Volume 10 Number 21 1982 Nucleic Acids Research

Hypoxanthine-guanine phosphoribosyltransferase genes of mouse and Chinese hamster: construction and sequence analysis of cDNA recombinants

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Received 4 August 1982; Accepted 1 October 1982

ABSTRACT

Recombinant plasmids containing DNA inserts complementary to mRNA coding for hypoxanthine-guanine phosphoribosyltransferase (HPRT) from mouse and Chinese hamster cell lines have been isolated from cDNA libraries and characterized by DNA sequence analysis. A total of 1292 nucleotides of the mouse cDNA sequence and 1301 nucleotides of the Chinese hamster cDNA sequence has been determined. Each of these sequences includes an open reading frame of 654 nucleotides (218 amino acids) corresponding to the HPRT protein coding region. The deduced amino acid sequences for the mouse and Chinese hamster enzymes are presented and compared to that of human HPRT. At least 95% of the amino acids are conserved in the three species. In addition, we present evidence that two species of HPRT mRNA, which differ in the site of polyadenylation that is utilized during processing of the RNA transcripts, exist in Chinese hamster cells.

INTRODUCTION

The elucidation of mutational events responsible for alterations in the expression of eucaryotic genes has now become possible through the use of molecular cloning and DNA sequencing techniques. In mammalian cells the hypoxanthine-guanine phosphoribosyltransferase (HPRT, EC 2.4.2.8) gene has been extensively used in mutation studies, primarily because it is a functionally haploid locus due to its location on the X-chromosome and because powerful selection techniques allow the isolation of mutants and revertants (1,2). In addition to genetic analyses, a variety of biochemical techniques have been applied to the study of induced and spontaneous mutations at this locus. These studies have focused on characterization of electrophoretic, immunological, kinetic and thermal sensitivity properties of the enzyme product, as well as on tryptic peptide analysis (3-12). The examination of mutational changes in the HPRT gene at the nucleic acid level has, until recently, been impossible due to the lack of cloned probes.

We have previously reported the isolation of partial cDNA recombinants complementary to HPRT mRNA from a mouse cell line which was found to overpro-

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duce HPRT mRNA by virtue of having amplified the HPRT gene (13,14). This report describes the construction of cDNA recombinants containing the full coding sequence for mouse HPRT and the isolation of similar sequences complementary to mRNA from a Chinese hamster cell line possessing amplified HPRT genomic sequences. These recombinants have been characterized by DNA sequence analysis. A high degree of homology is found between the mouse and hamster nucleotide sequences, particularly within the protein coding regions. The sequence analysis of Chinese hamster cDNA recombinants provides evidence that two species of mRNA, which differ in their site of polyadenylation, are present in hamster cells. The data also allow the prediction of the complete amino acid sequences for the mouse and hamster HPRT proteins.

MATERIALS AND METHODS

Purification of mRNA and preparation of double-stranded cDNA

Total cellular RNA was isolated from the mouse neuroblastoma cell line, NBR4 (14,15), and the V79 Chinese hamster cell line RJK159 (8) by guanidinium/HCl extraction (16). mRNA was partially purified by two passages through oligo dT cellulose (Collaborative Research) and recovered by ethanol precipitation (17).

Single stranded cDNA was synthesized from mRNA in a reaction mixture containing 50-100 μg/ml mRNA, 50 mM Tris/HCl pH 8.3, 40 mM KCl, 10 mM $MgCl_2$, 20 mM β-mercaptoethanol, 1 mM each of dATP, dCTP, dGTP and dTTP, 20-50 μg/ml oligo dT_{12-18} (Collaborative Research) and 1000 units/ml of AMV reverse transcriptase (Life Sciences). After 1 hr at 46° the reaction mixture was extracted with phenol and the mRNA was hydrolyzed with 0.1N KOH at 67°C for 1 hr. Following neutralization, Sephadex G50 chromatography and lyophilization, single-stranded cDNA was made double-stranded in a reaction mixture containing 10-50 μg/ml cDNA, 50 mM Tris/HCl pH 8.3, 10 mM $MgCl_2$, 20 mM β-mercaptoethanol, 1 mM each of dATP, dCTP, dGTP and dTTP and 1000 units/ml of AMV reverse transcriptase at 46°C for 3 hr. cDNA was recovered as described above. Blunt-ended double-stranded cDNA was then prepared by digestion with 1000 units/ml of Aspergillus oryzae S1 endonuclease (Miles) in a reaction mixture containing 300 mM NaCl, 4.5 mM Zn acetate and 30 mM Na acetate pH 4.5 and recovered as before.

Construction of DNA recombinants

Double-stranded cDNA molecules between 1000 and 2000 base pairs (bp) in length were isolated by electroelution (18) following fractionation by agarose gel electrophoresis. These fragments were "tailed" (19) with 10-20 cytosine

residues/3' terminus in 200 mM potassium cacodylate pH 7.2, 1 mM $CoCl_2$, 100 μCi ^{32}P-dCTP (2-3000 Ci/mMole), 5 μM dCTP, 1mM β-mercaptoethanol and 200 units/ml terminal deoxynucleotidyltransferase (Bethesda Research Laboratories) at 30° for approximately 2 min. pBR322 was digested with PstI and "tailed" with 10-20 guanine residues/3' terminus as described above, substituting dGTP for dCTP. The two DNAs were annealed in a molar ratio of 1:1 at a final DNA concentration of 1 μg/ml in 10 mM Tris/HCl pH 7.6, 200 mM NaCl, 1 mM NaEDTA at 65°C for 5 min followed by incubation at 42°C for 2 hr and gradual cooling to room temperature overnight. Annealed plasmids were precipitated with ethanol in the presence of 20 μg/ml E. coli tRNA as carrier and used to transform E. coli RR1 cells made competent by treatment with $CaCl_2$ (20). Transformants were selected on L agar plates containing 25 μg/ml tetracycline.

Identification and Characterization of HPRT cDNA recombinants

Bacterial colonies were grown overnight, in ordered arrays, on nitrocellulose filters overlayed on selective agar plates. HPRT cDNA recombinants were identified by colony hybridization. The probe was a 230 bp Hae III fragment which was isolated from pHPT2 and labelled by nick translation (13). Plasmid DNA was isolated using a Triton X-100 lysis procedure (21) following overnight plasmid amplification in the presence of chloramphenicol and purified by banding in gradients of CsCl containing ethidium bromide. The identity of the HPRT cDNA plasmids was confirmed by comparison of restriction endonuclease cleavage maps to those of pHPT1 and 2 (13) and by DNA sequence analysis.

DNA sequence determination

Nucleotide sequences were determined using the chemical sequencing method of Maxam and Gilbert (22,23). Restriction enzymes were used under conditions recommended by the supplier (New England Biolabs) and fragments were labelled at 5' termini using T4 polynucleotide kinase (New England Nuclear) and γ-^{32}P-ATP (Amersham). The analysis of labelled products obtained following base-specific modification reactions and subsequent strand cleavage was carried out on 8% and 25% polyacrylamide gels.

RESULTS

Construction and identification of cDNA recombinants

The results of a preliminary experiment carried out to determine optimal conditions for the synthesis of full-length mouse HPRT cDNA are shown in Figure 1. Lane A shows that HPRT mRNA from the NBR4 cell line is approximately 1550 nucleotides in length. Lane B contained single-stranded cDNA

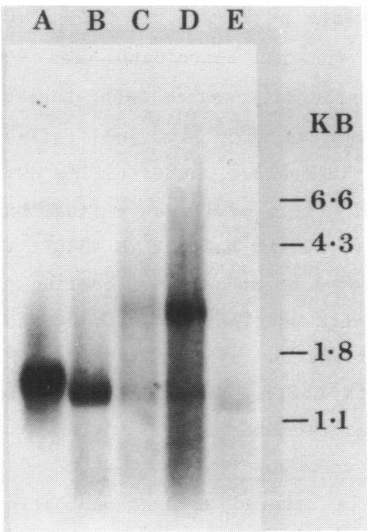

Figure 1. Gel electrophoretic analysis of HPRT mRNA and cDNA. Five µg of NBR4 mRNA was converted to double-stranded cDNA by a modification of the method of Wickens et al. (24). At each step an aliquot was removed and the products were glyoxalated (25), fractionated by agarose gel electrophoresis and transferred to nitrocellulose as described by Thomas (26). The filter was then probed with a radiolabelled cDNA fragment from pHPT2 under hybridization conditions reported previously (13). Lane A = polyA$^+$ mRNA, Lane B = single-stranded cDNA following first strand synthesis with AMV reverse transcriptase, Lanes C and D = DNA products following second strand synthesis with E. coli DNA polymerase I or AMV reverse transcriptase, respectively, and Lane E = final product obtained following treatment of double-stranded cDNA with S1 nuclease.

synthesized from NBR4 mRNA; the predominant product had a length of approximately 1375 nucleotides. The reason for the size discrepancy between HPRT mRNA and cDNA is unlikely to be due to incomplete synthesis resulting from mRNA secondary structure because identical results were obtained when cDNA was synthesized following denaturation of the mRNA by treatment with methyl mercury (data not shown). The high concentration of oligo dT primer used may have precluded extensive copying of the poly A tails on the mRNA resulting in the ostensibly shorter cDNA sequences. Both E. coli polymerase I (Lane C) and AMV reverse transcriptase (Lane D) were capable of HPRT second strand synthesis. The two strands of the cDNA were held together at this point by a hairpin loop (27) and gel electrophoresis under denaturing conditions showed a product of about 2700 nucleotides in length. Under the conditions used in this study, AMV reverse transcriptase (Lane D) was more efficient in the conversion of DNA to this size. Treatment of the double-stranded cDNA with S1 nuclease was used to cleave the loop and digest other single-stranded regions producing double-stranded HPRT cDNA of about 1300 nucleotides (Lane E). Similar results were obtained using Chinese hamster (RJK159) mRNA (data not shown).

Based on the results of the experiment shown in Figure 1, AMV reverse transcriptase was chosen for both first and second strand cDNA synthesis with mouse (NBR4) and hamster (RJK159) mRNA. cDNA libraries were then constructed and screened as described in the Materials and Methods section. Three HPRT

recombinants plasmids were identified among the 5000 recombinants constructed using mouse mRNA. These contained inserts of 500, 1200 and 1300 bp and were designated pHPT3, 4 and 5 respectively. Restriction endonuclease mapping and comparison to pHPT2 confirmed their identity as HPRT cDNA sequences. The end points of the cDNA inserts in these plasmids with respect to the mRNA sequence are shown in Figure 2. Seven HPRT recombinants were found among the 3200 recombinants in the hamster cDNA library and the two largest ones, designated pHPT11 and 12, have been characterized. Their identity as HPRT recombinants was verified by restriction endonuclease mapping and DNA sequence analysis. pHPT12 contains an insert of 1300 bp (Figure 2); the pHPT11 insert extends for an additional 338 bp at the 5' end and is 85 bp shorter at the 3' end (relative to the mRNA orientation).

Determination and analysis of nucleotide sequences

Nucleotide sequence determination was carried out by the Maxam-Gilbert method on fragments obtained either by secondary cleavage with restriction enzymes or by strand separation following 5'-end labelling (22,23). A total

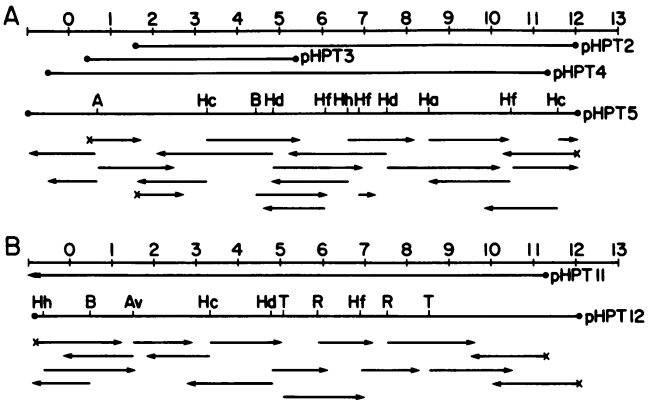

Figure 2. Partial restriction maps and DNA sequencing strategies for mouse (Panel A) and Chinese hamster (Panel B) cDNA inserts. The scale refers to nucleotide position (x 10^{-2}) relative to the predicted protein initiation codon beginning at position 0. The endpoints for cDNA inserts of the different recombinants are indicated. Restriction endonuclease cleavage sites used as labelling positions for nucleotide sequencing are indicated only on pHPT5 and pHPT12 for simplicity. The arrows beneath these maps indicate the direction and approximate extent of sequence determination for each fragment analyzed. Fragments with an x at the end were labelled at pBR322 vector sites outside the cDNA insert. The following abbreviations are used for restriction enzymes: A = AccI, Av = AvaI, B = BstNI, Ha = HaeIII, Hc = HincII, Hd = HindIII, Hf = HinfI, Hh = HhaI, R = RsaI and T = TaqI.

of 1292 nucleotides of the mouse HPRT cDNA was determined by the sequencing strategy summarized in Figure 2A. At least 70% of the sequence was determined from both strands; the remainder was obtained from at least two independent experiments using fragments labelled at different restriction sites and all sites used for end-labelling were read through from other positions. Several regions were checked by sequencing fragments from different recombinants and no sequence discrepancies resulting from cloning artifacts were found. The complete nucleotide sequence for the mouse HPRT cDNA is presented in Figure 3. This sequence includes an open reading frame extending from a methionine codon at positions 1-3 to a UAA termination codon at positions 655-657. Translation of this region to the amino acid sequence predicted by the genetic code gives a protein with the properties expected for HPRT, as discussed below. This sequence is preceded by 87 nucleotides of presumed 5' noncoding sequence and is followed by an additional 548 bases of 3' noncoding sequence and a stretch of poly A. The sequence AAUAAA, which is generally found 10-25 nucleotides before the poly A tail and is believed to be a signal for the polyadenylation of eucaryotic mRNA (28), occurs three times in the 3' noncoding region. Two of these sequences are overlapping and are located at the expected position while the third is an additional 77 bases upstream, as indicated in Figure 3.

From the two hamster cDNA recombinants, pHPT11 and pHPT12, a total of 1301 bases of nucleotide sequence has been determined. The sequencing strategy, outlined in Figure 2B, again employed either bidirectional or duplicate determinations. The hamster cDNA sequence, aligned relative to the mouse cDNA sequence to emphasize homology, is presented in Figure 3. The predicted protein coding sequence is of identical length to that for the mouse and there is 95.6% nucleotide sequence homology within this region. If gaps are introduced in the sequences as shown in Figure 3 there is approximately 80% sequence homology between the noncoding regions.

The nucleotide sequences found near the 3' end of the hamster cDNAs were particularly interesting. The sequence determined for pHPT12 contained the AAUAAA polyadenylation signal twice, in positions comparable to those found in the mouse cDNA sequence (see Figure 3). However, the sequence for pHPT11 extended only 22 bases past the first of these signals (at positions 1125-1130) before a stretch of A's was found. From these results we have tentatively concluded that there are two classes of hamster HPRT mRNA which differ in the site of addition of the poly A tails. Although this situation was not encountered in the mouse cDNA recombinants, it certainly cannot be ruled out on the basis of the limited number which have been sequenced in this

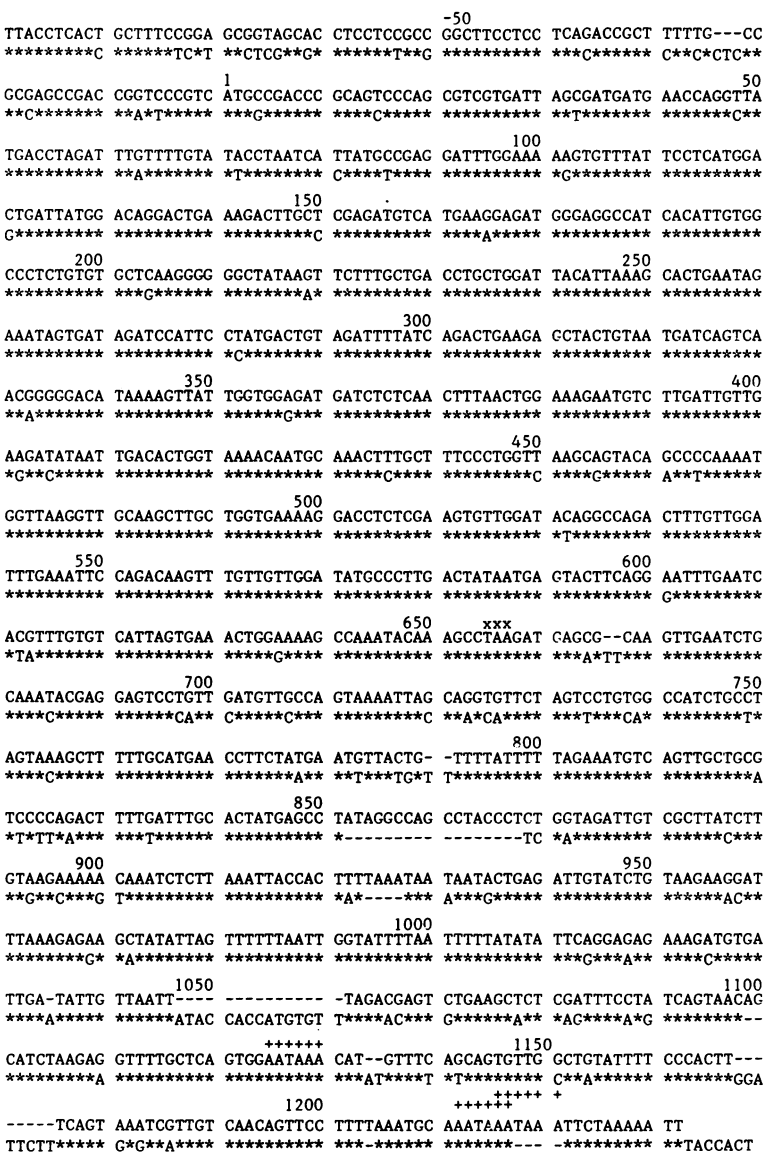

Figure 3. Complete nucleotide sequences of mouse (NBR4) and Chinese hamster (RJK159) cDNA. The top line represents the mouse DNA sequence and the bottom line represents the hamster DNA sequence, where nucleotides identical to those in the mouse sequence are indicated by asterisks. The sequences have been arbitrarily numbered such that position 1 is the first nucleotide of the methionine initiation codon for the protein coding sequence with gaps, represented by dashes, introduced to emphasize regions of sequence homology. The translational termination signal for the HPRT protein is indicated by x's and signals for polyadenylation are indicated by +'s above the sequence.

region.

Predicted amino acid sequences for mouse and hamster HPRT proteins

A single open reading frame was found in both the mouse and Chinese hamster cDNA sequences which appeared to correspond to the protein coding sequence for HPRT. This conclusion is supported by comparison of the predicted amino acid sequences to that recently determined by Wilson et al. (29) for human HPRT by protein sequencing methods (see Discussion). The complete amino acid sequences derived from this region for both the mouse (NBR4) and Chinese hamster (RJK159) proteins are shown in Figure 4. A total

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                                        10                                  20
Met Pro Thr Arg Ser Pro Ser Val Val Ile Ser Asp Asp Glu Pro Gly Tyr Asp Leu Asp
    Ala

                                        30                                  40
Leu Phe Cys Ile Pro Asn His Tyr Ala Glu Asp Leu Glu Lys Val Phe Ile Pro His Gly
                                    Val

Leu                                     50                                  60
Val Ile Met Asp Arg Thr Glu Arg Leu Ala Arg Asp Val Met Lys Glu Met Gly Gly His

                                        70                                  80
His Ile Val Ala Leu Cys Val Leu Lys Gly Gly Tyr Lys Phe Phe Ala Asp Leu Leu Asp

                                        90                                 100
Tyr Ile Lys Ala Leu Asn Arg Asn Ser Asp Arg Ser Ile Pro Met Thr Val Asp Phe Ile

                                       110                                 120
Arg Leu Lys Ser Tyr Cys Asn Asp Gln Ser Thr Gly Asp Ile Lys Val Ile Gly Gly Asp

                                       130                                 140
Asp Leu Ser Thr Leu Thr Gly Lys Asn Val Leu Ile Val Glu Asp Ile Ile Asp Thr Gly

                                       150                                 160
Lys Thr Met Gln Thr Leu Leu Ser Leu Val Lys Gln Tyr Ser Pro Lys Met Val Lys Val
                                        Arg     Asn Leu

                                       170                                 180
Ala Ser Leu Leu Val Lys Arg Thr Ser Arg Ser Val Gly Tyr Arg Pro Asp Phe Val Gly

                                       190                                 200
Phe Glu Ile Pro Asp Lys Phe Val Val Gly Tyr Ala Leu Asp Tyr Asn Glu Tyr Phe Arg

                                       210                            218
Asn                                 Val                                 Ala (UAA)
    Leu Asn His                     Ile Cys Val Ile Ser Glu Thr Gly Lys     Lys Tyr Lys
Asp
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Figure 4. Predicted amino acid sequences for mouse and Chinese hamster HPRT proteins. The sequences are identical except where two residues are shown; at these positions the amino acid above the line is that predicted by the nucleotide sequence of mouse cDNA while the residue below the line is that predicted from the Chinese hamster cDNA sequence. The amino acids are arbitrarily numbered from the methionine initiator although this residue may be absent in the mature protein (see Discussion).

of 210 of the 218 amino acids are identical and all of the differences found are a result of single base changes in the nucleotide sequences. None of the other 21 nucleotide differences found by comparing the cDNA sequences in this region affects the amino acid sequences predicted by the genetic code.

DISCUSSION

The cell lines used here for molecular cloning of rodent HPRT cDNA sequences were both obtained by reversion of HPRT mutants which show no detectable HPRT enzymic activity or production of immunologically-reactive material *in vivo* in spite of the fact that they appear to produce normal levels of HPRT mRNA (14, J.C. Fuscoe, R.G. Fenwick, Jr. and C.T. Caskey, manuscript in preparation). The NBR4 mouse cell line which arose as a revertant of NB^-, contains a thermosensitive HPRT enzyme which exhibits reduced binding properties for the substrates hypoxanthine and 5-phosphoribosyl-1-pyrophosphate (PRPP) and an altered migration in electrophoretic analyses (15). The hamster HPRT from RJK159, which arose as a spontaneous revertant of RJK10, has not been extensively characterized but does appear to have lost the high affinity antibody recognition site(s) present in wild-type protein (8). It has been proposed that NB^- and RJK10 contain altered enzymes resulting from missense mutations, and that the NBR4 and RJK159 cell lines represent phenotypic revertants which result from overproduction of variant HPRT as a result of gene amplification (30, Fuscoe *et al.*, in preparation). Although neither of the rodent HPRT sequences from the cloned cDNAs is expected to be identical to the sequences for wild type enzyme, they are not believed to have any major alterations in amino acid sequence. This conclusion is supported by comparison of the predicted rodent amino acid sequences with that of human HPRT determined by protein sequencing techniques (29). The human enzyme contains of 217 amino acids with an acetylated alanine at the amino terminus. The amino terminus of HPRT isolated from rodents has also been found to be blocked (unpublished data). Assuming that the translational products are processed in a similar way, one would predict that the total number of residues in the mouse and hamster proteins would be identical to that of human, with proline and alanine residues comprising the amino terminus of the mouse (NBR4) and Chinese hamster (RJK159) proteins respectively (see Figure 4). Based on amino acid compositions, both of the rodent proteins have a molecular weight equal to 24,500.

Table 1 summarizes all of the amino acid differences found by comparison of the predicted amino acid sequences for the NBR4 mouse HPRT, RJK159 Chinese

Table 1. Amino acid differences between HPRT proteins of Chinese hamster, mouse and man.

Position[1]	Hamster	Mouse	Human[2]
1	MET	MET	-
2	ALA	***PRO**	ALA
7	SER	SER	***GLY**
29	***VAL**	ALA	ALA
34	LYS	LYS	***ARG**
41	***VAL**	LEU	LEU
151	LYS	LYS	***ARG**
152	***ARG**	GLN	GLN
154	ASN	***SER**	ASN
155	***LEU**	PRO	PRO
169	SER	SER	***PRO**
175	ARG	ARG	***LYS**
201	ASP	***ASN**	ASP
205	***ILE**	VAL	VAL

[1]Amino acid positions correspond to those shown in Figure 4. The amino terminal residue in the human protein has been determined to be an alanine (29) which would thus correspond to position 2 by this convention. At each position noted, identical amino acids are found in 2 of the 3 proteins; the unique residue in each case is indicated by an asterisk.
[2]Data from Wilson et al. (29).

hamster HPRT and human HPRT. There is a remarkably high sequence conservation between the HPRT proteins from the different sources and many of the differences found represent conservative changes which should have little influence on the HPRT protein conformation. However, it is interesting to note that antisera raised against either native Chinese hamster or human HPRT do not exhibit cross-species reactivity (31; unpublished data). Although the precise identification of differences in the mouse and hamster sequences which result from mutations must await direct comparison with wild-type sequences, examination of the data in Table 1 can be used to predict the most likely candidates. In the hamster sequence the most dramatic differences would appear to be the presence of arginine at position 152 where both the mouse and human sequences contain a glutamine and the presence of leucine at position 155 where the other two species contain proline. The changes at the other positions shown appear to be more conservative and can be reasonably explained as differences resulting from evolutionary change when all three species are compared. For the mouse NBR4 protein sequence there are three places where differences may be most easily explained on the basis of mutation. These are at positions 2 (Pro), 154 (Ser) and 201 (Asn), where both the hamster and human sequences

show Ala, Asn and Asp, respectively. The latter difference would account for the altered electrophoretic properties of the mouse enzyme while the others would be expected to potentially affect only subunit conformation or assembly of the final multimeric enzyme. The possibility of multiple mutations cannot be ruled out at this point.

Studies on variant enzymes produced as a result of mutation at the HPRT locus have previously focused on either the examination of physical properties of the proteins or on tryptic peptide analysis. Both the results presented here and recent studies by Wilson et al. (32) indicate that the latter method has given misleading results due to experimental artifacts. For example, the number of rodent tryptic peptides terminating in arginine and lysine determined by analysis of in vivo labelled HPRT peptides resolved by high pressure ion exchange chromatography (9-11) appears to have been an underestimate. In addition, the conclusion that a methionine-containing tryptic peptide which did not possess an arginine or lysine residue represented the carboxyl terminus of the protein (9-11) must be abandoned given the predicted HPRT sequence shown in Figure 4. Thus, although the isolation of premature chain termination (PCT) mutants in the HPRT gene should be possible since approximately 30% of the codons could be mutated to termination codons by a single base change, previous identification of such mutants based on tryptic peptide analysis must now be questioned. Likewise the possibility of reversion of PCT mutations in eucaryotic cells through tRNA-mediated suppression should now be reanalyzed.

Further study will be required to completely characterize the HPRT mRNA. The cloned sequences probably do not extend to the extreme 5' end of the mRNA, even though some 90 bases preceding the apparent initiation codon are included. One of the hamster recombinants, pHPT11, appears to extend for an additional 338 bases in the 5' direction. However, preliminary S1 mapping experiments suggest that these sequences are not present in the mRNA. Additional analysis will be needed to determine whether this anomaly is a result of a cloning artifact or is, in fact, significant. On the other hand we have detected differences between two hamster recombinants at the 3' end which do appear to be significant. The results suggest that two different mRNA species are produced which vary in their site of polyadenylation due to differential recognition of RNA processing signals. A similar situation, where different mRNA species are apparently produced from the same transcript, has been found with dihydrofolate reductase mRNAs (33). S1 mapping experiments are in progress with the HPRT recombinants to examine the situation more closely to

determine if this is a general phenomenon or is a function of the particular cell line used.

The results presented in this paper should greatly facilitate the study of mutational alterations at the HPRT locus in cultured cell lines, either at the level of amino acid sequence or nucleotide sequence. In addition, the high level of interspecies sequence homology has already been exploited to identify DNA sequence polymorphisms near the HPRT locus in man (34) and studies are underway to use the cloned HPRT probes for the examination of the naturally-occurring mutations at the HPRT locus which result in gouty arthritis or Lesch-Nyhan syndrome in humans.

ACKNOWLEDGEMENTS

We are indebted to James Wilson and Dr. William Kelley for communication of results prior to publication. We also wish to thank Elsa Perez for her patient assistance in the preparation of the manuscript. J.B. was supported by a fellowship from the Arthritis Foundation and J.C.F. was supported by NIH Postdoctoral Training Grant #GM07526. This work was also supported by a grant from the Robert A. Welch Foundation (Q533) and by the Howard Hughes Medical Institute.

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REFERENCES

1. Caskey, C.T. and Kruh, G.D. (1979) Cell **16**, 1-9.
2. Fenwick, R.G., Jr., Konecki, D.S. and Caskey, C.T. (1982) in Somatic Cell Genetics, Caskey, C.T. and Robbins, D.C. Eds., pp. 19-42, Plenum Press, New York.
3. Sharp, J.D., Capecchi, N.E. and Capecchi, M.R. (1973) Proc. Natl. Acad. Sci. USA **70**, 3145-3149.
4. Fenwick, R.G., Jr. and Caskey, C.T. (1975) Cell **5**, 115-122.
5. Wahl, G.M., Hughes, S.H. and Capecchi, M.R. (1975) J. Cell Physiol. **85**, 307-320.
6. Milman, G., Lee, E., Ghangas, G.S., McLaughlin, J.R. and George, M., Jr. (1976) Proc. Natl. Acad. Sci. USA **73**, 4589-4593.
7. Fenwick, R.G., Jr., Sawyer, T.H., Kruh, G.D., Astrin, K.H. and Caskey, C.T. (1977) Cell **12**, 383-391.
8. Fenwick, R.G., Jr., Wasmuth, J.J. and Caskey, C.T. (1977) Somatic Cell Genet. **3**, 207-216.
9. Milman, G., Krauss, S.W. and Olsen, A.S. (1977) Proc. Natl. Acad. Sci. USA **74**, 926-930.
10. Capecchi, M.R., Vonder Haar, R.A., Capecchi, N.E. and Sveda, M.M. (1977) Cell **12**, 371-381.
11. Kruh, G.D., Fenwick, R.G., Jr. and Caskey, C.T. (1981) J. Biol. Chem. **256**, 2878-2886.
12. Wilson, J.M., Baugher, B.W., Landa, L. and Kelley, W.N. (1981) J. Biol. Chem. **256**, 10306-10312.

13. Brennand, J., Chinault, A.C., Konecki, D., Melton, D.W. and Caskey, C.T. (1982) Proc. Natl. Acad. Sci. USA **79**, 1950-1954.
14. Melton, D.W., Konecki, D.S., Ledbetter, D.H., Hejtmancik, J.F. and Caskey, C.T. (1981) Proc. Natl. Acad. Sci. USA **78**, 6977-6980.
15. Melton, D.W. (1981) Somatic Cell Genet. **7**, 331-344.
16. Przybla, A.E. MacDonald, R.J., Harding, J.D., Pictet, R.L. and Rutter, W.J. (1979) J. Biol. Chem. **254**, 2154-2159.
17. Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA **69**, 1408-1412.
18. Smith, H.O. (1980) Methods Enzymol. **65**, 371-380.
19. Nelson, T. and Brutlag, D. (1979) Methods Enzymol. **68**, 41-50.
20. Dagert, M. and Ehrlich, S.D. (1979) Gene **6**, 23-28.
21. Shepard, H.M. and Polisky, B. (1979) Methods Enzymol. **68**, 503-513.
22. Maxam, A.M. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA **74**, 560-564.
23. Maxam, A.M. and Gilbert, W. (1980) Methods Enzymol. **65**, 499-560.
24. Wickens, M.P., Buell, G.N., and Schimke, R.T. (1978) J. Biol. Chem. **253**, 2483-2495.
25. Carmichael G.G. and McMaster, G.K. (1980) Methods Enzymol. **65**, 380-391.
26. Thomas, P.S. (1980) Proc. Natl. Acad. Sci. USA **77**, 5201-5205.
27. Efstratiadis, A., Kafatos, F.C., Maxam, A.M. and Maniatis, T. (1976) Cell **7**, 279-288.
28. Proudfoot, N.J. and Brownlee, G.G. (1976) Nature **263**, 211-214.
29. Wilson, J.M., Tarr, G.E., Mahoney, W.C. and Kelley, W.N. (1982) J. Biol. Chem., **in press.**
30. Melton, D.W., Konecki, D.S., Ledbetter, D.H., Brennand, J., Chinault, A.C. and Caskey, C.T. (1981) in Gene Amplification, Schimke, R.T. and McElheny, V.K. Eds., pp 59-65, Cold Spring Harbor, New York.
31. Beaudet, A.L., Roufa, D.J. and Caskey, C.T. (1973) Proc. Natl. Acad. Sci. USA. **70**, 320-324.
32. Wilson, J.M., Landa, L.E. and Kelley, W.N. (1982), **submitted.**
33. Setzer, D.R., McGrogan, M., Nunberg, J.H. and Schimke, R.T. (1980) Cell **22**, 361-370.
34. Nussbaum, R.L., Caskey, C.T., Gilbert, F. and Nyhan, W. (1982) Proceedings of the IVth International Symposium on Human Purine and Pyrimidine Metabolism, De Bruyn, C.H.M.M., Ed., Plenum Press, New York, **in press.**