The organization of the human HPRT gene

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ABSTRACT

The organization of the X-linked gene for human hypoxanthine phosphoribosyltransferase (HPRT, EC 2.4.2.8.) has been determined by a combination of restriction endonuclease mapping, heteroduplex analysis and DNA sequence analysis of overlapping genomic clones. The entire gene is 42 kilobases in length and split into 9 exons. The sizes of the 7 internal exons and the exon-intron boundaries are identical to those of mouse HPRT gene. The 5' end of the gene lacks the prototypical 5' transcriptional regulatory sequence elements but contains extremely GC-rich sequences and five GC hexanucleotide motifs (5'-GGCGGG-3'). These structural features are very similar to those found in the mouse HPRT gene and to some of the regulatory signals common to a class of constitutively expressed "housekeeping" genes. Several transcriptional start sites have been identified by nuclease protection studies. Extensive sequence homology between the mouse and human genes is found in the 3' non-coding portion of the gene.

INTRODUCTION

Purine biosynthesis in higher eukaryotic cells occurs by two major pathways -- a de novo pathway and a salvage or reutilization pathway. In the latter, the enzyme HPRT catalyzes the conversion of preformed bases guanine and hypoxanthine to guanylic and inosinic acids in the presence of phosphoribosyl pyrophosphate (PRPP). The rate of purine synthesis is regulated partly through inhibition of the rate-limiting first step by the end products adenylic and guanylic acids in the de novo pathway. The disruption of the normal balanced regulation of the two pathways due to partial deficiency of salvage biosynthesis leads to excessive production of uric acid and to clinical gout, while complete enzyme deficiency also leads to the severe and bizarre central nervous system (CNS) defects found in the Lesch-Nyhan syndrome (1,2). Although some of the biochemical mechanisms responsible for the overproduction of uric acid are reasonably well understood, and despite the fact that this metabolic defect can be well treated

with the drug allopurinol, the molecular and biochemical basis of the neurological dysfunction in the CNS remain obscure and no effective treatment for the neurological symptoms is available. For these reasons, an understanding of the mechanisms regulating HPRT gene expression is essential and useful not only to clarify the molecular basis of neurological dysfunction but also to develop a model system for the study of genetic manipulation of human disease.

To initiate the molecular characterization of the cellular regulatory mechanisms of purine biosynthesis, we previously isolated and characterized a full length human HPRT cDNA (3). Full length rodent HPRT cDNAs have also been isolated independently (4,5), and the overall genomic organization of the mouse HPRT gene has been described (6). The mouse gene reveals several interesting features, including the absence of prototypical 5' transcriptional regulatory sequences such as TATA and CAAT boxes and the presence of extremely GC-rich sequences and several GC hexanucleotide motifs in the 5' end of the gene. These regulatory sequences differ considerably from those of many differentiated, tissue-specific genes, but are common to a variety of mammalian "housekeeping" genes. Most such genes share some general features including constitutive expression of ubiquitous or common cellular functions and include, among other functions, hamster hydroxymethylglutaryl coenzyme A reductase (7), human adenosine deaminase (8), human phosphoglycerate kinase (9), mouse dihydrofolate reductase (10) and human epidermal growth factor receptor genes (11).

We describe here the general features of the human HPRT gene and show that its structure and organization are very similar to those of the mouse gene and that its promoter region exhibits some of the characteristic features common to other housekeeping genes.

MATERIALS AND METHODS

Isolation and characterization of genomic clones.

The initial human HPRT genomic clone p6B (formerly p6B2aE2) was derived from HPRT-deficient mouse cells transfected with whole human DNA and was further subcloned into the EcoRI site of plasmid pBR322 (12). Genomic clones λ HA2-2 and λ β *1 were isolated from bacteriophage λ human genomic libraries kindly provided by Dr. Tom Maniatis (13,14), while the genomic clone cos5 was isolated from a cosmid human genomic library provided by Dr. George Brownlee. The

cosmid library had been constructed from a MboI partial digest of human 48, XXXX DNA and cloned into the BamHI site of cosmid vector pTM (15). To isolate the genomic clone λ RI2-4 containing the 5' region of the HPRT gene including the promoter region, a size-selected EcoRI library of human female placental DNA was constructed in bacteriophage vector λ EMBL4 (16).

In most studies, the 1.6kb BamHI fragment of the full length HPRT cDNA p4aA8 (3) was used as the probe to identify HPRT-positive plaques or colonies. The library screening and isolation of positive clones, restriction endonuclease mapping, blot hybridization and all other DNA manipulations were performed according to established techniques (15,17). Identification and orientation of the cloned genomic fragments containing exons were accomplished by hybridization of each genomic clone to the phage M13 subclones of HPRT cDNA (3). Restriction enzyme digests of genomic clones were sequenced by the chemical method of Maxam and Gilbert (18) or were cloned into phage vector M13mp8 or M13mp19 and sequenced by the dideoxy-chain termination method of Sanger (19).

Heteroduplex Analysis

The human HPRT cDNA plasmid p4aA8 was linearized with SalI, subcloned into the SalI site of bacteriophage vector λ Charon 30 and used for heteroduplex analysis with genomic clones. Heteroduplexes were prepared as described by Davis et al. (20) and examined using a Phillips EM 300 electron microscope.

Transfection of genomic clones and assay for HPRT activity

Cotransfections with three overlapping genomic clones (p6B, λ HA2-2, cos5) and with the binary combinations of these clones were performed by the calcium phosphate precipitation method (21) into mouse HPRT-deficient LA9 cells (22) grown in Dulbecco's modified Eagle's medium supplemented with 10% calf serum and 50 ug/ml gentamycin. In a typical cotransfection experiment, 20 ug of mouse carrier DNA, 1 ug of p6B, 3 ug of λ HA2-2 and 3 ug of cos5 were mixed and transfected into $0.5-1.0 \times 10^6$ subconfluent HPRT-deficient mouse LA9 cells. The HPRT-positive cells were selected by growth in medium containing 100 μ M hypoxanthine, 0.4 μ M aminopterin and 50 μ M thymidine (HAT). These cells were assayed for HPRT activity by isoelectric focusing gel electrophoresis followed by an in-situ enzyme assay (23).

RNAse Protection Analysis

The human lymphoblast cell line WI-L2 (24) was used as the

Figure 1. Restriction map of the human HPRT gene. The nine exons of the full human HPRT gene are shown as closed boxes in the top line in the 5' to 3' orientation above the recognition sites for 7 of the restriction enzymes used to map the gene. Six overlapping genomic clones covering the gene are indicated at the bottom of the figure.

source of HPRT-specific cytoplasmic messenger RNA. Cultured cells were suspended in cold 0.1M Tris (pH 7.5), 0.25M sucrose, 1.5mM $MgCl_2$, 30mM KCl, 3mM glutathione, 0.3% NP40 and 100ug/ml heparin. After 5 minutes on ice, the cells were disrupted by homogenization with a tight Dounce homogenizer. The cytoplasmic fraction was separated from the nuclei by centrifugation at 2500xg for 10 minutes at 4°C. The supernatant fraction was made 0.1% SDS and extracted 3 times with phenol-chloroform. Poly (A)⁺ mRNA selection was carried out by oligo(dT)-cellulose chromatography as described (17).

Uniformly labelled, high specific activity ^{32}P -RNA probe for the 5' region of human HPRT mRNA was made by transcription in vitro of HPRT sequences cloned into plasmid pSP65 (Promega Biotec) carrying the Sp6 RNA polymerase promoter (25) or into plasmid pGEM2 (Promega Biotec) containig both the Sp6 and T7 RNA polymerase promoters. Genomic HPRT sequences including the entire 1.5kb insert from pBR1.5 (12) and an EcoRII fragment derived from the 1.5kb fragment were cloned into the plasmids to produce pGEM2-BR1.5 and pSP65-RII respectively. The purified plasmids were linearized and transcribed with α - ^{32}P -CTP (400Ci/mmol) and Sp6 or T7 RNA polymerase, producing RNA probes complementary to the region extending from BamHI to XmaIII and from EcoRII to XmaIII, respectively, in the 5' end of the HPRT gene, as indicated in Fig. 5A.

EXON-INTRON JUNCTION SEQUENCES OF THE HUMAN HPRT GENE

EXON	LOCATION IN cDNA	LENGTH(bp)	SPLICE ACCEPTOR	SPLICE DONOR	INTRON SIZE(Kb)
1	1-133	>133		TCGTG GTGAGCA	13.3
2	134-240	107	ATATTTCTTTTCAG ATT	.GACAG GTAAGTA	1.63
3	241-424	184	CTGTAG GAC	.ATTGT GTGAGTA	10.9
4	425-490	66	TTTTTTTTTAAC TAG AAT	.GAAAG GTCATGT	3.7
5	491-508	18	CTCTTTTCTTCTAG AAT	.TGGAA GTAAGTT	3.4
6	509-591	83	TTCAATTTTTGAAAG GAT	.GCAAG GTATGTA	4.7
7	592-638	47	TTTTGTAATTAACAG CTT	.AGACT GTAAGTG	0.17
8	639-715	77	TATGATTCCTTTTAG TTG	.TGAAT GTAAGTA	1.3
9	716-1352	637	GATTTTTTTTTTAG CAT	.	

Consensus Sequence
from Literature

YYYYYYYYYY^{NC}_{NT}AG|G . . . ^AAG|GT^A_GAGT

Figure 2. Exon-intron organization of the human HPRT gene. Each row lists the exons of the human HPRT gene, numbered from the 5' transcription start site to the 3' end of exon 9 preceding the poly A sequence. The total exon and intron lengths and the nucleotide sequences of both the exons and the introns flanking the splice sites are shown. Splice junction sequences of the HPRT gene are compared to the consensus sequence(26).

These labelled probes and 10 ug of poly (A)⁺ mRNA were used in each RNA protection experiment by methods modified from those described by Melton (25). After hybridization, the samples were added to prewarmed RNase digestion buffer containing 60mM NaCl, 40ug/ml RNase A and 2ug/ml RNase T1 and incubated at 33°C for 45 minutes. The sizes of the protected fragments were estimated by electrophoresis in 8.5% polyacrylamide-8M urea and 33% formamide gels.

RESULTS

Isolation and characterization of HPRT genomic clones

The genomic clones were characterized by restriction endonuclease mapping, heteroduplex analysis and blot hybridization. The physical map of the human HPRT gene and the locations of selected restriction enzyme sites are shown in Fig. 1. The overlapping genomic clones p6B, λHA2-2, and cos5 span a total of 55 Kb.

Characterization of some of the intron and exon locations and sizes was performed partly by heteroduplex analysis and partly by

nucleotide sequence analysis. To define the precise locations of each of the 5' and 3' exon-intron boundaries, HaeIII and/or AluI fragments of genomic clones were subcloned into M13 phage vectors. Exon-containing subclones, identified by hybridization to the cDNA clone p4aA8 or to synthetic oligonucleotide probes, were sequenced and exon-intron junctions were localized by alignment with the cDNA sequence. Fig. 2 summarizes the exon-intron junction sequences of the human HPRT gene. The transcription unit is seen to be divided into 9 exons and 8 introns and spans 42 kb in length. The 9 exons range in size from 18 to 637 nucleotides separated by introns ranging from 0.17 to 13.3 kb in length. The locations of the exon-intron junctions of the human HPRT gene are identical to those of the mouse gene and the sequences at the 5' and 3' boundaries of each intron listed in Fig. 2 are shown to be consistent with the established consensus splice junction sequences (26). All the introns begin with the sequence GT at the 5' end and end with the sequence AG. Further flanking sequences beyond the splice sites themselves show no obvious homology to those of the mouse gene. In contrast to the strict conservation of the 7 internal exons and of the coding regions, the intron sizes of the human HPRT gene show some deviations from those of the mouse gene, as shown in Fig. 3, although the relative sizes of the introns in these two mammalian HPRT genes have been maintained.

Expression of human HPRT in transfected mouse cells

Evidence that the entire functional human HPRT gene including the promoter is present in the region spanned by the isolated genomic clones was obtained by cotransfection of three overlapping genomic clones (p6B, λ HA2-2 and cos5) into cultured mouse LA9 cells. The genomic clone p6B contains the putative promoter within

Figure 3. Comparison of the organization of the human and mouse HPRT genes. The exons are indicated as solid boxes and the locations and sizes of the introns of the two genes (in Kb) are indicated.

400 bp upstream from the translation initiation site. Cotransfection of these three genomic clones produced 10 HAT-resistant transformants from a total of approximately 4×10^6 recipient cells, giving a transfection frequency of 2×10^{-6} . None of the binary cotransfection experiments produced transformants. The cell lysates from HPRT-positive transformants were assayed for HPRT activity by isoelectric focusing gel electrophoresis followed by an in situ enzyme assay. Two independent HPRT-positive transformants showed bands of enzyme activity co-migrating with the authentic human HeLa HPRT, as shown in Fig. 4.

Figure 4. Isoelectric focusing assay of HPRT activity in cells transfected with three overlapping HPRT genomic clones (p6B, λ HA2-2, cos5). Cellular extracts were prepared from the HPRT-positive transformants, fractionated on 7.5% polyacrylamide gels and assayed for HPRT activity as described (23). Two independent HPRT-positive transformants (lane 1,2) show bands of enzyme activity that co-migrate with authentic human HeLa HPRT (lane 3) and that differ from mouse NIH 3T3 activity (lane 4).

Characterization of the 5' end of the human HPRT gene

The sequence of 400 nucleotides upstream from the HPRT translation initiation codon contained in the genomic clone pBR1.5 (12) is shown in Fig. 5A. Striking features of this region include the very high content of G and C residues (75%), the presence of five GC hexanucleotide motifs (5'-GGCGGG-3') starting at -214, -202, -183, -178 and -167 (Fig. 5A) and the absence of classical eukaryotic transcriptional control elements such as TATA and CAAT boxes (27). These GC clusters are imbedded within two copies of a 27 base pair repeated sequence beginning at positions -217 and -182 as shown in Fig. 5A, and are of the sort first described in the bidirectional promoter of simian virus 40 (28) and known to bind the cellular transcription factor Spl (29,30).

In addition to the sequences described above, there are two other regions in the 5' flanking portion of the human HPRT gene that display homology to the enhancer core sequence present in the SV40 72 base pair repeats. The sequence 5'-GGGAAAG-3' beginning at -316 (Fig. 5A) is similar to the SV40 enhancer core sequence 5'-GTGGAAAG-3' and to the consensus viral and cellular core sequences (31). Furthermore, the sequence extending from -274 to -253 in Fig. 5A is identical in 17 out of 22 positions to a different region in the SV40 72 base pair repeat. There are also two copies of the sequence 5'-CCTCCTCCT-3' beginning at positions -69 and -42.

In addition to these sets of direct repeats, there are extensive inverted repeat sequences compatible with the formation of a number of potentially stable stem-loop structures, two of the more stable of which are shown in Fig. 5B, extending from position -220 through -112 and from -33 through +63. The free energy of the stem-loop structures was -64 Kcal/mole and -65 Kcal/mole respectively (32). It remains to be proven, of course, whether these or similar structures are formed in vivo or what role they may play in HPRT gene expression.

In contrast to the reported single transcriptional start site in the mouse HPRT gene, our nuclease protection studies illustrated in Fig. 6 show a heterogeneous set of transcription initiation sites in the region extending from approximately 131 to 73 nucleotides upstream from the ATG translational initiation start site and are found not only in the human lymphoblast line WI-L2 but also in a mouse-human hybrid cell carrying the human X chromosome. The two

Figure 5. Nucleotide sequence (A) and two potential secondary structures (B) in the promoter region of the human HPRT gene. (A) Nucleotide sequence of the promoter region contained within a 470 bp XmaIII/EcoRII genomic fragment (see Methods). Nucleotide residues +1 denotes the A of the translation initiation codon ATG and residues preceding it are indicated by negative numbers. The repeated sequence 5'-GGCGGG-3' is enclosed by boxes within the two copies of the 27 bp repeats indicated by brackets. The wavy line indicates the sequence showing homology to the SV40 72 bp repeats (see Discussion), while the sequence showing the very close homology to the SV40 enhancer core sequence (see Discussion) is designated by the thick underline. The additional repeated sequences present in the 5' untranslated regions of both the human and the mouse gene are indicated by underlines. The several transcriptional start sites are designated by numbered bars above the nucleotide sequence. (B) Two of the more stable, potential secondary structures in the promoter region of the human HPRT gene. Base-paired regions are designated as a continuous line, whereas unpaired regions are shown as discontinuities and small loops. The positions of the 5'-GGCGGG-3' boxes are indicated by boxes and the asterisks indicate the approximate positions of the transcriptional start sites as determined by RNase protection analysis. The free energy value for each secondary structure is indicated.

Figure 6. RNase protection analysis of the 5' end of the human HPRT mRNA. Poly (A)⁻ RNA (10ug, lane 1) and poly (A)⁺ RNA (10ug, lane 2) prepared from WI-L2 cells were hybridized to the ³²P-RNA probe from genomic subclone pSP65-R11 as described in materials and methods. After RNase digestion, the protected products were electrophoresed in denaturing polyacrylamide gels (Methods) and detected by autoradiography. The protected fragments are numbered 1-7. The positions of ³²P-labelled HaeIII-cut pBR322 fragments used as size markers are shown in lane M. Lane 3 contains material from an RNase protection experiment performed without added cellular RNA.

major start sites are found at approximately positions -93 and -88. The start sites are present in the region downstream from the last of the five GC boxes on the upstream stem and in the potential inter-stem region shown in Fig. 5B. This analysis indicates that

the human HPRT promoter is present on the 0.4kb XmaIII/Eco RII fragment subcloned from the genomic fragment pBR1.5.

Homology between the human and mouse HPRT genes

Nucleotide sequence homology in the 5' flanking and 3' untranslated regions of the human and mouse HPRT genes was investigated by matrix analysis (33) with a required stringency of nucleotide identity of 12 or more out of 15 positions and by nucleotide sequence comparison (34). Fig. 7 illustrates the graphical representation of the homology in the (A) 5' flanking and (B) 3' untranslated regions of the two genes. These analyses show that the 5' flanking region has considerable homology (51%) up to approximately 299 bp upstream from the translation initiation codon. Within this region are the five GC hexanucleotide motifs in the human gene that occur three times in the analogous region of the mouse gene. The 10 bp direct repeats present in the mouse gene (6) which show homology to the SV40 72 bp repeat are not present in the human gene. However, the two copies of 5'-CCTCCTCT-3' sequence shown in Fig. 5A are partially represented in the homologous region of the mouse gene as two copies of 5'-CCTCCTC-3'.

Of particular interest is the very high sequence identity (74%) in the 3' untranslated region of two genes as shown in Fig. 7B. This homology extends from a position immediately after the translation termination codon almost to the poly A addition signal sequence AATAA, spanning approximately 550 bp.

DISCUSSION

This study describes the overall organization of the human HPRT gene and some of the characteristic features of the promoter region. X-linkage of the genomic clones reported in this study was established by gene dosage hybridization (data not shown). The full HPRT gene is approximately 42 Kb in length, in contrast to the estimated 34 Kb size of the mouse gene (6), and consists of 9 exons and 8 introns. Previous studies from our laboratory and from others (3,4,5) have shown that the nucleotide sequences of the coding portions of the human and mouse HPRT cDNAs demonstrate a more than 95% homology, encoding proteins that differ in only 7 of the amino acid positions. We report in this study that the similarity of the two genes extends to their overall genomic organization. There is precise conservation of the exon-intron boundaries of the two genes and even general conservation of the intron sizes along the gene,

Figure 7. Two dimensional matrix analysis comparison of the A.) 5' flanking sequences and B.) 3' untranslated sequences of the human and mouse HPRT genes. The 440 nucleotides of the human 5' flanking region upstream from the translation initiation codon and the 590 nucleotides of the human 3' untranslated region downstream from the translation termination codon were compared to the corresponding regions of the mouse gene. The location of fragments with sequence identity in 12 or more positions out of 15 is indicated by a dot on the matrix.

although intron sequences diverge immediately beyond the splice junctions. Such conservation of the coding and non-coding regions and also of exon-intron organization of two genes therefore may reflect not only constraints on the structure-function requirements of the protein but also the conservation of some important regulatory functions of the genes. Many previous studies with a variety of eukaryotic genes encoding differentiated cell functions have revealed several highly conserved sequences in the 5' flanking portions of the genes, including TATA and CAAT boxes and other sequences now known to be involved in transcriptional control of gene expression (27). Since both the human and mouse HPRT genes are missing those classical transcription signals but do both contain other conserved sequences in their 5' flanking regions that are also found in other housekeeping genes, we infer that such sequences play an important role in the regulation of HPRT and other gene expression. The strict sequence conservation (74%) of the 3' untranslated region of the human and mouse HPRT genes is unusual since such regions of most characterized eukaryotic genes of the same or different species usually show considerable sequence divergence (35,36). Although studies of the biological function of

3' untranslated regions are very limited, this region of the mouse DHFR and histone H4 genes seems to be involved in some aspects of growth-dependent or cell-cycle-dependent regulation of gene expression (37,38,39). These studies imply that there may be some important regulatory functions in the 3' untranslated regions of the mammalian HPRT genes.

Nucleotide sequence analysis of 400 bp upstream from the translation initiation site of the human HPRT gene reveals some characteristic features of mammalian housekeeping genes, including the absence of conventional 5' transcriptional regulatory sequences such as TATA and CAAT boxes and the presence of extremely GC-rich sequences and several GC hexanucleotide motifs in the 5' end of the gene. Although the exact locations of the transcription initiation sites still remain to be defined, our results indicate that there are multiple transcription start sites in the region downstream from the last of five GC hexanucleotide motifs (Fig. 5A). The conservation of these sequences and the presence of potential secondary structural features in this region suggest that they may provide some transcriptional initiation signals used for HPRT gene expression.

We have compared the distribution of the GC hexanucleotide motifs of the human HPRT promoter region with that of several other housekeeping genes. As can be seen in Fig. 8, the GC hexanucleotide motifs in several genes are clustered in a region up to about 200 bp upstream from the first transcription start site and are repeated from 3 to 6 times, although the location and number of these GC motifs differ among several of the housekeeping genes. These GC motifs in housekeeping genes are homologous to the six GC hexanucleotide sequences (5'-CCGCCC-3') present in the 21 bp repeat region of the SV40 promoter which serve as binding sites for cellular transcription factor Sp1 (29,30). This factor has been shown to interact with several SV40-like cellular sequences in the promoter regions of cloned monkey genomic DNA, the mouse dihydrofolate reductase gene and some viral promoters (30,40). Although the precise role of these GC motifs in the initiation or regulation of transcription of the HPRT gene is not clear, their similarity to the well defined GC motifs of the SV40 promoter suggests strongly that they may interact with cellular transcription factor(s) to provide constitutive gene expression.

The ability of three independent overlapping genomic clones

Figure 8. Comparison of the distribution of the conserved GC hexanucleotide motifs in the human HPRT gene with several other mammalian housekeeping genes. The locations of the repeated sequence 5'-GGCGGG-3' are shown as open boxes and the homologous sequence 5'-CCGCCC-3' is indicated by hatched boxes. Asterisks indicate the transcription initiation sites. The numbering of nucleotides is relative to the first transcription start site (+1).

cotransfected into HPRT-deficient mouse cells to express human HPRT activity provides evidence that these clones encompass the entire functional HPRT gene including the promoter region. We cannot, of course, exclude the possibility that other sequences not covered by these three clones may also play some role in HPRT gene expression. Any such sequences, however, are obviously not required for HPRT expression. Several groups have reported homologous recombination between two cloned sequences containing overlapping region after gene transfer into cultured cells (41,42,43). On the basis of these studies, we suggest that the three genomic clones are likely to have undergone homologous recombination to produce a complete functional HPRT gene.

Interestingly, the level of HPRT gene expression is highly elevated in the CNS, particularly in the basal ganglia (44), while the activity of amidophosphoribosyltransferase, the rate-limiting step in the de novo pathway of purine biosynthesis, is low in the CNS. It has been suggested that the brain may be unusually dependent on the salvage pathway for the synthesis of inosinic and guanylic acids and that the absence of HPRT may make it difficult for some neural cells to maintain appropriate intracellular concentration of cyclic nucleotides essential for normal CNS function (45). In this regard, it seems likely that there exist some sequences in the 5' and 3' flanking regions or even within the

HPRT gene itself that are important for aspects of the regulation of HPRT expression, especially in the CNS. It should be possible, by using a variety of in vitro and in vivo gene transfer and mutagenesis methods, to identify such regions and to characterize the sequence elements required for properly regulated and efficient HPRT gene expression in the CNS and other organs. Furthermore, identification of promoter sequences in this and other genes may allow the isolation of transcription factors and a study of their role in the enhanced expression in the CNS. These kinds of studies may provide useful information on the mechanisms of regulation of housekeeping gene expression and on the molecular basis of the neurological disorder in the Lesch-Nyhan syndrome.

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