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Sequence and functional characterization of the human purine nucleoside phosphorylase promoter

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ABSTRACT

Purine nucleoside phosphorylase (PNP) is a ubiquitously expressed enzyme which contributes to the catabolism and recycling of nucleotides. To characterize the promoter region of the human PNP gene, the nucleotide sequence from a *Bam*HI site located in the 5′ untranslated region extending 2237 bp upstream to an *Xba*I site was determined. The transcriptional start site as determined by primer extension was 119 bp upstream of the coding sequence and consisted of a 5′-CA-3′ dimer with A at +1. A TATA box was identified −24 to −29 bp upstream of the transcriptional start site. A CCAAT pentamer sequence in the inverted orientation was present at −51 to −55 bp and two GC rich regions were identified at −68 to −81 bp and −168 to −187 bp. Progressive 5′ deletions of the 5′ flanking region were fused to the chloramphenicol acetyltransferase (CAT) reporter gene and transient expression measured after transfection of murine NIH/3T3 fibroblasts. A 91 bp promoter (the shortest tested) provided CAT activity at 60% the level of a 216 bp promoter, possibly due to removal of the GC rich region between −168 and −187 bp. Longer promoters resulted in CAT expression at similar or lower levels than the 216 bp promoter indicating that this region contained all of the 5′ flanking sequences affecting transcription from the PNP promoter.

INTRODUCTION

Purine nucleoside phosphorylase (PNP; purine-nucleoside orthophosphate ribosyltransferase; EC 2.4.2.1) catalyzes the reversible phosphorolysis of inosine and guanosine and their respective deoxynucleosides, using inorganic phosphate as co-substrate to generate the free base and (deoxy)ribose-1-phosphate (1). Net flux of the reaction *in vivo* is in the catabolic direction. PNP is widely distributed in nature with activity demonstrated in bacteria, yeast, fish, chickens, and mammals (1). PNP is ubiquitously expressed in human tissues (2). Enzyme activity reportedly ranges from 10 nmol/min/mg protein in the brain to 120 nmol/min/mg protein in hematopoietic cells (2). The human PNP gene can therefore be considered a 'housekeeping gene', one which is constitutively expressed within a relatively narrow range in all tissues and whose product is an enzyme involved in basic cellular metabolism.

In humans, the absence of PNP activity is associated with T-cell immunodeficiency (3). This rare autosomal recessive disease becomes manifest between 4 months and 9 years of age as repeated viral and fungal infections, often in conjunction with neurological abnormalities (4). Lymphopenia and crippled T-cell function are present by the time symptoms appear. In contrast, B-cell function remains relatively intact. Molecular basis of the disease has been described for one patient, in which a glutamine to lysine substitution was observed at position 89 (5).

The human PNP gene consists of 6 exons including 870 bp of coding sequence dispersed across approximately 7.5 kb of chromosome 14 (5,6,7). As an initial study of transcriptional regulation of the human PNP gene, the nucleotide sequence of a 2.2 kb region from the 5′ flanking region was determined. Sequences typical of RNA polymerase II promoters in higher eukaryotes (TATA box, GC boxes and a reverse CCAAT box) were identified. Functional analysis of 5′ deletion constructs fused to the chloramphenicol acetyltransferase gene demonstrated promoter activity ascribable primarily to a region within 216 bp upstream of the transcriptional initiation site.

MATERIALS AND METHODS

DNA manipulations

All restriction enzymes were from New England Biolabs® (Beverly, MA). DNA polymerase (Klenow fragment) and T4 DNA ligase were from BRL (Life Technologies, Inc., Gaithersburg, MD). Plasmids were maintained in *Escherichia coli* K-12 strain 294, harvested by the alkaline lysis procedure and purified by banding twice on CsCl-ethidium bromide gradients (8). Concentrations of plasmid preparations were assessed spectrophotometrically (260/280 nm) and the proportion of form I DNA was estimated by electrophoresis of uncut plasmids in 0.7% agarose gels containing 0.5 μg/ml ethidium bromide (8). All preparations used for transfection contained greater than 50% supercoiled plasmid.

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DNA sequencing

Restriction fragments generated from the genomic clone λPNP1 (5,6) were cloned into M13 vectors mp18 and mp19 (9) (Fig. 1, solid arrows) and sequenced in both directions using the dideoxynucleotide chain termination method (10).

Primer extension

Poly(A)+ RNA was isolated from HeLa cells using the FastTrack™ system (Invitrogen Corp., San Diego, CA). A 30 base synthetic oligonucleotide (5′-CGCTCCGCTATGCTGAA-CTGAGCAAGGCTG-3′) complementary to bases +22 to +51 in the 5′ untranslated region of the human PNP message (6) (Fig. 1) was end-labelled with T4 polynucleotide kinase. Six μg of poly(A)+ HeLa RNA was hybridized to 10^5 CPM (0.7 ng) of labelled primer for two hours at 65°C in 20 μl of aqueous buffer containing 10 mM Tris pH 8.0, 250 mM KCl and 1 mM EDTA (protocol courtesy of Dr. Kathleen Conklin, University of Minnesota). Subsequently, 50 μl of primer extension mixture was added, resulting in final concentrations of 10 mM Tris pH 8.0, 10 mM $MgCl_2$, 5 mM dithiotreitol, and 200 μM of each deoxynucleotide. The labelled primer was extended using 400 units of murine leukemia virus (MLV) reverse transcriptase (BRL) for 30 minutes at 37°C. The reaction mixture was then ethanol precipitated, washed once with ice cold 70% ethanol and resuspended in 3 μl of TE buffer followed by addition of 4 μl of 95% formamide containing 20 mM EDTA (Sequenase® kit 'stop solution', United States Biochemical Corp., Cleveland, OH). Reaction sample (3 μl) was electrophoresed in a 6% polyacrylamide—8M urea gel. The gel was then fixed in methanol:acetic acid:water (7.5:7.5:85) followed by drying and autoradiography. Size markers for the primer extension reaction were generated by sequencing alkali-denatured (11) plasmid pPNPC4i1-5 (Foresman, M.D., et al., manuscript in preparation) using the same labelled primer and the Sequenase® dideoxynucleotide chain termination kit (United States Biochemical Corp.).

Mammalian cell culture and transfections

NIH/3T3 *tk*⁻ (lacking thymidine kinase activity) fibroblasts (12) were routinely cultivated at 37°C and 5% CO_2 in Dulbecco's modified Eagle medium (GIBCO Laboratories, Grand Island, NY) supplemented with 10% newborn calf serum (Armour Pharmaceutical Company, Kankakee, IL), 2 mM glutamine, penicillin (50 U/ml), and streptomycin (50 μg/ml) (GIBCO). Cells (5×10^5) were plated in 10 cm dishes (Falcon 3003, Becton Dickinson and Co., Paramus, NJ) 20 hours before transfection. Fresh medium was provided four hours prior to transfection. Transfections using the calcium phosphate-DNA coprecipitate technique (13) were performed essentially as described by Gorman (14). Ten μg of each pCAT3M (15) derived plasmid were used per transfection. In experiments using pCAT(An) derived plasmids (16) the molar equivalent of 10 μg of pCAT(An) was used per transfection. Ten μg of plasmid pHSV-106, containing a 3.4 kb sequence from the herpes simplex virus type 1 genome spanning the thymidine kinase gene (17) (BRL), was co-transfected as an 'internal standard' plasmid. Preliminary experiments were conducted to verify that quantities of plasmids were within the linear range of this transient expression system and that the co-transfecting pHSV-106 plasmid did not affect expression of transfected pCATC1 plasmid. Cells were exposed to precipitate overnight (16 hours), shocked with 1 ml of 15% glycerol in PBS for three minutes (18) and then provided with fresh medium. Forty eight hours later the cells were harvested by trypsinization, washed two times with PBS, resuspended in 50 mM Tris pH 7.8 (220 μl) and lysed by three cycles of freeze-thawing. Cytoplasmic extracts were collected after centrifugation at 16,000×g for 15 min (4°C) and stored at −20°C until assayed.

Enzyme assays

CAT activity was assayed by the method of Nordeen et al. (19) using 50 μl of cytoplasmic extract preincubated at 60°C for 10 minutes to inactivate thioesterases. Two aliquots were collected from the CAT reaction mixture after incubation at 37°C for 1−3 hours. CAT activity was computed from the change in radioactive product accumulated between the two time points.

CAT activity was normalized for herpes simplex virus thymidine kinase (HSV-TK) activity as determined using [*methyl*-^{3}H]thymidine as substrate in a polyethyleneimine thin layer plate binding assay (Jonsson, J.J. and McIvor, R.S., manuscript submitted).

RESULTS

5′ flanking sequence analysis

To initially characterize the promoter region of the human PNP gene, a 2237 bp DNA sequence from the 5′ flank was determined (Fig. 1). The 51 bp sequence immediately upstream of the *Bam*HI site (positions +1 to +51) was identical to the corresponding sequence at the 5′ end of cDNA clone pPNP1, previously characterized (6). In a primer extension experiment (see below), the transcriptional start site coincided with the 5′ end of the cDNA clone pPNP1. A TATA box was identified between −24 and −29 bp upstream of the transcriptional initiation site. Two GC rich regions were present (at positions −68 to −81 bp and −168 to −187 bp), including two overlapping GC boxes (GGGCGG, in reverse orientation at positions −174 to −179 bp and from −177 to −183) known to bind transcription factor SP-1 (20). A CCAAT pentasequence in the reverse orientation was located between positions −51 and −55 bp. Several AT-rich sequences were found, the most extensive of which was a 95 bp sequence at position −759 to −853 bp in which five single G's were interspersed between As and Ts.

Inspection of the PNP promoter unexpectedly revealed a nonamer sequence (5′-GGGGACTCC-3′) located at −48 to −40 bp that resembled the NF-κB binding site consensus sequence (21). To examine the functional capability of this sequence to bind NF-κB, two complementary synthetic 20-mers, representing sequences −34 to −53 bp in the PNP promoter, were end-labelled and incubated with both murine B-cell (lipopolysaccharide stimulated 70Z cells) and human myeloma cell nuclear extracts (IM-9) known to contain NF-κB binding activity (22). No binding to the PNP oligomers was observed in a mobility-shift assay (data not shown). We tested a range of experimental conditions which in concurrent experiments were shown to be permissive for binding of NF-κB to the murine kappa gene NF-κB binding site (22) (experiments performed in collaboration with Sue Christian in the laboratory of Dr. Brian Van Ness, University of Minnesota). The PNP sequence deviates from the NF-κB binding site consensus sequence in that the tenth base (position −39) is an A but should be a C or at least a pyrimidine according to the consensus sequence (21). The significance of this sequence in the PNP promoter, which is probably not an authentic NF-κB binding site, is unknown.

Identification of the transcriptional start site

The presence of an identifiable TATA box at position −24 to −29 suggested that the transcriptional initiation site must be located at or a few bases 5′ of base +1, corresponding to the first base of the cDNA clone pPNP1 (6). To determine the location of the transcriptional start site experimentally, a 30 base synthetic oligomer complementary to bases +22 to +51 in the 5′ untranslated region (Fig. 1) was end-labelled, hybridized with poly(A)+ RNA isolated from HeLa cells, and extended with MLV reverse transcriptase. The primer extension product was electrophoresed in a sequencing gel and its size compared to a corresponding DNA sequence ladder generated by extending the same primer on a cloned PNP genomic template (plasmid pPNPC4i1-5) (Fig. 2). A predominant primer extension product corresponding to the 5′ end of the originally isolated cDNA clone pPNP1 was seen (Fig. 2). Two minor bands were observed, one four bases shorter and the other twelve bases longer than the predominant band. The size of the predominant band positioned the TATA box −24 to −29 bp upstream of the transcription initiation site, which is in good agreement with the location of the TATA box in other genes (23,24). Transcription started at an A, and C was present at position −1, as is commonly observed in other genes (24). It is unlikely that the minor bands represented variant transcriptional initiation sites considering the exact spatial relationship observed for other promoters between a single transcriptional initiation site and the TATA box (23,25). It is not surprising that the cDNA clone pPNP1 (6) extended to the 5′ end of the PNP transcript because this clone was obtained by dCTP homopolymer tailing of the reverse transcription product using terminal transferase, an approach which can avoid any loss of 5′ sequences during second strand cDNA synthesis (8).

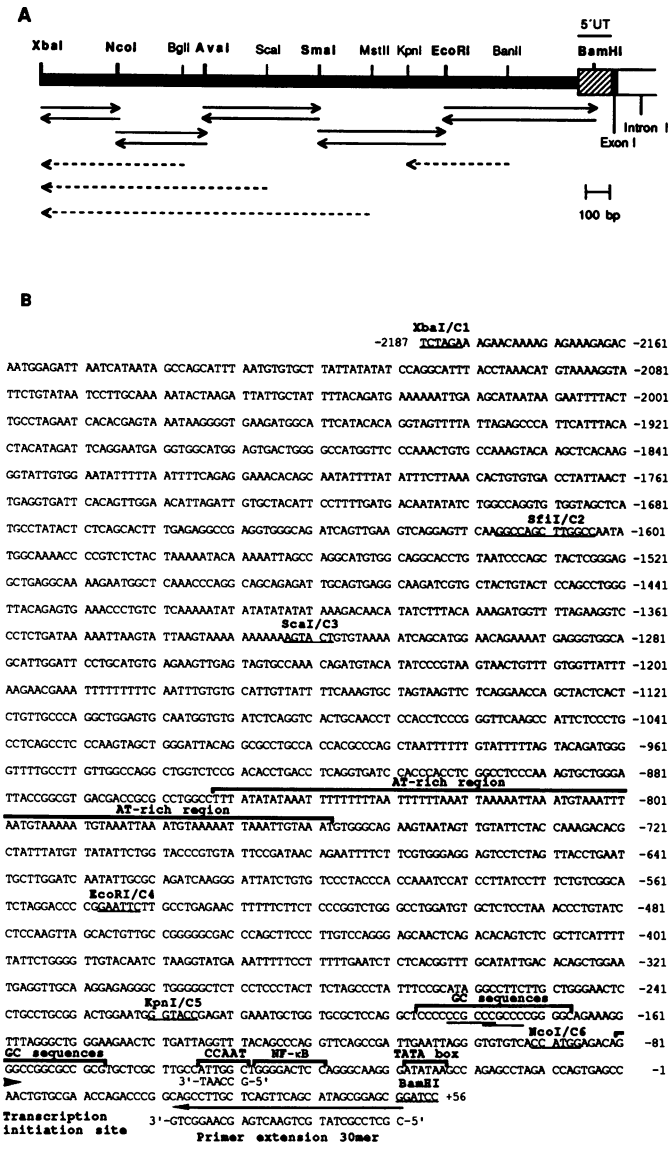


Figure 1. A. Strategy for determining the sequence of the human PNP gene 5′ flanking region. *Solid arrows:* Fragments from genomic clone λPNP1 (generated using primary restriction sites indicated in bold letters) were cloned into M13 vectors mp18 and 19 and sequenced in both directions using the dideoxy chain termination method. *Dashed arrows:* Template fragments used to sequence across the primary restriction sites in the direction indicated. **B.** DNA sequence of the 5′ flanking region of the human PNP gene. Nucleotides are numbered relative to the transcriptional start site (arrowhead). Recognized consensus sequences are bracketed and labelled: (i) TATA box; (ii) GC sequences, with GC-boxes (in inverted orientation) underlined; (iii) CCAAT box (in inverted orientation); (iv) Sequence resembling NF-κB binding site; (v) A-T rich region. Restriction sites used to construct the variable length PNP promoters C1 to C6 (Fig. 3 and 4) are underlined. The primer used to define the transcriptional initiation site (Fig. 2) is illustrated with an arrow.

Functional analysis of the PNP promoter

To examine the function of 5′ flanking sequences in regulating PNP transcript initiation, a series of 5′ deletion mutants of the 2237 bp *Xba*I to *Bam*HI sequence were constructed and used to

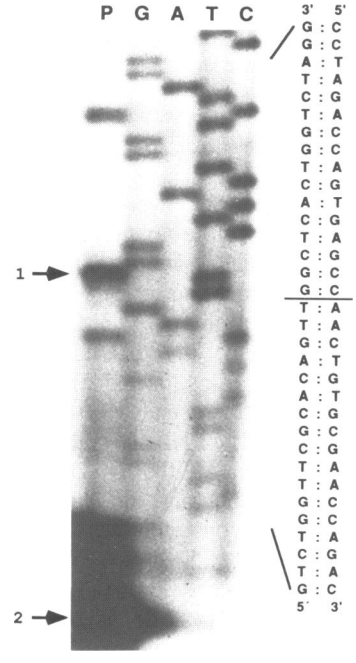
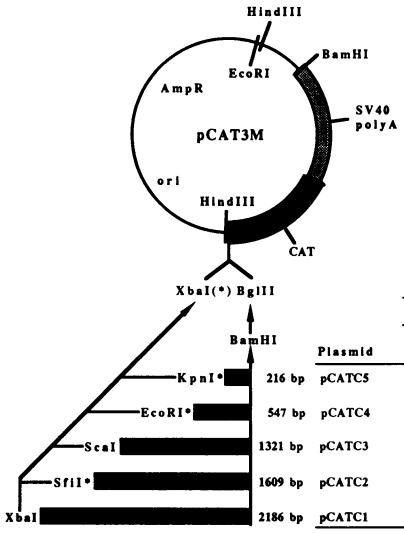
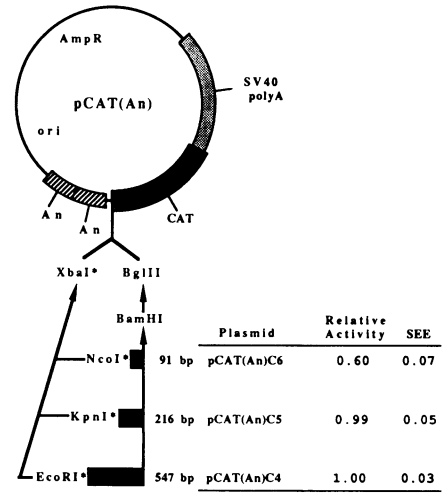


Figure 2. Primer extension to determine the human PNP gene transcriptional initiation site. An end-labelled synthetic primer complementary to bases +22 to +51 in the PNP 5′ untranslated region was hybridized with 6 μg of poly(A)+ HeLa cell RNA and extended using MLV reverse transcriptase as described in Materials and Methods. *Lanes:* (P) Primer extension reaction; (G, A, T, C) Sequencing ladder obtained by extending the same primer using cloned human PNP genomic sequences as template (dideoxy chain termination method). *Arrows:* (1) Predominant extension product; (2) Unextended primer. The genomic sequence observed and the complementary coding strand sequence (Fig. 1) are illustrated in the columns to the right of the sequencing ladder. The horizontal line delineates the transcriptional initiation site.



Plasmid	PLASMID FORM: Circular Relative Activity	Circular SEE	Linear Relative Activity	Linear SEE
pCATC5	0.92	0.12	0.60	0.02
pCATC4	1.00	0.13	1.00	0.28
pCATC3	1.04	0.13	1.21	0.15
pCATC2	0.58	0.19	0.49	0.09
pCATC1	0.90	0.13	0.91	0.09

Figure 3. Construction and expression of plasmids containing the PNP promoter region to initiate transcription of the CAT gene. All PNP promoters had a common 3′ end, the *Bam*HI restriction site at position +52 bp in the 5′ untranslated region, ligated to a *Bgl*II site 5′ of the CAT gene. The length of the PNP promoters upstream from the transcriptional initiation site is indicated as well as the restriction sites used to generate the fragments. Except for the *Xba*I to *Xba*I ligation in pCATC1, the 5′ restriction sites used to generate PNP fragments were ligated as blunt ends (*treated with the Klenow fragment of *E.coli* DNA polymerase I) into the blunted *Xba*I site in pCAT3M. Expression of 10 μg of each circular (uncut) plasmid was tested with two independent plasmid preparations. Average CAT activities from at least five independent transient transfections in NIH/3T3 *tk*⁻ fibroblasts are tabulated relative to plasmid pCATC4. Plasmids were also digested to completion with *Hind*III and 5 μg of each plasmid was transiently transfected in duplicate into NIH/3T3 *tk*⁻ fibroblasts. CAT activities are shown relative to linearized (cut) pCATC4. Expression levels of linearized plasmids were an average of 17% the level of their circular counterparts. All results were corrected for differences in plasmid molecular weights and normalized for co-transfected HSV-TK activity as described in Materials and Methods. SEE columns indicate the standard error of estimates.



Plasmid	Relative Activity	SEE
pCAT(An)C6	0.60	0.07
pCAT(An)C5	0.99	0.05
pCAT(An)C4	1.00	0.03

Figure 4. Construction and expression of plasmids containing short PNP promoters to initiate transcription of the CAT gene. A PNP fragment extending from an *Eco*RI site (blunted) 547 bp upstream of the PNP transcriptional start site to a *Bam*HI restriction site at position +52 bp in the 5′ untranslated region was inserted between *Xba*I (blunted) and *Bgl*II sites in the plasmid pCAT(An). The resulting plasmid pCAT(An)C4 was cut to completion at the regenerated *Xba*I site and then partially cut with either *Kpn*I or *Nco*I restriction enzymes, blunted with Klenow fragment of *E.coli* DNA polymerase I and religated to construct plasmids pCAT(An)C5 and pCAT(An)C6, respectively. Average CAT activities from at least five independent transient transfection experiments in NIH/3T3 *tk*⁻ fibroblasts using the molar equivalent of 10 μg of plasmid pCAT(An) are tabulated relative to the plasmid pCAT(An)C4. CAT activities were normalized for TK activity resulting from co-transfected HSV-TK activity. SEE column indicates the standard error of estimates.

initiate transcription of the bacterial chloramphenicol acetyltransferase gene (Figs. 3 and 4). For all PNP promoter deletion mutants, the *Bam*HI site at the 3′ end (position +52) was fused to a *Bgl*II site in the pCAT3M polylinker 5′ of the CAT translation initiation site. PNP inserts extending to various restriction sites upstream (Figs. 1 and 3) were ligated either directly (pCATC1) or as blunt ends into the *Xba*I site in the pCAT3M polylinker (15). The PNP promoter regions tested were selected partly on the basis of the availability of convenient restriction sites (Fig. 1), but also on the basis of sequence character. The first set of constructs were designed to test the function of sequences from +52 bp to −2186 bp upstream of the transcriptional initiation site. PNP-CAT plasmids were co-transfected into NIH/3T3 *tk*⁻ fibroblasts along with the thymidine kinase expression plasmid pHSV-106 (17) using the calcium phosphate-DNA coprecipitation technique. NIH/3T3 fibroblasts contain a moderate level of PNP activity (40 nmol/min/mg) (5) and were thus a suitable target cell population for analysis of the human PNP promoter.

Expression levels for all five constructs (pCATC1 to pCATC5) varied only twofold (Fig. 3, circular plasmids column). Constructs containing the 1321 bp C3 and 547 bp C4 promoters expressed the highest levels of CAT. Constructs containing the longest promoters, 2186 bp C1 and 1609 bp C2, gave somewhat lower levels of expression, 90% and 58% of pCATC4 respectively. Plasmid pCATC5, containing the shortest promoter (216 bp) tested in this series, retained 92% of the CAT expression observed with the 547 bp C4 promoter which was within the margin of experimental error. The PNP promoter was relatively weak in this experimental system. In comparison, the Rous sarcoma virus promoter (pRSVcat) (26) resulted in CAT activity approximately 40-fold higher than plasmid pCATC4 after transient expression in NIH/3T3 *tk*⁻ fibroblasts.

The parent plasmid pCAT3M (Fig. 3), when transiently transfected into NIH/3T3 *tk*⁻ fibroblasts, resulted in CAT activity which was 20% that of plasmid pCATC4. This background CAT expression was most likely attributable to bacterial vector sequences with fortuitous eukaryotic promoter activity, as reported by others (27,28,29,30). Background activity was not observed in CAT plasmids containing PNP promoter fragments inserted in reverse orientation (data not shown), suggesting that the CAT activity obtained using PNP promoter plasmids pCATC1-5 (Fig. 3) was not due to read-through transcription initiated from bacterial vector sequences. To further exclude that possibility, pCAT3M and the pCATC1-5 series of plasmids were cut to completion in the polylinker immediately 5′ of PNP promoter inserts with *Hind*III (Fig. 3). Linearized plasmids were transfected into NIH/3T3 *tk*⁻ fibroblasts and analyzed for transient CAT expression. *Hind*III digestion abrogated the ability of pCAT3M to provide CAT expression in transfected cells. However *Hind*III-digested plasmids of the pCATC1-5 series maintained similar relative levels of expression as uncut supercoiled plasmids, with the exception of linearized pCATC5 which in duplicate experiments resulted in only 60% the activity of linearized pCATC4. The higher relative expression

observed with the circular form of pCATC5 compared to the linearized plasmid could have been due to read-through transcription initiated in bacterial sequences (however see below). Expression levels of linearized plasmids on a molar basis were an average of 17% that of their circular counterparts, presumably because of the topological change from supercoiled to linearized plasmid (31). We concluded from experiments shown in Fig. 3 that 5′ flanking sequences of the PNP gene extending further than −547 bp upstream of the transcriptional start site did not appreciably affect transcriptional activity.

To further define the minimal PNP promoter, a series of plasmids containing short PNP promoters were constructed (Fig. 4). The parent plasmid of these constructs, pCAT(An), contained the CAT reporter gene fused to SV40 early region 3′ flanking sequences (16) as did pCAT3M. In addition, plasmid pCAT(An) contained two head to tail SV40 early region polyadenylation signals immediately upstream of the polylinker used for promoter insertion (29) to abolish background CAT transcription initiated in bacterial vector sequences. The parent plasmids pCAT3M and pCAT(An) have different sequences immediately 5′ of the CAT gene, including the polylinker for promoter insertion. CAT messages transcribed from PNP promoters inserted into pCAT3M or pCAT(An) thus have different 5′ untranslated regions which could affect CAT expression. Therefore, two plasmids pCAT(An)C4 and pCAT(An)C5 (Fig. 4) containing the previously tested 547 bp C4 and 216 bp C5 promoters were constructed so that a sufficient overlap between the two series of CAT vectors could be established. NIH/3T3 cells transfected with these two plasmids expressed similar levels of CAT activity (Fig. 4), confirming the results obtained with the circular plasmids pCATC4 and pCATC5 (Fig. 3). In addition, we tested a 91 bp promoter (C6). This promoter resulted in significantly lower expression levels (60%) than the longer, 547 bp C4 and 216 bp C5 promoters. This indicated that sequences between −91 to −216 bp upstream of the transcriptional initiation site were influential for optimum CAT expression from the PNP promoter.

DISCUSSION

The 5′ flanking region of the human PNP gene was characterized by determining its nucleotide sequence and assessing its ability to direct expression of a heterologous reporter gene. The transcriptional initiation site was also mapped by primer extension. Several canonical sequences were identified, which are known to be necessary for accurate and efficient initiation of transcription from RNA polymerase II promoters in higher eukaryotes. Two key structural motifs for transcription initiation site selection were identified: A TATA box at −24 to −29 bp and a 5′-CA-3′ initiator sequence with A at +1 (23,24,32). The presence of a TATA box in the PNP promoter contrasts with many housekeeping genes encoding enzymes involved in basic cellular metabolism (33). Canonical sequences associated with transcriptional regulation were also identified. A CCAAT pentameric sequence (34) at −51 to −55 bp was present in the inverted orientation as has been described for other genes (35,36). Two GC rich regions were noted at −68 to −81 bp and −168 to −187 bp including two overlapping GC boxes known to bind the transcription factor Sp1 (20). The function of these motifs was not tested directly in this paper. However the only identifiable canonical sequence which was present in the 216 bp C5 promoter but absent in the 91 bp C6 promoter was the upstream GC-rich region at −168 to −187 bp. Interestingly the 91 bp C6 promoter was only half as strong as the 216 bp C5 promoter, indicating that the GC-rich region at −168 to −187 bp, containing the two overlapping GC boxes, may affect transcription. Overall sequence analysis of the human PNP promoter suggested a modular organization as proposed for other well-characterized RNA polymerase II promoters (37). CAT expression from the PNP promoter was approximately forty-fold lower in comparison with the Rous sarcoma virus promoter, but this was not surprising given that PNP is an enzyme of basic cellular metabolism (1).

Sequences upstream of the 216 bp C5 promoter were shown not to significantly affect expression in the experimental system used i. e. transient transfection in NIH/3T3 fibroblasts. These sequences could however have regulatory function in the endogenous gene or when integrated into chromosomal DNA (38). Of note in this regard is the presence of extensive AT rich sequences far upstream of the transcriptional initiation site, the most prominent at position −759 to −853 bp (Fig. 1). Such oligo(dA-dT) tracts have different conformational flexibility than heteropolymeric DNA and are thought to be involved in the formation of nucleosome-free regions (39,40). It is tempting to speculate that these sequences might be involved in constitutive expression of the PNP gene as has been described for yeast genes (41,42). The poly(dA-dT) sequences in yeast, however, were much closer to the transcriptional initiation site (less than −130 bp upstream) than the poly(dA-dT) sequences observed in the human PNP promoter.

Identification of the PNP transcriptional initiation site clarifies our understanding of the PNP message. Sequencing of the cloned human genomic PNP 3′ flanking region has revealed a canonical poly(A) signal 28 to 33 bp downstream of the 3′ end of cDNA clone pPNP1 (Jonsson, J.J. and McIvor, R.S., unpublished observations). This poly(A) signal generates a 3′ untranslated region of approximately 500 bp, when combined with a 119 bp 5′ untranslated region and 870 bp coding sequence (total = 1.5 kb) should result in a polyadenylated message of 1.7 kb (assuming a 200 nucleotide polyA tail). This is the size observed for the mature PNP message in HeLa cells (43, Foresman, M.D., et al. manuscript in preparation).

Currently, the only PNP mutation which has been fully characterized at the molecular level was a single G to A transition at nucleotide 265 of the coding sequence (5). Characterization of the human PNP promoter forms the basis for examination of the potential role of PNP promoter dysfunction in human immune disease as well as for future studies into the regulation of PNP gene expression in different cells and tissues. Such regulated PNP expression may be important in the maintenance of appropriate purine nucleoside and nucleotide levels during normal immunodevelopment (4). The inclusion of regulatory elements (such as the promoter) from the natural gene in recombinant retroviruses or other gene transfer vectors might thus be important in experiments addressing the possibility of gene transfer in the treatment of PNP^- T-cell immunodeficiency.

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