Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 477–481, January 1983
Genetics

Isolation and characterization of a full-length expressible cDNA for human hypoxanthine phosphoribosyltransferase

(Lesch–Nyhan disease/gene cloning/transfection/DNA sequence analysis)

D. J. JOLLY*, H. OKAYAMA†, P. BERG†, A. C. ESTY*, D. FILPULA*, P. BOHLEN‡, G. G. JOHNSON§, J. E. SHIVELY¶, T. HUNKAPILLAR‖, AND T. FRIEDMANN*

*Department of Pediatrics, University of California at San Diego, La Jolla, California 92093; †Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305; ‡The Salk Institute, Post Office Box 85800, San Diego, California 92138; §Department of Biology, San Diego State University, San Diego, California 92182; ¶Department of Immunology, City of Hope Medical Research Center, Duarte, California 91010; and ‖Division of Biology, California Institute of Technology, Pasadena, California 91125

Communicated by J. Edwin Seegmiller, October 7, 1982

ABSTRACT We have cloned a full-length 1.6-kilobase cDNA of a human mRNA coding for hypoxanthine phosphoribosyltransferase (HPRT; IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8) into a simian virus 40-based expression vector and have determined its full nucleotide sequence. The inferred amino acid sequence agrees with a partial amino acid sequence determined for authentic human HPRT protein. Transfection of HPRT-deficient mouse LA9 cells with the purified plasmid leads to the expression of human HPRT enzyme activity in cells stably transfected and selected for enzyme activity in hypoxanthine/aminopterin/thymidine medium.

Current methods of molecular biology, including the techniques of recombinant DNA construction and cloning, rapid nucleotide sequence analysis, the design and construction of transducing vectors, and techniques of transfection of eukaryotic cells with foreign genes, have made it possible to clone and characterize a large number of eukaryotic genes. One gene of particular interest, not only for basic studies of eukaryotic gene regulation but also for understanding of several important human genetic diseases, is the gene encoding the enzyme hypoxanthine phosphoribosyltransferase (HPRT) (1). This enzyme catalyzes vital steps in the reutilization pathway for purine biosynthesis, and its deficiency leads to forms of gouty arthritis and to the devastating Lesch–Nyhan disease (2, 3). The HPRT locus is known to be X linked in the human and other mammalian genomes, and the availability of a cloned HPRT gene would facilitate studies of the organization of a particularly interesting region of the human X chromosome and of the mechanisms of inactivation of specific and well-mapped regions of the X chromosome. Recently, we succeeded in isolating a human genomic clone containing a portion of the HPRT gene together with some intervening sequence (4). This fragment of the HPRT gene has been used to isolate a full-length cDNA clone encoding the human HPRT enzyme from a human cDNA library. Nucleotide sequence analysis of the cloned cDNA segment established that it encodes the entire HPRT protein.

METHODS

Isolation of the HPRT Gene Fragment. A genomic segment of human DNA containing sequences from the extreme 5′ end of the HPRT gene together with a portion of an intervening sequence has been isolated and cloned as plasmid p6B2aE2 (4) by a combination of gene transfection into enzyme-deficient mouse cells and localization of human sequences by hybridization with probes of middle repetitive human (*Alu*) sequence, as described (4). A 1.5-kilobase (kb) *Eco*RI/*Bam*HI fragment derived from p6B2aE2 and free of repetitive human sequences has been subcloned into the *Bam*HI and *Eco*RI sites of pBR322 and shown to hybridize to a discrete human cytoplasmic poly(A)$^+$RNA approximately 1.5 kb long, presumably representing the mRNA for HPRT (4). DNA from this subcloned plasmid, called pBR1.5, was prepared by detergent lysis (5) of chloramphenicol-treated (6) transformed SF8 *Escherichia coli* (7) followed by cesium chloride/ethidium bromide ultracentrifugation (8). This DNA was cleaved with *Eco*RI/*Bam*HI and the cloned insert was isolated by trough electroelution onto a dialysis membrane (9) after agarose gel electrophoresis. It was labeled by nick-translation (10) with [^{32}P]dATP (3,000 Ci/mmol; 1 Ci = 37 GBq; Amersham) in the presence of unlabeled dCTP/dGTP/dTTP to a specific activity of approximately 2 × 10^8 cpm/μg and was thereafter used in hybridization studies to screen a human cDNA library.

Preparation and Screening of a Human cDNA Library. The preparation of the complete human cDNA library and the sublibraries containing different size cDNA inserts is described in detail elsewhere (11). Plasmid DNA representative of the complete and sublibraries was transformed into actively growing cultures of *E. coli* SF8 (OD_{600} = 0.6) by the method of Lerach *et al.* (12). Transformed colonies were selected by growth overnight at 37°C on Penassay base agar (Difco Laboratories) containing ampicillin at 25–50 μg/ml (Sigma) in 400-cm^2 Petri dishes (Nunc Bio-assay plates) at one dish per transformation. Approximately 300,000 transformants per library were grown and replica plated twice with velvet. *E. coli* SF8 containing the cloned HPRT fragment (p6B2aE2) was plated as a positive control.

The colonies on the replica plates were transferred to Whatman 541 paper, amplified on chloramphenicol plates overnight at 37°C by the method of Gergen *et al.* (13), denatured and fixed to the filters, and screened for HPRT cDNA sequences by hybridization with the gel-purified insert from pBR1.5 containing the HPRT fragment free of repeat sequences. The filter replicas of the random colony arrays from each library were prehybridized for 4 hr at 60°C in 30% formamide/0.5 M NaCl/20 mM Hepes, pH 7.4/0.1 mM EDTA/10 mM NaH_2PO_4/10 mM $Na_4P_2O_7$ containing denatured and sonicated herring sperm DNA at 500 μg/ml (buffer A) and then hybridized to the ^{32}P-labeled nick-translated pBR1.5 insert fragment overnight at 60°C in buffer A/10% dextran sulfate. The filter sheets were

---

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: HPRT, hypoxanthine phosphoribosyltransferase; kb, kilobase(s); HAT, hypoxanthine/aminopterin/thymidine; bp, base pair(s).

then washed twice with 0.30 M NaCl/0.030 M Na citrate, pH 7.4, and then with 60 mM NaCl/6 mM Na citrate, pH 7.4, once at 60°C and twice at room temperature. Filters were dried and autoradiographed on flashed Kodak XAR-5 film with an intensifying screen at -70°C.

Areas showing a putative positive signal on duplicate filters were identified, scraped with a toothpick onto 1 ml of Penassay broth, plated at various concentrations from the broth, and grown overnight. Single colonies were transferred with a toothpick into arrays, grown overnight, and rescreened on Whatman 541 paper as described above.

DNA "minipreps" (14) were prepared from apparently positive colonies derived from the one original positive area from the transformation with sublibrary 4 (11), which contains inserts of 1.5–2.0 kb of DNA. The cDNA inserts were screened with ^{32}P-labeled nick-translated purified insert DNA from pBR1.5 by blotting agarose gels containing whole DNA or *Bam*HI digests onto nitrocellulose according to Southern (15), treatment with HCl (16), and hybridization as described above for the cDNA library, with the addition of 5-fold concentrated Denhardt's additives (17) to the prehybridization and hybridization buffers.

All manipulations involving recombinant DNA were carried out in accordance with National Institutes of Health and institutional guidelines and requirements.

Transfection of Enzyme-Deficient Cells. The isolated full-length cDNA plasmid was introduced into enzyme-deficient mouse LA9 cells grown in Dulbecco's modified Eagle's medium/10% calf serum by calcium phosphate-mediated transfection as described (4, 18), and the resulting HPRT-positive cells were selected by growth in hypoxanthine/aminopterin/thymidine (HAT) selective medium (19). Colonies were picked through cloning cylinders and grown in the selective medium.

Gel Assay for HPRT Enzyme Activity. After isoelectric focusing in polyacrylamide gels, cytoplasmic extracts of transfected and control cells were assayed for HPRT activity by the method of Johnson *et al.* (20).

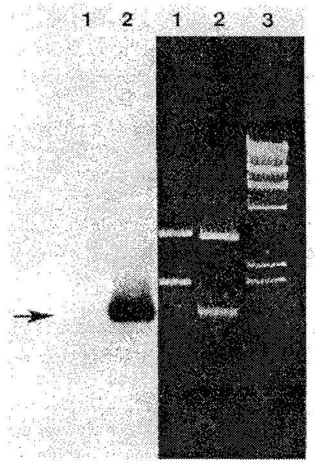

FIG. 1. Structure of the HPRT cDNA plasmid (p4aA8). Purified plasmid DNA was digested to completion with *Bam*HI, electrophoresed on a 1% agarose gel, stained, and photographed, and the DNA was blotted onto nitrocellulose paper. The paper was hybridized to the ^{32}P-labeled insert from pBR1.5 in 10% formamide/0.5 M NaCl/0.1 mM EDTA/50 mM Hepes, pH 7.4, at 60°C, washed, and exposed to x-ray film. (*Left*) X-ray film of the hybridized blot. (*Right*) Photograph of the ethidium bromide-stained gel. Lanes: 1, p4aB8 (a randomly picked control plasmid); 2, p4aA8 (the HPRT cDNA plasmid); 3, *Hin*dIII-digested bacteriophage markers (from top to bottom): 23.6, 9.6, 6.6, 4.3, 2.3, 2.0, and 0.55 kb (29). Arrow, the heavily hybridizing insert fragment from the HPRT cDNA plasmid.

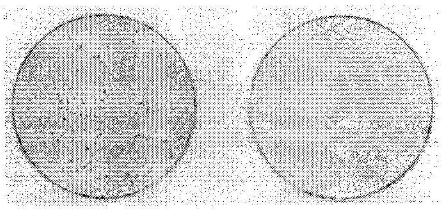

FIG. 2. Transfection of mouse LA9 cells with the HPRT clone p4aA8 (*Left*) and a control clone, p4aB8 (*Right*). Approximately 10^6 HPRT-deficient LA9 cells per plate were transfected with 1 μg of plasmid together with 20 μg of LA9 carrier DNA, grown in HAT medium after transfection, and stained with Giemsa.

Cloning of p4aA8 cDNA Fragments into M13. Plasmid p4aA8 was treated with *Bam*HI and electrophoresed on a preparative 1% agarose gel in Tris acetate buffer (21). Subclones in M13 mp8 were generated by the method of P. Deininger (personal communication). Briefly, the 1.6-kb *Bam*HI fragment from p4aA8 was electroeluted (9), ligated into a multimer, and sonicated to give random shear fragments of 200–800 base pairs (bp), which were ligated into the *Sma* I-cut phosphatase-treated M13 mp8 vector. The ligation mixture was used to transfect *E. coli* JM 101 (22).

DNA Sequence Analyses. Single-stranded template was prepared from white M13 plaques and used for sequence determination by the dideoxy chain-termination methods of Messing *et al.* (22) and Sanger *et al.* (23) using an M13 pentadecamer primer (New England BioLabs).

HPRT Amino Acid Sequence Determination. Authentic human HPRT enzyme was purified as described by Johnson *et al.* (24) and tryptic peptides were prepared and purified by HPLC (25). The amino acid sequences of several of the peptides were determined by microanalysis using a modified spinning cup method (26–28).

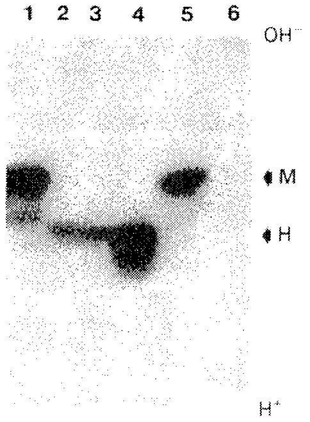

FIG. 3. Gel assay for HPRT activity of transfected mouse LA9 cells. Polyacrylamide isoelectric focusing gels loaded with cell lysates from different cell types were assayed for enzyme activity, and results were detected by autoradiography. OH$^-$ and H$^+$, alkaline and acid ends of the gel; M and H, major mouse and human HPRT activities. (Both human and mouse HPRT also display a minor band of activity at a lower pI.) Lanes: 1, 3T6 mouse cells; 2, a line of cells derived from LA9 cells transfected to HAT resistance with p4aA8 and making HPRT that has human isoelectric focusing properties; 3, as in lane 2 but a separately derived cell line; 4, HeLa (human) cells; 5, 3T6 mouse cells; 6, LA9 HPRT$^-$ cells, the parent of the cell lines in lanes 2 and 3.

RESULTS

Approximately 300,000 colonies of the cDNA library were transferred from nutrient-containing plates to filters and hybridized with the nick-translated insert from plasmid pBR1.5, isolated previously (4), thought to contain a portion of the HPRT coding sequence and some HPRT intervening sequence. One reproducibly hybridizing colony was found on duplicate filters, and it was picked, colony purified, and grown to prepare large

```
G G G G G G G G G G G G G G T C T T G C T G C G C C T C C G C C T C C T C C T C T G C T C C G C C A C C G G C T T C C T
                  10                  20                  30                  40                  50                  60
                                                                              met   ala   thr   arg   ser   pro   gly
C C T C C T G A G C A G T C A G C C C G C G C G C C G G C C G G C T C C G T T A T G G C G A C C C G C A G C C C T G G C
                  70                  80                  90                 100                 110                 120
val   val   ile   ser   asp   asp   glu   pro   gly   tyr   asp   leu   asp   leu   phe   cys   ile   pro   asn   his
G T C G T G A T T A G T G A T G A T G A A C C A G G T T A T G A C C T T G A T T T A T T T T G C A T A C C T A A T C A T
                 130                 140                 150                 160                 170                 180
tyr   ala   glu   asp   leu   glu   arg   (val  phe   ile   pro   his   gly   leu   ile)  met   (asp  arg   thr   glu
T A T G C T G A G G A T T T G G A A A G G G T G T T T A T T C C T C A T G G A C T A A T T A T G G A C A G G A C T G A A
                 190                 200                 210                 220                 230                 240
arg   leu   ala   arg   asp   val   met)  lys   glu   met   gly   gly   his   his   ile   val   ala   leu   cys   val
C G T C T T G C T C G A G A T G T G A T G A A G G A G A T G G G A G G C C A T C A C A T T G T A G C C C T C T G T G T G
                 250                 260                 270                 280                 290                 300
leu   lys   gly   gly   tyr   lys   phe   phe   ala   asp   leu   leu   asp   tyr   ile   lys   ala   leu   asn   arg
C T C A A G G G G G G C T A T A A A T T C T T T G C T G A C C T G C T G G A T T A C A T C A A A G C A C T G A A T A G A
                 310                 320                 330                 340                 350                 360
asn   ser   asp   arg   ser   ile   pro   met   thr   val   asp   phe   ile   arg   leu   lys   (ser  tyr   cys   asn
A A T A G T G A T A G A T C C A T T C C T A T G A C T G T A G A T T T T A T C A G A C T G A A G A G C T A T T G T A A T
                 370                 380                 390                 400                 410                 420
asp   gln   ser   thr   gly   asp   ile   lys)  val   ile   gly   gly   asp   asp   leu   ser   thr   leu   thr   gly
G A C C A G T C A A C A G G G G A C A T A A A A G T A A T T G G T G G A G A T G A T C T C T C A A C T T T A A C T G G A
                 430                 440                 450                 460                 470                 480
lys   (asn  val   leu   ile   val   glu   asp   ile   ile   asp   thr   gly   lys)  thr   met   gln   thr   leu   leu
A A G A A T G T C T T G A T T G T G G A A G A T A T A A T T G A C A C T G G C A A A A C A A T G C A G A C T T T G C T T
                 490                 500                 510                 520                 530                 540
ser   leu   val   arg   gln   tyr   asn   pro   lys   met   val   lys   val   ala   ser   leu   leu   val   lys   arg
T C C T T G G T C A G G C A G T A T A A T C C A A A G A T G G T C A A G G T C G C A A G C T T G C T G G T G A A A A G G
                 550                 560                 570                 580                 590                 600
thr   pro   arg   ser   val   gly   tyr   lys   pro   asp   phe   val   gly   phe   glu   ile   pro   asp   lys   phe
A C C C C A C G A A G T G T T G G A T A T A A G C C A G A C T T T G T T G G A T T T G A A A T T C C A G A C A A G T T T
                 610                 620                 630                 640                 650                 660
val   val   gly   tyr   ala   leu   asp   tyr   asn   glu   tyr   phe   arg   (asp  leu   asn   his   val   cys   val
G T T G T A G G A T A T G C C C T T G A C T A T A A T G A A T A C T T C A G G G A T T T G A A T C A T G T T T G T G T C
                 670                 680                 690                 700                 710                 720
ile   ser   glu   thr   gly   lys   ala   lys)  tyr   lys   ala   ***
A T T A G T G A A A C T G G A A A A G C A A A A T A C A A A G C C T A A G A T G A G A G T T C A A G T T G A G T T T G G
                 730                 740                 750                 760                 770                 780

A A A C A T C T G G A G T C C T A T T G A C A T C G C C A G T A A A A T T A T C A A T G T T C T A G T T C T G T G G C C
                 790                 800                 810                 820                 830                 840

A T C T G C T T A G T A G A G C T T T T T G C A T G T A T C T T C T A A G A A T T T T A T C T G T T T T G T A C T T T A
                 850                 860                 870                 880                 890                 900

G A A A T G T C A G T T G C T G C A T T C C T A A A C T G T T T A T T T G C A C T A T G A G C C T A T A G A C T A T C A
                 910                 920                 930                 940                 950                 960

G T T C C C T T T G G G C G G A T T G T T G T T T A A C T T G T A A A T G A A A A A A T T C T C T T A A A C C A C A G C
                 970                 980                 990                1000                1010                1020

A C T A T T G A G T G A A A C A T T G A A C T C A T A T C T G T A A G A A A T A A A G A G A A G A T A T A T T A G T T T
                1030                1040                1050                1060                1070                1080

T T T A A T T G G T A T T T T A A T T T T T A T A T A T G C A G G A A A G A A T A G A A G T G A T T G A A T A T T G T T
                1090                1100                1110                1120                1130                1140

A A T T A T A C C A C C G T G T G T T A G A A A A G T A A G A A G C A G T C A A T T T T C A C A T C A A A G A C A G C A
                1150                1160                1170                1180                1190                1200

T C T A A G A A G T T T T G T T C T G T C C T G G A A T T A T T T T A G T A G T G T T T C A G T A A T G T T G A C T G T
                1210                1220                1230                1240                1250                1260

A T T T T C C A A C T T G T T C A A A T T A T T A C C A G T G A A T C T T T G T C A G C A G T T C C C T T T T A A A T G
                1270                1280                1290                1300                1310                1320

C A A A T C A A T A A A T T C C C A A A A A T T T A A A A A A A A A A A
                1330                1340                1350
```

FIG. 4. Nucleotide sequence of human HPRT cDNA and inferred amino acid sequence. The cDNA sequence was determined by dideoxy sequence analysis of M13 mp8 recombinant clones. Fifty-three independent cloned fragments having a random distribution within the cDNA were analyzed and their sequences were assembled according to their overlapping regions. All of the DNA sequence was confirmed on independent clones and, for about 85% of the cDNA fragment, both strands of the sequence were analyzed. Verified amino acid sequences of isolated peptides from purified human HPRT are shown in parentheses. We have assumed that translation begins at the first available AUG codon (at base 86) and terminates at the first chain-termination codon in the single open reading frame (i.e., at the UAA codon marked by asterisks at base 740). A potential polyadenylylation site, A-A-T-A-A-A, occurs at base 1,334.

amounts of plasmid DNA. Digestion of this purified plasmid (p4aA8) DNA with *Bam*HI yielded an insert of approximately 1.6 kb (Fig. 1). The cloning vector has two *Bam*HI sites that flank the cloned cDNA segment. If the cloned cDNA segment does not contain a *Bam*HI restriction site, digestion with *Bam*HI will excise the complete cDNA sequence flanked at the 5′ end by about 100 bp derived from simian virus 40 DNA and the G·C joint and at the 3′ end by 100–200 bp containing the A·T joint and simian virus 40 DNA.

Transfection of mouse HPRT-deficient LA9 cells with the HPRT cDNA p4aA8 resulted in the appearance of HAT-resistant colonies at a frequency of approximately 1 per 10^3 cells when 1 μg of plasmid and LA9 carrier DNA was used (Fig. 2). Transfection of these cells with a control plasmid picked at random from the library (p4aB8) failed to produce any HAT-resistant colonies. Lysates from two HAT-resistant colonies were tested for HPRT activity by an isoelectric focusing gel assay (Fig. 3). Both colonies contained enzyme activities that cofocused with the major band of authentic human HeLa marker enzyme, as opposed to the major and minor bands of enzyme activity derived from mouse 3T6 cells.

Fragments of the isolated 1.6-kb insert were cloned into M13 mp8 and their sequences were determined. The nucleotide sequence of the insert spanning the G·C to the A·T tailing regions is presented in Fig. 4 together with the inferred amino acid sequence of the encoded polypeptide and the tryptic and cyanogen bromide peptides whose sequences were determined independently. In the five peptides (a total of 56 amino acids), there are no disagreements between the amino acid sequences and the nucleotide sequences determined.

DISCUSSION

Three important prerequisites were satisfied for the successful cloning of full-length human HPRT cDNA. First, a probe containing HPRT coding sequence was isolated by a combination of transfection of human DNA into enzyme-deficient mouse cells and identification of the human sequences in such transfected cells by their hybridization to human repetitive (*Alu*) sequences or to total human DNA. Second, a cDNA library, preferably one likely to contain a full-length cDNA segment was prepared by using the procedure outlined previously (30), which has been further developed to allow expression of full-length cDNA sequences in mammalian cells (11). The simian virus 40–pBR322-based cloning vector contains a simian virus 40 early region promoter and intron 5′ to the cDNA segment and a polyadenylation signal 3′ to the cDNA segment. These permit the expression of inserted cDNA sequences. In this instance, they permitted a direct test of the function of putative HPRT cDNA sequences in transfection assays and enabled us to identify and confirm full-length HPRT sequences. Third, it happened that pBR1.5 hybridizes to the 5′ end of the cDNA clone (this was shown by hybridization to M13 subclones) and hence its use as a hybridization probe selectively detected full-length or nearly full-length cDNA clones. This correlates with the observation that mouse sequences exist in this subcloned plasmid and at one end of the original genomic clone (p6B2aE2) and, hence, implies that the site of 5′ linkage of the exogenous human HPRT gene to mouse sequences is cloned in these plasmids.

When individual size classes of the cDNA library were hybridized with a fragment between positions 142 and 1,221 in the nearly full-length HPRT cDNA (Fig. 4), additional positive clones were found in the various sublibraries. The frequency of HPRT-positive clones in the library was about 2×10^{-5} or equivalent to approximately 2–5 copies of HPRT mRNA per cell. We estimate the frequency of full-length cDNA clones relative to the total number of positive clones in the complete library to be about 5%.

The sequence of the cDNA insert between the G·C and A·T linkers has been fully determined. The cDNA sequence is just under 1,350 nucleotides long, in good agreement with the size (approximately 1,600 bp) predicted from the RNA blots hybridized to pBR1.5 (4). It is not known whether some 5′ sequences are missing in the cDNA clone, but the 85-bp 5′-nontranslated region is in the usual size range for eukaryotic mRNA leader sequences. The coding sequence occupies 654 bp, slightly less than half of the full length of the cDNA, leaving a 3′-noncoding tail of approximately 600 nucleotides. The organization is typical of eukaryotic mRNA sequences, in which the major noncoding region is usually 3′ to the coding sequences (31).

The single open frame encodes a polypeptide 218 amino acids long, indicating a molecular weight of approximately 24,600. Post-translational removal of the NH_2-terminal methionine would result in a protein 217 amino acids long, with a molecular weight of 24,450. The purified human HPRT enzyme has been estimated to have a molecular weight of 24,000–26,000 (32, 33). In addition, the partial amino acid composition of the purified enzyme is in complete agreement with our inferred amino acid composition. Most impressively, the inferred amino acid sequence is in complete agreement with the full amino acid sequence of the human erythrocyte HPRT enzyme recently determined by Wilson *et al.* (34). This sequence indicates that the NH_2-terminal methionine is indeed cleaved in the fully processed mature enzyme.

The protein has no obvious large hydrophobic or hydrophilic domains but instead has small alternating regions of hydrophobic and hydrophilic regions, suggesting a compact and globular structure. We predict that human HPRT is not a membrane constituent.

Caskey and co-workers (35, 36) have used a different approach in their isolation of a cDNA clone of the mouse HPRT gene, taking advantage of cells overproducing HPRT (35, 36). They have determined the sequence of mouse HPRT cDNA, and comparison of the inferred mouse and human amino acid sequences shows only eight differences, and the lengths of the polypeptides are identical.

We expect that this cDNA clone will be useful for studying the organization of normal and mutant human HPRT genes, for examining the mechanisms of X-chromosome inactivation, for studying the fate and expression of HPRT cDNA in transfected HPRT-deficient mouse and human cells, and for examining the distribution and expression of HPRT sequences in mice after introduction of the gene into the male pronucleus of fertilized mouse eggs.

T.F. thanks Dr. Wm. Nyhan for his interest and support. We thank Dorothy Miller for her administrative assistance during this work. This work was supported by grants from the Kroc Foundation, Santa Ynez, CA, and the Leon Gould Foundation, Tamarac, FL, and by National Institutes of Health Grants GM28223 and 1 RP1 CA31928-01. D.F. was supported by Public Health Service Fellowship Training Grant CA09290.

1. Caskey, C. T. & Kruh, G. C. (1979) *Cell* 16, 1–9.
2. Becker, M. & Seegmiller, J. (1975) *Arthritis Rheum.* 18, 687–694.
3. Seegmiller, J. E., Rosenbloom, F. J. & Kelley, W. N. (1967) *Science* 155, 1682–1684.
4. Jolly, D. J., Esty, A. C., Bernard, H. U. & Friedmann, T. (1982) *Proc. Natl. Acad. Sci. USA* 79, 5038–5041.
5. Clewell, D. B. & Helinski, D. R. (1969) *Proc. Natl. Acad. Sci. USA* 62, 1159–1166.

6. Clewell, D. B. (1972) *J. Bacteriol.* **110,** 667–676.
7. Cameron, J. R., Panasenko, S. M., Lehman, I. R. & Davis, R. W. (1974) *Proc. Natl. Acad. Sci. USA* **72,** 3416–3421.
8. Radloff, R., Bauer, W. & Vinograd, J. (1967) *Proc. Natl. Acad. Sci. USA* **57,** 1514–1521.
9. Yang, R. C. A., Lis, J. & Wu, R. (1979) *Methods Enzymol.* **68,** 176–182.
10. Maniatis, T., Jeffrey, A. & Kleid, D. G. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1184–1188.
11. Okayama, H. & Berg, P. (1982) *Mol. Cell. Biol.,* in press.
12. Lerach, H., Frischhauf, A. M., Hanahan, D., Wozney, J., Fuller, F., Crkvenjakov, R., Boedtker, H. & Doty, P. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5417–5421.
13. Gergen, J. P., Stern, R. H. & Wensink, P. C. (1979) *Nucleic Acids Res.* **7,** 2115–2136.
14. Holmes, D. & Quigley, M. (1981) *Anal. Biochem.* **114,** 193–198.
15. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
16. Wahl, G. M., Stern, J. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3683–3687.
17. Denhardt, D. T. (1966) *Biochem. Biophys. Res. Commun.* **23,** 641–646.
18. Wigler, M., Pellicer, A., Silverstein, S. & Axel, R. (1979) *Cell* **14,** 725–731.
19. Littlefield, J. E. (1966) *Exp. Cell Res.* **41,** 190–196.
20. Johnson, G. G., Eisenberg, L. R. & Migeon, B. R. (1979) *Science* **203,** 174–176.
21. Hayward, G. S. & Smith, M. G. (1972) *J. Mol. Biol.* **63,** 383–395.
22. Messing, J., Crea, R. & Seeburg, P. H. (1981) *Nucleic Acids Res.* **9,** 309–321.
23. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
24. Johnson, G. G., Kamage, A. L., Littlefield, J. W. & Kazazian, H. H., Jr. (1982) *Biochemistry* **21,** 960–966.
25. Bohlen, P., Brazeau, T., Sesch, F., Ling, N. & Guillemin, R. (1981) *Regul. Peptides* **2,** 359–369.
26. Wittmann-Liebold, B. (1973) *Hoppe Seiler's Z. Physiol. Chem.* **354,** 1415–1431.
27. Hunkapiller, M. W. & Hood, L. E. (1978) *Biochemistry* **17,** 2124–2133.
28. Shively, J. E. (1981) *Methods Enzymol.* **79,** 31–48.
29. Philipsen, P., Kramer, R. A. & Davis, R. W. (1978) *J. Mol. Biol.* **123,** 371–386.
30. Okayama, H. & Berg, P. (1982) *Mol. Cell. Biol.* **2,** 161–170.
31. Breathnach, R. & Chambon, P. (1981) *Annu. Rev. Biochem.* **50,** 349–383.
32. Olsen, A. S. & Milman, G. (1977) *Biochemistry* **16,** 2501–2505.
33. Holden, J. A. & Kelley, W. N. (1978) *J. Biol. Chem.* **253,** 4459–4463.
34. Wilson, J. M., Tarr, G. E., Mahoney, W. C. & Kelley, W. N. (1982) *J. Biol. Chem.* **257,** 10978–10985.
35. Melton, D. W., Konechi, D. S., Ledbetter, D. M., Hejtmancik, J. F. & Caskey, C. T. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 6977–6980.
36. Brennand, J., Chinault, A. C., Konechi, D. S., Melton, C. W. & Caskey, C. T. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 1950–1956.