Minireview

Regulation of the Hypoxanthine Phosphoribosyltransferase Gene: *In Vitro* and *In Vivo* Approaches (43998)

SARN JIRALERSPONG* AND PRAGNA I. PATEL*,†,‡,§,[1]

Departments of Neurology, and Molecular and Human Genetics,† Human Genome Center,‡ and Division of Neuroscience,§ Baylor College of Medicine, Houston, Texas 77030-3411*

Abstract. **The hypoxanthine phosphoribosyltransferase (*HPRT*) locus is a constitutively expressed housekeeping gene characterized by a notably higher level of expression in the mammalian brain. The enzyme it encodes is key to purine salvage in humans and is the basis for the X-linked recessive disorder, Lesch-Nyhan syndrome (LNS). Methylation in the promoter plays a critical, if not fully understood, role in transcriptional silencing of the locus on the inactive chromosome, possibly by conferring structural stability. *In vivo* footprinting assays of the promoter region have shown protein interaction with multiple Sp1-binding sites, a possible AP2 site, and a potentially novel binding site. *In vitro* studies of *HPRT* promoter deletion constructs have identified a minimal promoter element necessary for maximal transcription and a position-dependent, orientation-independent repressor element (HPRT-NE) that functions on heterologous promoters. Regulatory intron elements have also been observed. Studies on transgenic mice bearing *HPRT* promoter constructs have shown that the minimal promoter element is insufficient for *in vivo* expression and that HPRT-NE is responsible for conferring neuronal specificity. *HPRT*$^-$ mice possess metabolic defects similar to LNS patients, but fail to develop human behavioral abnormalities, perhaps because of species differences in purine metabolism. A neuronal-specific protein complex appears to be necessary for activator function of HPRT-NE, while a ubiquitously expressed complex may be responsible for repression. Sequence analysis indicates that the latter complex may depend on the multifunctional transcription factor YY1 for binding. A fuller understanding of *HPRT* gene regulation will hopefully provide insight into the transcriptional mechanisms controlling the expression of housekeeping and brain-specific genes.** [P.S.E.B.M. 1996, Vol 212]

The gene encoding hypoxanthine phosphoribosyltransferase (*HPRT*) belongs to a class of constitutively expressed housekeeping genes which encode enzymes that perform basic metabolic functions. Characteristic of this group, which includes genes coding for adenosine deaminase (1), dihydrofolate reductase (2–5), and phosphoglycerate kinase (6), are a ubiquitous if relatively low level of expression in most cells and an overall shared promoter organization (7, 8). In comparison to promoters of tissue-specific genes, relatively little is known about transcriptional regulation of this important class of genes. HPRT enzyme activity has been measured at basal levels in most tissues, but, significantly, it is present at much higher levels in the brain, a distribution pattern observed in rodents and humans. The absence of HPRT

[1] To whom requests for reprints should be addressed at Department of Neurology, Baylor College of Medicine, Houston, TX 77030-3411.

0037-9727/96/2122-0116$10.50/0

in humans has been linked to Lesch-Nyhan syndrome (LNS) (9), a severe neurological disorder characterized by mental retardation, spasticity, and a compulsive form of self-mutilation (10–13), while a partial deficiency of the enzyme has been implicated in rare cases of severe gouty arthritis (14–17). A complete understanding of the molecular interactions in the *HPRT* promoter region that influence tissue-specific expression is an essential first step towards drawing a comprehensive picture of transcriptional control of this gene as well as other housekeeping genes, and in turn may point out regulatory sequences which are most vital to the success of gene replacement therapies.

Biochemistry

HPRT is an enzyme which catalyzes the conversion of the purines hypoxanthine and guanine to their respective 5′-mononucleotides by a reaction with 5-phosphoribosyl-1-pyrophosphate (18–20) (Fig. 1). In this capacity, HPRT is essential to the purine metabolic salvage pathway and supplements the supply of purine nucleotides synthesized by the *de novo* pathway (19, 21). Although there are other routes for recycling these purines in humans, HPRT bears the primary burden for this function (18). In 1967, Seegmiller

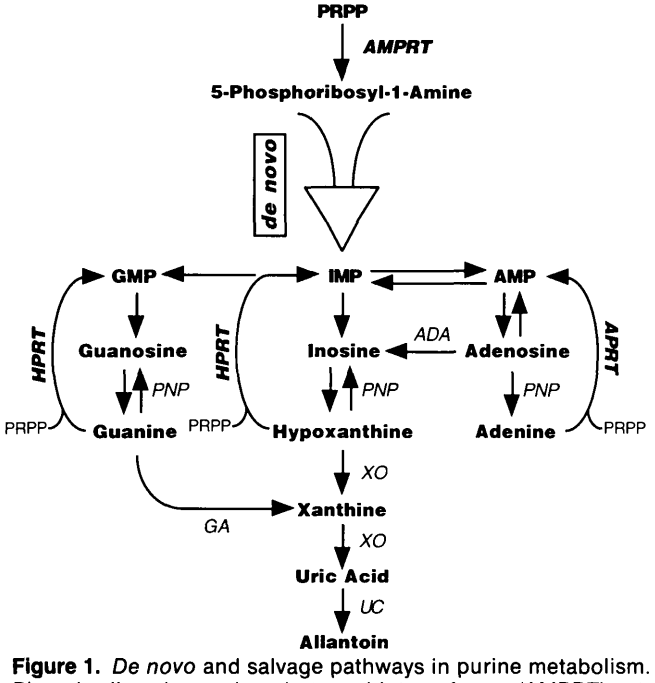

Figure 1. *De novo* and salvage pathways in purine metabolism. Phosphoribosyl pyrophosphate amidotransferase (AMPRT) catalyzes the committed step of *de novo* purine nucleotide synthesis. Hypoxanthine phosphoribosyltransferase (HPRT) and adenine phosphoribosyltransferase (APRT) are responsible for recycling purine bases into nucleotides. 5-phosphoribosyl-1-pyrophosphate (PRPP) levels regulate all of these reactions. Uricase (UC) prevents the buildup of uric acid in mice, but not in humans. Other important enzymes in the salvage pathway are adenosine deaminase (ADA), purine nucleoside phosphorylase (PNP), guanase (GA), and xanthine oxidase (XO).

et al. (9) demonstrated that a deficiency of HPRT was the biochemical basis for Lesch-Nyhan syndrome, an X-linked, recessive disorder (22–24) with an incidence of at least 1 in 200,000 males (25). The most striking metabolic features of LNS individuals are their abnormally high levels of uric acid excretion (10, 26), accelerated rate of *de novo* purine synthesis (26–28), and below average dopamine levels (70%–90% reduction) in the brain, especially in portions of the basal ganglia (29–31). Excess uric acid formation, which also leads to gouty arthritis in patients with partial HPRT deficiency (14, 16), has been attributed to the catabolization of accumulated hypoxanthine *via* xanthine into uric acid (9, 32, 33). This buildup is further exacerbated by the hyperactivity of the *de novo* pathway, which may be naturally upregulated by altered levels of salvage pathway regulatory products and substrates to assume a compensatory role in maintaining normal brain purine nucleotide levels (19, 21, 34–36). In a study by Jinnah *et al.* (37), lowered dopamine levels in *HPRT* knock-out mice have been found to correlate to reduced levels of tyrosine hydroxylase, and enzyme which catalyzes the rate-limiting step of dopamine synthesis. The caudoputamen, a dopaminergic region of the basal ganglia, underwent the most marked reduction in dopamine production in *HPRT* knock-out mice, and this study supports the hypothesis that reduced HPRT activity may indirectly reduce dopamine levels by altering purine metabolism in such a way as to lead to reduced arborization of dopamine fibers (29). A role for any of the above mentioned metabolic anomalies in the mental retardation, choreoathetosis, self-injurious behaviour, and other neurobehavioral defects commonly associated with LNS has yet to be defined, although biochemical changes in the basal ganglia could account for some of the symptoms seen (38–40).

Mutations

Mutations leading to an almost complete deficiency of HPRT enzyme activity are responsible for LNS, while partial deficiency results in gout (9, 14, 16, 41, 42). Point mutations, which typically produce less severe changes in enzyme structure, are the main cause of the latter disorder (42). Missense mutations are also the underlying basis for a large number of LNS cases, but, by and large, the mutations documented show a high degree of heterogeneity in terms of type and location within the gene locus (42–44). Novel missense mutations (45–49), nonsense mutations (50, 51), splice site mutations (46, 48, 52), deletions (51, 53), insertions, and other genetic anomalies (54) are continually added to the growing *HPRT* mutational spectrum. Instances in which the same mutation is found in unrelated LNS patients have been demonstrated (42–44) and may point to regions of par-

ticular genetic instability or of critical importance to enzyme function. For LNS, the incidence of new mutations is high, but for reasons unknown lower than would be predicted by Haldane's hypothesis for X-linked disorders in which affected males do not reproduce (55, 56). Finally, although the overwhelming majority of HPRT-related disorders afflict males, an exceptional case of a female LNS patient carrying an inactivated paternal allele and a deleted maternal allele has been documented (57–59).

Tissue Distribution

Early research efforts on HPRT have provided a firm foundation of biochemical and molecular information on which to build. The 217 residue, 24.5-kDa enzyme is highly conserved in rodents and humans (95% amino acid identity) (60–64). Conservation is also seen in the tissue distribution of HPRT in mammals. Enzyme activity assays of tissue extracts from humans (12, 65, 66), rhesus monkeys (67), and mice (68, 69), show that HPRT is constitutively expressed in most cells, but is especially abundant in the brain. RNase protection studies of lymphocyte mRNA indicate levels as low as one *HPRT* transcript per cell (70).

In human brains, HPRT activity seems to be particularly high in the basal ganglia (12, 16, 65, 66), but other species may not have the same expression pattern. The apparent lack of basal ganglia activity for phosphoribosyl-pyrophosphate amidotransferase (AMPRT) (12), an enzyme responsible for the rate-limiting step of *de novo* purine synthesis (34), could account for elevated HPRT levels, since there is evidence for an inverse relationship between the activity of the *de novo* and salvage purine metabolic pathways in some tissues (12, 71–76). Separate *in situ* hybridization studies of mouse brain have been able to localize high *HPRT* mRNA levels to neurons, excluding glial cells as enzyme sources, but gave conflicting results for relative expression levels in the basal ganglia. One study by Rincón-Limas *et al.* (77) indicates that, as is the case with humans, this region has higher levels of expression, a finding supported by reverse transcriptase–polymerase chain reaction (RT-PCR) data. The other study (78), while acknowledging higher than background levels of *HPRT* mRNA, showed by RNA *in situ* hybridization that expression in the basal ganglia was well below that of other regions of the brain. Studies of HPRT activity in rat brains would seem to confirm this result (76, 79). These differences may be due to technical variations in the studies or the use of different strains of wild-type mice. Certainly, given the fact that LNS patients and *HPRT* null mice show reduced dopamine levels in the basal ganglia as a major metabolic defect (37, 80–82), the results of the first study fit temptingly well with a simple model in which dopamine levels in a particular area of the brain are dependent on local HPRT activity. On the other hand, models in which HPRT and/or its metabolites are transported away from synthesizing cells (78), thereby exerting effects in distant areas, are equally plausible at this point. Moreover, species differences in basal ganglia expression may help explain the smaller reduction in dopamine levels seen in *HPRT* knock-out mice (48%–64%) (37) compared with LNS patients (70%–90%) (29, but additional research will be required to resolve this discrepancy.

In situ studies also show that the distribution of HPRT expression in the mouse brain does not match that for other housekeeping genes involved in purine metabolism (37), such as adenosine deaminase. Since many purines have been shown to act as neurotransmitters, this finding suggests an intricate and varied array of purine functions and distributions in the brain which is probably maintained by an equally complex system of biochemical regulation.

Genetic Mapping and Structure

Analysis of the *HPRT* gene by molecular genetic techniques has helped to delineate the gene structure and has defined putative regulatory elements to explain the distribution of HPRT in humans (Fig. 2). The expressed gene has been mapped to the X chromosome in rodents and human, specifically to band Xq26–q27 outside of the human pseudoautosomal region (83–88). Four pseudogenes are present in humans on chromosomes 11, 3, and 5 (89), while a single pseudogene has so far been identified in the rodent genome (90). The gene spans 39.8, 36, and 33 kb in the human (91), Chinese hamster (92), and mouse (93) genomes, respectively. The overall organization of *HPRT* is similar between species, each having nine exons with introns of varying sizes inserted at identical points within the coding sequence (92–95). Sequence homology approaches 89% among the coding regions for mouse, hamster, rat, and human (42). Comparison of two sequences of either mouse, human, or hamster shows 69%–76% homology in the 5′ untranslated region and 80%–88% homology in the 3′ untranslated region (42). This unusually high level of conservation in the downstream end of the transcript hints at an important regulatory function for this region which has yet to be studied fully.

The upstream promoter sequences have been subject to greater scrutiny, since accurately dissecting their functions is critical for successful *HPRT* gene replacement therapy. The human (94, 95) and mouse (93) *HPRT* gene have several elements in common with the promoter regions of other housekeeping genes as well as certain viral promoters, such as those for the SV40 early and late genes (96, 97). First, it lacks the consensus TATA and CAAT sequences found within 80 bp upstream of the ATG start codon in many eu-

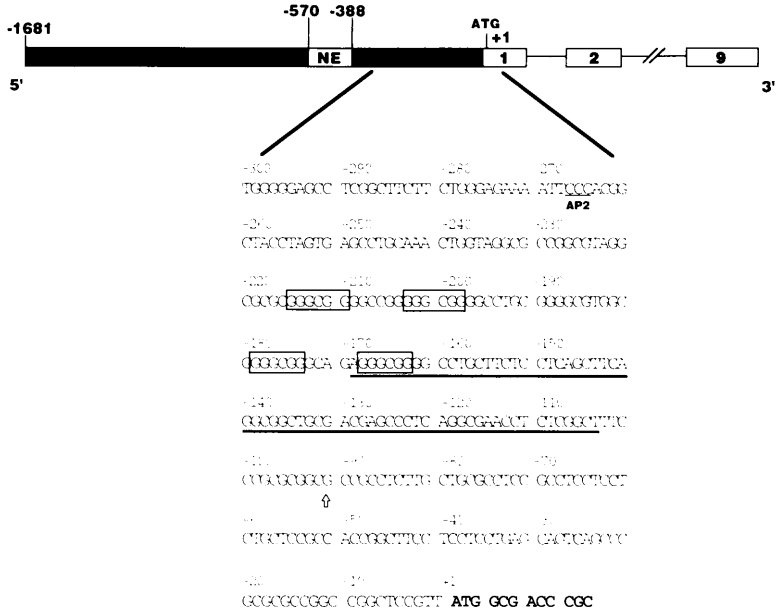

Figure 2. The human *HPRT* gene and its critical promoter region. The gene consists of nine exons, and tissue-specific expression is regulated by upstream sequences up to −1681 bp away from the translation start site (+1). The GC-rich proximal portion of the promoter contains several Sp transcription factor binding sites (boxed). Multiple transcriptional start sites are downstream of this region (heavy line). *In vivo* footprinting assays demonstrate protein interaction with the Sp sites, a possible AP2 site (indicated), and an unknown binding site (arrow). The region from −233 to −164 is hypomethylated on the inactive X chromosome.

karyotic genes transcribed by RNA polymerase II (13, 98). Second, the region is abnormally GC-rich and contains several closely spaced copies of a GGGCGG repeat which matches the consensus binding sequence (G/TGGGCGGG/AG/AC/T) (99) of members of the Sp family of transcriptional activators. Lastly, RNase protection and primer extension experiments have demonstrated that multiple transcriptional initiation sites are active in the human (94, 95) and mouse (100) promoters.

cis-Acting Sequences

Several *cis*-acting regulatory sequences have been delineated in the 5′ *HPRT* region by sequence comparison and creation of recombinant promoter constructs for *in vitro* and *in vivo* analysis.

Methylation. X chromosome inactivation accompanied by methylation of cytosine residues in CpG clusters appears to have a similar role in maintaining dosage balances of HPRT in females as has been demonstrated for other mammalian X-linked genes. The human (94, 95) and mouse (93) *HPRT* genes contain 46 and 35 CpG sites, respectively, within 400 bp of the translation initiation site (95). The importance of methylation to the inactive state is demonstrated by the fact that inactive *HPRT* alleles in somatic cell lines can be reactivated by treatment with the demethylating agent 5-azacytidine (101–106). Sequencing of mouse genomic DNA treated with bisulfite, which preferentially deaminates cytosine to the exclusion of methylcytosine, revealed no methylation of promoter CpG sites in *HPRT* genes from males or the active X chromosome of females (107). By contrast, an average of 70% of CpG sites were methylated in DNA from inactive X chromosomes. Although there was heterogeneity in the degree and location of methylation, CpG sites adjacent to the transcription start site showed the highest frequency of methylation in cell lines. This variability suggests that the broad pattern of methylation may be more significant than methylation of any specific site in transcriptional silencing (106, 107).

Similar overall patterns of methylation in active and inactive alleles of *HPRT* have been observed in humans with one important difference: a region of hypomethylation centered around the cluster of Sp-binding sites (106). Sp1 sites have been shown to confer methylation resistance in the *APRT* promoter (108, 109) and it is possible that they have a similar effect on the inactive X chromosome. A functional consequence of this hypomethylation might be reduced binding of the transcription factor. Sp1 shows unaltered (110, 111) or increased binding affinity for some sites, such as the stage selector element of the human γ-globin promoter (112), when they are methylated. *In vivo* footprinting studies, though, have failed to reveal binding of any factors to the human *HPRT* promoter on the inactive chromosome, while showing that the unmethylated Sp sites of the active allele are bound (113, 114).

Other regions in the mouse and human genes exhibit differential methylation in the active and inactive alleles (105, 115, 116). A region in intron 1 is completely unmethylated on the active X chromosome, but hypermethylated on the inactive chromosome. Conversely, several sites in the 3′ region of the gene methylated on the active X chromosome and unmodified on the inactive chromosome.

Whether methylation is the proximal cause of inactivation or plays a more secondary role in the maintenance of the condition is open to debate. The latter possibility is suggested by the fact that methylation of

the intron 1 region of the mouse *HPRT* gene occurs several days after general X inactivation in the embryo (117). Studies of the X-linked phosphoglycerate kinase gene, though, indicate that methylation of the promoter region occurs at approximately the same time as inactivation (118, 119). One reason for the different developmental timetables may be that the phosphoglycerate kinase gene lies closer to the X-inactivation center than the *HPRT* locus (119). Developmental studies focusing on the relationship between inactivation and methylation have been too few to draw any firm conclusions either way, and it may be that methylation serves both to initiate and maintain inactivation, depending on the site involved (120). Methylation patterns in the promoter region of the human *HPRT* gene during development remain a mystery.

The mechanism by which methylation induces transcriptional silencing is unknown as well. Ackerman *et al.* (121) have suggested that a stem-loop structure around the major transcription start site in CG-rich promoters may be a necessary prerequisite for increasing chromatin accessibility and inducing basal promoter function. Based on sequence analysis, the possibility of such structures has been deduced for regions flanking the major transcription initiation sites in humans (94) and mice (107). The fact that this region is also a consistent site for high degrees of methylation supports a model where methylation inhibits intrastrand base pairing, thereby stabilizing DNA and preventing the formation of transcriptional activation structures (106, 107).

***In Vitro* Studies.** *In vitro* experiments with various portions of the *HPRT* gene and its flanking sequences have contributed significantly to an understanding of its regulatory elements. Transfection experiments in mouse embryonic stem cells have revealed regulatory sequences outside of the traditional upstream promoter region (122). An element in intron 2 is necessary for HPRT expression in these cells, while a second element in intron 1 serves as a general enhancer of HPRT expression. Deletion analysis in which portions of the mouse *HPRT* promoter were used to drive expression of a *HPRT* minigene and the *neo* gene localized a 49-bp minimal promoter region which contains two Sp-binding sites (100).

In our laboratory, early minigene constructs bearing human 5′-flanking sequence up to 1.6 kb from the *HPRT* translational start site attached to human *HPRT* cDNA proved capable of expressing HPRT when transfected into *HPRT*$^-$ Chinese hamster cells (95). When subsequent deletion constructs of this 1.6-kb region were used to regulate expression of the chloramphenicol acetyltransferase (CAT) reporter gene, two distinct elements were revealed (123) (Fig. 3). The first (positions -219 to -122 relative to the translational start point) encompasses the clustered Sp-binding sites and functions as a putative minimal promoter required for gene expression. This minimal promoter, like many GC-rich promoters, displays limited bidirectional activity, possibly because of the functional symmetry resulting from the absence of TATA and CAAT elements. Work done in our laboratory suggests full orientation-independent functionality (123), while another study shows that promoter activity in the reverse orientation varies with the type and number of flanking reporter genes present (124). The second element (-570 to -388) is a repressor, dubbed the negative element (HPRT-NE), which is capable of reducing expression by at least an order of magnitude compared with constructs bearing the minimal promoter alone. Unlike other repressor elements such as that of the chicken γ1-crystallin gene (125), HPRT-NE continues to exhibit suppressing activity when combined with heterologous promoters for the *ADA* and *DHFR* genes. HPRT-NE behaves in a position-dependent and orientation-independent manner. In order to rule out the possibility that HPRT-NE functions by competing for a transcriptional activation factor that would normally bind to the minimal promoter, DNA competition studies were conducted in which constructs with and without HPRT-NE fused to the minimal promoter were separately cotransfected with recombinant DNA bearing the negative element. Reporter gene expression in cells with HPRT-NE in the regulatory region was increased by the presence of the competing HPRT-NE on another plasmid, while cells with constructs in which HPRT-NE was missing were unaffected. Therefore, the negative effect of HPRT-NE results from binding of a unique *trans*-acting factor and not from diversion of factors binding to the promoter.

***In Vivo* Studies.** Cultured cells can successfully model many aspects of the metabolic and chemical environment within a living organism, but *in vivo* studies ultimately confirm or refute a theoretical biological interaction. In the case of *HPRT*, subsequent studies done here (114) have shown that transgenic mice bearing the 1.6 kb human promoter fragment previously studied *in vitro* fused to the *lacZ* reporter gene exhibit a *lacZ* expression pattern similar to that normally seen for HPRT in mice. While X-gal staining of tissues from these mice revealed minimal *lacZ* expression in the heart, lung, liver, spleen, stomach, muscle, skin, pancreas, testis, kidney, or intestine, brain tissue showed high levels of expression, especially in the cerebral cortex, inferior colliculus, olfactory bulb, and basal ganglia. RT-PCR studies for the endogenous mouse *HPRT* gene revealed a similar pattern of expression in the brain. Analysis of deletion constructs of this promoter fragment in transgenic mice have both confirmed and countered conclusions drawn from *in vitro* data. Surprisingly, the minimal promoter identified by

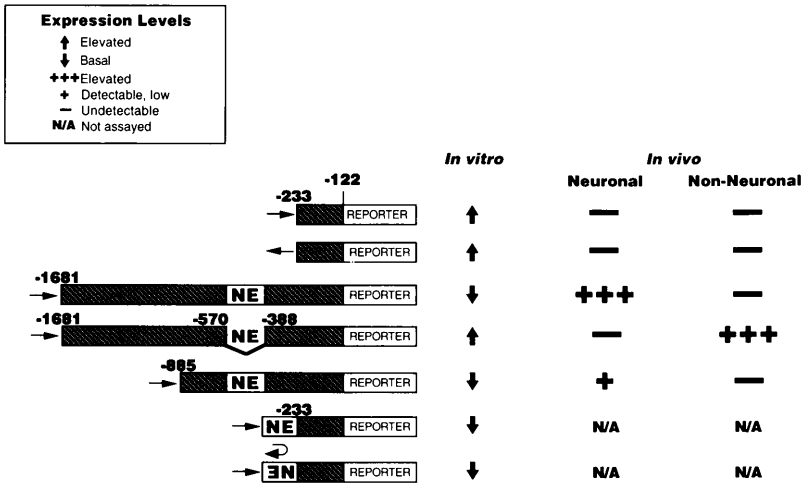

Figure 3. Relative expression levels of *HPRT* promoter deletion constructs used for *in vivo* and *in vitro* assays. A minimal promoter (−233 to −122) was able to drive expression *in vitro* but not *in vivo*. A negative element (NE) inhibited expression in cell cultures and non-neuronal mouse tissue, but was required for elevated expression in neuronal mouse tissue. The repressor function of NE is orientation independent. *In vivo* assays were performed with transgenic mice expressing the β-galactosidase reporter gene. *In vitro* assays were performed on a Chinese hamster fibroblast cell line (RJK88) expressing the chloramphenicol acetyltransferase reporter gene. Positions are given relative to the translation start codon (+1). Arrows (← →) indicate orientation of promoter fragments within the construct.

in vitro studies was unable to produce high expression levels in any tissues from multiple transgenic lines, although removal of HPRT-NE from the 1.6-kb promoter led to high levels of expression in non-neuronal tissue. This situation is reversed in those areas of the brain which show high expression with the 1.6-kb promoter: removal of HPRT-NE produces a dramatic reduction in the level of expression, strongly suggesting that HPRT-NE confers neuronal specificity to HPRT expression. A construct in which the downstream half (−855 to −122) of the 1.6-kb promoter fragment, a segment which includes HPRT-NE, serves as the regulatory sequences also leads to basal expression of reporter gene expression in brain tissue. It would seem, therefore, that expression in non-neuronal tissues depends on the presence of sequences upstream of HPRT-NE and probably the minimal promoter as well, while neuronal expression requires a combination of HPRT-NE, sequences upstream of HPRT-NE, though not necessarily the same as those required for non-neuronal expression, and possibly the minimal promoter. A study by Bonnerot *et al.* (126) suggests that spatiotemporal regulation of HPRT expression is strongly influenced by outside elements of the mouse genome. These conflicting results may be attributable to differences in the reporter constructs used, especially considering the fact that different *HPRT* promoter fragments were involved. Current work in our lab is focused on detailed analysis of the region upstream of HPRT-NE to isolate these additional *cis*-acting promoter sequences.

Other *in vivo HPRT* work has focused on creating an animal model for Lesch-Nyhan syndrome. *HPRT* knockout mice have been successfully created using various forms of the embryonic stem cell system (127, 128), but have shown a disappointing lack of neurobehavioral defects associated with HPRT deficiency in humans (80, 129). These animals do display many of the same metabolic anomalies as LNS patients, namely increased *de novo* purine synthesis and lowered levels of dopamine in the brain (36, 80–82). *HPRT*$^-$ mice fail to show the high levels of uric acid seen in LNS patients because of the presence of uricase, an enzyme responsible for conversion of uric acid to allantoin, in mice and not in humans (130). The reduction of this potential toxin may also explain the absence of obvious neurobehavioral defects in these mice. Another alternate possibility is that mice rely less heavily on HPRT for normal purine metabolism, and may effectively utilize APRT and other purine salvage enzymes to compensate for the loss of HPRT activity (131). The failure to replicate completely a human disease in a mouse model is not new (e.g., the absence of muscular impairment in *mdx* mice that share the dystrophin mutation of their human counterparts [132, 133]) and is not at all surprising given the substantial differences between the species.

Trans-Acting Factors

cis-acting sequences are only half the story of transcriptional regulation. Without an understanding of the protein factors associated with these sequences it is impossible to elucidate fully their function(s), if any. *HPRT cis*-acting sequences have been analyzed for protein binding capabilities to determine which of them may require *trans*-acting factors for regulatory activity.

***In Vivo* Footprinting.** *In vivo* footprint analysis shows that four of the Sp-binding sites in the *HPRT* promoter are subject to DNaseI protection consistent with protein binding (113). This proves that multiple Sp factors, or other factors recognizing the same sequence, interact with the region in some manner. Protection was also observed for a region (−265 to −267) spanning a possible AP2-binding site, as well as for a potentially novel DNA-binding site (−91).

HPRT-NE Binding Factors. Our lab has shown (114) that the HPRT-NE region appears to interact with two distinct sets of proteins in neuronal and non-neuronal cells (Fig. 4), which may explain the opposing functions it serves in these tissues. Incubation of the hHPRT-NE fragment with extracts from mouse brain or rat PC12 (adrenal pheochromocytoma-derived) cells revealed two different gel mobility shift products. The slower moving, larger product (complex II) is formed with extracts from neuronal and nonneuronal sources, including brain, liver, kidney, and HeLa cells. The smaller product (complex I) is formed exclusively with extracts from brain tissue. Neuronal specificity of complex I was confirmed by the finding that extracts from human NT2/D1 cells do not form complex I until treatment with retinoic acid induces their differentiation into neuronal cells. Furthermore, RT-PCR and RNase protection assays show that differentiation leads to a substantial increase in *HPRT* mRNA levels in these cells as well. Complex I would therefore appear to be involved in increasing expression of the *HPRT* locus, although direct evidence is lacking.

In order to localize the binding sites for complexes I and II more precisely, gel retardation assays were performed using overlapping subfragments of HPRT-NE and mouse brain and liver extracts. A 60-bp fragment (Ff) necessary for binding complexes I and II was identified and found to possess the same orientation-independent repression activity in homologous and heterologous promoters as the full negative element in non-neuronal cells. This makes complex II the most likely candidate as the *HPRT* repressor. The production of complex II from neuronal extracts may be due to the presence in the source tissue of brain cells, such as glia, that maintain a basal level of *HPRT* transcription.

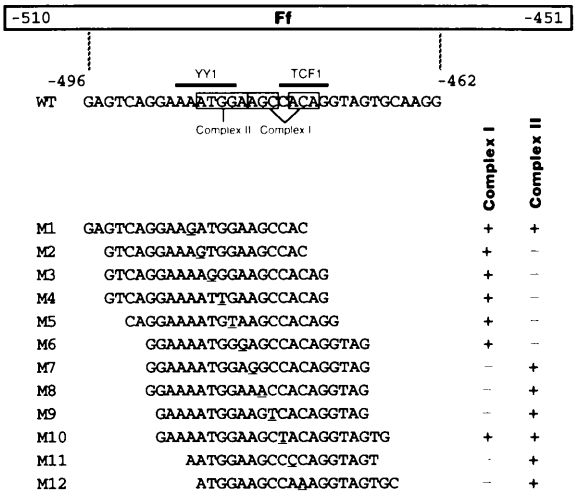

Figure 4. *In vitro* analysis of *HPRT* negative element point mutants. Thirteen point mutations (underlined) were introduced in a portion of the negative element (Ff) which has protein binding function. Protein extracts from non-neuronal cells formed complex II with Ff. Extracts from neuronal cells formed complex II and a faster moving complex I. +, strong complex formation; −, very weak or undetectable complex formation. M7–M9 and M11–M13 had reduced ability to form the neuronal-specific complex I. These mutants span a region (boxed) that matches the consensus binding sequence for transcription factor TCF1. M2—M6 showed reduced ability to bind complex II. The critical binding region for this complex overlaps the binding sequence of transcription factor YY1 (boxed).

Methylation interference studies further pinpointed the minimal site of interaction for both complexes to the heptamer 5′-GGAAGCC-3′ (−483 to −477). Gel shift analysis using clones with single point mutations in this region revealed that the binding site for complex II was a 5′-ATGGA-3′ (−485 to −481) sequence, while complex I could only be formed in the presence of nucleotides 5′-AGC-3′ (−480 to −478) and 5′-ACA-3′ (−476 to −474). *In vivo* footprinting confirmed binding of factors to position −485, −491, and −493 in non-neuronal cells.

Ultraviolet (UV) cross-linking studies show that both complexes are multiprotein structures. In this assay, each complex is separated from the bound DNA by UV cross-linking and then its component proteins are disassociated on a denaturing polyacrylamide gel. Complex I was found to have components of 51, 63, and possibly 110 kDa in size, while complex II is composed of 51-, 97-, and 200-kDa proteins. An obvious conclusion one can draw from this data is that the same 51-kDa protein is responsible for DNA interaction of both complexes and that the auxiliary proteins serve to modify binding specificity and function. It is equally possible, though, that each complex has a unique protein responsible for DNA-binding activity. Positions adjacent to the minimal binding area may produce conformational effects that are necessary for the complex to function properly.

Yin Yang-1. Determining the composition of complexes I and II is critical to understanding how HPRT-NE functions. Competition studies using oligonucleotides with binding sites for transcription factors Sp1, AP1, AP2, AP3, GRE, NF-kB, Oct-1, CREB, and CTF/NF1 failed to interfere with production of either complex, indicating that none of these factors is a component (114). Interestingly, the putative minimal binding site for complex II matches the consensus recognition sequence (ANATGG) of mouse transcription factor CF-1 or δ (134–136). This factor has many features which make it a likely participant in *HPRT* transcription. The highly conserved human homologue of CF-1, Yin Yang-1 (YY1) (137), has been shown to act as a repressor or activator for several genes, including some ubiquitously expressed housekeeping genes, such as dihydrofolate reductase (138). YY1 also may play some role in targeting expression, since it functions as a repressor of tissue-specific genes and an activator of genes with widespread expression (138).

The close proximity of the binding sites for the two HPRT-NE complexes is a motif also seen in many regulatory regions containing YY1 repressor–binding sites. For example, the YY1 site in the c-*fos* and α-actin promoters overlaps the binding site for the serum response factor (139, 140). In both cases, elevated expression of the serum response activator *in vivo* allows it to outcompete YY1 for binding to the site and in turn obviates transcriptional repression mediated by YY1 (139, 140). A similar mechanism may account for the brain-specific expression of HPRT. Incomplete competition would explain the formation of both complexes from brain extracts. In fact, sequence analysis shows that the suggested binding site for complex I matches the consensus binding site of factor TCF-1 (C/AAC/AAG) (136, 141), a transcriptional activator expressed primarily in lymphocytes (142–144). Unfortunately, TCF-1 does not appear to be neuronally expressed, and results in our laboratory show that a nonconsensus base substitution in this sequence was still able to bind complex I factors (114).

Despite this, current work in this laboratory focuses on providing direct evidence for the involvement of YY1 in *HPRT* transcription. Concurrent with these studies is an effort to isolate DNA-binding factors of both complexes through one-hybrid screening of neuronal and non-neuronal cDNA libraries. This approach and the related two-hybrid approach have proven to be powerful techniques for identifying DNA-protein and protein-protein interactions. The two-hybrid system will have to be employed at some point in the future, since both complexes are multiprotein units.

Conclusions

Our understanding of how HPRT expression is regulated in mammals has progressed significantly in terms of the gross mechanisms involved. Regulatory elements involved in X inactivation, basal promoter activity, transcriptional suppression, and neuronal-specific expression have been mapped out. Along the way, we have been reminded of the limitations of *in vitro* analysis in correctly modeling animals systems. The apparent behavioral normality of the *HPRT* knock-out mice also admonishes us to always be aware of the major metabolic and genetic differences that exist in the animals models we utilize. Despite our advances, many aspects of *HPRT* regulation merit further study. At the top of this list should be increasing the resolution of our current map of *HPRT* regulatory sequences, isolating the particular factors that interact with these elements from the large assortment of mammalian transcription factors, and correlating these elements with the spatiotemporal regulation of the *HPRT* locus. Understanding the DNA-protein and protein-protein interactions of these factors at the molecular and functional level should go hand in hand with this work. Although finding this information involves a great deal of effort, the results should increase our understanding—an understanding which lags severely behind that of the TATA and CAAT class of promoters—of transcriptional regulation in the broad class of housekeeping genes that share parts of the *HPRT* promoter structure, as well as provide us with valuable information on the mechanisms involved in brain-specific expression. The role of methylation in initiating/maintaining X inactivation for *HPRT* and other X-linked genes and the regulatory role of sequences outside the upstream promoter region, such as in the conserved portion of the 3′ UTR, are other areas that require study. In light of recent advances, we will hopefully be able to gain a thorough understanding of the *HPRT* regulatory system by the time viable gene replacement therapies for treating genetic disorders become available.

Work from the Patel laboratory was supported by National Institutes of Health Grant R01AR41996 and a Biomedical Science Award from the Arthritis Foundation.

1. Valerio D, Duyvesteyn MG, Dekker BM, Weeda G, Berkvens TM, van der Voorn L, van Ormondt H, van der Eb AJ. Adenosine deaminase: Characterization and expression of a gene with a remarkable promoter. EMBO J **4**:437–443, 1985.
2. Chen MJ, Shimada T, Moulton AD, Cline A, Humphries RK, Maizel J, Nienhuis AW. The functional human dihydrofolate reductase gene. J Biol Chem **259**:3933–3943, 1984.
3. Yang JK, Masters JN, Attardi G. Human dihydrofolate reductase gene organization. Extensive conservation of the G+C-rich 5′ non-coding sequence and strong intron size divergence from homologous mammalian genes. J Mol Biol **176**:169–187, 1984.
4. Crouse GF, Leys EJ, McEwan RN, Frayne EG, Kellems RE. Analysis of the mouse *DHFR* promoter region: Existence of a divergently transcribed gene. Mol Cell Biol **5**:1847–1858, 1985.
5. McGrogan M, Simonsen CC, Smouse DT, Farnham PJ, Schimke RT. Heterogeneity at the 5′ termini of mouse dihydrofolate reductase mRNAs. Evidence for multiple promoter regions. J Biol Chem **260**:2307–2314, 1985.
6. Singer-Sam J, Keith DH, Tani K, Simmer RL, Shively L, Lindsay S, Yoshida A, Riggs AD. Sequence of the promoter region of the gene for human X-linked 3-phosphoglycerate kinase. Gene **32**:409–417, 1984.
7. Dynan WS. Promoters for housekeeping genes. Trends Genet **2**:196–197, 1986.
8. Melton DW. Strategies and mechanisms for the control of transcriptional initiation of mammalian protein-coding genes. J Cell Sci **88**:267–270, 1987.
9. Seegmiller JE, Rosenbloom FM, Kelley WN. Enzyme defect associated with a sex-linked human neurological disorder and excessive purine synthesis. Science **155**:1682–1684, 1967.
10. Nyhan WL. The Lesch-Nyhan syndrome. Annu Rev Med **24**:41–60, 1973.
11. Emmerson BT, Thompson L. The spectrum of hypoxanthine-guanine phosphoribosyltransferase deficiency. Q J Med **166**:423–440, 1973.
12. Watts RWE, Spellacy E, Gibbs DA, Allsop J, McKeran RO, Slavin GE. Clinical, post-mortem, biochemical and therapeutic observations on the Lesch-Nyhan syndrome with particular reference to the neurological manifestations. Q J Med **201**:43–78, 1982.
13. Maniatis T, Goodbourn S, Fischer JA. Regulation of inducible

and tissue-specific gene expression. Science **236**:1237–1245, 1987.
14. Kelley WN, Rosenbloom FM, Henderson JF, Seegmiller JE. A specific enzyme defect in gout associated with overproduction of uric acid. Proc Natl Acad Sci USA **57**:1735–1739, 1967.
15. Yu TF, Balis ME, Krenitsky TA, Dancis J, Silvers DN, Elion GB, Gutman AB. Rarity of X-linked partial hypoxanthine-guanine phosphoribosyltransferase deficiency in a large gouty population. Ann Intern Med **76**:255–264, 1972.
16. Kelley WN, Greene ML, Rosenbloom FM, Henderson JF, Seegmiller JE. Hypoxanthine-guanine phosphoribosyltransferase deficiency in gout. Ann Intern Med **70**:155–206, 1969.
17. Seegmiller JE. Diseases of purine and pyrimidine metabolism. In: Bondy PK, Rosenberg LE, Eds. Metabolic Control and Disease (8th ed). Philadelphia: W. B. Saunders, pp777–937, 1980.
18. Murray AW, Elliott DC, Atkinson MR. Nucleotide biosynthesis from performed purines in mammalian cells: Regulatory mechanisms and biological significance. Prog Nucleic Acid Res Mol Biol **10**:87–119, 1970.
19. Murray AW. The biological significance of purine salvage. Annu Rev Biochem **40**:811–826, 1971.
20. Henderson JF, Paterson ARP. Nucleotide Metabolism: An Introduction. New York: Academic Press, pp124–135, 1973.
21. Puig JG, Jimenez MJ, Mateos FA, Fox IH. Adenine nucleotide turnover in hypoxanthine-guanine phosphoribosyltransferase deficiency: Evidence for an increased contribution of purine byosinthesis *de novo*. Metabolism **38**:410–418, 1989.
22. Hoefnagel D, Andrew ED, Mireault NG, Berndt WO. Hereditary choreoathetosis, self-mutilation and hyperuricemia in young males. N Engl J Med **273**:130–135, 1965.
23. Shapiro SL, Sheppard GL Jr., Dreifuss FE, Newcombe DS. X-linked recessive inheritance of a syndrome of mental retardation with hyperuricemia. Proc Soc Exp Biol Med **122**:609–611, 1966.
24. Nyhan WL, Pesek J, Sweetman L, Carpenter DG, Carter CH. Genetics of an X-linked disorder of uric acid metabolism and cerebral function. Pediatr Res **1**:5–13, 1967.
25. Crawhall JC, Henderson JF, Kelley WN. Diagnosis and treatment of the Lesch-Nyhan syndrome. Pediatr Res **6**:504–513, 1972.
26. Lesch M, Nyhan WL. A familial disorder of uric acid metabolism and central nervous system dysfunction. Am J Med **36**:561–570, 1964.
27. Rosenbloom FM, Henderson JF, Caldwell IC, Kelley WN, Seegmiller JE. Biochemical basis of accelerated purine synthesis *de novo* in human fibroblasts lacking hypoxanthine-gluanine phosphoribosyltransferase. J Biol Chem **243**:1166–1177, 1968.
28. Skaper SD, Seegmiller JE. Hypoxanthine-guanine phosphoribosyltransferase mutant glioma cells: Diminished monamine oxidase activity. Science **194**:1171–1173, 1976.
29. Lloyd KG, Hornykiewicz O, Davidson L, Shannak K, Farley I, Goldstein M, Shibuya M, Kelley WN, Fox HI. Biochemical evidence of dysfunction of brain neurotransmitters in the Lesch-Nyhan syndrome. N Engl J Med **5**:1106–1111, 1981.
30. Silverstein FS, Johnston MV, Hutchinson RJ, Edwards NL. Lesch-Nyhan syndrome: CSF neurotransmitter abnormalities. Neurology **35**:907–911, 1985.
31. Jankovic J, Caskey TC, Stout JT, Butler IJ. Lesch-Nyhan syndrome: A study of motor behavior and cerebrospinal fluid neurotransmitters. Ann Neurol **23**:466–469, 1988.
32. Balis ME, Krakoff IH, Berman PH, Dancis J. Urinary metabolites in congenital hyperuricosuria. Science **156**:1122–1123, 1967.
33. Balis ME. Seminars on Lesch-Nyhan syndrome. Aspects of purine metabolism. Fed Proc **27**:1067–1074, 1968.
34. Wyngaarden JB, Ashton DM. The regulation of activity of phosphoribosylepryophosphate amidotransferase by purine ribonucleotides: A potential feedback control of purine biosynthesis. J Biol Chem **234**:1492–1496, 1959.
35. Udom A, Holmes EW. Purification and characterization of human amidophosphoribosyltransferase. J Clin Chem Clin Biochem **20**:428, 1982.
36. Jinnah HA, Page T, Friedman T. Brain purines in a genetic mouse model of Lesch-Nyhan disease. J Neurochem **60**:2036–2045, 1993.
37. Jinnah HA, Wojcik BE, Hunt M, Narang N, Lee KY, Goldstein M, Wamsley JK, Langlais PJ, Friedmann T. Dopamine deficiency in a genetic mouse model of Lesch-Nyhan disease. J Neurosci **14**:1164–1175, 1994.
38. Baumeister AA, Frye GD. The biochemical basis of the behavioral disorder in the Lesch-Nyhan syndrome. Neurosci Biobehav Rev **9**:169–178, 1985.
39. Alpin RL, Young AB, Penney JB. The functional anatomy of basal ganglia disorders. Trends Neurosci **12**:366–375, 1987.
40. Stout JT, Caskey CT. Hypoxanthine phosphoribosyltransferase deficiency: The Lesch-Nyhan syndrome and gouty arthritis. In: Scriver CR, Beaudet AL, Sly WS, Valle D. Eds. The Metabolic Basis of Inherited Disease (6th ed). New York: McGraw-Hill, pp1007–1028, 1989.
41. Kelley WN. Hypoxanthine-guanine phosphoribosyltransferase deficiency in the Lesch-Nyhan syndrome and gout. Fed Proc **27**:1047–1052, 1968.
42. Rossiter BJF, Caskey CT. Hypoxanthine-guanine phosphoribosyltransferase deficiency: Lesch-Nyhan syndrome and gout. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Eds. The Metabolic and Molecular Bases of Inherited Disease (7th ed). New York: McGraw-Hill, pp1679–1706, 1995.
43. Cariello NF, Skopek TR. *In vivo* mutation at the human *HPRT* locus. Trends Genet **9**:322–326, 1993.
44. Sculley DG, Dawson PA, Emmerson BT, Gordon RB. A review of the molecular basis of hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency. Hum Genet **90**:195–207, 1992.
45. Fujimori S, Tagaya T, Kamatani N, Akaoka I. A germ line mutation within the coding sequence for the putative 5-phosphoribosyl-1-pyrophosphate binding site of hypoxanthine-guanine phosphoribosyltransferase (HPRT) in a Lesch-Nyhan patient: Missense mutations within a functionally important region probably cause disease. Hum Genet **90**:385–388, 1992.
46. Yamada Y, Goto H, Suzumori K, Adachi R, Ogasawara N. Molecular analysis of five independent Japanese mutant genes responsible for hypoxanthine guanine phosphoribosyltransferase (HPRT) deficiency. Hum Genet **90**:379–384, 1992.
47. Bouwens-Rombouts AG, van den Boogaard MJ, Puig JG, Mateos FA, Hennekam RC, Tilanus MG. Identification of two new nucleotide mutations ($HPRT_{Utrecht}$ and $HPRT_{Madrid}$) in exon 3 of the human hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) gene. Hum Genet **91**:451–454, 1993.
48. Marcus S, Christensen E, Malm G. Molecular analysis of the mutations in five unrelated patients with the Lesch-Nyhan syndrome. Hum Mutat **24**:473–477, 1993.
49. Tohyama J, Nanba E, Ohno K. Hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency: Identification of point mutations in Japanese patients with Lesch-Nyhan syndrome and hereditary gout and their permanent expression in an *HPRT*-deficient mouse cell line. Hum Genet **93**:175–181, 1994.
50. Marcus S, Steen AM, Andersson B, Lambert B, Kristoffersson U, Francke U. Mutation analysis and prenatal diagnosis in a Lesch-Nyhan family showing non-random X-inactivation interfering with carrier detection tests. Hum Genet **89**:395–400, 1992.
51. Sege-Peterson K, Chambers J, Page T, Jones OW, Nyhan WL. Characterization of mutations in phenotypic variants of hypoxanthine phosphoribosyltransferase deficiency. Hum Mol Genet **1**:427–432, 1992.
52. Yamada Y, Goto H, Tamura S, Ogasawara N. Molecular genetic study of a Japanese family with Lesch-Nyhan syndrome: A point mutation at the consensus region of RNA splicing ($HPRT_{Keio}$). Jpn J Hum Genet **38**:413–419, 1993.
53. Fuscoe JC, Nelsen AJ. Molecular description of a hypoxanthine phosphoribosyltransferase gene deletion in Lesch-Nyhan syndrome. Hum Mol Genet **3**:199–200, 1994.
54. Marcus S, Hellgren D, Lambert B, Fallstrom SP, Wahlstrom J. Duplication in the hypoxanthine phosphoribosyl-transferase

gene caused by Alu-Alu recombination in a patient with Lesch-Nyhan syndrome. Hum Genet **90**:477–482, 1993.
55. Haldane JBS. The rate of spontaneous mutation of a human gene. J Genet **31**:317, 1935.
56. Francke U, Winter RM, Lin D, Bakay B, Seegmiller JE, Nyhan WL. Use of carrier detection tests to estimate male to female ratio of mutation rates in Lesch-Nyhan disease. In: Hook EB, Porter IH, Eds. Population and Biological Aspects of Human Mutation. New York: Academic Press, pp117–130, 1981.
57. Hara K, Kashiwamata S, Ogasawara N, Ohishi H, Natsume R, Yamanaka T, Hakamada S, Miyazaki S, Watanabe K. A female case of the Lesch-Nyhan syndrome. Tohoku J Exp Med **137**:275–282, 1982.
58. Ogasawara N, Stout JT, Goto H, Sonta S, Matsumoto A, Caskey CT. Molecular analysis of a female Lesch-Nyhan patient. J Clin Invest **84**:1024–1027, 1989.
59. Ogasawara N, Yamada Y, Goto H. *HPRT* gene mutations in a female Lesch-Nyhan patient. Adv Exp Med Biol **309B**:109–112, 1991.
60. Wilson JM, Tarr GE, Mahoney WC, Kelly WN. Human hypoxanthine-guanine phosphoribosyltransferase. Complete amino acid sequence of the erythrocyte enzyme. J Biol Chem **257**:10978–10985, 1982.
61. Konecki DS, Brennand J, Fuscoe JC, Caskey CT, Chinault AC. Hypoxanthine-guanine phosphoribosyltransferase genes of mouse and Chinese hamster: Construction and sequence analysis of cDNA recombinants. Nucleic Acids Res **10**:6763–6775, 1982.
62. Yang TP, Patel PI, Chinault AC, Stout JT, Jackson LG, Hildebrand BM, Caskey CT. Molecular evidence of new mutation at the *HPRT* locus in Lesch-Nyhan patients. Nature **310**:412–414, 1984.
63. Rossiter BJF, Caskey CTC. Molecular scanning methods of mutation detection. J Biol Chem **265**:12753–12756, 1990.
64. Chiaverotti TA, Battula N, Monnat RJ Jr. Rat hypoxanthine phosphoribosyltransferase cDNA cloning and sequence analysis. Adv Exp Med Biol **309B**:117–120, 1991.
65. Rosenbloom FM, Kelley WN, Miller J, Henderson JF, Seegmiller JE. Inherited disorder of purine metabolism. JAMA **202**:103–105, 1967.
66. Adams A, Harkness RA. Developmental changes in purine phosphoribosyltransferases in human and rat tissues. Biochem J **160**:565–576, 1976.
67. Krenitsky TA. Tissue distribution of purine ribosyl- and phosphoribosyltransferases in the Rhesus monkey. Biochim Biophys Acta **179**:506–509, 1969.
68. Murray AW. Inhibition of purine phosphoribosyltransferases from Ehrlich ascites-tumor cells by purine nucleotides. Biochem J **100**:671–674, 1966.
69. Lo YV, Palmour RM. Developmental expression of murine HPRT. I. Activities, heat stabilities, and electrophoretic mobilities in adult tissues. Biochem Genet **17**:737–746, 1979.
70. Steen AM, Luthman H, Hellgren D, Lambert B. Levels of hypoxanthine phosphoribosyltransferase RNA in human cells. Exp Cell Res **186**:236–244, 1990.
71. Lajtha LG, Vane JR. Dependence of bone marrow cells on the liver for purine supply. Nature **182**:191–192, 1958.
72. Lowy BA, Ramot B, London JM. The biosynthesis of adenosine triphosphate and guanosine triphosphate in the rabbit erythrocyte *in vivo* and *in vitro*. J Biol Chem **235**:2920–2923, 1960.
73. Holmsen H, Rozenberg MC. Adenine nucleotide metabolism of blood platelets. 3. Adenine phosphoribosyl transferase and nucleotide formation from exogenous adenine. Biochem Biophys Acta **157**:266–279, 1968.
74. Fontenelle LJ, Henderson JF. An enzymatic basis for the inability of erythrocytes to synthesize purine ribonucleotides *de novo*. Biochim Biophys Acta **177**:175–176, 1969.
75. Howard WJ, Kerson LA, Appel SH. Synthesis *do novo* of purines in slices of rat brain and liver. J Neurochem **17**:121–123, 1970.
76. Allsop J, Watts RW. Activities of amidophosphoribosyltransferase (ec2.4.2.14) and the purine phosphoribosyltransferases (ec2.4.2.7 and 2.4.2.8), and the phosphoribosylpyrophosphate content of rat central nervous system at different stages of development. Their possible relationship to the neurological dysfunction in the Lesch-Nyhan syndrome. J Neurol Sci **46**:221–232, 1980.
77. Rincon-Limas DE, Geske RS, Xue J-J, Hsu CY, Overbeek PA, Patel PI. 5′-Flanking sequences of the human *HPRT* gene direct neuronal expression in the brain of transgenic mice. J Neurosci Res **38**:259–267, 1994.
78. Jinnah HA, Hess EJ, Wilson MC, Gage FH, Friedmann T. Localization of hypoxanthine-guanine phosphoribosyltransferase mRNA in the mouse brain by *in situ* hybridization. Mol Cell Neurosci **3**:64–78, 1992.
79. Gutensohn W, Guroff G. Hypoxanthine-guanine-phosphoribosyl-transferase from rat brain (purification, kinetic properties, development and distribution). J Neurochem **19**:2139–2150, 1972.
80. Finger S, Heavens RP, Sirinathsinghji DJ, Kuhen MR, Dunnet SB. Behavioral and neurochemical evaluation of a transgenic mouse model of Lesch-Nyhan syndrome. J Neurol Sci **86**:203–213, 1988.
81. Dunnett SB, Sirinathsinghji DJ, Heavens R, Rogers DC, Kuehn MR. Monoamine deficiency in a transgenic (*HPRT*$^-$) mouse model of Lesch-Nyhan syndrome. Brain Res **501**:401–406, 1989.
82. Williamson DJ, Sharkey J, Clarke AR, Jamieson A, Arbuthnott GW, Kelly P, Melton DW, Hooper ML. Analysis of forebrain dopaminergic pathways in *HPRT*$^-$ mice. Adv Exp Med Biol **309B**:269–272, 1991.
83. Shows TB, Brown JA. Localization of genes coding for *PGK, HPRT,* and *G6PD* on the long arm of the X chromosome in somatic cell hybrids. Cytogenet Cell Genet **14**:426–429, 1975.
84. Farrell SA, Worton RG. Chromosome loss is responsible for segregation at the *HPRT* locus in Chinese hamster cell hybrids. Somatic Cell Genet **3**:359–551, 1977.
85. Fenwick RG, Jr. Reversion of a mutation affecting the molecular weight of HGPRT: Intragenic suppression and localization of X-linked genes. Somatic Cell Genet **6**:477–494, 1980.
86. Francke U, Taggart RT. Comparative gene mapping: Order of loci on the X chromosome is different in mice and humans. Proc Natl Acad Sci USA **77**:3595–3599, 1980.
87. Pai GS, Sprenkle JA, Do TT, Mareni CE, Migeon BR. Localization of loci for hypoxanthine phosphoribosyltransferase and glucose-6-phosphate dehydrogenase and biochemical evidence of nonrandom X chromosome expression from studies of a human X-autosome translocation. Proc Natl Acad Sci USA **77**:2810–2813, 1980.
88. Lyon MF, Zenthon J, Burtenshaw MD, Evans EP. Localization of the *HPRT* locus by *in situ* hybridization and distribution of loci on the mouse X-chromosome. Cytogenet Cell Genet **44**:163–166, 1987.
89. Patel PI, Nussbaum RL, Framson PE, Ledbetter DH, Caskey CT, Chinault AC. Organization of the *HPRT* gene and related sequences in the human genome. Somat Cell Mol Genet **10**:483–493, 1984.
90. Isamat M, Macleod KF, King A, McEwan C, Melton DW. Characterization, evolutionary relationships, and chromosome location of processed mouse *HPRT* pseudogene. Somat Cell Mol Genet **14**:359–369, 1988.
91. Edwards A, Voss H, Rice P, Civitello A, Stegemamm J, Schwager C, Zimmermann J, Erfle H, Caskey CT, Ansorge W. Automated DNA sequencing of the human *HPRT* locus. Genomics **6**:593–608, 1990.
92. Rossiter BJ, Fuscoe JC, Muzny DM, Fox M, Caskey CT. The Chinese hamster *HPRT* gene: Restriction map, sequence analysis, and multiplex PCR deletion screen. Genomics **9**:247–256, 1991.
93. Melton DW, Konecki DS, Brennand J, Caskey CT. Structure, expression, and mutation of the hypoxanthine phosphoribosyltransferase gene. Proc Natl Acad Sci USA **81**:2147–2151, 1984.
94. Kim SH, Moores JC, David D, Respess JG, Jolly DJ, Friedmann T. The organization of the human *HPRT* gene. Nucleic Acids Res **14**:3103–3118, 1986.
95. Patel PI, Framson PE, Caskey CT, Chinault AC. Fine struc-

ture of the human hypoxanthine phosphoribosyltransferase gene. Mol Cell Biol **6**:393–403, 1986.
96. Elder JT, Spritz RA, Weissman SM. Simian virus 40 as a eukaryotic cloning vehicle. Annu Rev Genet **15**:295–340, 1981.
97. Hansen U, Sharp PA. Sequences controlling *in vitro* transcription of SV40 promoters. EMBO J **2**:2293–2303, 1983.
98. Breathnach R, Chambon P. Organization and expression of eucaryotic split genes coding for proteins. Annu Rev Biochem **50**:349–383, 1981.
99. Kadonaga JT, Jones KA, Tijian R. Promoter-specific activation of RNA polymerase II transcription of Sp1. Trends Biochem Sci **11**:20–23, 1986.
100. Melton DW, McEwan C, McKie AB, Reid AM. Expression of the mouse *HPRT* gene: Deletional analysis of the promoter region of an X-chromosome linked housekeeping gene. Cell **44**:319–328, 1986.
101. Mohandas T, Sparkes RS, Shapiro LJ. Reactivation of an inactive human X chromosome: Evidence for X inactivation by DNA methylation. Science **211**:393–396, 1981.
102. Graves JAM. 5-azacytidine-induced re-expression of alleles on the inactive X chromosome in a hybrid mouse cell line. Exp Cell Res **141**:99–105, 1982.
103. Hors-Cayala MC, Heuertz S, Frezal J. Coreactivation of four inactive X genes in a hamster × human hybrid and persistence of late replication of reactivated X chromosome. Somatic Cell Genet **9**:645–657, 1983.
104. Paterno GD, Adra CN, McBurney MW. X chromosome reactivation in mouse embryonal carcinoma cells. Mol Cell Biol **5**:2705–2712, 1985.
105. Lock LF, Melton DW, Caskey CT, Martin GR. Methylation of the mouse *HPRT* gene differs on the active and inactive X chromosomes. Mol Cell Biol **6**:914–924, 1986.
106. Hornstra IK, Yang TP. High-resolution methylation analysis of the human hypoxanthine phosphoribosyltransferase gene 5′ region on the active an inactive X chromosomes: Correlation with binding sites for transcription factors. Mol Cell Biol **14**:1419–1430, 1994.
107. Park JG, Chapman VM. CpG island promoter region methylation patterns of the inactive-X-chromosome hypoxanthine phosphoribosyltransferase (*HPRT*) gene. Mol Cell Biol **14**:7975–7983, 1994.
108. Brandeis M, Frank D, Keshet I, Siegfried Z, Mendelsohn M, Nemes A, Temper V, Razin A, Cedar H. Sp1 elements protect a CpG island from *de novo* methylation. Nature **371**:435–438, 1994.
109. Macleod D, Charlton J, Mullins J, Bird AP. Sp1 sites in the mouse *APRT* gene promoter are required to prevent methylation of the CpG island. Genes Dev **8**:2282–2292, 1994.
110. Harrington MA, Jones PA, Imagawa M, Karin M. Cytosine methylation does not affect binding of transcription factor Sp1. Proc Natl Acad Sci USA **85**:2066–2270, 1988.
111. Holler M, Westin G, Jiricny J, Schaffner W. Sp1 transcription factor binds DNA and activates transcription even when the binding site is CpG methylated. Genes Dev **2**:1127–1135, 1988.
112. Jane SM, Gumucio DL, Ney PA, Cunningham JM, Nienhuis AW. Methylation-enhanced binding of Sp1 to the stage selector element of the human γ-globin gene promoter may regulate development specificity of expression. Mol Cell Biol **13**:3272–3281, 1993.
113. Hornstra IK, Yang TP. Multiple *in vivo* footprints are specific to the active allele of the X-linked human hypoxanthine phosphoribosyltransferase gene 5′ region: Implications for X chromosome inactivation. Mol Cell Biol **12**:5345–5354, 1992.
114. Rincón-Limas DE, Amaya-Manzanares F, Nino-Rosales ML, Yu Y, Yang TP, Patel PI. Ubiquitous and neuronal DNA-binding proteins interact with a negative regulatory element of the human hypoxanthine phosphoribosyltransferase gene. Mol Cell Biol **15**:6561–6571, 1995.
115. Wolf SF, Jolly DJ, Lunnen KD, Friedmann T, Migeon BR. Methylation of the hypoxanthine phosphoribosyltransferase locus on the human X chromosome: Implications for X-chromosome inactivation. Proc Natl Acad Sci USA **81**:2806–2810, 1984.
116. Yen P, Patel PI, Chinault AC, Mohandas T, Shapiro LF. Differential methylation of *HPRT* genes on active and inactive human X chromosome. Proc Natl Acad Sci USA **81**:1759–1763, 1984.
117. Lock LF, Takagi N, Martin GR. Methylation of the *HPRT* gene on the inactive X occurs after chromosome inactivation. Cell **48**:39–46, 1987.
118. Singer-Sam J, Grant M, LeBon JM, Okuyama K, Chapman V, Monk M, Riggs AD. Use of a *Hpa*II-polymerase chain reaction assay to study DNA methylation in the *PGK-1* CpG island of mouse embryos at the time of X-chromosome inactivation. Mol Cell Biol **10**:4987–4989, 1990.
119. Grant M, Zuccotti M, Monk M. Methylation of CpG sites of two X-linked genes coincides with X-inactivation in the female mouse embryo but not in the germ line. Nature Genet **2**:161–166, 1992.
120. Monk M. The X chromosome in development in mouse and man. J Inherit Metab Dis **15**:499–513, 1992.
121. Ackerman SL, Minden AG, Yeung CY. The minimal self-sufficient element in a murine G + C-rich promoter is a large element with imperfect dyad symmetry. Proc Natl Acad Sci USA **90**:11865–11869, 1993.
122. Reid LH, Gregg RG, Smithies O, Koller BH. Regulatory elements in the introns of the human *HPRT* gene are necessary for its expression in embryonic stem cells. Proc Natl Acad Sci USA **87**:4299–4303, 1990.
123. Rincon-Limas D, Krueger D, Patel PI. Functional characterization of the human hypoxanthine phosphoribosyltransferase gene promoter. Evidence for a negative regulatory element. Mol Cell Biol **11**:4157–4164, 1991.
124. Johnson P, Friedmann T. Limited bidirectional activity of two housekeeping gene promoters: Human *HPRT* and *PGK*. Gene **88**:207–213, 1990.
125. Borras T, Peterson CA, Piatigorsky J. Evidence for positive and negative regulation in the promoter of the chicken δ1-crystallin gene. Dev Biol **127**:209–219, 1988.
126. Bonnerot C, Grimber G, Briand P, Nicolas JF. Patterns of expression of position-dependent integrated transgenes in mouse embryo. Proc Natl Acad Sci USA **87**:6331–6335, 1990.
127. Hooper M, Hardy K, Handyside A, Hunter S, Monk M. HPRT-deficient (Lesch-Nyhan) mouse embryos derived from germline colonization by cultured cells. Nature **326**:292–295, 1987.
128. Kuehn MR, Bradley A, Robertson EJ, Evans MJ. A potential animal model for Lesch-Nyhan syndrome through introduction of *HPRT* mutations into mice. Nature **326**:295–298, 1987.
129. Jinnah HA, Gage FH, Friedmann T. Animal models of Lesch-Nyhan syndrome. Br Res Bull **25**:467–475, 1990.
130. Keilin J. The biological significance of uric acid and guanine excretion. Biol Rev **34**:265–296, 1959.
131. Wu CL, Melton DW. Production of a model for Lesch-Nyhan syndrome in hypoxanthine phosphoribosyltransferase-deficient mice. Nat Genet **3**:235–240, 1993.
132. Tanabe Y, Esaki K, Nomura T. Skeletal muscle pathology in X chromosome-linked muscular dystrophy (*mdx*) mouse. Acta Neuropathol **69**:91–95, 1986.
133. Torres LF, Duchen LW. The mutant *mdx:* Inherited myopathy in the mouse. Morphological studies of nerves, muscles and end-plates. Brain **110**:269–299, 1987.
134. Hariharan N, Kelley DE, Perry RP. δ, a transcription factor that binds to downstream elements in several polymerase II promoters, is a functionally versatile zinc finger protein. Proc Natl Acad Sci USA **88**:9799–9803, 1991.
135. Riggs KJ, Merrell KT, Wilson G, Calame K. Common factor 1 is a transcriptional activator which binds in the *c-myc* promoter, the skeletal α-actin promoter, and the immunoglobulin heavy-chain enhancer. Mol Cell Biol **11**:1765–1769, 1991.
136. Faisst S, Meyer S. Compilation of vertebrate-encoded transcription factors. Nucleic Acids Res **20**:3–26, 1992.
137. Riggs KJ, Saleque S, Wong KK, Merrell KT, Lee JS, Shi Y, Calame K. Yin-Yang 1 activates the *c-myc* promoter. Mol Cell Biol **13**:7487–7495, 1993.
138. Shrivastava A, Calame K. An analysis of genes regulated by the multi-functional transcriptional regulator Yin Yang-1, Nucleic Acids Res **22**:5151–5155, 1994.

139. Gualberto A, LePage D, Pons G, Mader SL, Park K, Atchison ML, Walsh K. Functional antagonism between YY1 and the serum response factor. Mol Cell Biol **12**:4209–4214, 1992.
140. Lee TC, Shi Y, Schwartz RJ. Displacement of BrdUrd-induced YY1 by serum response factors activates skeletal α-actin transcription in embryonic myoblasts. Proc Natl Acad Sci USA **89**:9814–9818, 1992.
141. Oosterwegel MA, van de Wetering ML, Holstege FC, Prosser HM, Owen MJ, Clevers HC. TCF-1, a T cell-specific transcription factor of the HMG box family, interacts with sequence motifs in the TCRβ and TCRδ enhancers. Int Immunol **3**:1189–1192, 1991.
142. Oosterwegel M, van de Wetering M, Dooijes D, Klomp L, Winoto A, Georgopoulos K, Meijlink F, Clevers H. Cloning of murine TCF-1, a T cell-specific transcription factor interacting with functional motifs in the CD3-ϵ and T cell receptor α enhancers. J Exp Med **173**:1133–1142, 1991.
143. van de Wetering M, Oosterwegel M, Dooijes D, Clevers H. Identification and cloning of TCF-1, a T lymphocyte-specific transcription factor containing a sequence-specific HMG box, EMBO J **10**:123–132, 1991.
144. Waterman ML, Fischer WH, Jones KA. A thymus-specific member of the HMG protein family regulates the human T cell receptor Cα enhancer. Genes Dev **5**:656–669, 1991.