The αβα fold contains a central nine-stranded β-sheet and 10 flanking α-helices, and forms the core of the overall structure. The lid domain is composed of a five-stranded β-sheet flanked by two α-helices on the side exposed to the solvent. This structure differs from the RK structure [16, 29] in that AK has two additional α-helices and an additional β-strand in the lid domain. Overall, AK shows a remarkable structural homology to RK (compare Figs. 3A and 4A), despite low sequence identity.

When the structures of AK [14] and RK [16] are superimposed, the substrates and their interacting partners occupy similar three-dimensional space. The residues involved in binding of the sugar substrates show a moderate level of conservation between the two enzymes. Phe302 of AK and Phe257 of RK, which are involved in hydrophobic contacts with the substrates, are absolutely conserved in the RK family [14, 16]. On the contrary, Ala136, Leu16, and Phe170 of AK differ from the corresponding residues of RK, enabling adenosine binding instead of ribose [14, 16]. In AK, Ala136 replaces Ile112 of RK and allows for more room necessary for larger adenosine. Leu16 of AK replaces Asn14, forming part of the hydrophobic pocket for the purine base. Phe170 replaces Glu143 of RK and introduces stacking interaction with the adenine base of adenosine. The residues involved in

ATP binding, on the other hand, show a high level of identity between the two enzymes. These residues include Asn196, Asn223, Glu226, Thr265, and Gly267 of AK that correspond to Asn166, Asn187, Glu190, Thr223, and Gly225 in RK [14, 16]. These residues are also conserved in the other members of the RK family [14].

Further insight into AK structure and function was provided when the *Toxoplasma gondii* (a parasitic protozoan) AK structure was solved [15]. In this study, the structures of both the apo and substrate-bound forms were solved, and their comparison revealed drastic conformational alterations. The most striking change is the 30° hinge bending caused by the binding of adenosine, which brings the large and small domains together [15]. This bending is likely brought about by the GG switch formed by residues Gly68 and Gly69, which undergo large torsional changes as they interact with adenosine (Fig. 4B) [15]. A similar bending motion is observed in RK upon ribose binding, but of a smaller magnitude (17°) [16]. Subsequent ATP (AMP-PCP in the crystal structure) binding to the adenosine-enzyme complex induces less dramatic but nonetheless important conformational changes [15]. As a result, an anion hole is created around the β- and γ-phosphates of the ATP, which is further stabilized by residue Arg136 (Fig. 4B) [15].

Based on these observations, Schumacher et al. [15] have proposed a detailed catalytic mechanism for AK. The first step involves the binding of adenosine to AK in the open form. The binding of adenosine 'turns on' the GG switch, which results in the hinge bending and the subsequent burial of the substrate. The closing of the lid then increases the enzyme affinity towards ATP, which induces formation of the anion hole in the active site. The catalytic base Asp318 (Asp300 in human AK) deprotonates the 5′-hydroxyl group of adenosine, which then attacks the γ-phosphate of ATP. The anion hole induces and stabilizes the pentacovalent transition state, typical of an in-line S_N2 displacement reaction. Mg^{2+} bound with the ATP, on the other hand, functions to neutralize the negative charges on the nucleotide and to enhance the leaving group ability of ADP. Upon completion of the catalysis, the substrates part the enzyme in the order of ADP and AMP, since the adenosine-binding site is more deeply buried in the structure.

Biochemistry of RK and AK

Ionic requirements of RK and AK

Although their structures vary, carbohydrate kinases are thought to function by similar catalytic mechanisms. All known carbohydrate kinases require divalent cations, such as magnesium, which act as an electrophilic catalyst for the transfer of the phosphate group [31]. Divalent cations are thought to neutralize the negative charges on the phosphate groups of the nucleotide, and the actual substrate of these enzymes is the chelate of the metal and ATP [31]. RK, as a carbohydrate kinase, also requires divalent cations for its catalytic activity. It was shown that, besides magnesium, other metal ions such as Mn^{2+}, Co^{2+}, Ca^{2+}, Ni^{2+}, and Cu^{2+} could also satisfy the divalent cation requirement of RK [32]. For both of the mammalian (calf liver) and bacterial (*E. coli* and *Salmonella typhimurium*) forms of RK, no activity was seen in the absence of divalent metal ions [7, 32–35].

Many of the ATP-utilizing enzymes show an additional requirement for monovalent cations, such as potassium and cesium. Two general mechanisms have been proposed for this type of enzyme activation: i) direct (catalytic) and ii) indirect (conformational) mechanisms [36, 37]. RK also requires monovalent cations for activation. For instance, *E. coli* RK is activated by potassium and cesium with an apparent K_d of 5 mM and 17 mM, respectively [31]. This enzyme is also activated by NH_4^+ [33], and a similar pattern of ionic effects was observed in *S. typhimurium* RK [32]. A structural analysis of *E. coli* RK by Andersson et al. [31] suggests that RK is an example of an enzyme which is activated by monovalent cations via a conformational change (indirect mechanism) [37]. The structure of RK in the presence of cesium shows that the ion binds between the two loops adjacent to the anion hole of the active site (at residues 249–251 and 285–294). It is proposed that the interaction between the monovalent ion and these residues assists formation of the anion hole [31]. The enzyme is then activated for catalysis, as the anion hole functions to induce and stabilize the transition state.

The true phosphate-donating substrate of AK is also the complex of ATP and a divalent metal ion. In support of this concept, the absence of magnesium resulted in a lack of enzyme activity in human placental AK [38]. In the presence of magnesium, optimal activity appeared at pH values at which ATP and the divalent cation exist primarily in the complexed form [38]. It has been demonstrated that, as seen in RK, other divalent metal ions such as Mn^{2+}, Ca^{2+}, Co^{2+}, and Ni^{2+} can substitute Mg^{2+} for AK activity [39]. AK's preference for divalent cations, however, seems to be species- and/or tissue type-specific, as enzymes of different sources show dissimilar responses to varied metal ions [39].

Of universal observation in studies with enzymes of various sources is the effect of free magnesium on the activity of AK. In a reaction mixture, ATP exists in

Table 1. Substrate specificity of RK for various simple sugars.

Varied substrate	Relative enzyme activity				
	E. coli [a]	*S. typhimurium* [b]	*L. major* [a]	Calf [b]	Human[a]
D-Ribose	100 (309.7)	100	100 (36.0)	100	100 (40.58)
2-Deoxy-D-ribose	31	80	–[c]	75	–
D-Erythrose	–	< 3	0[d]	–	–
D-Threose	–	–	0	–	–
D-Arabinose	0.74	< 3	0	0	4
D-Ribulose	–	< 3	–	0	–
D-Xylulose	–	–	0	–	–
D-Xylose	1.06	< 3	–	0	0.4
D-Fructose	0.28	–	0	0	0.1

[a] The relative activity of these enzymes was calculated based on catalytic efficiency (k_{cat}/K_m), which was determined for each sugar substrate. The catalytic efficiency values at 100 % activity of these enzymes are shown in the parentheses (in $s^{-1}mM^{-1}$).
[b] The relative activity of these enzymes was calculated based on reaction rates measured at a single concentration of each substrate (10 mM and 1 mM for the *S. typhimurium* and calf liver enzymes, respectively).
[c] Compound not tested for the enzyme.
[d] In the original literature, authors reported 'no activity' instead of providing numeric values.

several different states of ionization depending on the pH of the solution. Mg^{2+} binds to ATP^{4-}, the fully dissociated species of ATP, and forms $MgATP^{2-}$, the true substrate of AK. An excess amount of $MgCl_2$ in a reaction results in saturation of available ATP^{4-}, and thus presence of free Mg^{2+} ions. Interestingly, the activity of AK increases as the level of free Mg^{2+} in the reaction increases [38–44]. Once passed the optimal concentration, however, free Mg^{2+} leads to inhibition of AK [38–44]. This observation suggests that AK represents an example of an ATP-utilizing enzyme, which requires an additional effect of divalent cation for optimal catalysis. Other examples of such enzymes include phosphoribosylpyrophosphate (PRPP) synthetase [45–47]. The secondary divalent ion in this group of enzymes is generally known to adjust the protein conformation and/or to interact with the other substrate at the active site [48].

Substrate specificity of RK and AK

RK shows high specificity towards its phosphate-accepting substrates. While the enzyme boasts extremely efficient phosphorylation of D-ribose and 2-deoxy-D-ribose, other simple sugars have shown to be poor substrates [7, 32–35]. Table 1 summarizes the results of the substrate specificity studies carried out with RKs of different sources. These data suggest that RK is not only inefficient in phosphorylating tetroses and hexoses, but is also highly sensitive to substitution or variation of the configuration except at the C-2 position of the pentoses. Other compounds tested include ribose-2-phosphate, ribose-3-phosphate, adenosine, thymidine, cytidine, 2-deoxyadenosine, 2-deoxycytidine, 2-deoxyguanosine, and 2-deoxyuridine, which have shown no substrate activity [7, 49]. Phosphoryl donor specificity of RK, in comparison to that for the sugar substrate, has not been as extensively studied. CTP, added instead of ATP to a reaction, resulted in comparable enzymatic activity in calf liver RK [7]. In *E. coli* RK, GTP displayed activity, but at a level which was two orders of magnitude lower versus ATP [33]. *S. typhimurium* RK, on the other hand, was shown to accept several ribonucleotides (ITP, GTP, CTP) and deoxyribonucleotides (dATP, dGTP, dCTP) as good phosphoryl donors, in addition to ATP [32]. Other compounds, including UTP, TTP, XTP, phosphoenolpyruvate, and acetyl phosphate, did not act as phosphate donors for this enzyme [32].

Unlike RK, AK shows remarkably broad specificity for the phosphate-accepting substrate. Alteration of the ribosyl moiety of adenosine is well tolerated, although this decreases catalytic efficiencies by two- to fivefold [50]. The 3′-hydroxyl and 4′-hydroxylmethyl groups may be in either *trans* or *cis* conformation, but the presence of a 2′-hydroxyl group in the *trans* position is an important structural requirement. For example, 2′-deoxyadenosine, which lacks the 2′-hydroxyl group, is a poor substrate of AK [50]. However, AK is still important for 2′-deoxyadenosine salvage in *Trypanosoma brucei* [51] and in mammalian cell lines deficient in deoxycytidine kinase [52]. Substitution or addition of functional groups in the purine base moiety can also be well accepted. In fact, compounds such as 8-azaadenosine show even higher phosphorylation efficiency than the natural substrate, adenosine [50]. The nucleosides and their analogues, which are efficiently phosphorylated by AK, generally differ from adenosine at the 2-, 6-, 7-, 8-, and 9-substituents [50, 53, 54]. With respect to the phosphate-donating substrate, ATP and GTP are the preferred nucleotides for AK, although a number of other compounds can substitute in the catalytic reaction [40, 43, 54, 55].

Substrate inhibition and reaction mechanism of AK

The AK reaction is unique because the substrate adenosine, which is phosphorylated by the enzyme, shares the same structural base as the phosphoryl group donor, ATP. Consequently, both adenosine and its phosphorylated product AMP bind to the ATP site [56], resulting in enzyme inhibition. As this complicates the inhibition pattern of the enzyme, AK represents an example of an enzyme whose catalytic mechanism is difficult to determine using biochemical methods. Indeed, numerous kinetic studies have failed to reach a consensus in this regard. Chang et al. [57], for instance, suggested a two-site ping-pong mechanism, while several others claimed ordered Bi Bi mechanisms [38, 58, 59]. The order of substrate binding and product release determined in each of these studies also differed from one another. The information obtained from the structures of human [14] and *T. gondii* AK [15], on the other hand, allowed clear understanding of the reaction mechanism of the enzyme. As previously described, an ordered Bi Bi mechanism was suggested, in which adenosine is the first substrate to bind, and AMP the last product to be released [15]. This reaction mechanism is in good agreement with the result of the biochemical studies by Palella et al. [38] and Rotllan et al. [43].

Adenosine-AMP exchange activity in AK

Another interesting aspect of AK is the adenosine-AMP exchange activity exhibited by this enzyme. In characterizing purified AK from rat liver, Mimouni et al. [59, 60] discovered that when radioactive adenosine and non-labeled AMP were added to the enzyme, the result was formation of labeled AMP. This exchange reaction, which was dependent on the presence of ADP and Mg^{2+} [59], was also seen with AK from other sources [61]. Although the exact mechanism of adenosine-AMP exchange activity is not clearly understood, it can be explained with the backward reaction of AK. In the exchange reaction, ADP serves as a carrier compound which shuttles a phosphate group from one adenosine to another, and thus is absolutely required [59]. Similar to the adenosine-phosphorylating activity of AK, the adenosine-AMP exchange activity of the enzyme also shows a dependence upon the presence of pentavalent ions and free Mg^{2+}, indicating that these two activities are related.

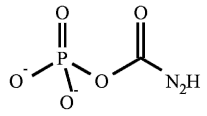
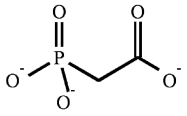
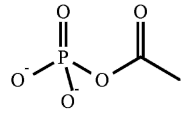
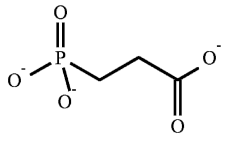
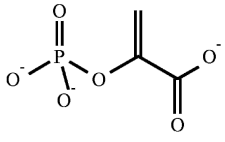
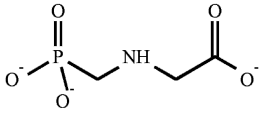
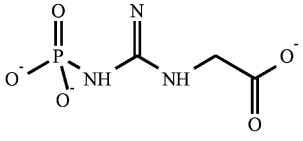
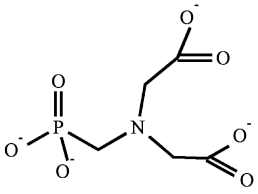
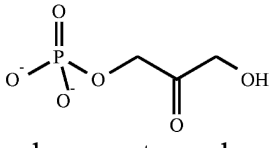
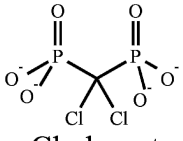
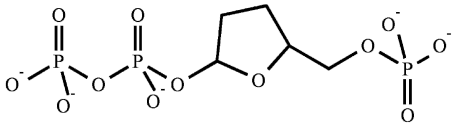
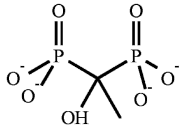
Regulation of RK and AK by inorganic phosphate

Phosphate or pentavalent ions dependency of RK and AK

Another important biochemical characteristic of RK and AK that has been discovered in recent years is that their catalytic activities show a nearly complete dependence upon the presence of pentavalent ions (PVIs) such as phosphate. This property was first demonstrated with Chinese hamster AK in 1996 [62] and with *E. coli* RK in 2001 [63]. The addition of inorganic phosphate in both cases led to an increase in the maximum velocity of the enzymes, as well as a decrease in the K_m for their substrates, adenosine and ribose [42, 44, 63]. Interestingly, other PVIs such as arsenate and vanadate were also able to substitute for phosphate as activators of these enzymes, showing similar kinetic effects [42, 44, 63]. AKs isolated from different sources (viz. human, Chinese hamster, spinach, yeast, *Leishmania donovani* and *Trypanosoma brucei*) were all stimulated by inorganic phosphate, indicating that PVIs dependency is a conserved property of the enzyme [42, 51, 64]. However, there can be some species variation in the kinetic details of PVI dependency, as phosphate was found to slightly increase (two-fold) the K_m for adenosine for *T. brucei* AK [51].

In addition to AK and RK, there are three other enzymes which have previously been reported to exhibit phosphate dependency: 6-phosphofructo-1-kinase (EC 2.7.1.11) [65], 6-phosphofructo-2-kinase (EC 2.7.1.105) [66], and phosphoribosylpyrophosphate synthetase (EC 2.7.6.1) [47]. Like AK and RK, these enzymes are all involved in the transfer of phosphate from ATP to a sugar-derived substrate. Furthermore, inorganic phosphate affects all of these enzymes in a manner similar as seen for AK and RK, resulting in increased V_{max} of the enzymes and reduced K_m for the sugar substrates within similar concentration ranges. It is interesting that all of these enzymes are involved in the production of essential metabolites, which is sensitive to the state of cellular energy. It fits then that the activities of these enzymes are regulated by inorganic phosphate, which provides an important index of the cellular energy status. The concentration of inorganic phosphate in guinea pig hearts, for example, was 1.7, 7.1, and 13.9 mM under normal, mildly hypoxic, and severely hypoxic conditions, respectively [67]. Inorganic phosphate results in half maximal velocity of AK and RK at about 2 mM, and maximal velocity at about 10 mM concentration [35, 68, 69]. The physiological level of inorganic phosphate therefore allows a full range of regulation for AK and RK.

Table 2. Molecular structures of AK activators and inhibitors.

Activators	Inhibitors
 Acetyl phosphate	 Phosphonoacetic acid
 Carbamoyl phosphate	 2-Carboxyethylphosphonic acid
 Phosphoenol pyruvate	 N-Phosphonomethylglycine
 Creatine phosphate	 N-Phosphonoimidodiacetic acid
 Dihydroxyacetone phosphate	 Clodronate
 Phosphoribosyl pyrophosphate	 Etidronate

Phosphorylated compounds that are activators and inhibitors of AK and RK

The activation of AK and RK by phosphate and other PVIs has led to an examination of the effect of other phosphorylated compounds on the activities of these enzymes. These studies have identified many phosphorylated compounds which activate AK and RK [35, 68, 69]. Some of these compounds are shown in Table 2. Kinetic studies with the activator compounds showed that they act by a mechanism similar to that seen with phosphate, resulting in increased V_{max} of the enzyme and reduced K_m for adenosine [35, 68, 69]. Most of the phosphorylated activators are important cellular metabolites (mostly high-energy metabolites), and thus may have a physiologically relevant role as regulators of phosphate-dependent enzymes. Interestingly, a number of other compounds that are structurally related to the activators were found to inhibit AK and RK (Table 2) [35, 68, 69]. The inhibitor compounds acted in a competitive manner with respect to the activating phosphate, indicating that they are binding to the same site on these enzymes [35, 68, 69].

Comparison of the chemical structures of the activators and inhibitors of AK and RK has identified certain distinguishing features [69]. The simplest activating compounds, such as phosphate, arsenate, and vanadate, contain a pentavalent core atom which

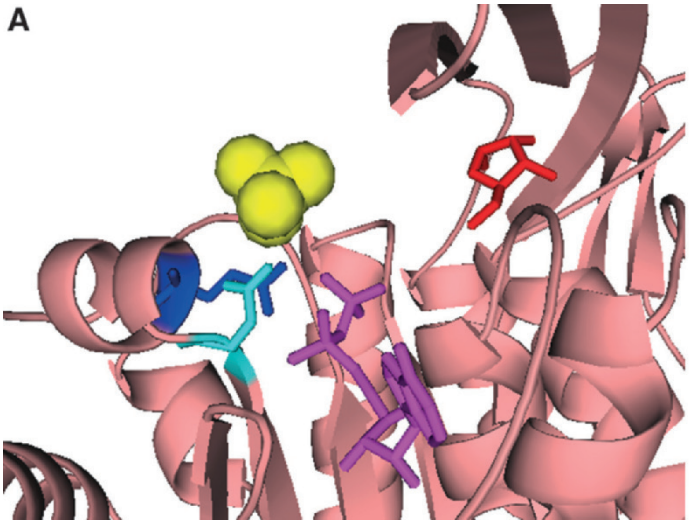


Figure 5. NXXE motif. (*A*) Close-up view of *E. coli* ribokinase showing Asn187 (cyan) and Glu190 (blue) from the NXXE motif, and the bound phosphate (yellow). This phosphate has shown to form direct and indirect hydrogen bonds with the residues of the NXXE motif. Ribose (red) and ADP (magenta) are also shown in the structure. (*B*) Sequence alignment showing the conserved region in various enzymes from the RK family.

is connected to four oxygen atoms. Higher electronegativity of oxygen results in a partial positive charge on the pentavalent atoms of these compounds. All of the other activators have the general structure O_3^{2-}P-X-R, in which the X atom is oxygen or nitrogen (Table 2). The electronegativity of the X atom in these compounds is further stabilized by the R group due to the inductive effect. As a result, these compounds also have a partial positive charge on the core pentavalent atoms (Table 2) [69]. In contrast to the activators, the inhibitors of AK have a methylene (-CH_2-) group adjacent to the core phosphorous atoms. Due to the electron-donating characteristic of this group, the partial positive charges on the core pentavalent atoms of these compounds are weaker than those in the activating compounds. The partial charge on the core pentavalent atom was calculated to be 1.29–1.37 for the activators and 0.95–1.17 for the inhibitors [69]. The hypothesis is then that both the activators and inhibitors, sharing similar structures, can bind to the phosphate regulatory site on AK and RK [69]. Although bound to the regulatory site, the inhibitor compounds cannot promote catalysis due to the low net positive charge on the core pentavalent atom.

The NXXE motif and its role in phosphate and magnesium activation of AK and RK

A crystal structure of RK has been solved with a bound phosphate in the active site, which makes close contacts (direct and indirect hydrogen bonds) with the residues Asn187 and Glu190 (Fig. 5A) [29]. These residues are highly conserved in the RK family (Fig. 5B) [42]. For instance, the corresponding residues are conserved in AK (Asn223 and Glu226 in human, and Asn239 and Glu242 in Chinese hamster), and occupy similar three-dimensional space [14, 29]. In the crystal structure of human AK these residues form hydrogen bonds with a bound magnesium ion through a bridging water molecule [14]. We have proposed that asparagine and glutamic acid residues at these positions constitute a new sequence motif, NXXE, which is a common characteristic of the RK family [42]. The importance of the NXXE motif in phosphate activation of AK has been studied by site-directed mutagenesis [42]. Unlike the wild-type enzyme, which showed negligible activity in the absence of phosphate, the N239Q and E242D mutants showed substantial activity without phosphate. Addition of phosphate did not cause significant stimulation in these cases. In contrast to these mutants, the N239L and E242L mutants of AK showed no significant activity both in the absence and presence of phosphate.

The dependence of AK activity on free magnesium, as well as the presence of a bound magnesium ion in the human AK structure [14], suggests a requirement of a catalytic Mg^{2+} at the active site. The E242D and N239Q mutants showed lower affinity for free Mg^{2+}, indicating that the effect of free Mg^{2+} on AK activity is closely related to that of the phosphate ion [42]. Recently, a mutation in the Glu190 residue of *E. coli* 6-phosphofructo-2-kinase, which corresponds to Glu242 in CHO AK, was also reported to result in an enzyme with an altered Mg^{2+} requirement [70]. These results indicate that the Asn and Glu residues of the NXXE motif are required for proper binding of the catalytic

Mg^{2+} ion at the active sites in these enzymes. Although the exact role of this magnesium ion is not yet clear, it is speculated that it helps in the formation of the transition state of the reaction by increasing the electrophilicity of the γ-phosphorous atom of the nucleotide [70, 71].

Based on these observations, a model describing the role of activating phosphate and free Mg^{2+} in AK catalysis has been proposed [68]. In this model (Fig. 6), when a phosphate or an activator compound binds to the NXXE motif, it facilitates binding of free Mg^{2+} and also the substrate adenosine to the active site. The binding of adenosine induces closing of the lid domain and increases the enzyme's affinity for the second substrate ATP. While the catalytic residue Asp316 deprotonates the 5′-hydroxyl end of adenosine, the activator compound enhances the electrophilicity of the β-phosphorous in ATP by withdrawing charge *via* its interaction with the non-bridging oxygen of the β-phosphate. Here, the net positive charge on the core pentavalent atom of the activator is important for its interaction with the oxygen atoms. The catalytic magnesium ion, on the other hand, may have a similar interaction with the γ-phosphate of ATP. Due to the increased electronegativity of the β- and γ-phosphorous atoms then, the electrons on the bridging oxygen are drawn towards the phosphorous atoms, making the bonds between them weak and elongated. The deprotonated 5′-hydroxyl end of adenosine subsequently attacks the positive center of the γ-phosphate, forming the transition state. In the final step, the γ-phosphate is transferred to adenosine, and the products AMP and ADP are released.

It is reasonable to assume that the catalytic steps of the RK reaction also proceed as described for AK, based on several observations: (i) Inorganic phosphate, arsenate, and vanadate all have very similar effects on the kinetics of both enzymes [44, 62, 63]. (ii) Other phosphorylated activators of AK also activate RK, whereas the structurally related inhibitors of AK all inhibit RK, without exception [68, 69]. (iii) The NXXE motif, which has shown to be important for phosphate activation in AK, is absolutely conserved in RK [42]. (iv) The tertiary structures of AK and RK are extremely similar, showing a high level of conservation especially at the catalytic site [14, 29]. The fact that the NXXE motif is conserved in members of the RK family suggests that other enzymes from this family might also share catalytic mechanisms in common with AK and RK.

Studies of mutants affected in AK and gene structures of AK and RK

Isolation and characterization of mutants affected in AK

As previously discussed, AK shows a very broad specificity for its nucleoside substrate, allowing phosphorylation of numerous adenosine analogues. Some of these compounds, such as toyocamycin and tubercidin, are highly toxic to various cell types, and a number of others exhibit antibacterial, antifungal, antiviral, and antineoplastic properties [72–75]. Based upon their chemical structures, the adenosine analogues can be grouped into two broad classes. Compounds such as toyocamycin and tubercidin, and also adenosine, contain a carbon-nitrogen bond in the ribosidic linkage, and are referred to as N-nucleosides. Other analogues such as formycin A, formycin B, and 9-deazaadenosine, where a carbon-carbon bond links the ribose sugar to the purine base, are referred to as C-nucleosides [76].

Mutants of mammalian cells resistant to toxic levels of adenosine analogues have been selected from several cell types and species [77–80] (see [73] for the summary of additional studies and references). The most commonly observed mutants from different cell lines are affected in AK, and they are unable to convert adenosine analogues into phosphorylated forms, which are correlated with their cytotoxicity [73]. Most of these mutants contain no detectable AK activity in cell extracts, and they exhibit high level of cross-resistance to various N- as well as C-adenosine analogues [73, 81]. The resistance phenotype of these mutants behaves recessively in cell hybrids formed with sensitive cells. Such mutants are referred to as Class A mutants. Interestingly, in the CHO cell, the spontaneous frequency of Class A mutants is unusually high (10^{-4} to 10^{-5} mutations/cell/generation which is about 100- to 10,000-fold higher than the frequency of similar mutants in other cell lines) [73, 82]. The genetic basis for this phenomenon is not fully understood, but this could be related to the differences in the number of functional AK gene copies in various cell lines [83]. It has been suggested that CHO cells, where such mutants were obtained at high rates, contain only a single functional AK gene copy [82].

Another interesting kind of mutant affected in AK has been obtained in CHO cells by selecting in the presence of C-nucleosides formycin A and B [81]. These novel mutants (referred to as Class B) exhibit cross-resistance to, and are unable to phosphorylate, various C-adenosine analogues, while remaining sensitive and fully capable of phosphorylating N-adenosine analogues [84]. The genetic lesion in these mutants thus specifically prevents the phosphoryla-

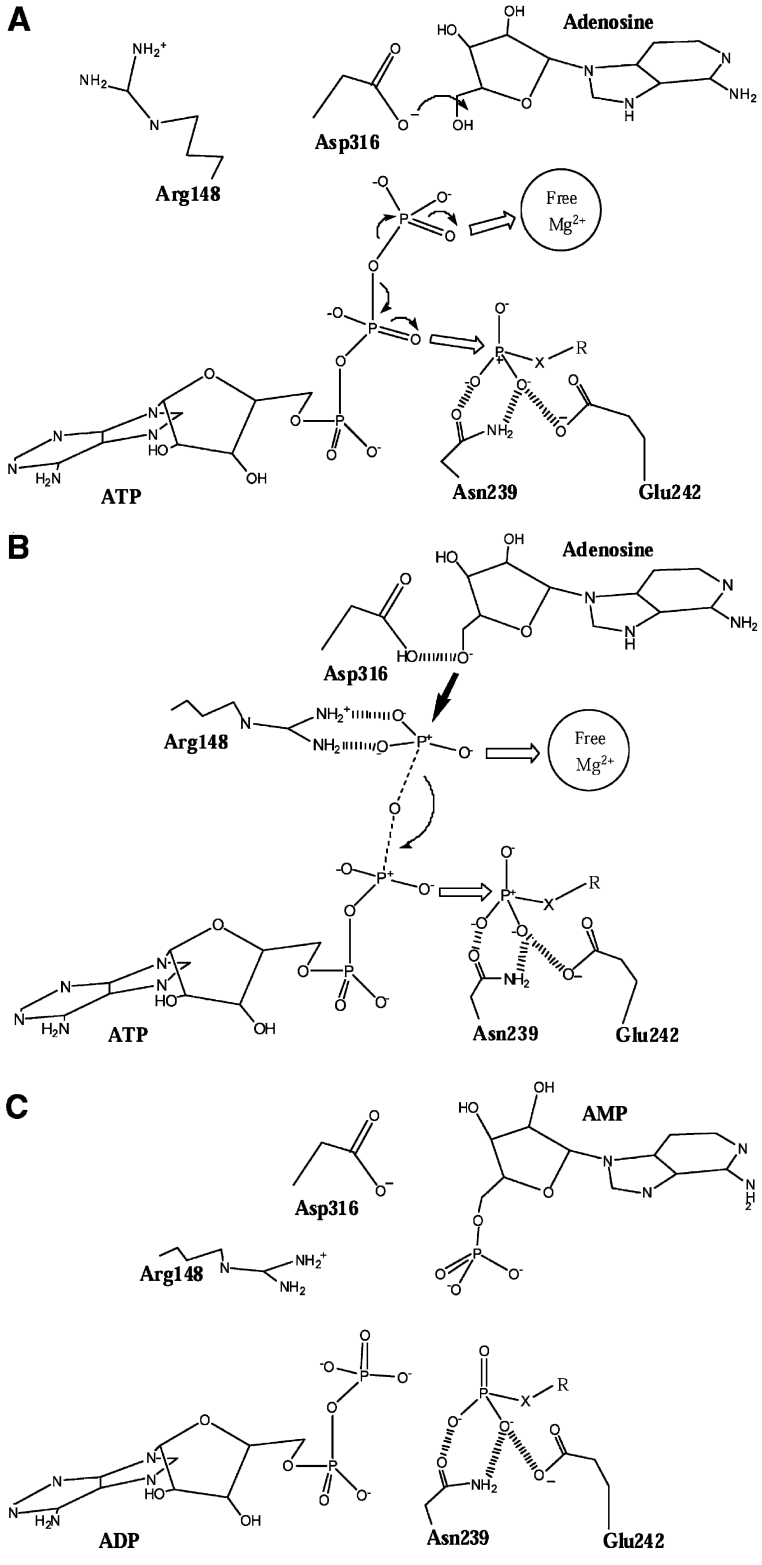


Figure 6. A model showing the activating effect of inorganic phosphate and interactions between different ligands at the active site of AK. Initially, binding of the activator facilitates binding of free Mg^{2+} as well as the substrate adenosine to the active site. This induces closing of the lid domain, which in turn increases the enzyme affinity for the second substrate ATP. (*A*) Once ATP is bound, the activator compound enhances the electrophilicity of the β-phosphorous atom by withdrawing charge *via* its interaction with the nonbridging oxygen of the β-phosphate. The net positive charge on the core pentavalent atom of the activator compound is important for its interaction with the oxygen atom. The catalytic magnesium ion, on the other hand, may have a similar interaction with the γ-phosphate of ATP. Due to the increased electronegativity of the β- and γ-phosphorous atoms, the electrons on the bridging oxygen are drawn towards the phosphorous atoms, making the bonds between the β- and γ-phosphate weak and elongated. In the meantime, the catalytic residue Asp316 deprotonates the 5′-hydroxyl end of adenosine. (*B*) The positive center of the γ-phosphate attracts the negative charge developed on the 5′-hydroxyl of adenosine after proton abstraction by the catalytic base Asp316. Arg148 helps stabilize the negative charges on the nonbridging oxygens of the γ-phosphate. (*C*) The γ-phosphate is transferred to adenosine. The arginine residue flips back, and the products ADP and AMP are released. The positions of the substrates, ligands, and amino acid residues in this figure are based on the structural data for AK and RK. The thick, hatched lines indicate hydrogen bonds. The X atom in the activating compound generally is oxygen or nitrogen.

tion of C-adenosine analogues without any effect on N-adenosine analogues. What is also intriguing with the Class B mutants is that, although these cells are sensitive to N-nucleosides, no AK activity (using adenosine, a N-nucleoside) could be detected in their cell extracts [84]. Some other mutants affected in AK (Class C) show reduced phosphorylation and affinity of certain adenosine analogues to which they exhibit increased resistance [73].

Gene structure of AK

The sequence of AK cDNA from human and Chinese hamster cells was first determined in 1996 [10, 11]. Subsequent work on AK gene structure has revealed that AK genes in mammalian organisms are extremely large [83, 85]. Human AK is estimated to be >550 Kb long, and the genes from rat, mouse, and Chinese hamster are also very large [83, 85]. Both human and Chinese hamster AK genes have identical structures, consisting of 11 exons whose length ranges from 36 to 173 bps and total 1.1 Kb [83, 85]. The lengths of the intervening introns in the human genome varies from 4.2 Kb to >126 Kb [83, 85]. The ratio of the noncoding to coding sequence for human AK (i.e. >550) is the highest known for any gene in mammalian or other organisms. In contrast to the mammalian genes, AK genes in other eukaryotic organisms are small [83]. *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, for instance, have no introns in their AK genes. In *Drosophila melanogaster* and *Caenorhabditis elegans*, the AK genes are only 1.5 and 1.3 Kb long, containing two and four small introns, respectively. *Arabidopsis thaliana*, on the other hand, has 10 small introns, which makes the overall length of the AK gene 2.4 Kb long [83].

Another unusual feature of AK gene structure in mammalian cells is that in various species (viz. human, mouse, and Chinese hamster) it is closely linked to the gene for the μ3A subunit of the clathrin adaptor protein-3 (AP-3 μ3A gene) [83, 85]. In all of these species, the genes for these two proteins are linked in a head-to-head fashion, and are transcribed from a single bi-directional promoter. The possible significance of the close linkage of these two genes and their sharing a common promoter is presently not clear.

The knowledge of AK gene structure has permitted characterization of mutants affected in AK at molecular levels. Southern and Northern blot analyses on a number of Class A mutants of CHO cells show that they contain large deletions leading to loss of several introns and exons [10, 83]. It is possible that the unusually large size of the AK gene and of its intervening introns is responsible for these deletions and also the high spontaneous frequency of the Class A mutants. Interestingly, in a number of these mutants, the deletion affecting the AK gene also extends into the neighboring AP-3 μ3A gene, leading to loss of all of its introns and exons [85]. The AP-3 μ3A adaptor protein is involved in vesicle-mediated protein sorting, and an alteration in this protein has been observed in Hermansky-Pudlak syndrome, a genetic disorder characterized by defective lysosome-related organelles [86, 87].

Gene structure of RK

Recently, the gene for RK in human cells has been characterized [35]. The human RK gene, which is located on chromosome 2, is approximately 110 Kb and comprises 8 exons that vary in length from 63 to 189 nucleotides. Interestingly, human RK shows a much higher degree of sequence identity to the RK sequences from protists species (viz. *Trypanosoma cruzi* and *Entamoeba histolytica*) and various bacteria (viz. *E. coli, Bukholderia cepacia*, etc.) in comparison with the homologues from plants (*Arabidopsis thaliana*) and fungi (*Saccharomyces cerevisiae*) [35]. These results are unexpected as fungi and plants are more closely related to the animals than bacteria [88]. It is thus possible that RKs from plant and fungi species have originated independently from those in animal and protist species. It is also possible that the plant and fungi RK sequences, which have not been characterized biochemically, may correspond to some other sugar kinases.

Studies with transgenic mice affected in AK

In contrast to other enzymes involved in the purine salvage pathway, such as adenosine deaminase, purine nucleoside phosphorylase, and hypoxanthine-guanine phosphoribosyltransferase (Fig. 7A), whose deficiency leads to a variety of genetic disorders in humans [89], no disease linked to the AK locus has been identified [90, 91]. However, in transgenic $AK^{-/-}$ mice, lack of AK has been shown to result in the development of acute neonatal hepatic steatosis, which leads to early postnatal mortality [92]. Two abnormalities of hepatic metabolism have been suggested to contribute in the development of steatosis in these mice: ATP depletion and inhibition of transmethylation reactions [91]. The absence of AK leads to loss of adenine nucleotides in favour of accumulation of adenosine. This leads to reduced metabolic capacity of mitochondria in the liver, which contributes to the development of steatosis [91]. Adenosine is also an important by-product of the transmethylation reactions catalyzed by various S-adenosylmethionine (SAM)-dependent methyltransferases (Fig. 7B) [91]. This reaction produces S-adenosylhomocysteine (SAH), which is hydrolyzed by SAH-hydrolase into homocysteine and adenosine. If adenosine is not

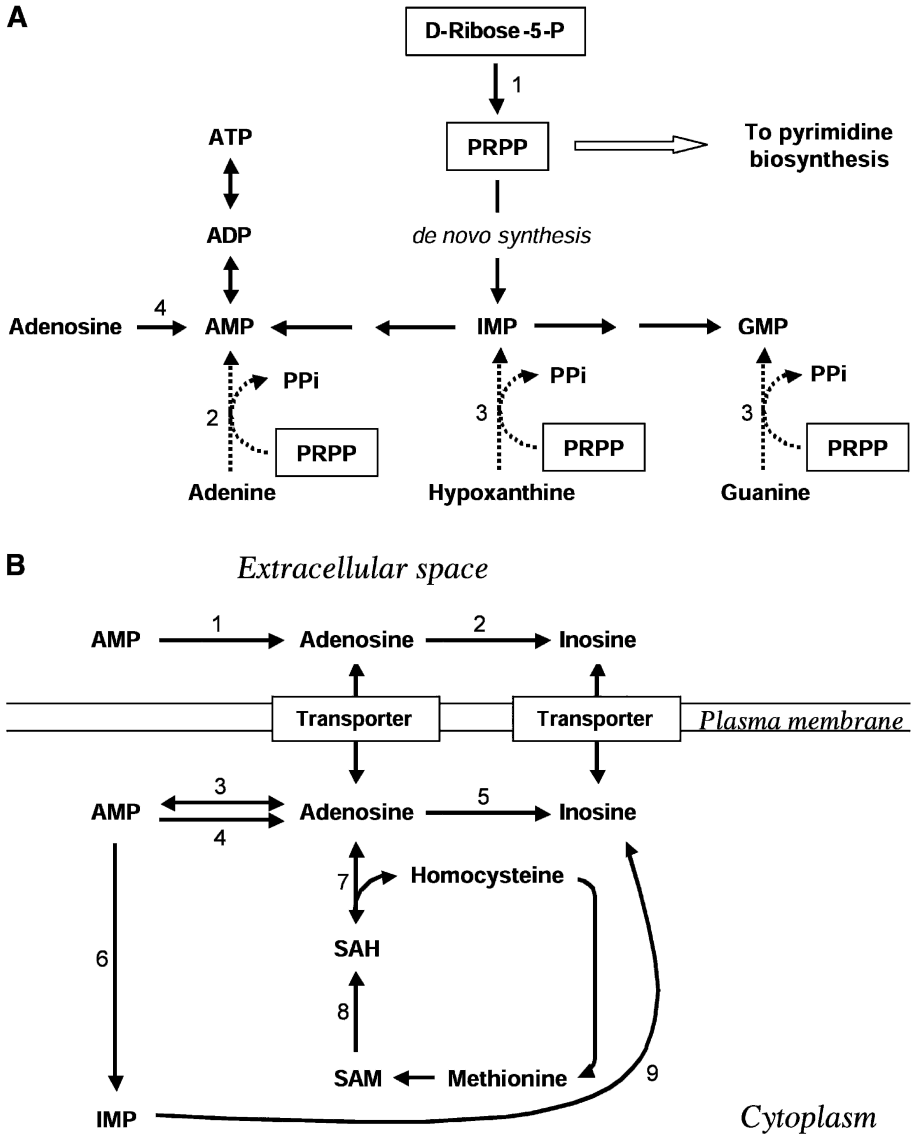


Figure 7. Metabolic pathways of purine nucleotides. (*A*) Synthesis of purine nucleotides. For both purine and pyrimidine biosynthesis, ribose-5-phosphate is pyrophosphorylated to form 5-phosphoribose-1-pyrophosphate (PRPP), by PRPP synthetase (enzyme 1). The detailed steps of *de novo* purine synthesis leading from PRPP to IMP are omitted. The enzymes of the salvage pathway, such as adenine phosphoribosyltransferase (enzyme 2) and hypoxanthine-guanine phosphoribosyltransferase (enzyme 3), regenerate nucleoside monophosphates from purine bases and PRPP (dotted arrows). AMP can be also formed by phosphorylation of adenosine by AK (enzyme 4). (*B*) Adenosine metabolism: pathways and enzymes involved. Enzymes: 1, ecto-5′-nucleotidase (e-N); 2, adenosine deaminase (both ecto- and plasma forms); 3, AK; 4, 5′-nucleotidase (c-NI); 5, adenosine deaminase; 6, AMP-deaminase; 7, SAH-hydrolase; 8, various methyl-transferases; 9, 5′-nucleotidase (c-NII) or alkaline phosphatase. Abbreviations: SAH, S-adenosylhomocysteine and SAM S-adenosylmethionine.

promptly removed by the action of AK, this will result in accumulation of SAH, which in turn inhibits various transmethylation reactions that are crucial for a wide variety of cellular processes [93, 94]. Thus, although AK is a salvage pathway enzyme, its cellular function is essential for the growth of organisms. This provides plausible explanations for the early postnatal lethality of $AK^{-/-}$ mice and also why mutants affected in AK have not been obtained in humans.

Boison and co-workers have carried out extensive work in recent years on the role of adenosine and AK in the development of epilepsy in a mouse model system [90, 95, 96]. According to these authors, the underlying pathogenic mechanism of epileptogenesis is astrocyte dysfunction that leads to overexpression of AK. The increased expression of AK in turn reduces ambient adenosine, which plays a central role as the inhibitory neuromodulator and endogenous anticonvulsant in neuronal cells. Their studies with transgenic mice that either overexpress or underexpress AK in the brain, where the incidence of epileptic seizure is either enhanced or greatly reduced, provide strong suggestive evidence for the AK hypothesis of epileptogenesis [90, 95, 97–99].

Ribose metabolism and its importance

Ribose metabolism

D-ribose is one of the most abundant and important sugars in biological systems [100]. Before entering the metabolic pathway, ribose needs to be phosphorylated by RK. The resulting ribose-5-phosphate (R-5-P) is then used for synthesis of nucleotides and other cofactors, or for energy production. Ribose has received much attention in recent years, as a metabolic supple-

ment for the heart [101]. Under normal conditions, energy-rich nucleotides are continuously produced in the heart to fulfill metabolic requirements. An absolutely essential compound in the production of purine and pyrimidine nucleotides is phosphoribosyl-5-pyrophosphate (PRPP), which is generated from R-5-P by the enzyme PRPP synthetase. In *de novo* synthesis (Fig. 7A), PRPP undergoes a series of reactions with other metabolic precursors, such as amino acids, CO_2, and NH_3, in order to produce nucleotides [102]. In the salvage pathway (Fig. 7A), PRPP is used to salvage and recycle the free purine and pyrimidine bases resulting from nucleotide catabolism [102]. Under pathologic cardiac conditions such as ischemia, however, the availability of PRPP is limited, thus hampering nucleotide synthesis [103, 104]. Nucleosides and bases produced *via* degradation of nucleotides then diffuse into the vascular space, and become lost from the tissue during reperfusion [105]. The repletion of nucleotides following ischemic insults has shown to be important in the recovery of mechanical function of the heart [102]. Supply of ribose, which is metabolized into R-5-P, the immediate precursor of PRPP, thus enhances the cells' ability to regenerate ATP and helps restore proper function.

The beneficial effects of supplemental ribose on the cardiovascular system have been extensively studied during the past three decades. In numerous animal models of cardiac and cerebral ischemia, cardiac hypertrophy, and cardiomyopathy, ribose administration has shown to recover and enhance diastolic function by replenishing myocardial adenine nucleotides [106–108]. Subsequent investigations with human subjects have also demonstrated cardio-protective effects of supplemental ribose. Patients with severe coronary artery disease have shown significantly increased tolerance to exercise-induced ischemia when treated with oral ribose [109]. In patients undergoing aortic valve replacement, supplementation of ribose prevented deterioration of the left ventricular function which was commonly seen in the untreated control group [110]. Furthermore, administration of ribose in congestive heart failure patients significantly improved diastolic function and ventilatory efficiency, leading to an improvement in quality of life [111, 112]. Showing no signs of adverse reactions in a number of toxicity studies [113, 114], the safety of ribose as a nutriceutical has been well accepted.

Ribose transport

Despite its importance, phosphorylation of free ribose following its transport into the cell is a little-investigated pathway in mammals. However, a substantial body of knowledge about ribose transport is available in a bacterial system. In *E. coli*, ribose transport is controlled by the *rbs* operon, which encodes six proteins, RbsDACBKR [115]. These proteins are normally expressed at a low level, but induced in the presence of ribose [1]. The general pathway is as follows [115]. (i) Ribose enters the periplasm through pores in the outer membrane. (ii) Ribose is recognized and bound by RbsB, which then binds to the RbsAC membrane complex. (iii) ATP hydrolysis by RbsA on the cytoplasmic side of the membrane brings ribose into the cell. Once in the cytoplasm, ribose undergoes a conformational change by the action of RbsD. This is a necessary step in ribose utilization, since RK (RbsK) binds to ribose in its furan form [29], whereas RbsB binds to the pyran form [116].

In eukaryotic cells, ribose transport has only been characterized in the parasitic protozoa *Leishmania* [117, 118]. In contrast to its bacterial counterpart, ribose transport in *Leishmania* species does not occur by an active transport mechanism, as it is insensitive to metabolic inhibitors and uncoupling agents [117]. Instead, a carrier-facilitated transport mechanism has been suggested, as free intracellular ribose exchanges diffusively with exogenous deoxyribose [117]. RK from *Leishmania major* was recently cloned, sequenced, and characterized as the first enzyme of its kind in the *Leishmania* genus [34].

Adenosine metabolism and related therapeutic applications

Adenosine metabolism

The purine nucleoside adenosine shows neuromodulating properties with a broad range of physiological responses in mammalian tissues *via* interaction with adenosine receptors. Much recent interest in adenosine metabolism has arisen from the discovery that adenosine receptor-mediated signals are involved in the protection of cells and tissues undergoing ischemic stress. Under normal oxygenated conditions, the rate of ATP catabolism and anabolism are equal, and the concentration of ATP is maintained at a much higher level (at least 10 times higher) than that of ADP [119, 120]. Under conditions of tissue stress or trauma, such as ischemia or hypoxia, however, the oxygen supply is inadequate for oxidative phosphorylation of ADP to regenerate ATP, and the ATP/ADP ratio is compromised. The increasing concentration of ADP results in an increase in the concentration of AMP, which in turn is hydrolyzed to adenosine [121, 122]. Due to the increased cytosolic concentration, adenosine is released into the extracellular space, where it can bind to adenosine receptors. The protective effects of adenosine receptor-mediated responses include coronary

vasodilation, neuronal activity reduction, platelet aggregation inhibition, anti-inflammatory activity, angiogenesis, and ischemic preconditioning [123–129]. For this reason, adenosine has been variously called as 'signal of life' and 'body's natural defense'. Interstitial concentrations of adenosine have been found as high as 30 μM within the myocardium [130], 30 μM in the brain [131], and 100 μM in the spinal cord [132], during a hypoxic episode. The cytosolic concentration of adenosine under normoxic conditions, on the other hand, was estimated to range between 0.01 and 0.1 μM [133, 134]. The concentration of adenosine is tightly regulated by AK and adenosine deaminase (ADA), which catalyze the phosphorylation and deamination of adenosine, respectively (Fig. 7B). The K_m of ADA for adenosine is in the range of 25–150 μM [135, 136], whereas that for AK is in the range of 25 nM – 1 μM [11, 137–139]. Since the K_m of AK for adenosine is much lower than that of ADA, AK is the principal enzyme responsible for regulating the level of adenosine.

While the AK and ADA are the main enzymes responsible for the catabolism of adenosine, there are three enzymes which are involved in the formation of adenosine. The most important source of adenosine quantitatively is the AMP-selective cytosolic 5′-nucleotidase (c-NI), which has been suggested to be responsible for approximately 76% of adenosine production in mammalian cells (Fig. 7B) [140, 141]. Another important enzyme responsible for the production of adenosine *via* hydrolysis of AMP is ecto-5′-nucleotidase (e-N) [142], a glycoprotein found primarily in the plasma membrane of eukaryotic cells [143]. The contribution of e-N to the total pool of adenosine in mammalian cells is estimated to be between 24 and 30% [144, 145]. In addition to these two enzymes, adenosine can also be produced from S-adenosylhomocysteine (SAH) *via* SAH-hydrolase (Fig. 7B). The reaction catalyzed by SAH-hydrolase is reversible, and the direction of its activity is dependent on the local concentrations of homocysteine [146]. The enzyme however, generally favours the hydrolysis of SAH as both homocysteine and adenosine are rapidly metabolized [147].

Adenosine transport

Because adenosine is hydrophilic, it requires specialized transport systems for its movement across the plasma membrane. There have been two major classes of nucleoside transport systems described in mammalian cells, the equilibrative (ENT) and the concentrative (CNT) nucleoside transporters [100, 148, 149]. The equilibrative system can mediate the transport of adenosine and other nucleosides in both directions, according to the nucleoside concentration gradient across the cell membrane. Thus far, four transporter proteins belonging to the equilibrative system have been cloned and identified, which are widely distributed in various cell types [149]. In contrast to the equilibrative system, the concentrative system mediates nucleoside transport in a uni-directional manner, coupled to that of the sodium ion. Six functionally different nucleoside transport activities belonging to this class have been identified, of which three have been characterized at the protein/gene level [148, 149]. The requirement for a Na^+ gradient is generally specific for these transporters, and their substrate specificity differs greatly from one another [148].

In contrast to the mammalian cells, all of the protozoan nucleoside transporters identified to date belong to the ENT family, showing sequence and topology homology to the members of mammalian ENTs [150]. Nucleoside transporters are important in the biochemistry of parasitic protozoa due to their absolute reliance upon the salvage of preformed purines from their vertebrate and invertebrate hosts. The first genes for protozoan nucleoside transporters were cloned from *Leishmania donovani* by functional complementation of an adenosine-pyrimidine transport-deficient mutant [151]. Since then, a number of additional adenosine/nucleoside transporters have been identified in organisms such as *Leishmania major*, *Trypanosoma brucei*, *Toxoplasma gondii*, and *Plasmodium falciparum* [150, 152].

Inhibitors of AK as therapeutic agents

As mentioned, adenosine shows a broad range of tissue-protective effects as an endogenous neuromodulator. The nucleoside is released into the extracellular space at specific sites during conditions of physiological stress and trauma, including ischemia, seizures, inflammation, and pain [153]. By activating various adenosine receptors on the cell surface, adenosine acts to limit tissue damaging and restore normal function [154]. Utilization of exogenous adenosine for pharmacological purposes, however, is difficult to achieve due to its short half-life in the interstitial fluid, which is of the order of seconds [155]. Inhibition of AK, on the other hand, provides a means to amplify the tissue-protective effects of adenosine by helping maintain its elevated level in the extracellular compartment. Since the release of endogenous adenosine is highly localized to tissues under stress, the effects of AK inhibition may only be pronounced at these specific sites [156]. In view of this potential as a pharmacological agent, there has been a great interest and effort to identify and optimize AK inhibitors.

One of the most extensively characterized groups of AK inhibitors consists of structural analogues of

adenosine, such as 5′-amino,5′-deoxyadenosine (5′NH_2dAdo), 5-iodotubercidin (5IT) and 5′-deoxy,5-iodotubercidin (5′d-5IT). With IC_{50} values on the order of several nanomoles, these compounds are highly potent in inhibiting AK *in vitro* [157]. Although these compounds have shown positive effects in several animal models of pain, seizure, and ischemia [158–161], their pharmacological utility is largely compromised due to short plasma half-lives, poor bio-availability, lack of target selectivity, and the potential to form cytotoxic metabolites [161, 162]. In an effort to optimize these AK inhibitors for better *in vivo* properties, a large series of 5′NH_2dAdo and 5IT derivatives were synthesized and tested [161, 163, 164]. As a result of rounds of structure-activity relationship studies, a number of diaryltubercidin analogues were identified as potent inhibitors of AK. Compounds such as GP790 and GP3966, for example, showed inhibition of human recombinant AK at low nanomolar concentrations [161, 163, 164]. When tested for anticonvulsant, anti-inflammatory, and analgesic properties in animal models, these compounds showed high efficacy with relatively mild adverse effects [161, 163, 164].

In addition to optimizing nucleoside analogue AK inhibitors, there has been a great effort in identifying novel, non-nucleoside AK inhibitors. Some of the most promising non-nucleoside AK inhibitors belong to two different classes, the pyridopyrimidine and the alkynylpyrimidine derivatives. These two groups of compounds were derived from an initial high-throughput screening lead [157], and have been subjected to rigorous rounds of structure-activity relationship studies [165–169]. Compound ABT-702, an example of pyridopyrimidine for instance, shows an IC_{50} value of 1.7 nM *in vitro*, and is comparably as efficient as morphine in animal models of pain [157]. An alkynylpyrimidine compound, 5-(4-(dimethylamino)-phenyl)-6-(6-morpholin-4-ylpyridin-3-ylethynyl)pyrimidin-4-ylamine, which shows levels of efficacy similar to ABT-702 [157], was recently crystallized with human AK [157]. Both the pyridopyrimidine and alkynylpyrimidine derivatives, in animal studies, showed little effect on motor coordination, mean arterial pressure, and heart rate, at effective doses for pain relief [165–169]. Other recently identified non-nucleoside AK inhibitors include N-(5,6-diphenyl-furo[2,3-d]pyrimidin-4-yl)-propionamide and 2-[2-(3,4-dihydroxy-phenyl)-5-phenyl-1H-imidazol-4-yl]-fluoren-9-one [64]. Both of these compounds potently inhibited human AK *in vitro* and in cultured cells in a concentration-dependent manner [64].

Conclusion and future directions

RK and AK were discovered more than 50 years ago, and there has been much progress in understanding these two important enzymes within the last decade or so. The structures of both RK and AK from various bacterial and eukaryotic sources have been solved, and have provided crucial information regarding ligand binding and reaction mechanisms. The mechanism of phosphate (or PVI) dependency, a unique catalytic property shared by RK and AK, is finally starting to emerge after recent biochemical studies. Both these enzymes carry out important metabolic reactions, and the therapeutic potentials of the substrates of these enzymes, ribose and adenosine, are now well recognized. The gene structures of RK and AK in mammalian systems have also become known within the last decade and have revealed several interesting features, opening the door for further studies. Recently, AK was identified as a key target for the prediction and prevention of epilepsy in a mouse model system, and these studies need to be extended to humans.

Although much work has been done with respect to ribose transport and its phosphorylation in bacterial systems, its eukaryotic counterpart is poorly understood. In light of the cardio-protective effects of supplemental ribose, ribose transport in mammalian systems, especially in humans, needs to be investigated in greater detail. In recent years, many inhibitors of AK have been identified which need to be further evaluated in clinical settings. If proven effective, AK inhibitors will represent a new group of drugs with analgesic, anti-inflammatory, and anti-epileptogenic properties, as well as cardio-protective properties. Another potentially interesting area for future studies is the phosphate (or PVI) dependency of other enzymes. As mentioned in this work, there are several enzymes which also show phosphate dependency, such as 6-phosphofructo-1-kinase, 6-phosphofructo-2-kinase, and phosphoribosylpyrophosphate synthetase, whose exact phosphate activation mechanisms are not known. Some of these enzymes, 6-phosphofructo-2-kinase in particular, belong to the RK family of enzymes, and share similar primary and tertiary structures with RK and AK. The phosphate activation mechanism of AK described in this work may apply to additional enzymes with a common structural basis.

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1 Anderson, A. and Cooper, R. A. (1969) The significance of ribokinase for ribose utilization by Escherichia coli. Biochim. Biophys. Acta1. 177, 163–165.
2 Lopilato, J. E., Garwin, J. L., Emr, S. D., Silhavy, T. J., and Beckwith, J. R. (1984) D-ribose metabolism in Escherichia coli K-12: genetics, regulation, and transport. J. Bacteriol. 158, 665–673.
3 Snyder, F. F. and Lukey, T. (1982) Kinetic considerations for the regulation of adenosine and deoxyadenosine metabolism in mouse and human tissues based on a thymocyte model. Biochim. Biophys. Acta. 696, 299–307.
4 Krenitsky, T. A., Miller, R. L., and Fyfe, J. A. (1974) Levels of nucleoside and nucleotide kinases in rhesus monkey tissues. Biochem. Pharmacol. 23, 70–72.
5 Dunwiddie, T. V. and Masino, S. A. (2001) The role and regulation of adenosine in the central nervous system. Annu. Rev. Neurosci. 24, 31–55.
6 Newby, A. C. (1985) The role of adenosine kinase in regulating adenosine concentration. Biochem. J., 226, 343–344.
7 Agranoff, B. W. and Brady, R. O. (1956) Purification and properties of calf liver ribokinase. J. Biol. Chem. 219, 221–229.
8 Caputto, R. (1951) The enzymatic synthesis of adenylic acid; adenosinekinase. J. Biol. Chem. 189, 801–814.
9 Bork, P., Sander, C., and Valencia, A. (1993) Convergent evolution of similar enzymatic function on different protein folds: the hexokinase, ribokinase, and galactokinase families of sugar kinases. Protein Sci. 2, 31–40.
10 Singh, B., Hao, W., Wu, Z., Eigl, B., and Gupta, R. S. (1996) Cloning and characterization of cDNA for adenosine kinase from mammalian (Chinese hamster, mouse, human and rat) species. High frequency mutants of Chinese hamster ovary cells involve structural alterations in the gene. Eur. J. Biochem. 241, 564–571.
11 Spychala, J., Datta, N. S., Takabayashi, K., Datta, M., Fox, I. H., Gribbin, T., and Mitchell, B. S. (1996) Cloning of human adenosine kinase cDNA: sequence similarity to microbial ribokinases and fructokinases. Proc. Natl. Acad. Sci. USA., 93, 1232–1237.
12 Hugouvieux-Cotte-Pattat, N., Nasser, W., and Robert-Baudouy, J. (1994) Molecular characterization of the Erwinia chrysanthemi kdgK gene involved in pectin degradation. J. Bacteriol. 176, 2386–2392.
13 Arnfors, L., Hansen, T., Schonheit, P., Ladenstein, R., and Meining, W. (2006) Structure of Methanocaldococcus jannaschii nucleoside kinase: an archaeal member of the RK family. Acta. Crystallogr. D. Biol. Crystallogr. 62, 1085–1097.
14 Mathews, I. I., Erion, M. D., and Ealick, S. E. (1998) Structure of human adenosine kinase at 1.5 A resolution. Biochemistry, 37, 15607–15620.
15 Schumacher, M. A., Scott, D. M., Mathews, I. I., Ealick, S. E., Roos, D. S., Ullman, B., and Brennan, R. G. (2000) Crystal structures of Toxoplasma gondii adenosine kinase reveal a novel catalytic mechanism and prodrug binding. J. Mol. Biol. 296, 549–567.
16 Sigrell, J. A., Cameron, A. D., and Mowbray, S. L. (1999) Induced fit on sugar binding activates ribokinase. J. Mol. Biol. 290, 1009–1018.
17 Bairoch, A. (1991) PROSITE: a dictionary of sites and patterns in proteins. Nucleic Acids Res. 19 Suppl, 2241–2245.
18 Hulo, N., Bairoch, A., Bulliard, V., Cerutti, L., Cuche, B. A., Castro, E. D., Lachaize, C., Langendijk-Genevaux, P. S., and Sigrist, C. J. (2008) The 20 years of PROSITE. Nucleic Acids Res. 36, D245-D249.
19 Holden, H. M., Thoden, J. B., Timson, D. J., and Reece, R. J. (2004) Galactokinase: structure, function and role in type II galactosemia. Cell. Mol. Life Sci. 61, 2471–2484.
20 Kawai, S., Mukai, T., Mori, S., Mikami, B., and Murata, K. (2005) Hypothesis: structures, evolution, and ancestor of glucose kinases in the hexokinase family. J. Biosci. Bioeng. 99, 320–330.
21 Luesink, E. J., Marugg, J. D., Kuipers, O. P., and de Vos, W. M. (1999) Characterization of the divergent sacBK and sacAR operons, involved in sucrose utilization by Lactococcus lactis. J. Bacteriol. 181, 1924–1926.
22 Sadaie, Y., Yata, K., Fujita, M., Sagai, H., Itaya, M., Kasahara, Y., and Ogasawara, N. (1997) Nucleotide sequence and analysis of the phoB-rrnE-groESL region of the Bacillus subtilis chromosome. Microbiology, 143 (Pt 6), 1861–1866.
23 Sato, Y., Yamamoto, Y., Kizaki, H., and Kuramitsu, H. K. (1993) Isolation, characterization and sequence analysis of the scrK gene encoding fructokinase of Streptococcus mutans. J. Gen. Microbiol. 139, 921–927.
24 Zembrzuski, B., Chilco, P., Liu, X. L., Liu, J., Conway, T., and Scopes, R. (1992) Cloning, sequencing, and expression of the Zymomonas mobilis fructokinase gene and structural comparison of the enzyme with other hexose kinases. J. Bacteriol. 174, 3455–3460.
25 Aulkemeyer, P., Ebner, R., Heilenmann, G., Jahreis, K., Schmid, K., Wrieden, S., and Lengeler, J. W. (1991) Molecular analysis of two fructokinases involved in sucrose metabolism of enteric bacteria. Mol. Microbiol. 5, 2913–2922.
26 Blatch, G. L., Scholle, R. R., and Woods, D. R. (1990) Nucleotide sequence and analysis of the Vibrio alginolyticus sucrose uptake-encoding region. Gene, 95, 17–23.
27 Smith, S. B., Taylor, M. A., Burch, L. R., and Davies, H. V. (1993) Primary structure and characterization of a cDNA clone of fructokinase from potato (Solanum tuberosum L. cv record). Plant Physiol. 102, 1043.
28 Long, M. C., Escuyer, V., and Parker, W. B. (2003) Identification and characterization of a unique adenosine kinase from Mycobacterium tuberculosis. J. Bacteriol. 185, 6548–6555.
29 Sigrell, J. A., Cameron, A. D., Jones, T. A., and Mowbray, S. L. (1998) Structure of Escherichia coli ribokinase in complex with ribose and dinucleotide determined to 1.8 A resolution: insights into a new family of kinase structures. Structure, 6, 183–193.
30 Sigrell, J. A., Cameron, A. D., Jones, T. A., and Mowbray, S. L. (1997) Purification, characterization, and crystallization of Escherichia coli ribokinase. Protein Sci. 6, 2474–2476.
31 Andersson, C. E. and Mowbray, S. L. (2002) Activation of ribokinase by monovalent cations. J. Mol. Biol. 315, 409–419.
32 Schimmel, S. D., Hoffee, P., and Horecker, B. L. (1974) Deoxyribokinase from Salmonella typhimurium. Purification and properties. Arch. Biochem. Biophys. 164, 560–570.
33 Chuvikovsky, D. V., Esipov, R. S., Skoblov, Y. S., Chupova, L. A., Muravyova, T. I., Miroshnikov, A. I., Lapinjoki, S., and Mikhailopulo, I. A. (2006) Ribokinase from E. coli: expression, purification, and substrate specificity. Bioorg. Med. Chem. 14, 6327–6332.
34 Ogbunude, P. O., Lamour, N., and Barrett, M. P. (2007) Molecular cloning, expression and characterization of ribokinase of Leishmania major. Acta Biochim. Biophys. Sin. (Shanghai), 39, 462–466.
35 Park, J., van Koeverden, P., Singh, B., and Gupta, R. S. (2007) Identification and characterization of human ribokinase and comparison of its properties with E. coli ribokinase and human adenosine kinase. FEBS Lett. 581, 3211–3216.
36 Suelter, C. H. (1970) Enzymes activated by monovalent cations. Science, 168, 789–795.
37 Di Cera, E. (2006) A structural perspective on enzymes activated by monovalent cations. J. Biol. Chem. 281, 1305–1308.
38 Palella, T. D., Andres, C. M., and Fox, I. H. (1980) Human placental adenosine kinase. Kinetic mechanism and inhibition. J. Biol. Chem. 255, 5264–5269.
39 Miller, R. L., Adamczyk, D. L., and Miller, W. H. (1979) Adenosine kinase from rabbit liver. I. Purification by affinity chromatography and properties. J. Biol. Chem. 254, 2339–2345.

40 Chang, C. H., Brockman, R. W., and Bennett, L. L., Jr. (1980) Adenosine kinase from L1210 cells. Purification and some properties of the enzyme. J. Biol. Chem. 255, 2366–2371.
41 Fisher, M. N. and Newsholme, E. A. (1984) Properties of rat heart adenosine kinase. Biochem. J., 221, 521–528.
42 Maj, M. C., Singh, B., and Gupta, R. S. (2002) Pentavalent ions dependency is a conserved property of adenosine kinase from diverse sources: identification of a novel motif implicated in phosphate and magnesium ion binding and substrate inhibition. Biochemistry, 41, 4059–4069.
43 Rotllan, P. and Miras Portugal, M. T. (1985) Adenosine kinase from bovine adrenal medulla. Eur. J. Biochem. 151, 365–371.
44 Maj, M., Singh, B., and Gupta, R. S. (2000) The influence of inorganic phosphate on the activity of adenosine kinase. Biochim. Biophys. Acta 1476, 33–42.
45 Arnvig, K., Hove-Jensen, B., and Switzer, R. L. (1990) Purification and properties of phosphoribosyl-diphosphate synthetase from Bacillus subtilis. Eur. J. Biochem. 192, 195–200.
46 Hove-Jensen, B., Harlow, K. W., King, C. J., and Switzer, R. L. (1986) Phosphoribosylpyrophosphate synthetase of Escherichia coli. Properties of the purified enzyme and primary structure of the prs gene. J. Biol. Chem. 261, 6765–6771.
47 Switzer, R. L. (1969) Regulation and mechanism of phosphoribosylpyrophosphate synthetase. I. Purification and properties of the enzyme from Salmonella typhimurium. J. Biol. Chem. 244, 2854–2863.
48 Mildvan, A. S. (1987) Role of magnesium and other divalent cations in ATP-utilizing enzymes. Magnesium, 6, 28–33.
49 Ginsburg, A. (1959) A deoxyribokinase from Lactobacillus plantarum. J. Biol. Chem. 234, 481–487.
50 Bennett, L. L., Jr. and Hill, D. L. (1975) Structural requirements for activity of nucleosides as substrates for adenosine kinase: orientation of substituents on the pentofuranosyl ring. Mol. Pharmacol. 11, 803–808.
51 Vodnala, M., Fijolek, A., Rofougaran, R., Mosimann, M., Mäser, P., and Hofer, A. (2008) Adenosine kinase mediates high affinity adenosine salvage in Trypanosoma brucei. J. Biol. Chem. 283, 5380–5388.
52 Hershfield, M. S., Fetter, J. E., Small, W. C., Bagnara, A. S., Williams, S. R., Ullman, B., Martin, D. W., Jr., Wasson, D. B., and Carson, D. A. (1982) Effects of mutational loss of adenosine kinase and deoxycytidine kinase on deoxyATP accumulation and deoxyadenosine toxicity in cultured CEM human T-lymphoblastoid cells. J. Biol. Chem. 257, 6380–6386.
53 Lindberg, B., Klenow, H., and Hansen, K. (1967) Some properties of partially purified mammalian adenosine kinase. J. Biol. Chem. 242, 350–356.
54 Miller, R. L., Adamczyk, D. L., Miller, W. H., Koszalka, G. W., Rideout, J. L., Beacham, L. M., III, Chao, E. Y., Haggerty, J. J., Krenitsky, T. A., and Elion, G. B. (1979) Adenosine kinase from rabbit liver. II. Substrate and inhibitor specificity. J. Biol. Chem. 254, 2346–2352.
55 Miller, R. L., Adamczyk, D. L., Rideout, J. L., and Krenitsky, T. A. (1982) Purification, characterization, substrate and inhibitor specificity of adenosine kinase from several Eimeria species. Mol. Biochem. Parasitol. 6, 209–223.
56 Pelicano, H., Maury, G., Elalaoui, A., Shafiee, M., Imbach, J. L., Goody, R. S., and Divita, G. (1997) Study of the substrate-binding properties of bovine liver adenosine kinase and inhibition by fluorescent nucleoside analogues. Eur. J. Biochem. 248, 930–937.
57 Chang, C. H., Cha, S., Brockman, R. W., and Bennett, L. L., Jr. (1983) Kinetic studies of adenosine kinase from L1210 cells: a model enzyme with a two-site ping-pong mechanism. Biochemistry, 22, 600–611.
58 Henderson, J. F., Mikoshiba, A., Chu, S. Y., and Caldwell, I. C. (1972) Kinetic studies of adenosine kinase from Ehrlich ascites tumor cells. J. Biol. Chem. 247, 1972–1975.
59 Mimouni, M., Bontemps, F., and Van den Berghe, G. (1994) Kinetic studies of rat liver adenosine kinase. Explanation of exchange reaction between adenosine and AMP. J. Biol. Chem. 269, 17820–17825.
60 Mimouni, M., Bontemps, F., and Van den Berghe, G. (1994) Production of adenosine and nucleoside analogues by an exchange reaction catalyzed by adenosine kinase. Adv. Exp. Med. Biol. 370, 613–616.
61 Gupta, R. S. (1996) Adenosine-AMP exchange activity is an integral part of the mammalian adenosine kinase. Biochem. Mol. Biol. Int. 39, 493–502.
62 Hao, W. and Gupta, R. S. (1996) Pentavalent ions dependency of mammalian adenosine kinase. Biochem. Mol. Biol. Int. 38, 889–899.
63 Maj, M. C. and Gupta, R. S. (2001) The effect of inorganic phosphate on the activity of bacterial ribokinase. J. Protein Chem. 20, 139–144.
64 Park, J., Vaidyanathan, G., Singh, B., and Gupta, R. S. (2007) Identification and biochemical studies on novel non-nucleoside inhibitors of the enzyme adenosine kinase. Protein J., 26, 203–212.
65 Hofer, H. W., Allen, B. L., Kaeini, M. R., Pette, D., and Harris, B. G. (1982) Phosphofructokinase from Ascaris suum. Regulatory kinetic studies and activity near physiological conditions. J. Biol. Chem. 257, 3801–3806.
66 Laloux, M., Van Schaftingen, E., Francois, J., and Hers, H. G. (1985) Phosphate dependency of phosphofructokinase 2. Eur. J. Biochem. 148, 155–159.
67 Gorman, M. W., He, M. X., Hall, C. S., and Sparks, H. V. (1997) Inorganic phosphate as regulator of adenosine formation in isolated guinea pig hearts. Am. J. Physiol. 272, H913-H920.
68 Park, J., Singh, B., Maj, M. C., and Gupta, R. S. (2004) Phosphorylated derivatives that activate or inhibit mammalian adenosine kinase provide insights into the role of pentavalent ions in AK catalysis. Protein J., 23, 167–177.
69 Park, J., Singh, B., and Gupta, R. S. (2006) Inhibition of adenosine kinase by phosphonate and bisphosphonate derivatives. Mol. Cell. Biochem. 283, 11–21.
70 Parducci, R. E., Cabrera, R., Baez, M., and Guixe, V. (2006) Evidence for a catalytic Mg2+ ion and effect of phosphate on the activity of Escherichia coli phosphofructokinase-2: regulatory properties of a RK family member. Biochemistry, 45, 9291–9299.
71 Matte, A., Tari, L. W., and Delbaere, L. T. (1998) How do kinases transfer phosphoryl groups? Structure, 6, 413–419.
72 Bloch, A. (1975) The structure of nucleosides in relation to their biological and and biochemical activity: a summary. Ann. N. Y. Acad. Sci. 255, 576–596.
73 Gupta, R. S. (1989) Purine nucleoside analogs. In: Drug Resistance in Mammalian Cells, Gupta, R. S. (ed.), pp. 89–110, CRC Press, Boca Raton, FL.
74 Robins, R. K. and Revankar, G. R. (1985) Purine analogs and related nucleosides and nucleotides as antitumor agents. Med. Res. Rev. 5, 273–296.
75 Suhadolnik, R. J. (1979) Naturally occurring nucleoside and nucleotide antibiotics. Prog. Nucleic Acid Res. Mol. Biol. 22, 193–291.
76 Daves, G. D., Jr. and Cheng, C. C. (1976) The chemistry and biochemistry of C-nucleosides. Prog. Med. Chem. 13, 303–349.
77 Caldwell, I. C., Henderson, J. F., and Patterson, A. R. (1967) Resistance to purine ribonucleoside analogues in an ascites tumor. Can. J. Biochem. 45, 735–744.
78 Suttle, D. P., Harkrader, R. J., and Jackson, R. C. (1981) Pyrazofurin-resistant hepatoma cells deficient in adenosine kinase. Eur. J. Cancer, 17, 43–51.
79 Chan, T. S., Creagan, R. P., and Reardon, M. P. (1978) Adenosine kinase as a new selective marker in somatic cell genetics: isolation of adenosine kinase-deficient mouse cell lines and human-mouse hybrid cell lines containing adenosine kinase. Somatic Cell Genet. 4, 1–12.

80 Cass, C. E., Selner, M., and Phillips, J. R. (1983) Resistance to 9-beta-D-arabinofuranosyladenine in cultured leukemia L 1210 cells. Cancer Res. 43, 4791–4798.
81 Mehta, K. D. and Gupta, R. S. (1985) Chinese hamster ovary cell mutants specifically affected in the phosphorylation of C-purine nucleosides. Can. J. Biochem. Cell. Biol. 63, 1044–1048.
82 Gupta, R. S. and Siminovitch, L. (1978) Genetic and biochemical studies with the adenosine analogs toyocamycin and tubercidin: mutation at the adenosine kinase locus in Chinese hamster cells. Somatic Cell Genet. 4, 715–735.
83 Singh, B., Lin, A., Wu, Z. C., and Gupta, R. S. (2001) Gene structure for adenosine kinase in Chinese hamster and human: high-frequency mutants of CHO cells involve deletions of several introns and exons. DNA Cell Biol. 20, 53–65.
84 Mehta, K. D. and Gupta, R. S. (1983) Formycin B-resistant mutants of Chinese hamster ovary cells: novel genetic and biochemical phenotype affecting adenosine kinase. Mol. Cell. Biol. 3, 1468–1477.
85 Singh, B. and Gupta, R. S. (2004) Genomic organization and linkage via a bidirectional promoter of the AP-3 (adaptor protein-3) mu3A and AK (adenosine kinase) genes: deletion mutants of AK in Chinese hamster cells extend into the AP-3 mu3A gene. Biochem. J., 378, 519–528.
86 Dell'Angelica, E. C., Mullins, C., Caplan, S., and Bonifacino, J. S. (2000) Lysosome-related organelles. FASEB J., 14, 1265–1278.
87 Dell'Angelica, E. C., Aguilar, R. C., Wolins, N., Hazelwood, S., Gahl, W. A., and Bonifacino, J. S. (2000) Molecular characterization of the protein encoded by the Hermansky-Pudlak syndrome type 1 gene. J. Biol. Chem. 275, 1300–1306.
88 Baldauf, S. L., Roger, A. J., Wenk-Siefert, I., and Doolittle, W. F. (2000) A kingdom-level phylogeny of eukaryotes based on combined protein data. Science 290, 972–977.
89 Boss, G. R. and Seegmiller, J. E. (1982) Genetic defects in human purine and pyrimidine metabolism. Annu. Rev. Genet. 16, 297–328.
90 Boison, D. (2008) The adenosine kinase hypothesis of epileptogenesis. Prog. Neurobiol. 84, 249–262.
91 Boison, D., Scheurer, L., Zumsteg, V., Rulicke, T., Litynski, P., Fowler, B., Brandner, S., and Mohler, H. (2002) Neonatal hepatic steatosis by disruption of the adenosine kinase gene. Proc. Natl. Acad. Sci. USA, 99, 6985–6990.
92 Satran, L., Sharp, H. L., Schenken, J. R., and Krivit, W. (1969) Fatal neonatal heptic steatosis: a new familial disorder. J. Pediatr. 75, 39–46.
93 Paik, W. K., Paik, D. C., and Kim, S. (2007) Historical review: the field of protein methylation. Trends Biochem. Sci. 32, 146–152.
94 Sulewska, A., Niklinska, W., Kozlowski, M., Minarowski, L., Naumnik, W., Niklinski, J., Dabrowska, K., and Chyczewski, L. (2007) DNA methylation in states of cell physiology and pathology. Folia. Histochem. Cytobiol. 45, 149–158.
95 Li, T., Ren, G., Lusardi, T., Wilz, A., Lan, J. Q., Iwasato, T., Itohara, S., Simon, R. P., and Boison, D. (2008) Adenosine kinase is a target for the prediction and prevention of epileptogenesis in mice. J. Clin. Invest. 118, 571–582.
96 Boison, D. (2006) Adenosine kinase, epilepsy and stroke: mechanisms and therapies. Trends Pharmacol. Sci. 27, 652–658.
97 Pignataro, G., Maysami, S., Studer, F. E., Wilz, A., Simon, R. P., and Boison, D. (2008) Downregulation of hippocampal adenosine kinase after focal ischemia as potential endogenous neuroprotective mechanism. J. Cereb. Blood Flow Metab. 28, 17–23.
98 Yee, B. K., Singer, P., Chen, J. F., Feldon, J., and Boison, D. (2007) Transgenic overexpression of adenosine kinase in brain leads to multiple learning impairments and altered sensitivity to psychomimetic drugs. Eur. J. Neurosci. 26, 3237–3252.
99 Gouder, N., Scheurer, L., Fritschy, J. M., and Boison, D. (2004) Overexpression of adenosine kinase in epileptic hippocampus contributes to epileptogenesis. J. Neurosci. 24, 692–701.
100 Tozzi, M. G., Camici, M., Mascia, L., Sgarrella, F., and Ipata, P. L. (2006) Pentose phosphates in nucleoside interconversion and catabolism. FEBS J., 273, 1089–1101.
101 Pauly, D. F., Johnson, C., and St Cyr, J. A. (2003) The benefits of ribose in cardiovascular disease. Med. Hypotheses 60, 149–151.
102 Taegtmeyer, H., Roberts, A. F., and Raine, A. E. (1985) Energy metabolism in reperfused heart muscle: metabolic correlates to return of function. J. Am. Coll. Cardiol. 6, 864–870.
103 Zimmer, H. G. (1983) Normalization of depressed heart function in rats by ribose. Science 220, 81–82.
104 Zimmer, H. G. and Ibel, H. (1984) Ribose accelerates the repletion of the ATP pool during recovery from reversible ischemia of the rat myocardium. J. Mol. Cell. Cardiol. 16, 863–866.
105 Pauly, D. F. and Pepine, C. J. (2000) D-Ribose as a supplement for cardiac energy metabolism. J. Cardiovasc. Pharmacol. Ther. 5, 249–258.
106 Lamberts, R. R., Caldenhoven, E., Lansink, M., Witte, G., Vaessen, R. J., St Cyr, J. A., and Stienen, G. J. (2007) Preservation of diastolic function in monocrotaline-induced right ventricular hypertrophy in rats. Am. J. Physiol. Heart Circ. Physiol. 293, H1869-H1876.
107 Pasque, M. K., Spray, T. L., Pellom, G. L., Van Trigt, P., Peyton, R. B., Currie, W. D., and Wechsler, A. S. (1982) Ribose-enhanced myocardial recovery following ischemia in the isolated working rat heart. J. Thorac. Cardiovasc. Surg. 83, 390–398.
108 St Cyr, J. A., Bianco, R. W., Schneider, J. R., Mahoney, J. R., Jr., Tveter, K., Einzig, S., and Foker, J. E. (1989) Enhanced high energy phosphate recovery with ribose infusion after global myocardial ischemia in a canine model. J. Surg. Res. 46, 157–162.
109 Pliml, W., von Arnim, T., Stablein, A., Hofmann, H., Zimmer, H. G., and Erdmann, E. (1992) Effects of ribose on exercise-induced ischaemia in stable coronary artery disease. Lancet, 340, 507–510.
110 Vance, R., Einzig, S., Kreisler, K., and St Cyr, J. (2007) D-ribose maintains ejection fraction following aortic valve surgery. FASEB J., 14, A419.
111 Omran, H., Illien, S., MacCarter, D., St Cyr, J., and Luderitz, B. (2003) D-Ribose improves diastolic function and quality of life in congestive heart failure patients: a prospective feasibility study. Eur. J. Heart Fail. 5, 615–619.
112 Vijay, N., Mac Cater, D., Washam, M., and St Cyr, J. (2005) Ventilatory efficiency improves with D-ribose in congestive heart failure patients. JMCC 38, 820.
113 Griffiths, J. C., Borzelleca, J. F., and St Cyr, J. (2007) Lack of oral embryotoxicity/teratogenicity with D-ribose in Wistar rats. Food Chem. Toxicol. 45, 388–395.
114 Griffiths, J. C., Borzelleca, J. F., and St Cyr, J. (2007) Subchronic (13-week) oral toxicity study with D-ribose in Wistar rats. Food Chem. Toxicol. 45, 144–152.
115 Iida, A., Harayama, S., Iino, T., and Hazelbauer, G. L. (1984) Molecular cloning and characterization of genes required for ribose transport and utilization in Escherichia coli K-12. J. Bacteriol. 158, 674–682.
116 Mowbray, S. L. and Cole, L. B. (1992) 1.7 A X-ray structure of the periplasmic ribose receptor from Escherichia coli. J. Mol. Biol. 225, 155–175.
117 Pastakia, K. B. and Dwyer, D. M. (1987) Identification and characterization of a ribose transport system in Leishmania donovani promastigotes. Mol. Biochem. Parasitol. 26, 175–181.
118 Maugeri, D. A., Cazzulo, J. J., Burchmore, R. J., Barrett, M. P., and Ogbunude, P. O. (2003) Pentose phosphate metabolism in Leishmania mexicana. Mol. Biochem. Parasitol. 130, 117–125.

119 Decking, U. K., Schlieper, G., Kroll, K., and Schrader, J. (1997) Hypoxia-induced inhibition of adenosine kinase potentiates cardiac adenosine release. Circ. Res. 81, 154–164.
120 Traut, T. W. (1994) Physiological concentrations of purines and pyrimidines. Mol. Cell. Biochem. 140, 1–22.
121 Bak, M. I. and Ingwall, J. S. (1998) Regulation of cardiac AMP-specific 5'-nucleotidase during ischemia mediates ATP resynthesis on reflow. Am. J. Physiol. 274, C992–1001.
122 Gustafson, L. A. and Kroll, K. (1998) Downregulation of 5'-nucleotidase in rabbit heart during coronary underperfusion. Am. J. Physiol. 274, H529-H538.
123 Berne, R. M. (1980) The role of adenosine in the regulation of coronary blood flow. Circ. Res. 47, 807–813.
124 Dawicki, D. D., Agarwal, K. C., and Parks, R. E., Jr. (1985) Role of adenosine uptake and metabolism by blood cells in the antiplatelet actions of dipyridamole, dilazep and nitrobenzylthioinosine. Biochem. Pharmacol. 34, 3965–3972.
125 Grisham, M. B., Hernandez, L. A., and Granger, D. N. (1989) Adenosine inhibits ischemia-reperfusion-induced leukocyte adherence and extravasation. Am. J. Physiol. 257, H1334-H1339.
126 Grover, G. J., Sleph, P. G., and Dzwonczyk, S. (1992) Role of myocardial ATP-sensitive potassium channels in mediating preconditioning in the dog heart and their possible interaction with adenosine A1-receptors. Circulation 86, 1310–1316.
127 Gunther, G. R. and Herring, M. B. (1991) Inhibition of neutrophil superoxide production by adenosine released from vascular endothelial cells. Ann. Vasc. Surg. 5, 325–330.
128 Schrader, J., Haddy, F. J., and Gerlach, E. (1977) Release of adenosine, inosine and hypoxanthine from the isolated guinea pig heart during hypoxia, flow-autoregulation and reactive hyperemia. Pflugers. Arch. 369, 1–6.
129 Schrader, J., Baumann, G., and Gerlach, E. (1977) Adenosine as inhibitor of myocardial effects of catecholamines. Pflugers Arch. 372, 29–35.
130 Manthei, S. A., Reiling, C. M., and Van Wylen, D. G. (1998) Dual cardiac microdialysis to assess drug-induced changes in interstitial purine metabolites: adenosine deaminase inhibition versus adenosine kinase inhibition. Cardiovasc. Res. 37, 171–178.
131 Latini, S., Bordoni, F., Pedata, F., and Corradetti, R. (1999) Extracellular adenosine concentrations during in vitro ischaemia in rat hippocampal slices. Br. J. Pharmacol. 127, 729–739.
132 McAdoo, D. J., Robak, G., Xu, G. Y., and Hughes, M. G. (2000) Adenosine release upon spinal cord injury. Brain Res. 854, 152–157.
133 Deussen, A., Stappert, M., Schafer, S., and Kelm, M. (1999) Quantification of extracellular and intracellular adenosine production: understanding the transmembranous concentration gradient. Circulation 99, 2041–2047.
134 Kroll, K., Deussen, A., and Sweet, I. R. (1992) Comprehensive model of transport and metabolism of adenosine and S-adenosylhomocysteine in the guinea pig heart. Circ. Res. 71, 590–604.
135 Singh, L. S. and Sharma, R. (2000) Purification and characterization of intestinal adenosine deaminase from mice. Mol. Cell. Biochem. 204, 127–134.
136 Ford, H., Jr., Dai, F., Mu, L., Siddiqui, M. A., Nicklaus, M. C., Anderson, L., Marquez, V. E., and Barchi, J. J., Jr. (2000) Adenosine deaminase prefers a distinct sugar ring conformation for binding and catalysis: kinetic and structural studies. Biochemistry 39, 2581–2592.
137 De Jong, J. W. (1977) Partial purification and properties of rat-heart adenosine kinase. Arch. Int. Physiol. Biochim. 85, 557–569.
138 Drabikowska, A. K., Halec, L., and Shugar, D. (1985) Purification and properties of adenosine kinase from rat liver: separation from deoxyadenosine kinase activity. Z. Naturforsch. [C], 40, 34–41.
139 Sahin, B., Kansy, J. W., Nairn, A. C., Spychala, J., Ealick, S. E., Fienberg, A. A., Greene, R. W., and Bibb, J. A. (2004) Molecular characterization of recombinant mouse adenosine kinase and evaluation as a target for protein phosphorylation. Eur. J. Biochem. 271, 3547–3555.
140 Sala-Newby, G. B., Skladanowski, A. C., and Newby, A. C. (1999) The mechanism of adenosine formation in cells. Cloning of cytosolic 5'-nucleotidase-I. J. Biol. Chem. 274, 17789–17793.
141 Garvey, E. P. and Prus, K. L. (1999) A specific inhibitor of heart cytosolic 5'-nucleotidase I attenuates hydrolysis of adenosine 5'-monophosphate in primary rat myocytes. Arch. Biochem. Biophys. 364, 235–240.
142 Darvish, A., Pomerantz, R. W., Zografides, P. G., and Metting, P. J. (1996) Contribution of cytosolic and membrane-bound 5'-nucleotidases to cardiac adenosine production. Am. J. Physiol. 271, H2162-H2167.
143 Misumi, Y., Ogata, S., Ohkubo, K., Hirose, S., and Ikehara, Y. (1990) Primary structure of human placental 5'-nucleotidase and identification of the glycolipid anchor in the mature form. Eur. J. Biochem. 191, 563–569.
144 Deussen, A., Bading, B., Kelm, M., and Schrader, J. (1993) Formation and salvage of adenosine by macrovascular endothelial cells. Am. J. Physiol. 264, H692-H700.
145 Mattig, S. and Deussen, A. (2001) Significance of adenosine metabolism of coronary smooth muscle cells. Am. J. Physiol. Heart Circ. Physiol. 280, H117-H124.
146 Loncar, R., Flesche, C. W., and Deussen, A. (1997) Determinants of the S-adenosylhomocysteine (SAH) technique for the local assessment of cardiac free cytosolic adenosine. J. Mol. Cell. Cardiol. 29, 1289–1305.
147 Deussen, A., Borst, M., and Schrader, J. (1988) Formation of S-adenosylhomocysteine in the heart. I: An index of free intracellular adenosine. Circ. Res. 63, 240–249.
148 Thorn, J. A. and Jarvis, S. M. (1996) Adenosine transporters. Gen. Pharmacol. 27, 613–620.
149 Podgorska, M., Kocbuch, K., and Pawelczyk, T. (2005) Recent advances in studies on biochemical and structural properties of equilibrative and concentrative nucleoside transporters. Acta. Biochim. Pol. 52, 749–758.
150 Landfear, S. M., Ullman, B., Carter, N. S., and Sanchez, M. A. (2004) Nucleoside and nucleobase transporters in parasitic protozoa. Eukaryot. Cell 3, 245–254.
151 Vasudevan, G., Carter, N. S., Drew, M. E., Beverley, S. M., Sanchez, M. A., Seyfang, A., Ullman, B., and Landfear, S. M. (1998) Cloning of Leishmania nucleoside transporter genes by rescue of a transport-deficient mutant. Proc. Natl. Acad. Sci. USA, 95, 9873–9878.
152 Carter, N. S., Landfear, S. M., and Ullman, B. (2001) Nucleoside transporters of parasitic protozoa. Trends Parasitol. 17, 142–145.
153 Williams, M. and Jarvis, M. F. (2000) Purinergic and pyrimidinergic receptors as potential drug targets. Biochem. Pharmacol. 59, 1173–1185.
154 Ralevic, V. and Burnstock, G. (1998) Receptors for purines and pyrimidines. Pharmacol. Rev. 50, 413–492.
155 Moser, G. H., Schrader, J., and Deussen, A. (1989) Turnover of adenosine in plasma of human and dog blood. Am. J. Physiol. 256, C799-C806.
156 Kowaluk, E. A. and Jarvis, M. F. (2000) Therapeutic potential of adenosine kinase inhibitors. Expert. Opin. Investig. Drugs, 9, 551–564.
157 Jarvis, M. F., Yu, H., Kohlhaas, K., Alexander, K., Lee, C. H., Jiang, M., Bhagwat, S. S., Williams, M., and Kowaluk, E. A. (2000) ABT-702 (4-amino-5-(3-bromophenyl)-7-(6-morpholinopyridin-3-yl)pyrido[2, 3-d]pyrimidine), a novel orally effective adenosine kinase inhibitor with analgesic and anti-inflammatory properties: I. In vitro characterization and acute antinociceptive effects in the mouse. J. Pharmacol. Exp. Ther. 295, 1156–1164.
158 Jiang, N., Kowaluk, E. A., Lee, C. H., Mazdiyasni, H., and Chopp, M. (1997) Adenosine kinase inhibition protects brain against transient focal ischemia in rats. Eur. J. Pharmacol. 320, 131–137.

159 Kowaluk, E. A., Kohlhaas, K. L., Bannon, A., Gunther, K., Lynch, J. J., III, and Jarvis, M. F. (1999) Characterization of the effects of adenosine kinase inhibitors on acute thermal nociception in mice. Pharmacol. Biochem. Behav. 63, 83–91.
160 Miller, L. P., Jelovich, L. A., Yao, L., DaRe, J., Ugarkar, B., and Foster, A. C. (1996) Pre- and peristroke treatment with the adenosine kinase inhibitor, 5'-deoxyiodotubercidin, significantly reduces infarct volume after temporary occlusion of the middle cerebral artery in rats. Neurosci. Lett. 220, 73–76.
161 Wiesner, J. B., Ugarkar, B. G., Castellino, A. J., Barankiewicz, J., Dumas, D. P., Gruber, H. E., Foster, A. C., and Erion, M. D. (1999) Adenosine kinase inhibitors as a novel approach to anticonvulsant therapy. J. Pharmacol. Exp. Ther. 289, 1669–1677.
162 Cottam, H. B., Wasson, D. B., Shih, H. C., Raychaudhuri, A., Di Pasquale, G., and Carson, D. A. (1993) New adenosine kinase inhibitors with oral antiinflammatory activity: synthesis and biological evaluation. J. Med. Chem. 36, 3424–3430.
163 Boyer, S. H., Ugarkar, B. G., Solbach, J., Kopcho, J., Matelich, M. C., Ollis, K., Gomez-Galeno, J. E., Mendonca, R., Tsuchiya, M., Nagahisa, A. et al. (2005) Adenosine kinase inhibitors. 5. Synthesis, enzyme inhibition, and analgesic activity of diaryl-erythro-furanosyltubercidin analogues. J. Med. Chem. 48, 6430–6441.
164 Ugarkar, B. G., Castellino, A. J., DaRe, J. S., Ramirez-Weinhouse, M., Kopcho, J. J., Rosengren, S., and Erion, M. D. (2003) Adenosine kinase inhibitors. 3. Synthesis, SAR, and antiinflammatory activity of a series of l-lyxofuranosyl nucleosides. J. Med. Chem. 46, 4750–4760.
165 Matulenko, M. A., Lee, C. H., Jiang, M., Frey, R. R., Cowart, M. D., Bayburt, E. K., DiDomenico, S., Gfesser, G. A., Gomtsyan, A., Zheng, G. Z. et al. (2005) 5-(3-Bromophenyl)-7-(6-morpholin-4-ylpyridin-3-yl)pyrido[2,3-d]pyrimidin-4-ylamine: structure-activity relationships of 7-substituted heteroaryl analogs as non-nucleoside adenosine kinase inhibitors. Bioorg. Med. Chem. 13, 3705–3720.
166 Zheng, G. Z., Mao, Y., Lee, C. H., Pratt, J. K., Koenig, J. R., Perner, R. J., Cowart, M. D., Gfesser, G. A., McGaraughty, S., Chu, K. L. et al. (2003) Adenosine kinase inhibitors: polar 7-substitutent of pyridopyrimidine derivatives improving their locomotor selectivity. Bioorg. Med. Chem. Lett. 13, 3041–3044.
167 Muchmore, S. W., Smith, R. A., Stewart, A. O., Cowart, M. D., Gomtsyan, A., Matulenko, M. A., Yu, H., Severin, J. M., Bhagwat, S. S., Lee, C. H. et al. (2006) Crystal structures of human adenosine kinase inhibitor complexes reveal two distinct binding modes. J. Med. Chem. 49, 6726–6731.
168 Gomtsyan, A., DiDomenico, S., Lee, C. H., Matulenko, M. A., Kim, K., Kowaluk, E. A., Wismer, C. T., Mikusa, J., Yu, H., Kohlhaas, K., Jarvis, M. F. et al. (2002) Design, synthesis, and structure-activity relationship of 6-alkynylpyrimidines as potent adenosine kinase inhibitors. J. Med. Chem. 45, 3639–3648.
169 Lee, C. H., Jiang, M., Cowart, M., Gfesser, G., Perner, R., Kim, K. H., Gu, Y. G., Williams, M., Jarvis, M. F., Kowaluk, E. A. et al. (2001) Discovery of 4-amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3-d]pyrimi dine, an orally active, non-nucleoside adenosine kinase inhibitor. J. Med. Chem. 44, 2133–2138.

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