Cloning and Characterization of the Multisubstrate Deoxyribonucleoside Kinase of *Drosophila melanogaster*

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A *Drosophila melanogaster* deoxyribonucleoside kinase (Dm-dNK) was reported to phosphorylate all four natural deoxyribonucleosides as well as several nucleoside analogs (Munch-Petersen, B., Piskur, J., and Sondergaard, L. (1998) *J. Biol. Chem.* 273, 3926–3931). The broad substrate specificity of this enzyme together with a high catalytic rate makes it unique among the nucleoside kinases. We have in the present study cloned the *Dm*-dNK cDNA, expressed the 29-kDa protein in *Escherichia coli*, and characterized the recombinant enzyme for the phosphorylation of nucleosides and clinically important nucleoside analogs. The recombinant enzyme preferentially phosphorylated the pyrimidine nucleosides dThd, dCyd, and dUrd, but phosphorylation of the purine nucleosides dAdo and dGuo was also efficiently catalyzed. *Dm*-dNK is closely related to human and herpes simplex virus deoxyribonucleoside kinases. The highest level of sequence similarity was noted with human mitochondrial thymidine kinase 2, and these enzymes also share many substrates. The cDNA cloning and characterization of *Dm*-dNK will be the basis for studies on the use of this multisubstrate nucleoside kinase as a suicide gene in combined gene/chemotherapy of cancer.

Deoxyribonucleoside kinases catalyze the phosphorylation of 2’-deoxyribonucleosides to 2’-deoxyribonucleoside monophosphates. The human deoxyribonucleoside kinases and the herpes simplex virus type 1 thymidine kinase (HSV-1 TK) have been intensively studied because they catalyze the rate-limiting step in the pharmacological activation of many nucleoside analogs (1). The nucleoside kinases have distinct, although partially overlapping, substrate specificities. Human mitochondrial deoxyguanosine kinase (dGK) is a purine nucleoside kinase that phosphorylates dGuo, dAdo, and dIno (2, 3). dGuo and dAdo are also phosphorylated by deoxycytidine kinase (dCK) in addition to dCyd, which is the main substrate of this enzyme (4, 5). dCK and dGK are sequence-related to mitochondrial thymidine kinase 2 (TK2) and to HSV-1 TK (6, 7). The latter two enzymes phosphorylate the pyrimidine nucleosides dThd, dUrd, and dCyd (8). The S-phase-specific cytosolic thymidine kinase 1 (TK1) phosphorylates dThd and dUrd (8), but TK1 is not sequence-related to the other mammalian or herpes simplex virus nucleoside kinases.

Munch-Petersen *et al.* (9) recently reported that *Drosophila melanogaster* embryonic S-2 cells contain a single major deoxyribonucleoside kinase. The *D. melanogaster* deoxyribonucleoside kinase (*Dm*-dNK) is, in contrast to the other enzymes, a multisubstrate nucleoside kinase. Although pyrimidine nucleosides are the preferred substrates of the enzyme, it catalyzes phosphorylation of all the natural pyrimidine and purine deoxyribonucleosides. The enzyme also efficiently phosphorylates several anti-viral and anti-cancer nucleoside analogs. The catalytic rate of deoxyribonucleoside phosphorylation by *Dm*-dNK is, depending on the substrate, 10- to 100-fold higher than what has been reported for the mammalian enzymes. The broad substrate specificity and the high catalytic rate make *Dm*-dNK unique among the members of the deoxyribonucleoside kinase enzyme family.

In recent years, the use of nucleoside kinases as suicide genes in gene therapy of cancer has been intensively studied. The prototype for combined gene/chemotherapy of malignant tumors is transduction of tumor cells with the gene encoding HSV-1 TK and subsequent systemic chemotherapy with the nucleoside analog ganciclovir (10). However, transduction of tumor cells with the cDNAs encoding other nucleoside kinases, such as human dCK and dGK, has also been demonstrated to increase the effects of cytotoxic nucleoside analogs (11–13). Because the initial activation step is rate-limiting for the phosphorylation of the majority of nucleoside analogs, the properties of the enzyme catalyzing this step are important for the efficiency of gene therapy. In an effort to find better suicide genes for gene therapy, mutants of HSV-1 TK have been genetically engineered with improved kinetic properties for nucleoside analog phosphorylation (14). Cancer cells transfected with these mutant nucleoside kinase genes become more sensitive to cytotoxic nucleoside analogs compared with cells transfected with the wild-type enzyme. Accordingly, the kinetic properties of the nucleoside kinases constitute a limiting factor in the efficiency of suicide gene therapy, and there is a need to identify better enzymes for further development of this therapeutic strategy.

The unique kinetic properties of *Dm*-dNK make it a candidate suicide gene for combined gene- and chemotherapy of cancer. We have in the present study cloned the cDNA of *Dm*-dNK to enable characterization of the enzyme with regard to phosphorylation of nucleoside analogs and compared its properties to those of the sequence-related human and herpes simplex virus nucleoside kinases. The sequence and substrate specificity of *Dm*-dNK indicated that the enzyme had evolved.© 1999 by The American Society for Biochemistry and Molecular Biology, Inc.
close to human TK2, but that it also exhibited sequence similarity and overlapping substrate specificity with dCK, dGK, and HSV-1 TK. The identification and cloning of a novel enzyme with broad substrate specificity and high catalytic activity for phosphorylation of nucleoside analogs will be the basis for evaluation of its potential use in combined gene/chemotherapy of cancer.

**EXPERIMENTAL PROCEDURES**

**Cloning of Dm-dNK cDNA**—We searched the expressed sequence tag library of the GenBank data base at the National Institute for Biotechnology Information with the Basic Local Alignment Search Tool (BLAST) (15) to identify D. melanogaster cDNA clones that encode enzymes similar to human dCK, dGK, and TK2 (6, 7, 16). The expressed sequence tag identified was obtained from D. Harvey (Howard Hughes Medical Institute, University of California). The DNA sequence of the plasmid was determined with the automatic laser fluorescent (A.L.F.) sequencer (Amersham Pharmacia Biotech). The protein was expressed and purified as described (7). The size and purity of the recombinant protein was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Phast system, Amersham Pharmacia Biotech). The protein concentration was determined with Bradford protein assay (Bio-Rad), and bovine serum albumin was used as the concentration standard.

**Enzyme Assays**—The phosphoryl transfer assay was performed using [γ-32P]ATP (3000 Ci/mmol, Amersham Pharmacia Biotech) (17). The nucleosides were added to a final concentration of 5 μM in a 10-μl reaction mixture containing 50 mM Tris-HCl, pH 8.0, 5 μM MgCl₂, 1 mM unlabeled ATP, 100 μCi of [γ-32P]ATP, and 1 μg of recombinant Dm-dNK. The samples were incubated for 30 min at 37 °C. Two μl of the reaction mixtures were spotted on polyethyleneimine-cellulose F thin layer chromatography sheets (Merck), and the nucleotides were separated in a buffer containing NH₄OH, isobuturic acid, and distilled H₂O (1.66:33). The sheets were autoradiographed using phosphorimaging plates (BAB 1000, Fuji, Japan).

The radiolabeled substrates [methyl-3H]-dThd (70 Ci/mmol), [5-3H]dCyd (21.1 Ci/mmol), [8-3H]dGuo (6.1 Ci/mmol), and [8-3H]dAdo (13 Ci/mmol) were obtained from Amersham Pharmacia Biotech or from Moravek Biochemicals (Brea, CA). The cDNAs of TK1, TK2, dCK, dGK, and HSV-1 TK were inserted in the pGEX-5X-1 vector, expressed as fusion proteins to glutathione-S-transferase, and purified as described (7). The activity of the purified recombinant nucleoside kinases was assayed in a 50-μl reaction mixture containing 50 mM Tris-HCl, pH 8.0 (22 °C), 2.5 mM MgCl₂, 10 mM dithiothreitol, 0.5 mM CHAPS, 3 mg/ml bovine serum albumin, 2.5 mM ATP, indicated concentrations of [methyl-3H]-dThd, [5-3H]dCyd, [8-3H]dGuo, or [8-3H]dAdo, and recombinant Dm-dNK. The samples were incubated at 37 °C for 30 min in the presence or absence of different concentrations of the test compounds. Aliquots of 45 μl of the reaction mixtures were spotted on Whatman DE-81 filter paper disks. The filters were washed 3 times for 5 min in 1 mM ammonium formate, 1 time for 1 min in H₂O, and 1 time for 5 min in ethanol. The radioactivity was determined by scintillation counting. The Kₘ and Vₘₐₓ values were derived from Lineweaver-Burk plots.

**Phylogenetic Analysis**—The software package TRECON (18) was used for the phylogenetic analysis. We performed distance calculations with Poisson correction. Insertions and deletions were taken into account, and bootstrap analysis was performed with value 100. The neighbor-joining method with bootstrap was used to infer the tree topology.

**RESULTS**

**Cloning and Expression of the Dm-dNK Multisubstrate Deoxyribonucleoside Kinase**—The multisubstrate deoxyribonucleoside kinase of D. melanogaster purified by Munch-Petersen et al. (9) has several biochemical features in common with the human deoxyribonucleoside kinases. Therefore, we hypothesized that Dm-dNK may belong to this enzyme family. We used the amino acid sequences of human dCK, dGK, and TK2 to search the expressed sequence tag library of the GenBank data base for D. melanogaster cDNA clones that encoded sequence homologues proteins. An expressed sequence tag clone that encoded a protein ~30% identical to the human nucleoside kinases was identified (LD15983, D. Harvey and co-workers). DNA sequence determination of the 1001-base pair clone showed that it contained an open reading frame encoding a 250-amino acid residue protein with a predicted molecular mass of 29 kDa (Fig. 1).

We expressed the cDNA-encoded enzyme to study the activity of this putative nucleoside kinase. Among the natural deoxyribonucleosides, the enzyme phosphorylated the pyrimidine nucleosides dThd, dUrd, and dCyd as well as the purine nucleosides dAdo and dGuo (data not shown). The enzyme did not phosphorylate ribonucleosides, nor did it phosphorylate the nucleoside monophosphate dTMP (data not shown). The enzymatic activity, assayed with 1 μM deoxyribonucleosides, showed relative phosphorylation efficiencies of 100% for dThd, 83% for dCyd, 1.8% for dAdo, and 0.14% for dGuo. The specificity of nucleoside phosphorylation, as well as the molecular mass of the protein, were thus similar to what has been described for Dm-dNK (9). Therefore, we conclude that the cloned D. melanogaster cDNA encoded the multisubstrate Dm-dNK.

**Nucleoside and Nucleoside Analog Phosphorylation by Deoxyribonucleoside Kinases**—The Michaelis-Menten kinetic properties of recombinant Dm-dNK were determined for the natural substrates dThd, dCyd, dAdo, and dGuo (Table I). The pyrimidine nucleosides dThd and dCyd were the preferred substrates of Dm-dNK, and these nucleosides showed similar affinities and catalytic rates. Although dAdo and dGuo phosphorylation was catalyzed by the enzyme, the affinities for these substrates were ~100- to 1000-fold lower as compared with the affinity of the pyrimidine nucleosides. However, the maximal catalytic rates of all four deoxyribonucleosides were similar, ranging from 220 to 910 nmol/μg/h. Although we performed the assay at similar conditions as described by Munch-Petersen et al. (9), the absolute Vₘₐₓ values of native Dm-dNK are reported to be ~2- to 10-fold higher than those determined for the recombinant enzyme. In spite of the difference in maximal catalytic velocity, the similarities in affinity to the nucleoside substrates support the conclusion that the cloned cDNA encodes Dm-dNK.

To compare the different nucleoside kinases for the phosphorylation of nucleosides and nucleoside analogs, we assayed the inhibitory activity of the natural nucleosides and nucleoside analogs on the phosphorylation of their preferred natural substrates. Recombinant Dm-dNK, HSV-1 TK, and human dCK, dGK, and TK2 were included in this comparative study, and the IC₅₀ values of the phosphorylation of 1 μM substrates were determined. We also included the S-phase specific TK1, although it does not share conserved sequences with the other nucleoside kinases nor exhibit as broad a substrate specificity as the other enzymes. The experiments with natural nucleosides showed that the pyrimidine nucleosides dCyd, dUrd, and dThd were able to efficiently compete with dCyd and dThd for phosphorylation by Dm-dNK and human TK2 (Table II). TK1 showed, as expected, a narrower substrate specificity than the other thymidine kinases, since only dUrd, and not dCyd, competed with dThd phosphorylation. dCyd was not an efficient competitor of HSV-1 TK-catalyzed dThd phosphorylation, but in contrast to the other thymidine kinases, the purine nucleoside dGuo slightly inhibited the phosphorylation of both dThd and dCyd catalyzed by HSV-1 TK. The human nucleoside kinases, dCK and dGK, showed strong preference for dCyd and...
dGuo, respectively. However, dIno was able to compete with dGuo phosphorylation catalyzed by dGK.

We further tested 19 nucleoside analogs to determine their ability to compete with natural deoxyribonucleosides (Table III). The compounds tested have been shown to be active as either anti-viral or anti-cancer agents. Most of the investigated dThd and dUrd analogs inhibited dCyd and dThd phosphorylation catalyzed by Dm-dNK, TK2, and HSV-1 TK. Among the pyrimidine nucleoside analogs, the anti-herpetic compound (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) was the most efficient substrate to compete with dThd for phosphorylation by Dm-dNK as well as for TK2 and HSV-1 TK. Phosphorylation of dThd and dCyd by Dm-dNK was also efficiently inhibited by all tested dCyd analogs. Although TK2 activity was inhibited by a few dCyd analogs, the degree of inhibition was less pronounced. Similarly, none of the dCyd analogs inhibited HSV-1 TK-catalyzed phosphorylation. dCk was the enzyme most efficiently inhibited by dCyd analogs. As expected, dAdo phosphorylation catalyzed by dCK was more efficiently inhibited than dCyd phosphorylation, because dAdo exhibits lower affinity to dCK than dCyd. dCk also phosphorylates several purine nucleosides and purine nucleoside analogs. However, among the investigated purine nucleoside analogs, only 2-chloro-2'-deoxyadenosine was able to efficiently inhibit dCyd and dAdo phosphorylation. Interestingly, 2-chloro-2'-deoxyadenosine was also the only purine nucleoside analog that efficiently inhibited dCyd and dThd phosphorylation catalyzed by Dm-dNK. The human purine nucleoside kinase dGK showed a narrow specificity regarding the inhibition of dGuo phosphorylation, since only the deoxyguanosine analogs araG and dFdG were inhibitors of the reaction. HSV-1 TK is also a purine nucleoside kinase, and it phosphorylates the acyclic guanosine analogs acyclovir and ganciclovir. In the present study, we detected inhibition by ganciclovir, and dFdG of HSV-1 TK catalyzed dThd and dCyd phosphorylation.

Sequence Analysis—Alignment of the predicted amino acid sequences of the cloned D. melanogaster deoxyribonucleoside kinase.

**Table I**

|        | $K_m$ (mM) | $V_{max}$ (nmol/mg/h) | $V_{max}/K_m$ |
|--------|------------|------------------------|---------------|
| dThd   | 1.6        | 240                    | 150           |
| dCyd   | 2.6        | 370                    | 140           |
| dGuo   | 2000       | 220                    | 0.11          |
| dAdo   | 373        | 910                    | 2.4           |

Fig. 1. cDNA and predicted amino acid sequences of the cloned D. melanogaster deoxyribonucleoside kinase.

**TABLE I**

| Kinetic properties of recombinant Dm-dNK fused to GST for the natural 2'-deoxyribonucleosides |
|---|---|---|---|
| dThd | dCyd | dGuo | dAdo |
| $K_m$ (mM) | 1.6 | 2.6 | 2000 | 373 |
| $V_{max}$ (nmol/mg/h) | 240 | 370 | 220 | 910 |
| $V_{max}/K_m$ | 150 | 140 | 0.11 | 2.4 |
sequence of Dm-dNK with the sequences of the human deoxyri-
bonucleoside kinases and HSV-1 TK showed that Dm-dNK was
38% identical to human TK2, 28% identical to dCK and dGK,
and 14% identical to HSV-1 TK (Fig. 2). Dm-dNK was of similar
length as the human deoxyribonucleoside kinases, whereas
HSV-1 TK was larger than the other enzymes due to an ex-
tended C-terminal domain. Dm-dNK showed a similar align-
ment as TK2, because both these enzymes lacked three regions
(starting at amino acids 63, 93, and 190) as compared with
dCK, dGK, and HSV-1 TK.

The crystal structure of HSV-1 TK in complex with nucleo-
sides and nucleoside analogs has been solved, and several
regions involved in substrate binding and catalysis have been
identified (19, 20). The ATP binding glycine-rich loop located in
the N-terminal domain of the enzyme was highly conserved in
Dm-dNK, as well as in the other nucleoside kinases. HSV-1 TK
amino acid residues involved in binding of the nucleoside sugar
moiety include Glu-83 and Arg-163, which bind to the 5’ OH
group, and Tyr-101 and Glu-225, which bind to the 3’ OH
group. Except Tyr-101, these residues are absolutely conserved
in all the five enzymes. Tyr-101 is located in a part of HSV-1 TK
that is not present in Dm-dNK or TK2, and the corresponding
regions of dCK and dGK exhibit low levels of sequence conser-
vation. HSV-1 TK amino acid residues involved in binding of
the thymine base include Ile-100, Gln-125, Met-128, and Tyr-
172. Met-128 binds to the thymine N3 group with support from
Ile-100. Gln-125 forms hydrogen bonds with the 4-carbonyl
group and with the 3-NH group. Tyr-172 stacks against the
thymine base on the opposite side to Ile-100 and Met-128.
Gln-125 is absolutely conserved in all kinases, and Tyr-172 is
also highly conserved in all enzymes, except in Dm-dNK, where
it is replaced by an arginine. A methionine at the position
equivalent to HSV-1 TK Met-128 is not present in any of the
other enzymes. However, the physical properties at this posi-
tion are conserved, since all enzymes have an uncharged amino
acid residue at this position. Similar to HSV-1 TK Tyr-101,
which is involved in binding of the sugar moiety, Ile-100 is
located in a region that is not present in Dm-dNK or TK2 and
not conserved in dCK and dGK. Other HSV-1 residues located
in close association to the substrate or phosphate donor sites
are Pro-84, Thr-88, Arg-216, Arg-220, and Arg-222. These res-
ides are conserved in all the five nucleoside kinases.

The close sequence relation of the nucleoside kinases suggest
an evolutionary relationship. We generated a prediction of
their phylogenetic relation, and the analysis suggested that the
enzyme family is divided in two separate evolutionary
branches (Fig. 3). One branch contained TK2, Dm-dNK, and
HSV-1 TK, and the second branch dCK and dGK.

**DISCUSSION**

We have identified, cloned, and recombinantly expressed the
cDNA of the multisubstrate deoxyribonucleoside kinase of *D.
melanogaster*. Although the pyrimidine nucleosides dCyd,
dThd, and dUrd are the preferred substrates of the recombi-
nant enzyme, it also catalyzed the phosphorylation of the pu-
rine nucleosides dAdo and dGua. We thereby provided further
evidence that Dm-dNK purified by Munch-Peterson et al. (9) is
a single protein with the ability to phosphorylate all the natu-
The sequence of Dm-dNK showed that the enzyme is closely related to the mammalian deoxyribonucleoside kinase enzyme family and to herpesvirus thymidine kinases. The partially overlapping substrate specificities and sequence similarities of the deoxyribonucleoside kinases suggest a common ancestor for these enzymes. The phylogenetic analysis in our study, comprising the human deoxyribonucleoside kinases and HSV-1 TK, suggests that the enzyme family is divided in two separate evolutionary branches. Dm-dNK showed closest sequence similarity to human TK2, and these two enzymes also exhibited similar patterns of substrate specificity for the phosphorylation of pyrimidine nucleosides and pyrimidine nucleoside analogs. However, a major difference compared with TK2 is the broader substrate specificity of Dm-dNK with its ability to phosphorylate purine nucleosides and purine nucleoside analogs. The substrate specificity of Dm-dNK for purine nucleosides was most similar to dCK. Taken together, the multisubstrate nucleoside kinase of D. melanogaster combines the activity of the mammalian enzymes TK2 and dCK and, thus, phosphorylates a broad range of substrates. The mammalian dGK showed narrower substrate specificity and accordingly shared less substrates with Dm-dNK, although dGK is as closely sequence-related to the enzyme as dCK. HSV-1 TK also belongs to the same evolutionary branch as Dm-dNK, and this is supported by the similarities in substrate specificity toward dThd and dUrd analogs. Major differences between HSV-1 TK and Dm-dNK were the higher affinity toward dCyd analogs for Dm-dNK and the higher affinity of HSV-1 TK toward the guanosine analogs ganciclovir and 2',2'-difluorodeoxyguanosine compared with Dm-dNK. A third difference between these enzymes is the dTMP kinase activity of HSV-1 TK, which was not present in the Dm-dNK nor in any of the mammalian deoxyribonucleoside kinases.

Mammalian cells contain four major deoxyribonucleoside kinase activities that have been identified as TK1, TK2, dCK, and dGK. Many bacteria, such as E. coli, only have a thymidine kinase that is partly similar to the eukaryotic TK1. It appears that these organisms lack enzymes related to dCK, dGK, TK2, or HSV-1 TK. However, certain bacteria such as Lactobacillus acidophilus R-26 contain nucleoside kinases that belong to the family of mammalian and herpesvirus enzymes (21). In D. melanogaster cells a single deoxyribonucleoside kinase activity was detected (9). The lack of other nucleoside kinases in this organism is further supported by the apparent absence of expressed sequence tag cDNAs encoding other D. melanogaster enzymes related to Dm-dNK or to human TK1. Accordingly, it appears that the enzymes involved in the pathways of deoxyribonucleoside salvage are highly organism-specific. A general feature for several nucleoside kinases is their broad substrate specificity, although Dm-dNK is the first enzyme identified that phosphorylates all four natural deoxyribonucleotides. The mammalian enzymes expressed throughout the cell cycle are believed to be important for the supply of dNTP for DNA repair and mitochondrial DNA replication. Together the mammalian enzymes phosphorylate all four natural deoxyribonucleotides required for DNA synthesis although with different affinities for the different deoxyribonucleotides.
ent deoxyribonucleosides. The kinetic data indicate a single substrate binding site in the nucleoside kinases, including the multisubstrate Dm-dNK. In situ, where all deoxyribonucleosides are present, it is likely that the Dm-dNK would preferentially phosphorylate dThd and dCyd compared with dGuo and dAdo. However, little is known about the pathways of dNTP synthesis and its regulation in Drosophila cells, and further studies have to be done to elucidate any difference compared with mammalian cells.

The crystal structure of HSV-1 TK has provided important information on the mechanism of substrate recognition and catalysis (19, 20, 22). However, there are at the present time no solved crystallographic structure available for any mammalian nucleoside kinase, and thus, the structural basis for their diverse substrate specificities is not known. A comparison of the primary structure alignment and the HSV-1 TK structure shows features that distinguish Dm-dNK from other members of the deoxyribonucleoside kinase family. The HSV-1 TK amino acid residues Ile-100 and Tyr-101 have important roles in substrate recognition by this viral enzyme. However, this region is lacking in both Dm-dNK and TK2, and it is poorly conserved in dCK and dGK. This suggests that the binding of the base may be different compared with the cellular kinases. Another difference is found at HSV-1 TK Tyr-172, which stacks against the thymine base and also contributes to binding of several anti-herpetic nucleoside analogs. In HSV-1 TK, this residue can only be functionally replaced with phenylalanine (23). In dCK, dGK, and TK2, the corresponding residue is either a tyrosine or a phenylalanine, whereas the corresponding residue in Dm-dNK is an arginine. Studies on the importance of these residues for the substrate specificity of Dm-dNK are currently initiated. However, the crystal structure of Dm-dNK and the mammalian enzymes will be necessary to elucidate mechanisms of substrate recognition and to clarify the basis for the unique broad substrate specificity of Dm-dNK.

The role of suicide gene therapy using nucleoside kinases in clinical practice remains to be established. There are a few studies that show promising results, although several technical problems have to be solved. One possibility to enhance the efficiency of nucleoside kinase gene therapy is to use nucleoside kinases with enhanced kinetic properties that generate larger amounts of phosphorylated nucleoside analogs to kill the transfected cells or to induce cell death of neighboring cells via the so-called bystander effect. HSV-1 TK mutants have been engineered using random mutagenesis, and enzymes with enhanced kinetic properties make tumor cells more sensitive to cytotoxic nucleoside analogs (14). Recently, Christians et al. (24) used DNA family shuffling to create chimeras between HSV-1 and HSV-2 TK and subsequently selected mutant enzymes with enhanced ability to phosphorylate the anti-HIV nucleoside analog AZT. The broad substrate specificity and high catalytic rate of Dm-dNK makes this enzyme an interesting candidate for suicide gene therapy.

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