Guanylate kinase catalyzes the phosphorylation of either GMP to GDP or dGMP to dGDP and is an essential enzyme in nucleotide metabolism pathways. Despite its involvement in antiviral drug activation in humans and in mouse model systems and as a target for chemotherapy, the human and mouse primary structures have never been elucidated. Full-length cDNA clones encoding enzymatically active guanylate kinase were isolated from mouse B-cell lymphoma and human peripheral blood lymphocyte cDNA libraries. Multiple tissue Northern blots demonstrated an mRNA species of approximately 1 kilobase for both mice and humans in all tissue types examined. The mouse cDNA is predicted to encode a 197-amino acid protein with a molecular mass of 21,904 daltons. The human cDNA is predicted to encode a 197-amino acid protein with a molecular mass of 21,696 daltons. These proteins share 88% sequence identity with each other and 52-54% identity with the yeast guanylate kinase. Molecular modeling using the yeast diffraction coordinates indicates a high degree of conservation within the active site and maintenance of the overall structural integrity, despite a lack of similarity along the periphery of the enzyme.

**Guanylate Kinase (GMK,1 ATP:GMP phosphotransferase; EC 2.7.4.8) catalyzes the reaction (d)GMP + ATP → (d)GDP + ADP where (d)GMP indicates GMP or dGMP. In addition to being a critical enzyme in the biosynthesis of GTP and dGTP, guanylate kinase functions in the recovery of cGMP (cGMP → GDP → GTP → cGMP) and is, therefore, thought to regulate the supply of guanine nucleotides to signal transduction pathway components (1, 2).

As with other enzymes involved in nucleotide metabolism, guanylate kinase is a target for cancer chemotherapy and is inhibited by the potent antitumor drug, 6-thioguanine (3-5). Guanylate kinase activity is also required for the potentiation of antiviral drug activity in virus-infected cells. Activation of the anti-herpes guanosine nucleoside analogs, acyclovir and famciclovir, is carried out by guanylate kinase (6, 7).

Although guanylate kinase is a key enzyme for cancer chemotherapy and antiviral drug activation in humans, the gene encoding this important enzyme has not been described. In this study, we describe the isolation, expression, and functional analysis of both human and mouse guanylate kinases in an effort to aid improvements in antiviral and tumor chemotherapy approaches through molecular modeling and rational drug design.

**EXPERIMENTAL PROCEDURES**

Materials—Restriction enzymes, deoxyribonucleoside triphosphates, and random-primed DNA labeling kits were purchased from Boehringer Mannheim. Reagents for enzyme assays were purchased from Sigma. [32P]ATP (3000 Ci/mmol) and [14C]-labeled protein molecular weight markers were purchased from Amersham Corp. 35S Express protein labeling mix was purchased from DuPont NEN. Biotinylated thrombin, pET23d vector, and streptavidin beads were obtained from Novagen (Madison, WI). Multiple tissue Northern blots were purchased from Clontech (Palo Alto, CA, catalog nos. 7760-1 and 7762-1). Bradford assay reagents, chromatography columns, electrophoresis cuvettes, and prestained protein molecular weight markers were purchased from Bio-Rad. Ni-NTA resin, Qiaprep, spin kits and plasmid purification reagents were purchased from Qiagen (Chatsworth, CA). All other reagents were purchased from Sigma unless otherwise indicated.

**Bacterial Strains—** Escherichia coli strain M522 (F' lac pro A B' supE thi d [lac-proAB] [hsdM S B (rB mB) Mo BC]) was used as a recipient for certain cloning procedures. BL21 (DE3) tk+, a gift from Dr. William C. Summers (Yale University; F ompT [lon] hsdS rB mB [rB mB Mo BC]) was used as a recipient for plating the λ library.

**PCR Amplification and Cloning of Human Guanylate Kinase cDNA—** The PCR primers 5' ACTACTGGATCCATCGGGGGGCCCAGGCTCGTG3' (DMO437) and 5'-TACTACGATCTCCAGGGCCGGTGTCCTTGAGC3' (DMO438) were synthesized by Genset (La Jolla, CA). Both primers contain BamHI restriction sites near their respective 5' ends. DMO437 also contains an NcoI site. reverse transcription-PCR was performed as described by Kawasaki (8). The resulting 600-bp amplification product was cloned into the BamHI site of pUC118, and the resulting plasmid was designated pUC118/hgmk.

**Human gmk Sequencing—** Primers used to sequence both strands of the cDNA were M13 forward (−20 nucleotides) and reverse primers (−48 nucleotides) which anneal to pUC118 vector sequences adjacent to the insert cloning sites; 5'-CTGCTCAAGAGGCTGTTC3' (DM0512); 5'-ACACAGATGGCCTTACG3' (DM0513); 5'-CTGAGCTTACGCGCACT3' (DM0514); and 5'-CTTGATGACCCACACATG3' (DM0515). All reactions (dye-terminator) were performed on double-stranded DNA and run on ABI 373A automated sequencing machines.

**PCR Amplification and Cloning of Mouse Guanylate Kinase cDNA—** Approximately 500,000 phage from a random-primed cDNA library of the mouse cell line 70 Z/3 were plated and screened by the method of Sambrook et al. (9). Hybridization was carried out with a 3P-labeled random-primed human guanylate kinase 600-bp BamHI fragment. The Eco RI fragments from nine positive clones were cloned into the Eco RI site of pUC118.

**Mouse gmk Sequencing—** Sequence analysis of the mouse cDNAs was performed using the following primers: M13 forward (−20 nucleotides) and reverse primers (−48 nucleotides); 5'-TGCTGGCATACTGACAG3' (DM0592); 5'-TGAAAGCTAGCTAGCTTC3' (DM0594); 5'-TCTGAGCTGGCAGGCGCACT3' (DM0598); and 5'-ACCTGGGATAAGCGC3'.
TATG-3' (DMO674); 5'-AAGCGACCGCTCTCTGA-3' (DMO675); 5'-TCCCCACCTCCACGGC-3' (DMO478); 5'-CTCGATTGTTCCCGGC-3' (DMO749); and 5'-GCGAAAGACGTCTGCTG-3' (DMO750). All reactions were performed on double-stranded DNA and run on ABI sequencing machines for analysis.

Northern Blots—Total cellular RNA (10 µg) isolated from SP2 mouse myeloma cells according to the method of Chomczynski and Sacchi (10) was denatured in 75% formamide, 1× formaldehyde, and 1.5 M MOPS buffer (1× MOPS: 20 mM MOPS, pH 7, 5 mM NaAc, and 10 µM EDTA, pH 8) at 60°C for 10 min. The samples were electrophoresed on a 1.2% agarose/MOPS gel containing 0.25% ethidium bromide (11). The RNA was transferred to nitrocellulose in 10× SSC and UV-cross-linked. The filter was prehybridized at 68°C in 0.5 M NaPO4, 7 M urea, 1× SSC, and 0.1% SDS and hybridized with 20 ng of random-primed purified hgmk PCR product (600 bp). After hybridization, the blot was washed for 1 h at 55°C in 2× SSC (1× SSC: 150 mM NaCl, 15 mM sodium citrate)/0.1% SDS and exposed to film.

For the human multiple tissue Northern blot, the random-primed purified hgmk PCR product (600 bp) described above was used as a probe. For the mouse multiple tissue blot, the mouse gmk probe was generated by asymmetric PCR using 10 ng of EcoRI fragment from pUC18:mgmk as a template in the presence of 150 µCi of [α-32P]dCTP and 100 pmol of primer 5′-ACTACTACTGGATCCCTGGC-3′ (DMO856) in a standard polymerase chain reaction for 40 cycles. Hybridization conditions were as described above. The filters were subsequently stripped and probed with β-actin.

Vector Constructions—The Ncol/BamH I fragment from pUC18: hgmk was gel purified and ligated to Ncol/BamH I-digested pET23d and used to transform BL21(DE3) tk− cells. The resulting plasmid was designated pET23dhgmk.

An Ncol site was introduced at the mouse gmk initiation codon by PCR amplification using the following two primers: 5′-CGCGGAGCGCATTGGGCACTAG-3′ (DMO855) and 5′-ACTACTACTGATTCGACGCGACTTCCAGGCCTG-3′ (DMO856). PCR conditions were identical to the reverse transcription-PCR protocol listed above, except that the template was 2 ng of pUC18:mgmk DNA. The resulting fragment was cloned with Ncol and BamH I and directly cloned into the Ncol and BamH I sites of pET23d. The entire insertion and fusion sites were sequenced, and the resulting plasmid was designated pET23dmgmk.

Construction of pETHT Bacterial Expression Vector—pET23d was modified by PCR using overlapping oligonucleotides to create amino-terminal tag encoding a stretch of six histidine residues, followed by a 2-amino acid spacer and a consensus sequence for a thrombin proteolytic cleavage site, followed by another 2-amino acid spacer. The primer 5′-ACTACTACTGATTCGACGCGACTTCCAGGCCTG-3′ (DMO604) was used to prime the sense strand starting at a unique 5′ site, at the conserved region in the cDNA of bovine retina (nucleotides 82 bases upstream of the initiating methionine, encoded by the vector, and 95 bases downstream of the initiating methionine). The primer 5′-CTCAGTGTTGCGCAGT-3′ (DMO605), 5′-CGGCACAGGCCGCTGCTATG-3′ (DMO606), and 5′-AGTAGATTCGACGCGACTTCCAGGCCTG-3′ (DMO607) were used in consecutive PCR amplifications. The first PCR was performed on 2 ng of pET23d DNA in 0.1 ml with 0.2 mM of each deoxynucleotide triphosphate, 0.5 µM each of DMO604 and DMO605, and 0.5 units Taq DNA polymerase. The 110-bp PCR product was isolated and used as the template in a second PCR under the same conditions, except 0.01 µM DMO606 and 1 µM DMO607 were used instead of DMO605. The resulting 185-bp product was digested with Ncol and Bgl II, gel isolated, and ligated to Bgl II/Ncol-digested pET23d. The resulting plasmid was sequenced and designated pETHT.

Both human and mouse guanylate kinase cDNAs were cloned as Ncol/BamH I fragments isolated from pET23dhgmk or pET23dmgmk into the Ncol/BamH I sites of pETHT. Sequence analysis confirmed 5′ and 3′ ligation sites.

In Vivo Transcription and Translation—Plasmid DNAs (pET23d: hgmk, pET23d:mgmk, and pETHT:mgmk) were subject to in vitro transcription as described by Black et al. (13). The resulting transcripts and a control (no RNA) were then used to program a rabbit reticulocyte cell-free translation, as described by Black et al. (13). A small fraction of each translation carried out in the presence of [35S]methionine was then heat denatured and subjected to electrophoresis on polyacrylamide containing SDS gels. After drying, the gel was exposed to film.

Overexpression of Guanylate Kinase in E. coli—pETHT:mgmk and pETHT:mgmk were electroporated into competent E. coli BL21(DE3)

**RESULTS**

Isolation of the Human Guanylate Kinase cDNA—To ascertain whether any new guanylate kinase cDNAs had been isolated since the publication of the bovine sequence in 1993, the nucleotide and protein data bases were searched using bovine sequences. A BLAST search for homology to a sequence from a conserved region in the cDNA of bovine retina (nucleotides 303–333) revealed a nearly perfect match to a sequence with the

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the accession number a11042. This is described as a human hematopoietic cell growth-potentiating factor (nucleotide sequence 7 from patent EPO274560). Indeed, an alignment of the bovine and putative human guanylate kinase sequences displayed 90.4% identity at the amino acid level. Primers (DMO437 and DMO438) were designed from this sequence to amplify the coding region of the putative human guanylate kinase (hgmk) by reverse transcriptase-coupled PCR of total cellular RNA isolated from human peripheral blood lymphocytes. A single band of 600 bp was amplified and cloned into the BamHI site of pUC118. Complete double-stranded DNA sequencing was done on two subclones, and both were found to be identical to the a11042 sequence. A protein of 197 amino acids with a molecular mass of 21,696 daltons is predicted from a cDNA open reading frame as well as 5′- and 3′-untranslated regions. One of the clones contained the entire mgmk open reading frame as well as 50–100 bp of both 5′- and 3′-untranslated regions. One of the clones contained the entire 3′-untranslated region as well as a portion of the poly(A) tail. DNA sequence analysis of both strands demonstrated that the nucleotide sequence was identical in the overlapping regions of all nine clones. The nucleotide and deduced amino acid sequences are shown in Fig. 2. The mgmk encodes a 198-amino acid polypeptide with a calculated molecular mass of 21,904 daltons.

Sequence Analysis and Comparison—Translations of the cDNA open reading frames were aligned with each other as well as the peptide sequences of bovine, porcine, yeast, and E. coli guanylate kinases (Fig. 3). The percentage of identity and similarity were calculated using the GCG program Bestfit with a gap weight of 1.0 and are shown in Table I. Human, mouse, bovine, and porcine guanylate kinases share nearly 90% identity, with the highest similarity value of 99.5% between bovine and porcine enzymes. Comparisons between mammalian and yeast GMKs reveal much less conservation, with amino acid identities on the order of 52 to 54%. The yeast and E. coli guanylate kinases share 48.6% identity.
Multiple Tissue Northern Blots—The human or mouse gmk probes hybridized strongly to a single ~1-kilobase transcript in the respective Northern blot (human or mouse RNA). Guanylate kinase transcripts are expressed in all tissue types examined and is indicative of ubiquitous mRNA expression (Fig. 5, A and B). This type of expression pattern is not unexpected for an essential “housekeeping” gene. Fig. 5, C and D, show the same Northern blots probed with β-actin and demonstrate the integrity of the RNA and the relative amounts loaded per lane. Heart and skeletal muscle contain two forms of β-actin, 2 and 1.8 kilobases (Fig. 5, C and D, lanes 1 and 6).

In Vitro Expression of Human and Mouse gmk—The coding regions of both hgmk and mgmk cDNAs were directionally subcloned into the bacterial expression vectors pET23d and pETHT as described under “Experimental Procedures.” In vitro transcription and translation of these constructs were performed as described in Black et al. (13). Radiolabeled translation products were subjected to acrylamide gel electrophoresis in the presence of SDS, and the dried gel was exposed to x-ray film. The resulting autoradiograph shows an ~23-kDa band for both the mouse and human pET23d constructs and shows a slightly larger ~25-kDa band for the histidine-tagged mouse and human GMKs (Fig. 6), indicative of full length in vitro translation products. Enzyme assays of the mgmk cell-free translations gave very marginal activity over a high background, and assays of hgmk cell-free translations did not display any detectable activity above background levels.

Protein Overexpression and Purification—To facilitate protein purification, the pETHT vector that encodes an initiating methionine followed by six histidine residues, a thrombin cleavage site, and an Nco restriction site for cloning the cDNAs in frame was used. Thus, the human and mouse guanylate kinase cDNAs are expressed with an amino-terminal histidine-thrombin (HT) tag. HT-tagged proteins were then purified on nickel resin columns, and the amino-terminal tag was removed by cleavage with biotinylated thrombin. The thrombin was removed with streptavidin beads, and the resulting supernatant was removed from the nickel resin, resulting in only cleaved, purified guanylate kinase. The thrombin cleavage leaves three amino acids at the amino terminus before the initiator methionine (Gly-Ser-Ser).

Relative to mouse gmk expression, the human cDNA was expressed at a very high level in several inductions. Unfortunately, the human GMK was mostly partitioned to an insoluble fraction. Mouse GMK did not share this property and was found predominately in the soluble fraction. Binding of MG MK to the Ni-NTA beads in batch required only 4–5 mM imidazole for effective blocking of nonspecific binding. An imidazole concentration of 25–30 mM was necessary to block the HGMK lysate, with virtually no proteins eluting up to 75 mM imidazole. Both GMK proteins eluted in 125 mM imidazole. Prior to thrombin cleavage, the imidazole was removed by dialysis in 20 mM Tris, pH 8.0, and 150 mM NaCl. Initial purifications were estimated from Coomassie-stained polyacrylamide gels to be 50% pure for HGMK and 80% pure for MG MK. A gel of fractions from various steps during the mouse GMK purification is shown in Fig. 7A. Despite modifications to the lysis, binding, wash, and elution conditions, the presence of minor contaminating proteins persisted. However, after thrombin cleavage and Ni-NTA chromatography, the HT tag and contaminating proteins were removed from the resulting purified GMK. The thrombin-cleaved MG MK migrated to the expected position in polyacrylamide/SDS gels relative to known molecular weight markers (Fig. 7B). The final preparation of MG MK protein was greater than 99% pure. Attempts to purify HGMK to greater than 50% under native conditions were unsuccessful. However,
99% purity was obtained using the denaturing lysis and purification protocols described by Qiagen (data not shown).

**Determination of Activities**—Because the human GMK did not display activity in E. coli lysates or cell-free translations, we sought to express active gmk cDNAs in mammalian cells. As described under “Experimental Procedures,” stable clones of BHK tk2(ts13) cells transfected with pREP8Δ7, pREP:hgmk, or pREP:mgmk were isolated. Guanylate kinase protein expression was confirmed by immunoblot analysis of transfected cell lysates using antisera directed against MGMK (Fig. 8).

>Percent amino acid identity and similarity between various guanylate kinases

|            | Human | Mouse | Bovine | Porcine | Yeast | E. coli |
|------------|-------|-------|--------|---------|-------|---------|
| Human      | 100   | 88.3  | 90.4   | 90.3    | 54.4  | 42.9    |
| Mouse      | —     | (91.9)* | (93.9) | (93.8)  | (69.6) | (62.5)  |
| Bovine     | 100   | —     | (94.95)| (95.4)  | (67.6) | (65.2)  |
| Porcine    | —     | 100   | —      | (99.5)  | (68.1) | (64.2)  |
| Yeast      | 100   | —     | —      | —       | 51.6  | 42.1    |
| E. coli    | —     | —     | —      | —       | (67.4) | (62.6)  |

*Numbers in parentheses indicate percent similarity.

**Fig. 4.** Computer modeling of mouse and human guanylate kinases with bound GMP based on the yeast structure. Using the Look program (Molecular Applications Group), the identical residues (purple), similar residues (light purple), and nonconserved residues (yellow) between the mouse, human, and yeast guanylate kinase sequences are displayed on the molecular model for yeast GMK that was elucidated by Stehle and Schulz (17). Certain amino acids are shown to aid in identification of particular regions discussed in the text. The amino and carboxyl termini are denoted by N and C, respectively.

**Fig. 5.** Multiple tissue Northern blot analysis of human and mouse gmk and β-actin levels in poly(A)-selected mRNA. Northern blots (Clontech) contain approximately 2 μg of poly(A)+ RNA from each different tissue run on a denaturing formaldehyde 1.2% agarose gel; the blots were transferred to nylon membranes, and the RNA was fixed by UV cross-linking. A, human tissue Northern blot. Lanes contain RNA from: lane 1, human heart; lane 2, brain; lane 3, placenta; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; and lane 8, pancreas. This blot was hybridized with the 600-bp human gmk probe. B, mouse tissue Northern blot. Lanes contain RNA from: lane 1, mouse heart; lane 2, brain; lane 3, spleen; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; and lane 8, testis. This blot was hybridized with the asymmetric PCR-generated mouse gmk probe. C, human tissue Northern blot hybridized with a β-actin probe provided by Clontech. D, mouse tissue Northern blot hybridized with the β-actin probe. The position of RNA size markers is shown.
Mouse and Human Guanylate Kinases

GUANYLATE KINASE

Guanylate kinase plays an essential role in generating the nucleotide precursors, GDP and dGDP, for RNA and DNA metabolism, respectively. Despite the importance of guanylate kinase in nucleotide metabolism, the only identified eukaryotic guanylate kinase nucleotide sequences are those from yeast (18, 20), rat liver (16), and human erythrocytes (16). Guanylate kinase activity has been observed in a variety of tissues and has been purified from many eukaryotic sources including bovine retina (1), hog brain (16), bakers’ yeast (18, 20), rat liver (16), and human erythrocytes (16). Although the human gmk gene (spor) has also been cloned and was found to share significant similarity with the amino acid sequence of the yeast and the yeast GMK was 52–54%, the overall structure is highly conserved.

Deployed enzyme activity was demonstrated with the mouse GMK when it was expressed in cell-free translations, crude bacterial lysates purified from E. coli, and in lysates from mammalian transfected cells. Preliminary kinetic determinations of mouse guanylate kinase purified from E. coli indicates a $K_m$ of $-25 \mu M$ when GMP is used as the substrate (data not shown). This is similar to that reported for porcine GMK ($K_m = 32 \mu M$; Ref. 22) and human erythrocyte GMK ($K_m = 15-24 \mu M$; Ref. 7). Activity from the human GMK was only detected in crude bacterially expressed lysates purified from BHK tk" (ts13)-transfected cell lysates; attempts to purify human GMK from E. coli resulted in only ~50% purification, and the enzyme was inactive. It is unclear what the bases of these differences between human and mouse GMK are, especially since they share 88% amino acid sequence identity.

In the last several years, sequence data from a number of novel genes have revealed that they encode a motif approxi-

**DISCUSSION**

Guanylate kinase has been observed to be present in a variety of tissues and has been purified from many eukaryotic sources including bovine retina (1), hog brain (16), bakers’ yeast (18, 20), rat liver (16), and human erythrocytes (16). Although the human gmk gene (GUK1) was mapped to chromosome 1q32–43 over 15 years ago (21), no primary sequence has been identified.

In this report, we describe the isolation of the cDNA for both human and mouse guanylate kinases. Guanylate kinase mRNA is ubiquitously expressed, as would be anticipated from

**FIG. 6. Autoradiograph of rabbit reticulocyte lysate cell-free translation products of gmk transcripts subjected to gel electrophoresis.** Transcripts were synthesized in vitro from pET23d and pETHT constructs and used to program a rabbit reticulocyte cell-free translation system. Translation products were subjected to denaturing polyacrylamide gel electrophoresis, followed by autoradiography. Lane 1, molecular weight markers with sizes listed in kDa; lane 2, E. coli lysate; lane 3, column flow-through fraction; lane 4, column wash fraction; lanes 5–8, imidazole eluate fractions 1–4. B, eluate containing visible amounts of protein were pooled, dialyzed, and incubated with biotinylated thrombin to cleave off the amino-terminal protein tag. After removal of thrombin using streptavidin and the amino acid tag using Ni-NTA resin, a fraction of the supernatant was subjected to gel electrophoresis and Coomassie Blue staining. Lane 1, molecular weight markers (in kDa); lane 2, thrombin-deaived mouse guanylate kinase.

**FIG. 7. Purification of recombinant mouse guanylate kinase from E. coli.** A, protein was purified from BL21(DE3) tk" cells after induction by isopropyl-1-thio-β-D-galactopyranoside as described, separated on a SDS/16% polyacrylamide gel, and stained with Coomassie Blue. Lane 1, molecular weight markers (in kDa); lane 2, E. coli lysate; lane 3, column flow-through fraction; lane 4, column wash fraction; lanes 5–8, imidazole eluate fractions 1–4. B, eluate containing visible amounts of protein were pooled, dialyzed, and incubated with biotinylated thrombin to cleave off the amino-terminal protein tag. After removal of thrombin using streptavidin and the amino acid tag using Ni-NTA resin, a fraction of the supernatant was subjected to gel electrophoresis and Coomassie Blue staining. Lane 1, molecular weight markers (in kDa); lane 2, thrombin-deaived mouse guanylate kinase.

**FIG. 8. Immunoblot of transfected cell lysates.** The equivalent of approximately 8000 cells of BHK tk" (ts13)-transfected cell lysates was separated on an SDS/16% polyacrylamide gel. The proteins were transferred to nitrocellulose, and an immunoblot was performed using rabbit-anti-MGMK serum. Lane 1, molecular weight markers (in kDa); lane 2, lysate from pREP:hgmk transfected cells; lane 3, lysate from pREP:mgmk-transfected cells; lane 4, lysate from pREP817-transfected cells; lane 5, purified thrombin-deaived MGMK.

**FIG. 9. Guanylate kinase assays of transfected cell lysates.** Ten μl of each BHK tk" (ts13)-transfected cell lysate (pREP837, pREP:hgmk, and pREP:mgmk) was assayed in the presence of 100 mM GMP for enzyme activity over time. The experiment was repeated with similar results. ○, pREP837; ●, pREP:hgmk; ■, pREP:mgmk.
and SAP97, proteins restricted to rat synaptic vesicles (25, 26, human erythrocytes (24); and two homologs, SAP90 or PSD-95 closely related proteins which are associated with tight junctions (23); p55, a major palmitoylated membrane protein from human erythrocytes (24); and two homologs, SAP90 or PSD-95, proteins restricted to rat synaptic vesicles (25, 26, 27). Although it has not been directly demonstrated that these guanylate kinase domains are functional, Mueller et al. (27) have indicated that SAP90 retains the ability to bind GMP, GDP, ATP, and ADP in vitro, despite the lack of a canonical ATP binding site. This suggests that this class of proteins does encode functional guanylate kinase enzymes, although why a guanylate kinase activity is associated with these proteins has not been established. One possibility might be that they serve to supply GDP at the cell membrane to regulate GTPase signal transduction proteins, such as Ras.

A more direct role for guanylate kinase activity in signal transduction comes from its function in guanine nucleotide metabolism. Guanylate kinase participates in the recovery of cGMP and is, therefore, thought to regulate the supply of guanine nucleotides to signal transduction pathways. Little is known about how extensive this role is, but such studies may be facilitated by the preliminary study reported here.

At least two other key areas of study will be aided by the elucidation of human and mouse guanylate kinase sequences. Guanylate kinase is required for certain antiviral drug activation pathways. Of particular note are the well studied antiviral drugs, acyclovir and ganciclovir, which must be initially phosphorylated by the herpes-encoded thymidine kinase. Once phosphorylated, these drugs require further phosphorylation to their triphosphate state to become activated and able to act as chain terminators during DNA synthesis. Both subsequent phosphorylation steps are carried out by cellular enzymes: guanylate kinase (monophosphate to diphosphate) and a number of nonspecific nucleoside diphosphokinases (diphosphate to triphosphate). More recently, this activation pathway has been exploited for ablative gene therapy of cancer, where the HSV-1 TK gene is introduced into tumor cells that are specifically killed after administration of ganciclovir. In a similar fashion, guanylate kinase is involved in activation of chemotherapeutic drugs such as 6-thioguanine (4, 6, 7). Development of novel antiviral drug or chemotherapeutic agents will be greatly aided by rational drug design based on molecular modeling techniques.

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