HPRT: GENE STRUCTURE, EXPRESSION, AND MUTATION

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INTRODUCTION AND PERSPECTIVES

The biosynthesis of purine and pyrimidine nucleotides is a crucial process for all cells since these molecules are the direct precursors of DNA and RNA and frequently participate as coenzymes in enzymatic reactions. With few exceptions, organisms are able to synthesize purines and pyrimidines via de novo
Figure 1  Pathways of purine interconversion in mammalian cells. APRT, adenine phosphoribosyltransferase; ADA, adenosine deaminase; HPRT, hypoxanthine phosphoribosyl transferase; PNP, purine nucleoside phosphorylase.

pathways. Since bacteria and humans have the ability to "salvage" free purine and pyrimidine bases by nucleotide conversion, cellular pools are economically maintained. Ninety percent of free purines in humans are recycled (60). The enzymes hypoxanthine phosphoribosyltransferase (HPRT) and adenine phosphoribosyltransferase (APRT) catalyze these activities in vertebrates (Figure 1) (54, 55). In 1967, Seegmiller et al demonstrated the importance of purine salvage when they identified HPRT deficiency as the defect in the Lesch-Nyhan syndrome (61, 92). The HPRT gene has now been cloned and characterized (14, 46, 47, 69). This permits the molecular analysis of mutations resulting in Lesch-Nyhan and gouty arthritis secondary to HPRT deficiency (51).

The HPRT gene has been used extensively in the study of cultured mammalian cells because of its location on the X chromosome (functional or true hemizygosity) and because simple selective media allow for growth of HPRT\(^+\) and HPRT\(^-\) cells. Thus the HPRT gene has been the most actively studied locus in investigations of mutational agents. Littlefield exploited the HAT counterselective medium for selection of somatic cell hybrids used extensively for human gene mapping (62). Similarly Köhler & Milstein used this selection for hybridoma fusions (52). More recently this selection has been used by Shapiro for the study of X inactivation mechanisms (74). In this review we will focus on the more recent molecular studies of mutation and refer the reader to a variety of earlier reviews of HPRT for cellular behavior (16, 51, 110).
NORMAL ENZYME FUNCTION AND CHARACTERISTICS

Enzymology

Hypoxanthine phosphoribosyltransferase (HPRT, EC 2.4.2.8) catalyzes the condensation of 5'-phosphoribosyl-1-pyrophosphate (PRPP) and the purine bases hypoxanthine and guanine in the formation of 5'-IMP and 5'-GMP respectively (58). Adenine phosphoribosyltransferase (APRT) catalyzes a similar salvage reaction with adenine (51). These enzymes provide cells with an alternative to the energy-expensive de novo synthesis of nucleotides and play a critical role in the maintenance of intracellular purine nucleotide pools in cells that have a decreased ability to synthesize new nucleotides [e.g. erythrocytes (24)].

HPRT is found in all cells as a soluble, cytoplasmic enzyme and accounts for 0.005–0.04% of total proteins. Wilson et al have determined the amino acid sequence for human erythrocyte HPRT and have shown that each monomer contains 217 amino acids and has a molecular weight of 24,470 (108). Original estimates of quaternary structure, based on gel filtration chromatography and analytical sedimentation, predicted dimeric and trimeric structures for functional HPRT, while cross-linking studies and isoelectric focusing of human-mouse heteropolymers identified tetrameric structures (5, 41, 45, 81, 82, 83).

In addition to hypoxanthine and guanine, HPRT can bind and ribosylate a wide range of toxic purine analogues, a characteristic first exploited by somatic cell geneticists when HPRT− cells were isolated by their resistance to 6-thioguanine (6-TG) and 8-azaguanine (8-AG) (95). In 1962 Szybalski & Szybalska developed a counterselective method for the isolation of HPRT+ cells using media containing hypoxanthine (a purine source), aminopterin (an inhibitor of purine and thymidine synthesis), and thymidine, HAT media (97). The power of these forward and reverse selection procedures has established HPRT as an indispensable tool in the development of mutagen testing, hybridoma, and somatic cell hybrid techniques.

The phosphoribosyl moiety is provided by 5'-phosphoribosyl-1-pyrophosphate in a transferase reaction. Human erythrocyte HPRT Michaelis constants for guanine and hypoxanthine are $5 \times 10^{-6}$ M and $1.7 \times 10^{-5}$ M respectively; $K_m$ values for PRPP range from $2 \times 10^{-4}$ to $5.5 \times 10^{-5}$ M depending upon enzyme source (35, 37, 58, 66). HPRT requires magnesium, and the specific mechanism of catalysis is greatly influenced by the $\text{Mg}^{2+} / \text{PRPP}$ ratio (89). Under physiologic conditions, the enzyme first binds PRPP and then the purine base in the establishment of a short-lived ternary complex. The phosphoribosyl moiety is transferred to the base and the enzyme and nucleotide dissociate, presumably with the release of pyrophosphate.

HPRT is one of ten catalytically related phosphoribosyltransferases found in many organisms. These transferases are involved in the biosynthesis of purines,
pyrimidines, and the aromatic amino acids histidine and tryptophan. While these enzymes recognize a wide range of substrates, they all require divalent metal cations for activity and can use only PRPP as the ribosyl donor (78). Functionally identical HPRT proteins have been purified from hamster, mouse, rat, and human sources (5, 18, 33, 44, 81). Yeast HPRT has also been purified and is a single enzyme, capable of recognizing guanine, hypoxanthine, and xanthine (22, 90). The protozoan Leishmania donovani has one enzyme that recognizes hypoxanthine and guanine and another that recognizes xanthine (99). Leishmania lacks de novo purine synthesis and thus is dependent on "salvage" reactions for purine nucleotides. The bacteria Salmonella typhimurium and Escherichia coli use independent enzymes for conversion of hypoxanthine and guanine to their respective nucleotides (20, 32, 73). Based on presumed substrate and catalytic similarities between these enzymes, Musick predicted that common structural features may represent shared substrate binding or active sites (78). Argos et al subsequently reported sequence comparison of three transferases (bacterial and human enzymes) and described a potential nucleotide binding domain based on secondary and tertiary structural homology (4).

**Tissue Distribution**

HPRT has been detected in all somatic tissues at low levels (0.005–0.01% of total mRNA) (51). Significantly higher levels are found in the central nervous system (0.02–0.04%) (51, 70). In normal mice, rats, monkeys, and humans, direct enzyme assay has been used to demonstrate high HPRT-specific activity in brain tissues (43, 49, 57). Furthermore, assay of proteins synthesized in vitro from mRNA isolated from Chinese hamster brain, testes, and liver tissues demonstrated a sevenfold elevation in brain HPRT activity over other tissues (70). Studies of regional central nervous system (CNS) levels of HPRT activity indicate that in rats and humans, the basal ganglia have the highest level (40, 49, 88). The de novo synthesis of purines is lowest in the caudate nucleus (the major component of the basal ganglia), suggesting this CNS region may be dependent upon salvage of preformed bases for nucleotide pool maintenance (101). This inverse relationship between de novo synthesis and salvage occurs in other tissues as well. Erythrocytes, platelets, and bone marrow cells produce little amidophosphoribosyltransferase (AMPRT, the rate limiting enzyme in de novo purine synthesis) yet are rich in HPRT (24, 42, 59, 65). These tissue differences suggest that the role for this enzyme in different tissues is more than simple "housekeeping" and that it may be important in the pathogenesis of the Lesch-Nyhan syndrome.

**HPRT Gene Structure**

Human, mouse, and hamster HPRT proteins are encoded by a single X-linked structural gene. The first evidence that the HPRT gene was X linked came from
pedigree analysis of families that had inborn deficiencies of HPRT (61). Ricciuti & Ruddle demonstrated, via mouse-human somatic cell hybrids, synteny between HPRT and two genes on the long arm of the X chromosome—glucose 6-phosphate dehydrogenase (G6PD) and phosphoglycerate kinase (PGK) (87). Subsequent mapping by Pai and Sprenkle localized the human HPRT gene to Xq26–27 (84).

Isolation of cloned sequences complementary to HPRT mRNA was originally made difficult by the low levels of normal expression of the gene. HPRT mRNA accounts for no more than 0.04% of the total brain message produced (70). In 1981, the isolation of a mouse neuroblastoma cell line (NBR4) that expresses high levels of a variant HPRT protein provided the mRNA source from which murine HPRT cDNA was first cloned (68). Reversion of a 6TG resistant, HPRT− mouse neuroblastoma cell line led to the isolation of HAT-resistant, HPRT+ cells that had elevated levels of an unstable HPRT mutant enzyme. While immunoprecipitation studies of cellular extracts indicated a tenfold elevation of HPRT protein, enzyme activity was only 10–25% of wild-type levels. In vitro translation of mRNA isolated from this cell line indicated a 20–50-fold increase of HPRT mRNA (70). NBR4 HPRT was shown to have a decreased affinity for both hypoxanthine and PRPP relative to wild-type HPRT. Sequence comparison of HPRT cDNA from NBR4 and wild-type cDNA has shown that a point mutation is responsible for this unstable character. NBR4 survival in HAT media was achieved by amplification of a mutant gene.

HPRT cDNA recombinants were identified by differential hybridization procedures using NBR4 cDNA (50 copy amplification) and cDNA from the parental cell line (1 copy) (14). The identity of HPRT cDNA clones was confirmed by in vitro translation of mRNA selected by hybridization to candidate HPRT cDNA recombinants. Probes generated in this fashion were used to isolate a recombinant, pHPT5, that contains an open reading frame of 654 nucleotides preceded by 100 nucleotides of 5' untranslated sequence and followed by 550 nucleotides of 3' untranslated sequence (53). Cross-species sequence homology facilitated the isolation of human and hamster HPRT cDNA recombinants using pHPT5 as a probe (15). In an alternative approach, Jolly et al used a human HPRT genomic fragment, isolated from transfected mouse cells, as a probe to isolate a functional HPRT cDNA clone (46, 47). Nucleic acid sequence of these clones was in agreement with amino acid sequence of human erythrocyte HPRT (109).

The human cDNA open reading frame begins with an ATG initiation codon, codes for a protein of 218 amino acids, and ends with a typical TAA termination codon (47). Cleavage of the initial methionine from the nascent polypeptide had previously been shown by Wilson et al to be a post-translational event that resulted in the 217 amino acid monomer (109). Northern analysis of human
poly(A)$^+$ RNA using these cDNA isolates has confirmed a message size of 1600 nucleotides (112).

Sequence comparison between mouse, hamster, and human cDNAs indicates that homology between these three species is >95% in coding regions and falls to ≈80% in 5' and 3' nontranslated regions (19). The differences between the mouse and human sequences result in seven amino acid substitutions, five of which are conserved changes with respect to amino acid class.

The isolation of these cDNA recombinants has facilitated the characterization of the mouse and human HPRT gene structure. Analysis of overlapping λ recombinants has indicated that human and mouse HPRT genes have nine exons within a 44 kb expanse of genomic DNA (Figure 2; see also 69, 85). (Patel et al, paper submitted). The intron/exon junctions for both species are identical. The nine exons range in length from 18 to 593 bp in mice and from 18 to 637 in humans.

In addition to the functional X-linked HPRT gene, four homologous autosomal sequences have been detected and localized in man (85). These sequences are processed pseudogenes presumably derived from intronless RNA intermediates. Southern analysis of DNA from a panel of human-Chinese hamster somatic cell hybrids indicated that two of these sequences were localized to chromosome 11, one to chromosome 3, and another to the region between p13 and q11 on chromosome 5. Single autosomal sequences have been identified in mouse and hamster DNA but have not been characterized. Size and transcription data suggest they are unexpressed intronless pseudogenes (28).

**HPRT Gene Expression**

As stated previously, HPRT is expressed in all tissues, albeit at different levels. Melton et al have shown that in the mouse HPRT gene there is no evidence for the presence of a CAAT box and that the nearest sequence corresponding to a "TATAA" box (normally located 20–30 bp upstream from structural genes) occurs >700 bp 5' to the cap site (Figure 2; see also 64). The nucleic acid sequences 5' to the start site for both the mouse and human HPRT genes are extremely G-C rich (≈80%) (Figure 3). The human HPRT gene also lacks CAAT and TATAA sequences immediately 5' to the initiation site (P. I. Patel, and A. C. Chinault, personal communication). Sequences homologous to the SV40 promoter are found in the 5' flanking region of the HPRT gene but their role in transcription is unresolved. In a description of the hamster HMG CoA reductase gene, Reynolds et al noted a similar lack of CAAT and TATAA sequences and the presence of a G-C rich promoter region containing three 6 bp (CGCCGG) repeats (86). By means of deletion and mutation analysis (11, 27), this signal has been shown to have promoter function for the SV40 early gene and the herpes thymidine kinase gene. The human adenosine deaminase gene
Figure 2  Structural organization of human and mouse HPRT genes. The stippled boxes represent 5'- and 3'-untranslated regions of the genes.
Figure 3  Promoter sequences of housekeeping genes characterized to date. The mRNA initiation site(s) are indicated by a bar above the sequence. The sequence GGGCGG, or its inverted complement CCCGCC, present in each of these promoters, is shown.
also lacks 5' CAAT and TATAA sequences and contains a G-C rich region with five GGGCGGG repeats (100). Analogous promoter structures have been reported for the human DHFR and the X-linked PGK genes (17, 93). It is interesting that each of these promoters contains the sequence 5' GGGCGGG 3' (or its complement) from 32 to 42 bp upstream from the cap site. Each of these housekeeping genes is expressed in all tissues, and each appears to share common nucleic acid sequences, possibly regulatory sequences (67). Two X-linked genes for clotting factors VIII and IX show no appreciable nucleic acid sequence homology in their promoter regions to the HPRT gene (3, 29). All of these genes contain TATAA-like sequences and are expressed in specific tissues.

Since selective media permit isolation of HPRT+ and HPRT- cells, this locus provides an excellent opportunity to examine structural differences between active and inactive X chromosomes. Yen and coworkers have suggested that patterns of methylation, rather than methylation of specific sites, correlate with maintenance of X-chromosome inactivation (113). By using methylation-sensitive restriction enzymes to analyze DNA from somatic hybrids containing active or inactive X chromosomes, they have demonstrated hypomethylation of active X chromosomes relative to inactive X chromosomes in the region 5' to the HPRT structural gene. They found no specific restriction site whose methylation was directly correlated with gene activity. Wolf et al described a "consensus methylation pattern" for active and inactive HPRT genes using a similar approach (111). Both studies associated 5' hypomethylation and 3' hypermethylation with active HPRT genes. While these studies have suggested that differences in methylation patterns exist between active and inactive X chromosomes, no cause and effect relationship has been established and many questions concerning transcriptional repression vs chromosome inactivation remain unanswered.

The molecular basis of tissue variability in the expression of HPRT has recently been addressed by studies of transgenic mice that express a human HPRT minigene. Recombinant molecules containing the human HPRT cDNA flanked by the mouse metallothionein promoter (5'), and the human growth hormone polyadenylation signal (3'), were injected into single cell mouse embryos. These embryos were implanted into pseudopregnant foster mothers and the resulting pups examined for expression of human HPRT. Upon Cd2+ induction, transgenic mice expressed the human enzyme variably in many tissues; however, expression was consistently elevated in brain tissue. Since the metallothionein promoter and the growth hormone polyadenylation signal do not normally influence CNS expression, this study suggests that sequences within the human HPRT transcript (cDNA) influence CNS expression presumably via increased mRNA synthesis or stability (94a).
Pathogenesis of HPRT Deficiency

Deficiency of HPRT in man results in a spectrum of disease, the severity of which is dependent upon the extent of the deficiency. Complete deficiency of the enzyme is associated with the Lesch-Nyhan syndrome (incidence 1 in 100,000), while partial deficiency is associated with gout (incidence 1 in 200 among males with gout) (51). The Lesch-Nyhan syndrome, first described in 1964, is clinically characterized by hyperuricemia, choreoathetoid movements, spasticity, hyperreflexia, mental retardation, and compulsive self-injurious behavior (92). Developmental delay is evident at three to six months of age while pyramidal and extrapyramidal involvements become apparent within a year of birth (91). The relationship between HPRT deficiency and CNS dysfunction in Lesch-Nyhan patients remains unclear. Recent biochemical and histologic studies suggest that inappropriate development of nigrostriatal dopaminergic neurons may be important (31). Postmortem analysis of brain tissue from three Lesch-Nyhan patients revealed that indexes of dopamine function were reduced by 70–90% in the basal ganglia (64). During normal development, arborization of these neuronal pathways occurs at the same time as increased HPRT levels in the CNS (2, 63). Moreover, behavioral changes including self-mutilation occur when drugs are used to arrest development of these terminal dopaminergic neurons (7, 13). Since it has been shown that dopamine receptor binding is regulated by guanine triphosphate and diphosphate nucleotides, perhaps purine imbalance during development results in inappropriate synaptogenesis in the basal ganglia (21).

Hyperuricemia in these patients is associated with an increase in the rate of de novo purine synthesis (51). This increase is probably due to the combined effects of decreased feedback inhibition by nucleotide end products and cellular loss of hypoxanthine. Lymphoblasts from both normal and Lesch-Nyhan patients are capable of accelerated de novo synthesis when cultured in hypoxanthine-depleted media, but only normal cells reduce de novo synthesis upon addition of exogenous hypoxanthine (39). The inability of Lesch-Nyhan cells to convert intracellular hypoxanthine to IMP leads to cellular loss and subsequent conversion of hypoxanthine to uric acid by hepatocyte xanthine oxidase.

In patients with partial HPRT deficiency, excessive production of uric acid leads to hyperuricemia, nephrolithiasis, and a severe form of gout. Evidence of spasticity, cerebellar ataxia, and mild mental retardation can be found in 20% of patients with partial HPRT deficiency, but these individuals do not become self-mutilating. Allopurinol, an inhibitor of xanthine oxidase, is helpful in reducing the uric acid production in these patients but has no effect on the neurological manifestations of disease. Fluphenazine, an antagonist of postsynaptic dopamine receptors, is purportedly effective in reducing self-mutilating behavior in these patients, presumably via interaction with D₁ nigrostriatal receptors.
MUTATIONS AT THE HPRT LOCUS

Mutations in Cultured Cells

Three factors have contributed to the development of the HPRT gene as an important test system in the study of mammalian cell mutagenesis. First, as an X-linked gene, HPRT is hemizygous in male cells and functionally hemizygous in female cells, making this locus particularly useful in the examination of recessive mutations. Secondly, HPRT is a nonessential enzyme for cells growing in culture since purines can be provided by de novo synthesis. Finally, powerful selection procedures exist for the isolation of HPRT⁻ or HPRT⁺ cells, making measurement of mutation and reversion rate possible (95, 97).

Purine analogue resistance in mammalian cells results from an inability to convert these analogues to toxic nucleotides, an activity dependent upon functional HPRT. Thus quantitation of 6-thioguanine or 8-azaguanine resistant cells provides a simple measure of mutation frequency (76). HPRT antibodies and cDNA probes permit the detailed characterization of individual mutations and mechanisms of mutation (8).

Such strategies were utilized by Fuscoe et al in their analysis of 19 HPRT Chinese hamster mutants (28). Southern blots of DNA from ten spontaneous and nine UV-induced mutants detected two subclones (one spontaneous, one UV irradiated) with major deletions at the HPRT locus. Cytogenetic analysis of these cell lines showed the deletions to be associated with chromosomal translocations. DNA from the remaining 17 subclones appeared normal in Southern blots. None of these mutant lines showed HPRT activity, and only one produced material that could cross-react with antibodies raised against HPRT. Subsequent analysis of mRNA synthesized in 20 HPRT⁻, CRM⁻ Chinese hamster lines, in which no genomic alterations were detected by Southern blots, indicated that 18 of these mutants produced normal levels of HPRT mRNA (79). Cumulatively these data suggest that point mutations in the HPRT locus account for the majority of spontaneous or UV-induced mutants. Recent work by Simons has shown that 12 of 19 mutant Chinese hamster cell lines generated by exposure to ionizing radiation (600 rads) contained deletions detectable by Southern analysis (J. Simons, personal communication). Crawford found four of six α-irradiated human fibroblast lines selected for 6-TG resistance to have HPRT gene deletions (B. Crawford, personal communication). These studies demonstrate the usefulness of recombinant DNA techniques in the characterization of mutational mechanisms.

An approach for differentiating between base pair substitution and frameshift mutants of the HPRT gene has recently been described by Stone-Wolff & Rossman (94a). This method utilizes a reversion system dependent upon single base pair substitution reversion following N-methyl-N'-nitro-nitrosoguanidine (MNNG) treatment, or frameshift reversion following ICR-191 treatment.
Distinction between these two types of mutations is predicated on the idea that frameshift mutations most frequently revert by a second frameshift mutation and that base substitution mutations most frequently revert by another substitution mutation. Thus frameshift mutations conferring 6-TG resistance will more frequently revert to the 6-TG sensitive phenotype following treatment with ICR-191 (a frameshift mutagen) than with MNNG (a substitution mutagen), the converse being true for base substitution mutations. Different reversion frequencies have been observed for different HPRT− cell lines treated with these compounds (94). DNA sequence analysis will be needed to specify the nature of these mutants and revertants, although such analysis might be difficult given the large size of the HPRT gene and the difficulties inherent in cloning and sequencing HPRT mutants.

Two developments may make the HPRT locus more amenable to rapid molecular analysis. First, somatic cell lines have been developed in which a single intronless HPRT minigene functions in place of a deleted endogenous gene, thus effectively reducing the target of mutation from 44 kb to 1 kb in size (S. M. W. Chang, personal communication). Second, Myers et al have developed a simple method of identifying and locating point mutations based on novel denaturation properties of wild-type:mutant heteroduplexes during gradient electrophoresis (71). These developments may overcome the disadvantage of the large size of the HPRT gene in the analysis of point mutants.

To examine the in vivo mutagenicity of toxic compounds in mice, Jones et al have developed a clonogenic assay to quantify 6-TG resistant spleen lymphocytes isolated from mice that have been treated with various drugs (48). These investigators have demonstrated a linear dose-related increase of 6-TG colonies from mice treated with ethynitrosourea (ENU). Fifteen days following intraperitoneal injections of ENU, isolated lymphocytes were plated at high density (4–8 × 10^5 cells/250 μl well) in the presence of 6-TG and concanavalin A. 6-TG resistant colonies were scored after 8 days in selective media. This system offers the advantages of a direct method of measuring in vivo mutagenicity of many compounds and the ability to expand cloned cells for mutant characterization at the genetic level.

Albertini (1) and Morley (75) have reported the ability to measure the rate of 6-TG resistance from peripheral T cells grown in culture. Turner et al reports that a relatively high proportion (57%) of spontaneous mutations in human lymphocytes involve substantial gene alterations (deletions, exon amplifications, etc) that are not evident cytogenetically (98). Twelve of 21 6-TG resistant clones had altered HPRT Southern patterns, while none of the unselected clones from the same individual showed alteration. Since stringent 6-TG selection conditions were used, however, mutations resulting in undetectable HPRT activity were favored. Genes with deletions and/or other major gene rearrangements may have appeared at an artificially high frequency. These results are...
interesting in light of observations that only 18% of patients with the Lesch-Nyhan syndrome and 10% of 6-TG resistant hamster cells have major gene rearrangements (79, 112). The data suggest that in vivo mutations of somatic cells may occur more commonly by gene deletion. This may be relevant to the deletion mutations associated with the neoplastic transformation of retinoblastoma and Wilms’ tumor (9, 23, 56).

**Mutations in Man**

Since the description of the familial nature of the Lesch-Nyhan syndrome and the biochemical elucidation of its etiology, HPRT deficiency has been a model for the study of X-linked disease. Advances in our knowledge of HPRT genomic and protein structure have allowed characterization of a number of heterogeneous mutant alleles.

First reports characterizing HPRT deficiency in human cell lysates described enzymatic data suggestive of mutational heterogeneity (66, 92). These changes included altered sensitivity to product inhibition, thermolability, $K_m$ value alterations, and changes in electrophoretic mobility (6, 10, 25, 34, 38, 50, 66, 92, 96). With the development of effective purification and protein sequencing protocols, Wilson et al have been able to compare amino acid sequence data from three HPRT deficient patients with gouty arthritis and one Lesch-Nyhan patient (Figure 4; see also 104, 107, 108, 110).

One of the patients with gout, HPRT$_{Toronto}$, has a mutation resulting in the replacement of an arginine residue with glycine at position 50 (107). A single C to G base change in the arginine codon can explain this substitution and would result in the loss of a TaqI restriction site, a prediction confirmed by Southern analysis (105).

HPRT$_{London}$ and HPRT$_{Munich}$ have serine to leucine (position 109) and serine to arginine (position 103) changes respectively (108, 110). Each of these mutations results in gouty arthritis. HPRT$_{Kinston}$ occurs in association with the Lesch-Nyhan syndrome and has a mutation that substitutes asparagine for arginine at position 193 (106). While this method of mutant analysis has proved valuable in the description of pathogenic mutations, it is limited to those that result in the production of a protein product. A recent survey of cell extracts from HPRT-deficient patients indicates that 11 of 15 Lesch-Nyhan patients produced no material recognized by HPRT antibodies (J. T. Stout, and J. M. Wilson, in preparation). Mutations that inhibit transcription, RNA processing, or involve major rearrangements of the HPRT gene generally fail to produce detectable protein and are not amenable to this type of examination.

Studies by Yang et al examined the organization of the HPRT gene in 28 unrelated Lesch-Nyhan patients (112). Five (18%) of the patients screened showed unique Southern patterns suggestive of major gene alterations. These alterations include a total gene deletion (RJK 853), two 3' deletions (RJK 849,
Figure 4  Rodent and human HPRT mutants. Point mutations identified to date are indicated within respective exons at the top while mutations involving major gene alterations are depicted in the lower half.
GM 3467), a potential internal insertion (GM 2227), and an exon duplication (GM 1662). All of these patients are cytogenetically normal and fail to produce a stable protein product. None of the deletion patients produce a stable mRNA. GM 1662 produces an HPRT mRNA approximately 200 nt larger than wild type. This is consistent with Southern and sequence data that have confirmed a perfect duplication of exons 2 and 3 (D. S. Konecki, in preparation). While this patient produces a stable mRNA, enzyme activity is undetectable and no cross-reactive material can be detected in Western blots.

Family study of the Lesch-Nyhan patient GM 1662 permitted the identification of the origin of this mutational event. An abnormal 4.1 kb Bgl II band, characteristic of the duplication mutation, was identified in the propositus, his mother, and two sisters but was absent in his maternal grandmother. These data indicate that this mutation originated in the germ line of a maternal grandparent and gave rise to an asymptomatic carrier female.

Two additional family studies provide data on the gametic origin of mutations (J. T. Stout, in preparation). The Lesch-Nyhan patient RJK 853 has no X-specific HPRT sequences while his mother has two normal copies of the HPRT gene, indicating that the deletion event occurred in a maternal gamete. In a second example, the patient (RJK 983) has a partial 3' deletion with the breakpoint 3' to the fourth exon. The mother of this patient has two normal copies of the HPRT gene, again identifying a maternal gametic deletion event as the origin of the new mutation.

These findings support Haldane's prediction that lethal, X-linked mutations that confer no selective advantage to heterozygotes will frequently arise as spontaneous new mutations (36). HPRT deficiency syndromes show no ethnic predilection. Males with the Lesch-Nyhan syndrome fail to reproduce, and female heterozygotes have no selective advantage. The maintenance of the Lesch-Nyhan syndrome in the population is thus dependent upon the sporadic occurrence of these heterogenous mutations.

In earlier studies, Francke et al compiled the results of carrier detection tests on mothers and maternal grandmothers from 54 families with a single affected child (26). These tests suggest that 11 of 54 affected males were the result of new mutations. In addition, 10 of the heterozygous mothers were shown to be carriers of new mutations. Molecular analysis of the origins of mutations in such families will permit identification of paternal and maternal gametic mutations as well as determine mechanisms of mutation for each.

To address the question of genetic heterogeneity among mutations of the HPRT gene, Southern, Northern, Western, kinetic, and immunoquantitative data have been compiled for 24 unrelated patients with HPRT deficiency. Using these molecular parameters to create a composite picture for each mutant, it was found that 14 of 24 patients have unique genetic backgrounds, suggestive of extensive mutant heterogeneity.
Indirect evidence for genomic heterogeneity in this population came from limited haplotype analysis. Two HPRT-linked restriction fragment length polymorphisms (RFLPs) have been described (12, 80). In 1983, Nussbaum described a three-allele BamHI RFLP that occurs within the HPRT gene. These alleles are expressed phenotypically on Southern blots as three distinct pairs of fragments: (a) a 22 kb/25 kb pair, (b) a 12 kb/25 kb pair, and (c) a 22 kb/18 kb pair. An additional two-allele TaqI RFLP was reported for an anonymous sequence (DXS-10) separate from, but closely linked to (95% confidence limits, 0<15cM), the HPRT gene. These alleles are represented as 5 kb or 7 kb bands on Southern blots. These RFLP patterns can be combined to establish six haplotypes, four of which are represented in this group of 24 patients. The most frequent haplotype pattern (68%) was that combining the 22 kb/25 kb BamHI allele and the 5 kb TaqI allele (22/25/5 pattern). The 22/25/7 pattern represented 9% of this group, and the 12/25/5 and 22/18/5 patterns were each seen in a single patient. Patients having unique, readily identifiable major gene alterations accounted for 9% surveyed (112; J. T. Stout, and J. M. Wilson, in preparation).

This study reports that a minority of HPRT deficient patients are able to produce a protein product detectable by immunologic methods. The majority of those that are CRM+ show the less severe, gouty phenotype. Only 15% of patients surveyed failed to produce a detectable HPRT mRNA and, as expected, those patients exhibited the severe Lesch-Nyhan phenotype. In general, the more severe the molecular defect (i.e. major gene alteration, HPRT mRNA−, etc) the more severe the clinical phenotype.

GENE TRANSFER AND THERAPEUTIC PROSPECTS

The transfer of an expressible HPRT cDNA molecule into HPRT-deficient mouse cells was first described by Jolly et al (47). Transfection of mouse LA 9 cells with a plasmid containing the human HPRT cDNA resulted in the appearance of HAT-resistant colonies at a frequency of approximately $1 \times 10^{-3}$. Brennand and coworkers subsequently reported the expression of human and Chinese hamster cDNA recombinants in fibroblasts from Lesch-Nyhan patients and HPRT-deficient Chinese hamster cells (15). These recombinants were introduced into cells by calcium phosphate coprecipitation, and HAT-resistant colonies were recovered at frequencies of $1.9 \times 10^{-4}$ (hamster cDNA in human cells) and $5 \times 10^{-4}$ (human cDNA into human cells). These frequencies are comparable to those obtained by Mulligan and Berg, who introduced into a similar Lesch-Nyhan cell line a chimeric plasmid containing the bacterial guanine phosphoribosyltransferase (gpt) gene and SV40 promoter elements (77).
Microinjection techniques have substantially enhanced gene transfer efficiencies. When HPRT cDNA molecules, under the control of a variety of promoters, are microinjected into HPRT- cells, HAT-resistant colonies appear at frequencies $10^2$--$10^3$ fold greater than frequencies obtained by calcium phosphate precipitation (S. M. W. Chang, personal communication). Studies involving the introduction of human HPRT cDNA sequences into mouse embryos via microinjection suggest that human minigenes can become stable components of the mouse genome. These genes can be expressed in a variety of tissues and can be transmitted to progeny in a Mendelian fashion. Alternatives to these relatively inefficient and labor-intensive methods of gene transfer have been found in the form of naturally occurring RNA and DNA viruses.

The unique structure and capabilities of viruses may make them the ideal gene transfer system in mammalian cells. Retroviruses are vectors whose life cycle depends upon the integration, replication, and expression of their genetic material in host cells (30, 102). The retroviral system is very efficient (100% of cells infected carry an integrated copy of the gene), and many cell types refractory to CaPO$_4$ transfection can be infected with retroviruses.

Infection of cultured Lesch-Nyhan cells with amphotrophic retroviruses containing human HPRT cDNA sequences was shown by Willis et al to result in the production of enzymatically active human HPRT (103). Concomitant with expression of HPRT in these cells was the partial correction of other aberrant metabolic parameters, i.e. elevated purine excretion and increased intracellular hypoxanthine. A. Miller et al have reported using similar viruses to infect mouse bone marrow cells that were subsequently transplanted into lethally irradiated mice (72). In these studies, HPRT-virus production was detected in mouse spleen and bone marrow cells as long as 133 days after transplantation. Human HPRT protein was detected in spleen cells for two months. These studies suggest that retroviral vectors may serve as an efficient mechanism for somatic gene transfer.

The ultimate goal of medical geneticists involved with gene regulation and transfer is the management of inborn errors of metabolism at the genetic rather than symptomatic level. Gene therapy will depend upon the development of effective delivery systems, capable of safely introducing new genetic material that can be expressed at the appropriate times and in the appropriate tissues. Lesch-Nyhan syndrome may be amenable to gene therapy but available data do not assure success.

It is not clear if gene introduction into bone marrow cells will influence CNS dysfunction in these patients since therapeutic bone marrow transplantation for the Lesch-Nyhan syndrome has not been reported. Many aspects concerning the delivery of genes via retroviral vectors remain unclear. Questions concerning tissue requirements and temporal restraints on HPRT expression need to be answered. The devastating nature of this disease, combined with the lack of
therapeutic alternatives, warrants the investigation of gene transfer as a potential treatment for Lesch-Nyhan patients.

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