Molecular Characterization of a Human DNA Kinase*

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Human polydeoxyribonucleotide kinase is an enzyme that has the capacity to phosphorylate DNA at 5′-hydroxyl termini and dephosphorylate 3′-phosphate termini and, therefore, can be considered a putative DNA repair enzyme. The enzyme was purified from HeLa cells. Amino acid sequence was obtained using several trypic fragments by mass spectrometry. The sequences were matched through the dbEST database with an incomplete human cDNA clone, which was used as a probe to retrieve the 5′-end of the cDNA sequence from a separate cDNA library. The complete cDNA, which codes for a 521-amino acid protein (57.1 kDa), was expressed in Escherichia coli, and the recombinant protein was shown to possess the kinase and phosphatase activities. Comparison with other sequenced proteins identified a P-loop motif, indicative of an ATP-binding domain, and a second motif associated with several different phosphatases. There is reasonable sequence similarity to putative open reading frames in the genomes of Caenorhabditis elegans and Schizosaccharomyces pombe, but similarity to bacteriophage T4 polynucleotide kinase is limited to the kinase and phosphatase domains noted above. Northern hybridization revealed a major transcript of approximately 2.3 kilobases and a minor transcript of approximately 7 kilobases. Pancreas, heart, and kidney appear to have higher levels of mRNA than brain, lung, or liver. Confocal microscopy of human A549 cells indicated that the kinase resides predominantly in the nucleus. The gene encoding the enzyme was mapped to chromosome band 19q13.4.

Transient DNA strand breaks and short gaps are frequently observed in cellular DNA. Many arise during regular cellular activity such as DNA replication, recombination, or differentiation. Others occur as a consequence of exposure to endogenous or exogenous DNA damaging agents. Repair of these strand interruptions is usually mediated by DNA ligases and polymerases. Both of these classes of enzymes require 3′-hydroxyl DNA termini, and the DNA ligases also require 5′-phosphate termini. However, the termini generated by nuclease, such as DNase II and many produced by ionizing radiation bear 3′-phosphate and 5′-hydroxyl groups (1–4), and therefore must be processed before they can be acted upon by DNA ligases or polymerases.

One enzyme that possesses the capacity to both phosphorylate 5′-hydroxyl termini and dephosphorylate 3′-phosphate termini is polynucleotide kinase (PNK). The PNK from T4 phage has found widespread application in molecular biology, especially for radiolabeling DNA and oligonucleotides (5). It can act on DNA and RNA and even phosphorylate nucleoside 3′-monophosphates. However, the main cellular function of the T4 enzyme is not to repair DNA, but rather to counter the action of a phage endoribonuclease that cleaves tRNA (6). Eukaryotic PNKs fall into two categories depending on whether their preferred substrate is DNA or RNA (7). While both can phosphorylate 5′-termini, only the former have an associated 3′-phosphatase activity (8–12).

Mammalian DNA kinases have been purified from a variety of sources including rat liver and testes and calf thymus (8–19). The isolated enzymes share similar properties with regard to the kinase activity including an acidic pH (5.5–6.0) optimum (8–18), and the minimum size of oligonucleotide that can be phosphorylated is in the range of 8–12 nucleotides (11, 15). The only significant discrepancy has been the molecular mass assigned to the polypeptides. Earlier reports regarding the PNK purified from rat organs indicated that the protein may be an 80-kDa homodimer composed of 40-kDa polypeptides (9, 10, 18), but PNK activity in tissue extracts detected on activity gels migrated as a 60-kDa polypeptide (20). Estimates for the size of calf thymus PNK have ranged from 56 to 70 kDa (16, 17). We and others have recently purified the DNA kinases from calf thymus and rat liver to near homogeneity, making use of a broad spectrum of proteolysis inhibitors (11, 12). The major protein band migrated as a 60-kDa peptide on polyacrylamide gels, but a minor band was observed at 40 kDa in the rat liver preparation.

‡‡Part of this work was carried out during a sabbatical stay at the ICRF Clare Hall Laboratories. The costs of publication were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF125807. §§Part of this work was carried out during a sabbatical stay at the ICRF Clare Hall Laboratories. To whom correspondence should be addressed: Experimental Oncology, Cancer Research Inst., 11560 University Ave., Edmonton, Alberta T6G 1Z2, Canada. Tel.: 780-432-8438; Fax: 780-432-8428; E-mail: mweinfeld@gpu.srv.ualberta.ca.

* The abbreviations used are: PNK, polynucleotide kinase or polydeoxyribonucleotide kinase; p21p, a 21-mer oligonucleotide phosphorylated at the 3′- and 5′-termini; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; MALDI, matrix-assisted laser desorption ionization; SMA, N-succinimidyl-2-morpholine acetate; bp, base pair(s); UTR, untranslated region; PCR, polymerase chain reaction.

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At present, the cellular function of mammalian DNA kinases has not been elucidated. Clearly, one possibility is participation in the repair of strand breaks induced by DNA damaging agents, such as ionizing radiation or topoisomerase inhibitors (21, 22). We have shown that, unlike T4 phage PNK, calf thymus PNK is able to efficiently phosphorylate the 5′-OH terminus at a nick and a one-nucleotide gap in a double-stranded DNA substrate (11). Alternatively, PNK could participate in a more regular function. For example, it has been observed that a proportion of Okazaki fragments have 5′-OH termini (23), which would have to be phosphorylated prior to ligation. As part of our ongoing study to address the question of the role of eukaryotic PNKs, this paper describes the molecular cloning, sequencing, cellular localization, and chromosomal mapping of human PNK.

**EXPERIMENTAL PROCEDURES**

**Phosphorylation Assay**—The DNA substrate containing 5′-OH termini was prepared by digestion of calf thymus DNA with micrococcal nuclease as described by Richardson (24). Each 5′-phosphorylation reaction mixture (20 μl), containing 10 μg of DNA substrate, 3 μCi of [γ-32P]ATP (3000 Ci/mmol, Amersham Pharmacia Biotech), 500 nM unlabeled ATP, 80 mM sucinic acid, pH 5.5, 10 mM MgCl₂, 1 mM dithiothreitol, 1 mM EGTA, 2 μg of bovine serum albumin, and protein fraction (typically 4 μl), was incubated for 20 min at 37 °C. The reaction was stopped and the DNA precipitated by addition of 200 μl of 20% trichloroacetic acid. This wash step was repeated once more before the radioactivity of the pellet was determined. As a control for kinase specificity (i.e. DNA versus protein), parallel reactions were carried out in the absence of the DNA substrate.

**3′-Phosphatase Assay**—The 3′-dephosphorylation of a 21-mer oligonucleotide (p21p) catalyzed by recombinant human PNK in Escherichia coli cell extracts was assayed by gel electrophoresis as described previously (21).

**Partial Purification of Polydeoxyribonucleotide Kinase from HeLa Cells**—A pellet of frozen HeLa S3 cells (3 × 10⁹, approximately 50 ml packed cell volumes) was thawed in 200 ml of hypotonic buffer (10 mM Tris-HCl, pH 7.5, 2 mM MgCl₂, 5 mM dithiothreitol, and 0.5 mM EDTA) containing a mixture of protease inhibitors (25 μg/ml N'-tosyl-l-lysine chloromethyl ketone, 5 μg/ml chymotrypsin, 1 μg/ml aprotinin, 0.5 μg/ml leupeptin, 0.5 μg/ml pepstatin, and 1 mM α-toluenesulfonyl fluoride) and held for 20 min at 0 °C before disruption in a Dounce glass homogenizer (15 strokes). Nuclei were collected by low speed centrifugation, and a protein extract was prepared in the presence of 0.3 M KCl.

**Experimental Procedures**

**FIG. 1.** PNK activity eluting from a gel filtration column. Extracts from HeLa nuclei were applied in 0.5 M NaCl to an AcA 34 Ultragel size exclusion column, eluted with 0.5 M NaCl, collected in 6-ml fractions, and assayed as described under “Experimental Procedures.” The void volume for the column was 180 ml.

**FIG. 2.** Penultimate purification step of the HeLa PNK by elution from a Mono S PC 1.6/5 column. A, DNA kinase activity in each fraction. B, analysis by SDS-PAGE electrophoresis and silver staining of the protein content in the material loaded on the column, L, and in several of the eluted fractions (numbered above). Mobility of protein size markers (in kDa) is indicated on the left. The protein most clearly associated with kinase activity is marked by the arrow on the right.

**TABLE I**

| Number | Sequence* | Source of PNK |
|--------|-----------|---------------|
| 1      | LWLESPAGGAPPIFLPTGQLVLGR | calf thymus |
| 2      | EMTDSSHPV   | calf thymus |
| 3      | VAGFD[LI]DGT[LI][LI]TTR  | HeLa cells |
| 4      | VA[LI]DNTNPDAAR      | HeLa cells |
| 5      | E[LI]EAEQV      | HeLa cells |
| 6      | GP[LI]TQTVDR      | HeLa cells |
| 7      | TQVE[LI]VADPETR    | HeLa cells |
| 8      | [LI][LI]YPE[LI]PR  | HeLa cells |

* Leucine and isoleucine [LI] cannot be distinguished by low energy collision-activated dissociation as they are isomers.

as described previously (25). Sequential chromatography of the extract on a phosphocellulose P11 column (Whatman, Clifton, NJ) and an Ultragel AcA34 gel filtration column (Sepracor/IBF, Marlborough, MA), and ammonium sulfate precipitation steps were carried out as described by Robins and Lindahl (26), except that the elution buffer for the first column contained 0.6 M KCl and the elution buffer for the second column contained 0.5 M NaCl. The active fractions in the second peak from the gel filtration column were pooled (63 mg of protein in a total volume of 54 ml), dialyzed against buffer A (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM dithiothreitol, 1 mM potassium phosphate, and 10% glycerol). The specific activity at this stage of purification was approximately 0.06 units/mg of protein, where one unit of enzyme is the amount required to incorporate 1 nmol of phosphate from ATP into micrococcal nuclease-treated DNA in 30 min at 37 °C (27). The pooled material was loaded onto a column (2.5 × 5.0 cm) of Bio-Gel HT hydroxyapatite (Bio-Rad) pre-equilibrated with buffer A. The column was washed with five volumes of buffer A before eluting bound protein with a 200-ml linear gradient of 50–500 mM potassium phosphate in buffer A collecting in 5-ml fractions. The active fractions, 28–33, were pooled and dialyzed against buffer B (10 mM potassium phosphate, pH 6.8, 4 mM 2-mercaptoethanol, and 10% glycerol) containing 50 mM KCl. The material was loaded onto a 1-ml HiTrap SP column (Amersham Pharmacia Biotech), washed with 10 column volumes of buffer B and eluted with a 30-ml linear gradient of 50–600 mM KCl in 30-ml fractions. A peak of kinase activity eluted at fractions 10–12. The contents of fraction 11 were dialyzed against buffer C (50 mM Tris-HCl,
pH 7.5, 1 mM dithiothreitol, 1 mM potassium phosphate, and 10% glycerol) containing 50 mM NaCl, and loaded onto a Mono S PC 1.6/5 column attached to a SMART micropurification chromatography system (Amersham Pharmacia Biotech). Protein was eluted with a 2-ml linear salt gradient of 50–450 mM NaCl at a flow rate of 100 ml/min in 20 100-ml fractions. After assaying the fractions for DNA kinase activity, a small quantity of each fraction was examined by SDS-PAGE to determine which polypeptide correlated with activity. The remaining contents of the fraction with the peak of kinase activity (fraction 12) was further fractionated by gel electrophoresis and electroblotted onto polyvinylidene difluoride membrane.

Amino Acid Sequencing—The electroblotted HeLa protein was stained with sulforhodamine B (0.05% w/v in 30% v/v aqueous methanol, 0.1% v/v acetic acid) using a rapid-staining protocol (28). The dried, stained protein was then digested in situ on the polyvinylidene difluoride membrane with trypsin (Roche Molecular Biochemicals, modified) for 18 h at 30 °C and the peptides extracted with 1:1 v/v formic acid/ethanol (29). Aliquots were sampled and directly analyzed by matrix-assisted laser desorption ionization (MALDI) time-of-flight mass spectrometry using a LaserMat 2000 mass spectrometer (Thermo Bioanalysis, UK) (30). Additional aliquots were quantitatively esterified using 1% v/v thionyl chloride in methanol and also analyzed by MALDI to provide acidic residue composition (31). Native and esterified peptide masses were then screened against the MOWSE peptide mass fingerprint data base (32). The remaining digested peptides (>90% of total digest) were then reacted with N-succinimidyl-2-morpholine ace...
tate (SMA) in order to enhance b-ion abundance and facilitate sequence analysis by tandem mass spectrometry (33). Dried peptide fractions were treated with 7 μl of freshly prepared, ice-cold 1% w/v N-succinimidy-2-morpholine acetate in 1.0 M HEPES (pH 7.8 with NaOH) containing 2% v/v acetonitrile. Following reaction for 20 min on ice, the reaction was terminated by the addition of 1 μl of heptfluorobutyric acid and diluted with an equal volume of water. The solution was then injected in three 5-μl aliquots onto a capillary reverse-phase column (300 μm x 15 cm) packed with POROS R2/H material (Perseptive Biosystems, MA) equilibrated with 2% v/v methanol, 0.05% v/v trifluoroacetic acid running at 3 μl/min. The adsorbed peptides were washed isocratically with 15% v/v methanol, 0.05% v/v trifluoroacetic acid for 30 min at 3 μl/min to elute the excess reagent and HEPES buffer. Derivatized peptides were eluted with a single step gradient to 75% v/v methanol, 0.1% v/v formic acid and collected in two 3-μl fractions. The derivatized peptides were then sequenced by low energy collision-activated dissociation using a Finnigan MAT TSQ7000 tandem triple quadrupole mass spectrometer and a Finnigan MAT LCQ ion-trap mass spectrometer, both instruments fitted with nanoelectrospray sources (34, 35). Collision-activated dissociation was typically performed with collisional offset voltages between ~18 and ~30 V.

Two tryptic peptides from previously purified calf thymus PNK (11) were sequenced by the Harvard Microchemistry Facility (Cambridge, MA) using either an ABI 477A protein sequencer (Applied Biosystems, Foster City, CA) or an HP G1000A (Hewlett Packard, Palo Alto, CA). Confirmation of sequence was obtained by MALDI time-of-flight mass spectrometry on a LaserMat 2000 mass spectrometer.

Isolation and Sequencing of Polynucleotide Kinase cDNA—DNA sequences derived from the peptide sequences were used to screen the dbEST data base (NIH). A cDNA clone from infant brain (clone number 32798 inserted in Iafmid BA) was identified and obtained from the L.M.A.G.E. Consortium. The cDNA insert (1548 bp) was fully sequenced, using an automated ABI Prism 377 DNA analysis system (Applied Biosystems), and confirmed the presence of the poly(A) tail, and a large open reading frame, but no clearly identifiable start codon. A 609-bp probe, prepared by digestion of clone 32798 with HindIII and PstI (New England Biolabs, Beverley, MA), was subsequently used to screen a Agt11 HeLa cell 5'-STRETCH PLUS cDNA library (CLONTECH, Palo Alto, CA) by a standard protocol (36). Ten positive clones were isolated, none of which contained a poly(A) tail. The largest insert (1.5 kilobase pairs) was amplified by PCR using the λ forward and reverse primers with Pfu DNA polymerase (Stratagene, La Jolla, CA), and then sequenced. Putative full-length cDNA was reconstituted as follows: (i) the PCR-amplified product was digested with SacI and shattedal kinase phosphatase (Amersham Pharmacia Biotech), and the larger fragment (1.1 kilobase pairs) isolated by agarose gel electrophoresis, (ii) the DNA of clone 32798 was digested with SacI, (iii) the DNA molecules were ligated using phage T4 DNA ligase (Amersham Pharmacia Biotech), and (iv) the ligation product was digested with EcoRI.

Expression of PNK cDNA in E. coli—The cDNA was amplified by PCR using Pfu DNA polymerase and primers with tails that provided cleavage sites for Ndel (5'-TTTGAATTCCTAATGCGGAGGTGAGGACCGGGGCGG-3') and BamHI (5'-CGCGGATCCTCGAGCGCTCCCGAGA-3') and then subcloned into the expression plasmid pET-16b (Novagen Inc., Madison, WI). The new plasmid (pPNK-His), which codes for a His-tagged derivative of PNK, was transfected into host E. coli bacterial strain BLR/DE3 (Novagen). The bacteria were grown at 37 °C to an OD600 of 0.6 in 100 ml of LB medium containing 50 μg/ml ampicillin and 12.5 μg/ml tetracycline. Zinc chloride was then added to the medium to a final concentration of 0.015 mM, and PNK expression was induced at 30 °C for 3 h by addition of 0.4 mM (final concentration) isopropyl-thio-β-D-galactopyranoside (Sigma). After harvesting the cells by centrifugation at 5000 × g at 4 °C for 5 min, they were resuspended in 10 ml of extraction buffer (50 mM Tris-HCl, pH 7.5, 0.015 mM ZnCl2, 6 mM mercaptoethanol). Lysozyme was then added to a concentration of 100 μg/ml together with Triton X-100 (final concentration, 0.1%), and, after incubation at 30 °C for 15 min, the bacteria were disrupted by sonication. The soluble fraction was separated from the insoluble fraction by centrifugation at 12,000 × g for 15 min at 4 °C. The insoluble fraction was resuspended in 1 ml of extraction buffer.

Northern (RNA) Hybridization Analysis—The 609-bp HindIII/PstI fragment used to screen the HeLa cDNA library was also used to probe a human multiple tissue Northern blot (CLONTECH) containing 2 μg (per lane) of polyadenylated RNA isolated from eight different human tissues. Hybridization was performed at 68 °C for 1 h under conditions described by the manufacturer. As a control for the amounts of mRNA in each lane, the membrane was reprobed with a sequence of β-actin

![Fig. 4. Activities of recombinant PNK.](image)

**Antibodies and Confocal Microscopy**—A synthetic peptide antigen was prepared commercially (SSPEQ, Quebec) from the first 17 amino acids of peptide sequence 1 (Table I) conjugated to a four-branch multiple antigenic peptide carrier. Rabbit polyclonal antibodies were raised by standard protocol (37).

The human malignant lung cell line A549 (ATCC no. CCL-185) was
grown as a monolayer on glass microscope slides to 80% confluence. Following rinsing in PBS, the cells were fixed in 95% ethanol at 2 °C for 15 min. The slides were allowed to dry, and were incubated for 1 h at room temperature with 1% skim milk powder in PBS to minimize nonspecific binding of the immunoreagents. Following extensive PBS rinsing, the slides were incubated overnight at 4 °C in the rabbit polyclonal antiserum (diluted 1/30 in PBS), in a humidified atmosphere. The cells were then rinsed extensively with PBS, and rhodamine-conjugated goat anti-rabbit IgG (H+L, Cappel Laboratories, Durham, NC) was applied at a dilution of 1/30 in PBS for a 1-h incubation at 37 °C in a water-saturated atmosphere. The unbound fluorescent antibody was removed by extensive washing in PBS, and the slides were covered with coverslips for confocal microscopy using PBS/glycerol, 1:1 as a mounting medium. The instrumentation and the procedures for the confocal laser scanning microscopy have been described previously (38).

RESULTS

Partial Purification and Peptide Sequencing of Human PNK—Fractions of a crude extract of HeLa cells that was passed down an AcA 34 Ultragel size exclusion column in the presence of 0.5 M NaCl were shown to contain DNA kinase activity (Fig. 1). Two peaks of activity were apparent, the first migrating with the bulk of the higher molecular weight protein, which may suggest that PNK is bound in a complex to other proteins, and the second eluting with proteins in the range of 40–100 kDa. Initial steps in the purification were carried out by conventional chromatography using gel filtration, hydroxyapatite, and cation exchange media. For the final step, the protein was applied on a SMART system precision column and eluted in 20 100-ml fractions with a 2-ml salt gradient (50–450 mM NaCl). The kinase assay revealed a peak of activity centering on fraction 12 (Fig. 2A). Correlation of the intensities of the protein bands in fractions 10–14 (Fig. 2B) with kinase activity
**Human Polydeoxyribonucleotide Kinase**

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**Bacterial Expression of Human PNK**—After splicing together the full sequence shown in Fig. 3, the translated region was subcloned into an expression vector (pET-16b), to generate the plasmid pPNK-His, and expressed in a strain of *E. coli* as a His-tagged protein. After sonication of the bacteria harboring pPNK-His, we isolated and purified the His-tagged protein from the inclusion bodies because the non-specific *E. coli* phosphatases were present in the soluble fraction. The 3′-phosphatase activity was measured by monitoring the removal of a 3′-phosphate group from a synthetic oligonucleotide (21). The 60-kDa band is the major protein in the insoluble fraction (Fig. 4a). The 60-kDa band is the major protein in the insoluble fraction. The 3′-phosphatase activity was measured by monitoring the removal of a 3′-phosphate group from a synthetic oligonucleotide.

**Expression of PNK in Human Tissues**—Northern blot analysis of RNA isolated from a number of human tissues (Fig. 5) indicated a major transcript of approximately 2.3 kilobases, although in some tissues a second less abundant but considerably larger (7.5 kilobases) transcript was observed. There were also notable differences in the levels of mRNA expression in the tissues examined.

**Cellular Localization of PNK**—Rabbit polyclonal antibodies were raised against a synthetic peptide composed of the first 17 amino acids in peptide 1 (Table I). These antibodies were used to visualize PNK in human A549 lung carcinoma cells by fluorescence microscopy. The results, shown in Fig. 6, indicate that the protein accumulates in the cell nuclei.

**Chromosomal Location of Human PNK**—To localize the PNK gene, we performed fluorescence *in situ* hybridization of biotin-labeled PNK cDNA probes to normal human metaphase chromosomes. Co-hybridization of two probes, EST clone 32978 and a 440-bp sequence stretching from the 5′-untranslated region into the 5′-end of the translated sequence, resulted in specific labeling only of chromosome 19 (Fig. 7). Specific labeling of 19q13.3–13.4 was observed on four (2 cells), three (9 cells), two (9 cells), or one (5 cells) chromatid(s) of the chromosome 19 homologues in 25 cells examined. Of 61 signals observed, 58 (95%) were located at 19q13.3–13.4. Of these, 11 (19%) signals were located at q13.3 and 47 (81%) signals were located at q13.4. Three single background signals were observed at other chromosomal sites (3p24.1, 4q21.2, and 1q43). We observed specific signal at 19q13.3–13.4 in additional experiments using...
the two cDNA probes hybridized individually. These results suggest that the PNK gene is localized to chromosome 19, band q13.4.

**DISCUSSION**

Gel electrophoresis of the partially purified DNA kinase activity from HeLa cells indicated that the enzyme is a ∼60-kDa polypeptide, and thus has a molecular mass similar to that of the proteins isolated from rat liver and calf thymus (11, 12, 20). The molecular mass of the 521-amino acid polypeptide encoded by the sequenced cDNA is 57,102 Da. The same computer program (ExPASy, Swiss Institute of Bioinformatics) also predicted a pI for the protein of 8.6 and 8.5, respectively (10, 11). The demonstration of its kinase and phosphatase activities (Fig. 4), confirmed that we had indeed cloned the cDNA for human PNK.

The amino acid sequence (Fig. 3) indicates that this is a novel protein. However, as shown in Fig. 8, predicted proteins of *Caenorhabditis elegans* and *Schizosaccharomyces pombe* have ∼30% similarity to human PNK. There are several large blocks of high similarity. Two in particular are probably associated with the two known activities of PNK. Other proteins possess these consensuses sequences. The first, residues 372–380, conforms to the sequence pattern \((A/G)X_2G-K-(S/T)\) of the P-loop consensus sequence (40), which is an ATP/GTP binding domain found in many kinases, including nuclease kinases such as adenylate and uridylate kinases (Table II). The second, residues 170–176, is a sequence found in several phosphatases (Table II), including carbohydrate-phosphate phosphatases and 

| A. ATP-binding (P-loop) domains |
|---------------------------------|
| Human polynucleotide kinase     |
| Phage T4 polynucleotide kinase  |
| NPVAC* putative PNK/RNA ligase  |
| *Bacillus subtilis* adenylate kinase |
| *Porcine uridylate kinase*      |
| Human heteronuclear ribonucleoprotein U protein |

| B. Phosphatase-associated domains |
|----------------------------------|
| Human polynucleotide kinase      |
| Phage T4 polynucleotide kinase   |
| NPVAC putative PNK/RNA ligase    |
| Haemophilus influenzae phosphoglycolate phosphatase |
| Rhodobacter sphaeroides phosphoglycolate phosphatase |
| *Aquifex aeolicus* phosphoglycolate phosphatase |
| *Saccharomyces cerevisiae* glycero-3-phosphatase |
| B. subtilis HPr(Ser(P)) phosphatase |
| Human phosphomannomutase 1      |

| C. 5′-UTR DNA sequences          |
|----------------------------------|
| Human polynucleotide kinase      |
| Human DNase II (complementary strand) |
| Human polynucleotide kinase      |
| Human DNase II (complementary strand) |

* NPVAC, nuclear polyhedrosis virus.

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We have mapped the location of the gene for PNK to chromosome 19q13.4. Among the other genes that have been mapped to this locus are DNA polymerase \(\delta\), the apoptosis regulator gene BAX, protein kinase C, and several zinc finger proteins. The DNA repair enzymes, DNA ligase I and ERCC1, are located at 19q13.3 and DNase II at 19q13.2.

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Note Added in Proof—The accompanying article by Jilani et al. (48) describes the independent identification and characterization of the same human DNA kinase, which they term PNKP.

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