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Cloning of human purine-nucleoside phosphorylase cDNA sequences by complementation in *Escherichia coli*

(polysome immunoadsorption/cDNA cloning/phenotypic expression/genetic disease/immunodeficiency)

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ABSTRACT **We have obtained cDNA clones that contain the entire coding region of the human purine-nucleoside phosphorylase (PNP; EC 2.4.2.1) mRNA. The cDNA sequences were generated by reverse transcription of PNP-enriched mRNA obtained by immunoadsorption of HeLa cell polyribosomes with monospecific antibody to human PNP. cDNA molecules that were close in length to PNP mRNA were separated by agarose gel electrophoresis and inserted into the *Pst* I site of the plasmid pBR322. Plasmid DNA from the pooled clones was used to transform PNP-deficient *Escherichia coli* cells, and those transformants that phenotypically expressed PNP were isolated on selective media. The presence of human PNP in the selected bacterial cells was detected by immunoprecipitation with human PNP antibody.**

Purine-nucleoside phosphorylase (PNP; EC 2.4.2.1) reversibly catalyzes the conversion of purine nucleosides and deoxynucleosides (guanosine, deoxyguanosine, inosine, and deoxyinosine) to their respective purine bases and pentose 1-phosphates. The enzyme has been purified to homogeneity from human erythrocytes (1–3) and shown to be a trimer composed of subunits of identical molecular weight (29,700) derived from a single genetic locus (3, 4). Deficiency of PNP is inherited in an autosomal recessive manner and results in a severe T-cell immunodeficiency disease in children (5, 6). Studies with S49 mouse T-lymphoma cells suggest that the loss of T-cell function is due to inhibition of ribonucleotide reductase by the high levels of dGTP that accumulate in the thymus of PNP-deficient patients (7, 8). Although in most cases there is little or no detectable PNP protein present in cells of PNP-deficient patients (9, 10), analysis of the heterozygous parents of one patient indicates that the defective protein contains an internal insertion of several amino acid residues (9, 11).

In order to gain further insights into the molecular nature of inherited PNP defects, it was necessary to obtain cDNA clones encoding PNP sequences. By using a selection system based on complementation in bacteria, we have isolated cDNA clones that contain the entire coding region of the PNP gene.

EXPERIMENTAL PROCEDURES

Materials. Avian myeloblastoma virus reverse transcriptase was obtained from J. Beard (Life Sciences, St. Petersburg, FL); terminal deoxynucleotidyltransferase (terminal transferase) was from Bethesda Research Laboratories; *Aspergillus oryzae* S1 nuclease was from Miles; and DNA polymerase I (*Escherichia coli*) was from Boehringer Mannheim. Restriction endonucleases were purchased from New England BioLabs. Monospecific PNP antibody was a generous gift of William Osborne (University of Washington, Seattle). Oligo(dT)-cellulose (type 3), oligo(dC)-cellulose, oligo(dG)-cellulose, $(dG)_{12\text{-}18}$ and $(dT)_{12\text{-}18}$ were purchased from Collaborative Research (Waltham, MA). GeneScreen membranes are a product of New England Nuclear.

Bacterial Strains and Media. All bacterial strains used were derivatives of *E. coli* K-12. MC1061 (*araD139*, Δ[*ara–leu*]7697, Δ*lac* X74, *galU*, *galK*, hsr^-, hsm^+, *strA*) (12) and MH-1 (a derivative of MC1061 that lacks the *ara–leu* deletion) were obtained from Michael Hall (University of California, San Francisco); the PNP-deficient Sφ312 (*metB*, *strA*, *purE*, *pup*) (13) was a generous gift of Per Nygaard (University Institute of Biological Chemistry B, Copenhagen, Denmark) provided by Olle Karlstrom (Universitetes Mikrobiologiske Institut, Copenhagen, Denmark). LB rich medium and M63 minimal medium were prepared as described (14). Unless otherwise indicated, tetracycline (Tc) was added at 15–20 μg/ml to all bacterial cultures and agar plates. Deoxyinosine selective medium consisted of M63 minimal medium plus 0.1% Casamino acids, deoxyinosine at 50 μg/ml, and Tc at 15 μg/ml.

Preparation of Polyribosomes and Purification of mRNA. Polyribosomes were prepared from logarithmically growing HeLa cells in the presence of cycloheximide (1 μg/ml) and heparin (40 units/ml) by a modification of the procedure of Efstratiadis and Kafatos (15). After 15 min in lysis buffer at 0°C, the cell suspension was adjusted to 0.05% Nonidet P-40, incubated another 5 min, and then centrifuged at 12,000 × *g* for 5 min. The supernatant was adjusted to 0.1 M $MgCl_2$, 2% Triton X-100, incubated 1 hr at 0°C, and then pelleted through a cushion of 1 M sucrose in lysis buffer. The resuspended polyribosomes were divided into aliquots and stored at −70°C. The average yield was 15–20 A_{260} units/ml of packed cells. The integrity of each preparation was determined by analytical centrifugation through a 15–40% linear sucrose gradient (16).

Polyribosomes were immunoadsorbed by using staphylococcal protein A-Sepharose (Pharmacia) as described by Kraus and Rosenberg (17) except that the first flow-through fraction from the protein A-Sepharose column was reincubated with PNP antibody and then reapplied to the column. Poly$(A)^+$ RNA was obtained from the column eluate by chromatography on oligo(dT)-cellulose (18). The yield of mRNA, estimated by comparing trichloroacetic acid-precipitable radioactive material after *in vitro* translation with that obtained from a known amount of total HeLa cell poly$(A)^+$ RNA, was 600–700 ng from 400 A_{260} units of polyribosomes.

Total poly$(A)^+$ RNA preparations were obtained from cultures of HeLa cells harvested by centrifugation for 5 min at

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Abbreviations: PNP, purine nucleoside phosphorylase; Tc, tetracycline; ds, double-stranded.

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3,000 rpm in an IEC PR-J centrifuge. The cell pellet was resuspended in 3 vol of buffer containing 0.15 M NaCl, 10 mM Tris·HCl at pH 7.5, and 0.5% Nonidet P-40. After Vortex mixing for 1 min, the nuclei and debris were removed by centrifugation again at 3,000 rpm for 5 min. The supernatant was adjusted to 0.5% $NaDodSO_4$, extracted twice with phenol and chloroform, and precipitated with 0.15 M sodium acetate and 2 vol of ethanol at −20°C. $Poly(A)^+$ RNA was isolated by one cycle of chromatography on oligo(dT)-cellulose.

In Vitro Translation and Immunoprecipitation of Protein Products. *In vitro* translations of mRNA preparations were carried out in the presence of 1.6 μCi of $[^{35}S]$methionine per μl (Amersham, 1,300 Ci/mmol; 1 Ci = 3.7×10^{10} Bq), using the rabbit reticulocyte *in vitro* translation system from Bethesda Research Laboratories. After incubation for 80 min at 30°C, the reaction mixtures were immunoprecipitated with PNP antibody and formalin-fixed *Staphylococcus aureus* strain A cells (Bethesda Research Laboratories) as described by Firestone *et al.* (19).

Polyacrylamide Gel Electrophoresis. Denaturing 12.5% polyacrylamide gels containing $NaDodSO_4$ were prepared and run as described (20). After fixing and staining, the gels were washed in water for 30 min and then prepared for fluorography by incubation in 1 M sodium salicylate for 30 min before drying.

Synthesis and Cloning of Double-Stranded (ds) cDNA. Reverse transcription of the PNP-enriched mRNA, tailing of the cDNA with terminal transferase, and synthesis of ds cDNA were carried out under conditions previously described (21) except for the following modifications. Fifty nanograms of PNP-enriched mRNA was incubated with 200 ng of $(dT)_{12\text{-}18}$ and 13 units of reverse transcriptase for 20 min at 46°C in a reaction volume of 10 μl. After the reaction had been stopped with 20 mM EDTA and 0.2% $NaDodSO_4$, unincorporated deoxynucleotides were removed by centrifugal column chromatography (22) through Bio-Gel P-10 (Bio-Rad). The cDNA and the $(dT)_{12\text{-}18}$ were recovered in 12 μl and then tailed at the 3′ end by using 1 mM dCTP and 10 units of terminal transferase for 30 min at 37°C in a 20-μl reaction volume. These conditions had been predetermined to give 10–20 dCMP residues per 3′ end with 200 ng of $(dT)_{12\text{-}18}$ as substrate. RNA was hydrolyzed in 0.3 M NaOH for 30 min at 65°C. After neutralization with 75 mM Tris·HCl, pH 8.0, and 0.3 M acetic acid, the mixture was extracted with phenol and chloroform and subjected to Bio-Gel P-10 centrifugal column chromatography. Oligo(dC)-tailed DNA molecules were selected by oligo(dG)-cellulose chromatography. Twenty nanograms of cDNA was recovered at this point from the original 50 ng of mRNA.

Synthesis of the second strand cDNA was carried out as described (21), using $(dG)_{12\text{-}18}$ (4 μg/ml) as a primer and 26 units of reverse transcriptase in a 50-μl reaction volume. Incubation was for 1 hr at 46°C. Single-stranded ends were removed with S1 nuclease (2,000 units/ml) in the presence of 0.5% $NaDodSO_4$ for 20 min at 37°C. The reaction mixture was adjusted to 5 mM EDTA and 100 mM Tris·HCl at pH 8.0, extracted with phenol, precipitated with ethanol, and subjected to Bio-Gel P-10 centrifugal column chromatography. Oligo(dC)-tailing of the ds cDNA molecules was carried out as described above; the DNA was then extracted with phenol and precipitated with ethanol.

The tailed ds cDNA (10 ng) was sized by electrophoresis through a 1.2% agarose gel (low melt, Marine Colloids, Rockland, ME) containing 1 mM methylmercury hydroxide to prevent degradation of the oligo(dC) tails. The fraction of molecules approximately 1,500–2,200 base pairs in length was eluted, extracted with phenol (23), and purified by chromatography over oligo(dG)-cellulose. Approximately 1 ng of sized cDNA was obtained.

The pBR322 vector DNA was linearized with *Pst* I and oligo(dG)-tailed as described above, using 1 μg of DNA, 100 μM dGTP, and 5 units of terminal transferase in a volume of 10 μl. Tailed molecules were selected by chromatography over oligo(dC)-cellulose. Equimolar amounts of oligo(dG)-tailed pBR322 and oligo(dC)-tailed ds cDNA were annealed in 0.2 M NaCl/10 mM Tris·HCl, pH 7.8/1 mM EDTA at a final concentration of 0.15 μg/ml. The annealing mixture was heated at 65°C for 1 min and slowly cooled to room temperature over a period of 2 hr.

Transformation was carried out with *E. coli* K-12 MC1061 cells made competent by using rubidium chloride (ref. 24; procedure modified by Michael Scott, University of California, San Francisco, personal communication). The transformation efficiency was 5×10^8 transformants per μg of supercoiled pBR322 DNA and $2\text{–}5 \times 10^6$ transformants per μg of recombinant plasmid DNA.

Bacterial Complementation. Plasmid DNA was prepared (25) from overnight cultures of an ampicillin-resistant control clone and pooled cDNA clones. Fifty nanograms of each DNA was used to transform 100 μl of competent (26) PNP-deficient Sϕ312 bacterial cells. The transformed cells were plated onto either rich agar medium (LB) or deoxyinosine selective agar medium containing Tc at 15 μg/ml.

Immunoprecipitation of Proteins from Bacterial Cells. Five-milliliter bacterial cultures were grown in M63 minimal medium containing 0.1% Casamino acids and Tc at 15 μg/ml to an OD_{590} of 0.6. The cells were harvested by centrifugation, resuspended in M63 medium without Casamino acids, and labeled with $[^{35}S]$methionine (Amersham; 1,300 Ci/mmol) at 50 μCi/ml for 5 min. After chilling on ice, the cells were pelleted and lysed by incubation in a lysozyme/detergent solution (27). The suspension was centrifuged at $15,000 \times g$ for 10 min, and the resulting supernatant was immunoprecipitated with human PNP-antibody and formalin-fixed *S. aureus* A cells as described above.

Nick-Translation. DNA (500 ng) was incubated under standard conditions (28) in the presence of 250 μCi of $[\alpha\text{-}^{32}P]$dCTP (3,000 Ci/mmol; Amersham) and 12 units of DNA polymerase I in a reaction volume of 50 μl for 2 hr at 23°C. After extraction with phenol and chloroform, the unincorporated nucleotides were removed by two cycles of precipitation with 2 M NH_4OAc and 2 vol of ethanol. The final specific activity of the DNA was approximately 6×10^7 cpm/μg of DNA.

RESULTS

PNP is estimated to make up only 0.04% of the total protein in human erythrocytes (3) and 0.05% in HeLa tissue culture cells (unpublished observations). Because of this low abundance, enrichment of PNP-specific mRNA was undertaken to facilitate the molecular cloning of the cDNA and subsequent screening procedures. Polyribosomes were prepared from HeLa cells (15) and then purified by immunoadsorption utilizing monospecific antibody to human PNP (17). Poly(A)-containing RNA was obtained from the protein A-Sepharose column eluate by chromatography on oligo(dT)-cellulose and assayed for PNP mRNA activity by *in vitro* translation in the presence of $[^{35}S]$-methionine. Protein products were analyzed by $NaDodSO_4$/polyacrylamide electrophoresis (20) before and after immunoprecipitation with specific PNP antibody (Fig. 1). Although the translation of total HeLa cell mRNA yields many distinct products (lane c), one major protein is found in the *in vitro* translation products from the PNP-enriched mRNA (lane b). This protein migrates at the same position as that obtained after immunoprecipitation with the PNP antibody (lanes d and e). From analysis of the densitometric tracings of the band pattern ob-

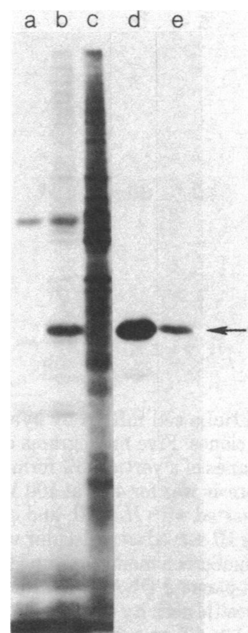

FIG. 1. Electrophoretic analysis of *in vitro* translation products of mRNA purified from immunoadsorbed HeLa cell polyribosomes. Approximately 3 ng of the immunopurified mRNA and 75 ng of total HeLa cell poly(A)$^+$ RNA were translated *in vitro* in the presence of [^{35}S]methionine. Five percent of the translation product from each sample was loaded directly onto a denaturing 12.5% polyacrylamide gel (20): lane a, no mRNA; lane b, immunopurified mRNA; lane c, total mRNA. The remainder of each sample was immunoprecipitated by incubation with PNP antibody and adsorption to formalin-fixed *S. aureus* A cells: lane d, immunopurified mRNA; lane e, total mRNA. Electrophoresis was at 5 mA overnight. The arrow indicates the position of PNP determined by coelectrophoresis of purified human erythrocyte PNP (11).

tained from the *in vitro* translation products, it was estimated that the specific PNP mRNA represents approximately 20% of the total polyribosome purified mRNA preparation. This estimation is based on the assumption that all proteins contain the same number of methionine residues, that the intensity of all bands is within the linear range of the densitometer reading, and that the contribution of proteins at the gel front is negligible.

PNP-enriched mRNA was used as a template to construct a cDNA library in the *Pst* I site of pBR322. In order to increase the probability of obtaining full-length cDNA molecules, the oligo(dC)-tailed ds cDNA molecules were sized by agarose gel electrophoresis. Because it had been previously determined that the PNP mRNA migrates slightly faster than 18S rRNA (approximately 2,100 nucleotides) in denaturing agarose gels and denaturing sucrose gradients (unpublished observations), those molecules that were approximately 1,500 to 2,200 base pairs in length were eluted, annealed with oligo(dG)-tailed pBR322, and used to transform *E. coli* MC1061 cells. Colonies that grew on plates containing Tc were pooled and grown in suspension overnight in L broth plus Tc. DNA was prepared from these cultures (25) and used to transform PNP-deficient Sϕ312 bacteria. Transformed cells were plated onto either LB/Tc plates or deoxyinosine selective medium. The selection for PNP-competent clones is based on the fact that, in bacteria, purine deoxynucleosides cannot be phosphorylated and thus cannot serve as the sole purine source unless PNP is available for cleavage to the free purine bases. Because *de novo* purine synthesis in Sϕ312 bacteria is blocked by the *purE* mutation (13), growth on deoxyinosine as sole purine source requires the presence of PNP.

Table 1. Selection of PNP cDNA clones by expression in PNP-deficient *E. coli*

	Number of transformants			
	Direct selection		Replica plating	
Source of DNA	LB	dIno	LB	dIno
Amp^R clone	30,000	0	1,100	0
cDNA library	30,000	6	1,100	4

Plasmid DNA from an ampicillin-resistant (Amp^R) control clone and pooled cDNA clones was used to transform PNP-deficient Sϕ312 bacterial cells. For direct selection, cells were plated onto either LB or deoxyinosine (dIno) selective medium; for replica plating, cells were plated onto LB agar, incubated overnight at 37°C, and then transferred onto dIno selective medium by replica plating. All media contained Tc at 15 μg/ml. Incubation of cells on deoxyinosine selective medium was for 3 days at 37°C.

After the transformation, 10 colonies were observed on the selective plates, 6 of which grew after plating directly onto deoxyinosine selective plates (pPNP1–pPNP6), and 4 of which appeared after replica plating of colonies from LB plates onto deoxyinosine selective plates (pPNP7–pPNP10) (Table 1). The reversion frequency for growth of PNP-deficient Sϕ312 bacteria on deoxyinosine selective media has been determined to be approximately 1×10^{-6} (unpublished data). When these 10 clones were analyzed by colony hybridization (29) with ^{32}P-labeled cDNA probe made from the PNP-enriched mRNA preparation, all clones except one (pPNP6, which exhibited the slowest growth) were positive (data not shown). Finally, DNA from two of the clones obtained after plating directly onto selective plates (pPNP1 and pPNP2, the two fastest-growing clones) and two obtained after replica plating from LB plates onto selective plates (pPNP7 and pPNP10) was used to transform the PNP-deficient Sϕ312 bacteria (Table 2). Within 3 days bacterial growth was observed on all plates, but the size of the colonies was markedly different. The largest colonies, those transformed by pPNP1, were approximately 2 mm in diameter. Growth of the other three clones was variable, as indicated in Table 2. The growth characteristics of transformed PNP-deficient Sϕ312 bacteria thus clearly appear to be a function of the transforming plasmid DNA. Variation in the ability of cDNA clones to promote growth of bacteria on selective medium has also been observed for pBR322 clones containing cDNA sequences of mouse dihydrofolate reductase. In this case it was suggested that the level of expression may be related to the distance between the putative ribosome binding site and the

Table 2. Transformation of PNP-deficient *E. coli* by DNA from selected PNP clones

	Transformants $\times 10^{-5}$ per μg of DNA		Colony diameter on dIno selective plates, mm
Source of DNA	LB	dIno	
Amp^R clone	6.7	0	—
pPNP1	7.2	5.2	2
pPNP2	13.8	11.6	1
pPNP7	8.0	7.6	<1
pPNP10	6.0	9.6	<1

Plasmid DNA was prepared from an ampicillin-resistant (Amp^R) control clone, from two of the six cDNA clones obtained by direct selection (pPNP1 and pPNP2), and from two of the four obtained by replica plating (pPNP7 and pPNP10) and was used to transform PNP-deficient Sϕ312 bacterial cells. Cells were plated onto either LB or deoxyinosine (dIno) selective medium and incubated at 37°C overnight (LB) or for 3 days (dIno). All media contained Tc at 15 μg/ml.

ATG initiation codon of the cDNA (30).

Restriction enzyme analyses of DNA prepared from pPNP1 and pPNP2 indicate that the two inserts are approximately 1,400 and 1,200 base pairs, respectively. Two internal *Pst* I sites are present in the DNA of both inserts, generating one common fragment approximately 600 base pairs long and two additional fragments, both of which are larger in the pPNP1 clone. Unique *Bam*HI and *Ava* I sites are found within 100 base pairs of the 5′ and 3′ ends, respectively, of the pPNP1 insert but not in the pPNP2 insert. From these results and those using other restriction enzymes (data not shown), it appears that the two clones contain identical internal sequences but that pPNP1 contains additional sequences (approximately 100 base pairs) of DNA at each end.

To demonstrate the presence of human PNP protein in transformed bacterial cells, logarithmically growing cultures of MH-1 cells containing pPNP1, pPNP2, or pBR322 plasmid DNA were labeled with [^{35}S]methionine, harvested by centrifugation, lysed, and subjected to immunoprecipitation with human PNP antibody and formalin-fixed *S. aureus* A cells. The labeled, immunoprecipitated protein products separated by $NaDodSO_4$/polyacrylamide electrophoresis are shown in Fig. 2. No protein bands are present in samples from MH-1 cells containing pBR322 (lane c). However, in both of the MH-1 cultures containing the pPNP1 and pPNP2 samples (lanes a and b) there is a prominent band that migrates at the position of human PNP as well as other bands both larger and smaller than human PNP. These additional bands could represent fusion proteins formed between β-lactamase and PNP sequences as well as corresponding breakdown products. The highest molecular weight forms are approximately 50,000–55,000, which is consistent with a protein formed between PNP sequences and the 181 amino acids of β-lactamase that precede the *Pst* I site in pBR322 (31).

Additional evidence that the two clones contain sequences for the same protein (PNP) comes from an analysis of total HeLa cell poly(A)$^+$ RNA by blot hybridization. After fractionation on

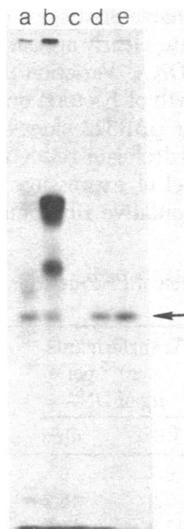

FIG. 2. Electrophoretic analysis of immunoprecipitated proteins from bacterial cells. Lanes a and b, MH-1 cells containing pPNP1 and pPNP2, respectively. Lane c, MH-1 cells containing pBR322. Lane d, MH-1 cells containing pBR322 to which had been added just before cell lysis an aliquot of [^{35}S]methionine-labeled *in vitro* translation products of HeLa cell mRNA. Lane e, ^{35}S-labeled proteins from *in vitro* translated HeLa cell mRNA. Electrophoresis was at 5 mA overnight. The arrow indicates the position of PNP determined by coelectrophoresis of purified human erythrocyte PNP (11).

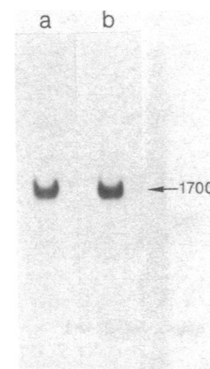

FIG. 3. Analysis of HeLa cell mRNA by hybridization with nick-translated PNP cDNA clones. Five micrograms of poly(A)$^+$ RNA was applied to each of two lanes of a vertical 6% formaldehyde/1.5% agarose gel (32). Electrophoresis was for 4 hr at 100 V. HeLa cell rRNA, λ bacteriophage DNA digested with *Hin*dIII, and φX174 bacteriophage DNA digested with *Hae* III served as molecular weight markers. RNA was transferred to a GeneScreen membrane and then probed with ^{32}P-labeled nick-translated plasmid DNA from either pPNP1 (lane a) or pPNP2 (lane b). The specific activity of the probes was approximately 6×10^7 cpm/μg DNA; 1×10^6 cpm/ml was used in the hybridization solution (GeneScreen protocol). The arrow indicates the position of an approximately 1,700-nucleotide sequence.

a formaldehyde/agarose gel (32), the poly(A)$^+$ RNA was transferred to a GeneScreen membrane and probed with ^{32}P-labeled nick-translated plasmid DNA (28). From the autoradiogram in Fig. 3, it is apparent that both clones hybridize to a species of mRNA that is approximately 1,700 base pairs in size. This is consistent with other experiments (unpublished) that, as mentioned above, indicate that the PNP mRNA migrates slightly faster than the 18S rRNA marker in denaturing sucrose gradients and denaturing agarose gels. Because the amino acid coding region of PNP is estimated from its amino acid composition (33) to be approximately 800 nucleotides, about 900 nucleotides of the PNP mRNA must be present in noncoding regions.

DISCUSSION

The expression of eukaryotic genes in *E. coli* has been used to identify specific DNA sequences from both lower eukaryotic and mammalian systems. Although assays based on bacterial complementation have been reported for DNA clones encoding proteins of yeast (34, 35) and *Neurospora* (36), mammalian protein products have been detected primarily by immunological techniques (37–40) or selection based on drug resistance (30). Selections with immunological assays, however, do not require the presence of the entire coding region of the gene, and screening by resistance to specific drugs has limited applicability to other proteins. The cloning strategy described here appears to be a general method for obtaining full-length or nearly full-length cDNA clones encoding proteins whose enzymatic activities might phenotypically complement inactive or deleted bacterial gene products. Because the screening of the cDNA clones is rapid and of such high specificity, the procedure may be especially useful for proteins present in eukaryotic cells in low abundance. As little as 20–50 ng of enriched mRNA can be used to generate several thousand cDNA clones, and, although the enrichment of the mRNA is not a strict prerequisite, it does allow screening of low numbers of transformants, thus eliminating complications due to reversion of bacterial mutations.

The success of the approach described here is due in large part to work of the last two decades on biochemical genetics of bacteria. The wide spectrum of available mutants now provides

an extremely rapid and powerful system for obtaining cDNA clones for many mammalian enzymes and could be especially important in the case of those enzymes for which an inherited deficiency results in human disease.

Examination of PNP in heterozygous parents of PNP-deficient children indicates that in all cases the protein is catalytically less active and is aberrant with respect to either its conformational stability or its electrophoretic mobility (6, 10). In one family the change has been attributed to an internal insertion of several amino acid residues, possibly the result of an unequal genetic crossover or an abnormal intron processing event after transcription (9, 11). The availability of the PNP cDNA clones should provide a means for distinguishing between these mechanisms and permit identification of the specific genetic aberration in the PNP gene of other affected children as well. In addition, the full-length cDNA clones make feasible the development of possible gene therapy procedures directed at the correction of the usually lethal mutant phenotype that results from the selective inhibition of normal thymic development.

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