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Functional Characterization of the Human Hypoxanthine Phosphoribosyltransferase Gene Promoter: Evidence for a Negative Regulatory Element

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The enzyme hypoxanthine phosphoribosyltransferase (HPRT) catalyzes the metabolic salvage of the purine bases hypoxanthine and guanine. We previously characterized the genomic structure of the human *HPRT* gene and described its promoter sequence. In this report, we identify *cis*-acting transcriptional control regions of the human *HPRT* gene by linking various 5′-flanking sequences to the bacterial chloramphenicol acetyltransferase gene. The sequence from positions −219 to −122 relative to the translation initiation site is required for maximal expression of this gene, and it functions equally in both normal and reverse orientations. In addition, a *cis*-acting negative element is present in the region spanning from positions −570 to −388. This negative element can also repress promoters of heterologous genes, such as those of adenosine deaminase and dihydrofolate reductase, which are structurally and functionally similar to the human HPRT promoter. Furthermore, this repressor element functions independently of its orientation but appears to be distance dependent. In vivo competition assays demonstrated that the *trans*-acting factor(s) that binds to this negative element specifically inhibits human HPRT promoter activity. Taken together, these data localize *cis*-acting sequences important in the regulation of human *HPRT* gene expression and should allow the study of protein-DNA interactions which modulate the transcription of this gene.

Hypoxanthine phosphoribosyltransferase (HPRT; IMP: pyrophosphate phosphoribosyltransferase; EC 2.4.2.8) catalyzes the conversion of hypoxanthine and guanine to their respective 5′-mononucleotides and plays a crucial role in the metabolic salvage of purines in mammalian cells. A partial deficiency of the enzyme in humans causes gouty arthritis, whereas a virtually complete deficiency leads to a devastating neurological disorder, Lesch-Nyhan syndrome (31).

The HPRT enzyme is approximately 24.5 kDa and is highly conserved among hamsters, mice, and humans (4). The functional gene is constitutively expressed in a wide variety of tissues, including cultured cells. Studies of tissue distribution of the enzyme in mammals have indicated that the enzyme activity is approximately sevenfold higher in the brain than in other tissues (4, 22, 31). The mechanism for increased HPRT expression in the brain with the maintenance of a constitutive low level of HPRT expression in other tissues is largely unknown.

The human HPRT (hHPRT) promoter is extremely GC rich, lacks a consensus TATA or CAAT sequence, and contains several copies of the sequence 5′-CCGCCC-3′ or its inverted complement (17, 27). Previously, we demonstrated that sequences within 245 bp of the initiation codon were sufficient for the transcription of a human *HPRT* minigene and suggested the presence of a negative element upstream of this region (28). As a first step toward understanding the regulation of the human *HPRT* gene, we constructed and analyzed a number of deletion mutations within the 5′-flanking region of the gene to define the important *cis*-acting elements. Our analyses revealed that the sequence from positions −219 to −122 directs a relatively high level of expression and also exhibits bidirectional activity. In addition, an upstream regulatory element negatively modulates this expression. This negative element is able to function in a position-dependent and orientation-independent fashion and is capable of repressing heterologous promoters. The tandem organization of the negative and positive elements suggests that the interplay of these regulatory domains may regulate the tissue-differential and/or constitutive expression of the human *HPRT* gene. Further characterization of the factor(s) that interacts with the negative element should lead to the identification of transcriptional regulators important in the expression of the human *HPRT* gene.

MATERIALS AND METHODS

Enzymes and radioisotopes. The Klenow fragment, calf intestinal alkaline phosphatase, and T4 DNA ligase were obtained from Boehringer Mannheim. Restriction endonucleases were obtained from New England BioLabs or Bethesda Research Laboratories and used under the conditions specified by the supplier. Radioisotopes, including [α-^{32}P]dCTP, [α-^{35}S]dATP, and [^{14}C]acetylchloramphenicol, were purchased from New England Nuclear.

Miscellaneous expression vectors. pSV0CAT is a promoterless expression vector containing the gene encoding bacterial chloramphenicol acetyltransferase (CAT) (12). All CAT expression plasmids were derived from pSV0CAT. pSV2CAT expresses the CAT gene under the control of the enhancer and promoter of simian virus 40 (12). pXN.8CAT and pEN.24CAT (14) contain 800-bp *Xba*I-*Nco*I and 240-bp *Eco*RI-*Nco*I fragments, respectively, from the murine adenosine deaminase (ADA) promoter driving CAT gene expression in pSV0CAT. pD35CAT (20) has a 297-bp *Nae*I-*Taq*I (−291 to +6) fragment containing the mouse dihydrofolate reductase (DHFR) major promoter inserted into the *Hin*dIII site of pSV0CAT. pAVE1hGH (29) contains the promoterless human growth hormone (hGH) gene downstream of the

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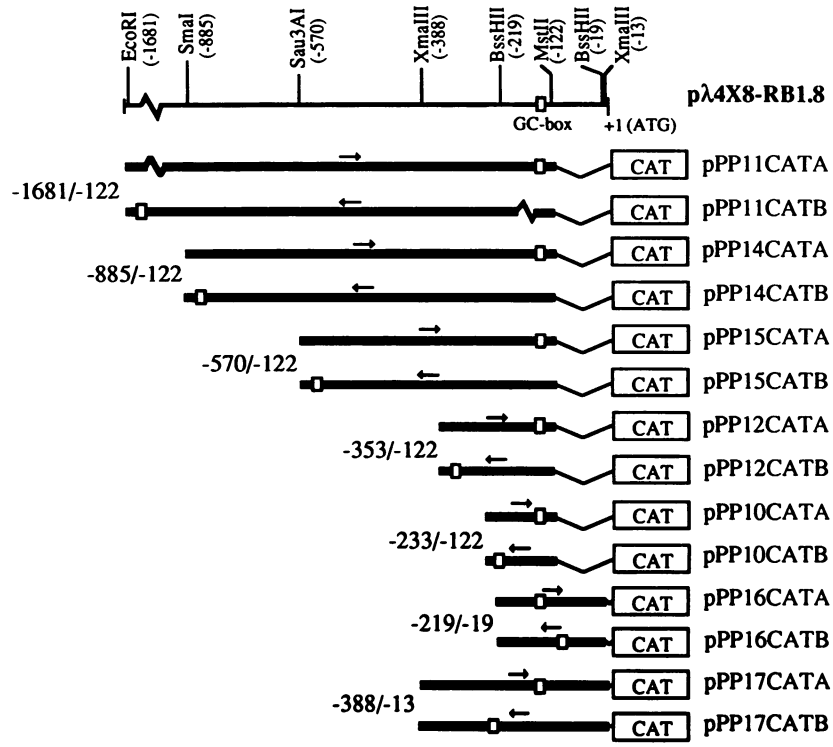

FIG. 1. Deletion constructs of the hHPRT promoter. The structural organization of the hHPRT promoter and the deletions generated by restriction enzymes and *Bal*31 exonuclease are depicted. The numbers on the left show the lengths of the fragments relative to the translation initiation site (+1). The open box corresponds to the GC-rich region, and the arrows indicate the orientations of the fragments within the constructs. Black bars correspond to human *HPRT* gene-related sequences, and thin lines indicate deleted areas. The backbone of the various constructs is pSV0CAT.

enhancer-promoter unit of the immediate-early gene of human cytomegalovirus.

Expression plasmids derived from the human *HPRT* gene 5′-flanking sequences. The 5′-flanking sequence of the human *HPRT* gene was obtained from a genomic clone, pλ4X8-RB1.8 (27). To construct 5′-flanking deletion mutations of the human *HPRT* gene, we used restriction enzyme sites in the 5′-flanking region of the gene together with an *Mst*II site located at position −122 relative to the translation initiation site to isolate the desired fragments. The *Eco*RI, *Sma*I, and *Sau*3AI restriction enzyme sites were used to generate the −1681 to −122, −885 to −122, and −570 to −122 deletions. After filling in of recessed ends with Klenow polymerase, *Hin*dIII linkers were added to each of these fragments, and the fragments were subcloned into the *Hin*dIII site of pSV0CAT to generate plasmids pPP11CAT, pPP14CAT, and pPP15CAT, respectively.

pPP16CAT and pPP17CAT were also derived from pλ4X8-RB1.8 by digestion with *Bss*HII and *Xma*III to obtain fragments containing sequences spanning from −219 to −19 and −388 to −13, respectively. The Klenow polymerase was used to fill in the ends, and the fragments were ligated into the similarly treated *Hin*dIII site of pSV0CAT.

Plasmids pPP12CAT and pPP10CAT were obtained by cleavage of pλ4X8-RB1.8 at a unique *Sma*I site and digestion with *Bal*31 exonuclease and *Mst*II. *Hin*dIII linkers were ligated to the newly generated ends of the fragments, and the fragments were subcloned into the *Hin*dIII site of pSV0CAT. These constructs contained the sequences spanning from −353 to −122 and −233 to −122, respectively. Exact deletion endpoints were determined by nucleotide sequencing (30).

For each expression vector, a construct containing the hHPRT promoter in the normal or reverse orientation with respect to the wild-type gene was identified and is described by the letter A or B following the name of the plasmid, respectively (Fig. 1).

pPP11ΔNE, which has an internal deletion of the region from −570 to −388, was constructed as follows. pPP11CATA was digested with *Sma*I (−885) and *Xma*III (−388), and 497- and 5,603-bp fragments were isolated. The 497-bp fragment was partially digested with *Sau*3AI to isolate a 315-bp fragment. After generation of blunt ends with the Klenow polymerase, the 315-bp *Sma*I-*Sau*3AI fragment spanning from −885 to −570 was religated with the 5,603-bp *Sma*I-*Xma*III fragment isolated from pPP11CATA.

For the in vivo competition experiments, the *Sau*3AI-*Xma*III DNA fragment containing the 5′-flanking region between −570 to −388 was subcloned into the *Sma*I site of pTZ19R by blunt-end ligation to generate plasmid pTZNE. Similarly, the same sequence was inserted in either orientation into the unique *Nde*I site of the following plasmids: pPP10CATA to generate pPP10NEA and pPP10NEB; pXN.8CAT to generate pXN.8NEA and pXN.8NEB; pEN.24CAT to generate pEN.24NEA and pEN.24NEB; and pD35RCAT to generate pD35NEA and pD35NEB. Clones were screened by restriction analysis to identify constructs containing single inserts in either orientation. All plasmid DNAs were prepared by banding twice to equilibrium in cesium chloride gradients (21).

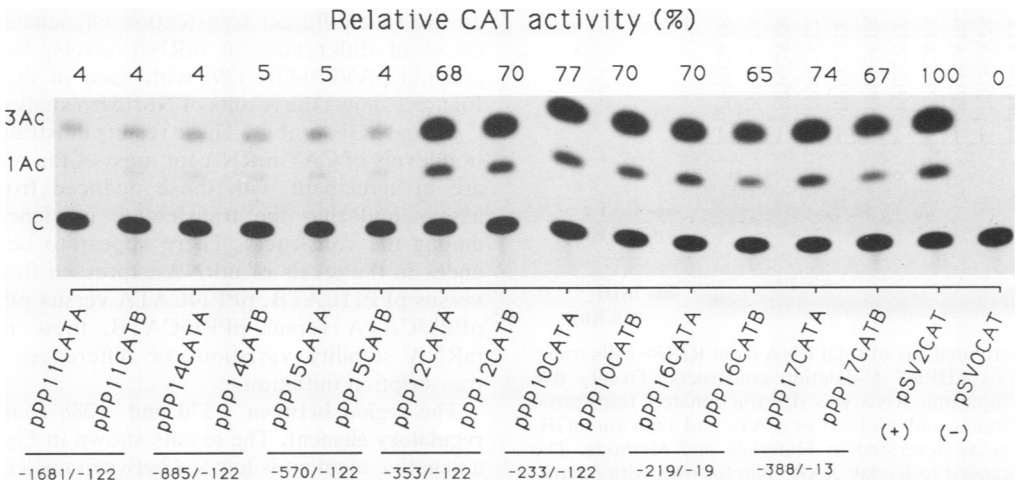

FIG. 2. Functional assay of the 5'-flanking sequences of the human *HPRT* gene. RJK88 cells were transfected with 10 μg of each expression plasmid, and CAT activity was assayed as described in Materials and Methods. The extent of the 5'-flanking sequences of the human *HPRT* gene included in each construct is indicated below the name of the corresponding construct. CAT activities relative to that of pSV2CAT are shown at the top as percentages. The values represent averages of four separate transfections, at least one of which contained a cotransfected hGH expression plasmid to normalize for differences in transfection efficiencies. Plasmid pSV0CAT served as a negative control. The positions of the substrate chloramphenicol (C) and its acetylated products (1Ac and 3Ac) are indicated.

Cell cultures and DNA transfections. RJK88, a hamster fibroblast cell line with a deletion of the entire *HPRT* gene (10), was used as the recipient for DNA transfections. This cell line was selected for most transfections because of its relatively short doubling time of 12 h when compared with that of a human cell line such as HeLa. Cells were grown in Dulbecco's modified Eagle medium (GIBCO) containing 10% (vol/vol) fetal calf serum, along with 100 μg of streptomycin and 100 U of penicillin per ml, under an atmosphere of 5% CO_2–95% O_2. Approximately 24 h before transfection, RJK88 cells were plated at a density of 5×10^5 cells per 100-mm plate. Transfection of plasmid DNA into the cells was carried out by the calcium phosphate precipitation technique (13). After 5 h of incubation, the DNA-calcium phosphate mixture was removed, the cells were shocked for 2 min by treatment with 15% glycerol (26), and fresh medium was added. RJK88 cells were transfected with 10 μg of the test plasmids and, in some cases, 1 μg of pAVE1hGH (29) was also cotransfected to normalize transfection efficiencies. The amount of DNA used for the competition assays varied as indicated in the figure legends. Each transfection experiment was repeated three to four times with at least two different plasmid preparations.

CAT assays. CAT assays were performed essentially as described by Gorman et al. (12). Forty eight hours after transfection, cells were harvested and lysed by three cycles of freezing and thawing and cellular debris was removed by centrifugation. Equal amounts of protein, as determined by the Bio-Rad Bradford assay, were used for each CAT assay, which was done in a total reaction volume of 190 μl containing 200 μg of protein extract, 0.05 μCi of [^{14}C]chloramphenicol (60 mCi/mmol), and 0.05 mM acetyl coenzyme A. Incubation was done at 37°C for 1 h. The percentage of [^{14}C]chloramphenicol converted to acetylated product (percent conversion) was determined by quantitating the radioactivity on thin-layer chromatography plates with a liquid scintillation counter. All assays were performed within the range of linear relation of activities with respect to incubation time and sample concentration. The percent conversion of chloramphenicol to acetylated product for each construct was determined by averaging the results of at least four separate transfections.

Northern (RNA) analysis. Northern analysis (21) to determine the transcription level of the 5'-deletion constructs was performed with 25 μg of total RNA preparations from cells cotransfected with the test plasmids and pAVE1hGH (29). Total RNA was isolated by the thiocyanate-phenol-chloroform technique (5). Cytoplasmic RNA was size fractionated in a 1.2% denaturing phosphate-agarose gel and transferred to a nylon membrane. The probes were either a 1,632-bp *Hin*dIII-*Bam*HI fragment from pSV0CAT or a 640-bp *Aat*II-*Sma*I fragment from hGH cDNA (29). The probes were labeled with [α-^{32}P]dCTP by random priming (Pharmacia) to a specific activity of about 10^9 cpm/μg. The filter was hybridized and washed by standard procedures (21). After hybridization with the CAT probe, the filter was stripped and rehybridized with the radiolabeled hGH cDNA probe.

RESULTS

Functional analysis of the 5'-flanking sequences of the human *HPRT* gene. To determine the extent of 5'-flanking sequence required for transcription of the human *HPRT* gene, we subcloned various lengths of 5'-flanking sequences of this gene in either orientation into a CAT reporter vector (12). Each of the plasmids depicted in Fig. 1 was introduced by the calcium phosphate procedure (13) into RJK88 cells (10), and the production of CAT was examined 48 h post-transfection. For each transfection, CAT activity was expressed relative to the activity obtained with the pSV2CAT vector transfected into duplicate plates of RJK88 cells.

The results of the transfection experiments are shown in Fig. 2. When plasmid pPP11CATA, which bears the sequence from −1681 to −122, was transfected into RJK88 cells and the cell extracts were assayed for CAT activity, a low level of activity was observed. Deletion of the sequences from −1681 to −885 and −1681 to −570 led to no remarkable change in CAT activity over that seen with pPP11CATA.

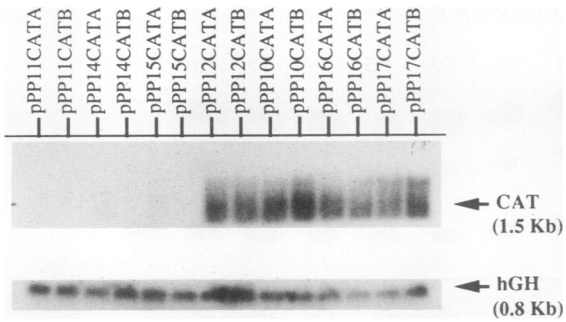

FIG. 3. Northern analysis of total RNA from RJK88 cells transfected with various hHPRT 5′-deletion constructs. Twenty five micrograms of cytoplasmic RNA was size fractionated, transferred to a nylon membrane, and probed for CAT- and then for hGH-specific transcripts as described in Materials and Methods. The membrane was exposed to Kodak XAR5 film for 60 h with intensifying screens. Only the relevant parts of the autoradiogram are shown.

Deletion of the sequence from −1681 to −353 caused a 13-fold increase in activity, although further deletion from −1681 to −233 did not significantly alter this increase. In addition, comparable levels of CAT activity were observed with sequences spanning from −219 to −19 and −388 to −13, thus allowing delineation of promoter function to the sequence between −219 and −122. Since CAT activity was similar when the sequences were tested in both normal and reverse orientations, these results suggest that the hHPRT promoter has bidirectional activity. These results also suggest that there is at least one *cis*-acting negative regulatory element located within the region from −570 to −388. Similar results were observed when HeLa cells were used as the recipient for transfections (data not shown).

To confirm that the observed results were due to an effect on the transcription of the reporter gene and not to an effect on translation, we examined the steady-state levels of CAT mRNA produced by each chimeric plasmid. Each deletion construct was transfected into RJK88 cells (10), total RNA was isolated (5), and Northern analysis was done (21). To ensure that different transfection efficiencies were not the cause of differences in mRNA levels, we cotransfected plasmid pAVE1hGH (29) with each of the test plasmids. Figure 3 shows the results of Northern analysis with both the CAT and hGH probes. These results illustrate that transcription levels of CAT mRNA for most of the reporter plasmids are in agreement with those deduced from the enzyme assays and that the transfection efficiencies are similar among the constructs. There appear to be notable differences in the levels of mRNA expressed from pPP11CATA versus pPP11CATB, pPP14CATA versus pPP14CATB, and pPP15CATA versus pPP15CATB; these may be due to mRNA stability variations or differences in the rate of transcription initiation.

The region between −570 and −388 contains a negative regulatory element. The results shown in Fig. 2 suggest that a negative element is located between nucleotides −570 and −388. To further confirm this suggestion, we sought to (i) specifically remove sequences contributing to the repressor function in plasmid pPP11CATA and (ii) introduce such sequences into plasmid pPP10CATA, which expresses CAT activity at a 13-fold-higher level than pPP11CATA. To accomplish the first objective, we constructed plasmid pPP11ΔNE by making an internal deletion in plasmid pPP11CATA. To address the second objective, we introduced the putative repressor region in both normal and reverse orientations into the unique *Nde*I site of pPP10CATA, in which CAT expression is driven by the sequence between −233 and −122, to create plasmids pPP10NEA and pPP10NEB, respectively.

When the 5′-flanking sequence between −570 and −388 was deleted from pPP11CATA, CAT expression increased 11-fold, indicating that a negative element lies within this sequence (Fig. 4). Furthermore, the insertion of the sequence corresponding to −570 to −388 upstream of the hHPRT core promoter (−233 to −122) in either orientation repressed the activity of the promoter ninefold. These results clearly demonstrate that the region spanning from −570 to −388 contains a bona fide negative element that can function regardless of its orientation.

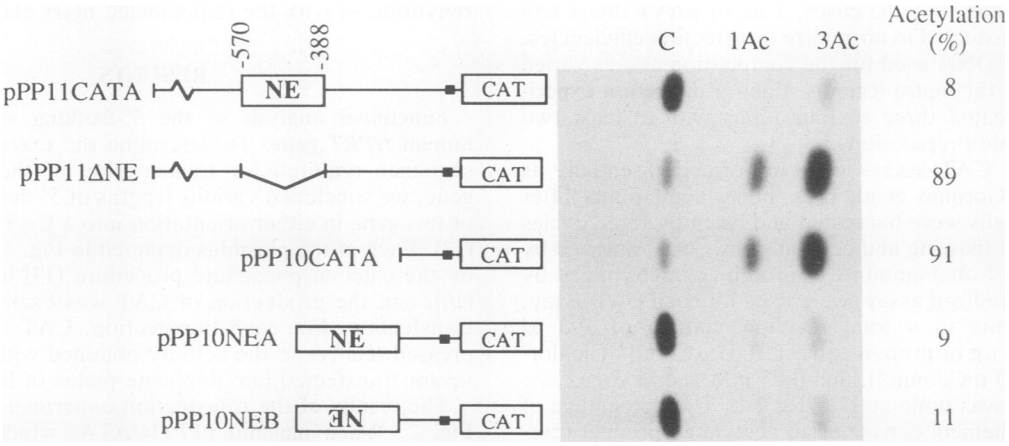

FIG. 4. Effect of the negative element (NE) on the transcriptional activity of the hHPRT promoter. The region from −570 to −388 was deleted from plasmid pPP11CATA and cloned into pPP10CATA in both orientations. The resulting plasmids were assayed for CAT expression after transfection of RJK88 cells as described in Materials and Methods. The plasmids are depicted on the left, and the results of the CAT assay are shown on the right. Percent acetylation represents the extent of conversion of chloramphenicol (C) to 1-acetyl (1Ac) and 3-acetyl (3Ac) derivatives of chloramphenicol. The data are representative of four independent experiments.

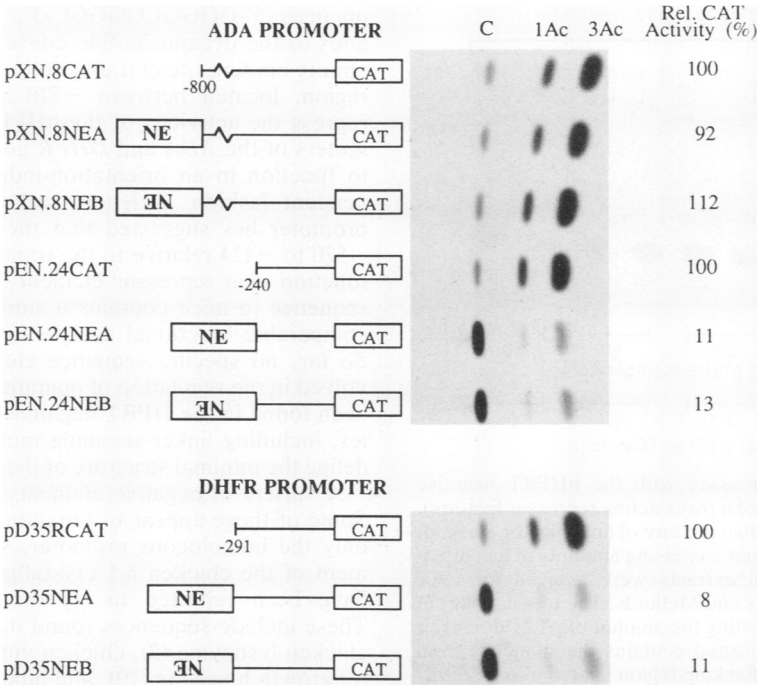

FIG. 5. Effect of the hHPRT negative element (NE) on heterologous promoters. The hHPRT negative element was inserted in both orientations into the *Nde*I site of plasmids pXN.8CAT and pEN.24CAT, which contain 800 and 240 bp of the murine ADA promoter, respectively, and pD35RCAT, which contains 297 bp of the murine DHFR promoter. These constructs were assayed for CAT expression as described in Materials and Methods. CAT activity is expressed as a percentage of the activity in the respective parental clone (Rel., relative). Values represent averages of at least three separate experiments. The positions of the substrate chloramphenicol (C) and its acetylated products (1Ac and 3Ac) are indicated.

The sequence between −570 and −388 inhibits the activity of heterologous promoters. The data from the preceding experiments indicate the presence of a negative regulatory element in the hHPRT promoter. We next determined whether the region from −570 to −388 could also repress the transcriptional activity of heterologous promoters such as those of the *ADA* and *DHFR* genes, which are structurally and functionally similar to the *HPRT* gene. To study the promoter specificity, we inserted the repressor region upstream of the *Nde*I site of both plasmids pXN.8CAT and pEN.24CAT (14), which contain 800 and 240 bp of the murine ADA promoter, respectively. Similarly, we introduced the negative element into the *Nde*I site of pD35RCAT (20), which contains 297 bp of the murine DHFR major promoter. The repressor region was inserted in both orientations, and the constructs were assayed for CAT activity in RJK88 cells (Fig. 5). The insertion of this sequence into pXN.8CAT in either orientation did not affect the CAT expression level. However, when the same sequence was inserted into pEN.24CAT, enzyme activity was reduced eightfold. Furthermore, the negative element also repressed the activity of the murine DHFR major promoter 10-fold when inserted into pD35RCAT in either orientation. These results indicate that the hHPRT negative element can repress heterologous promoters and suggest that it functions in a position-dependent and orientation-independent fashion.

In vivo competition assay for a *trans*-acting repressor factor(s). We sought to determine whether the repressed activity of pPP11CATA could be alleviated by an in vivo competition assay designed to neutralize the negative effect of the repressor sequence. RJK88 cells were transfected with a mixture of an indicator plasmid, pPP11CATA (−1681 to −122), and increasing amounts of a competitor plasmid, pTZNE, containing the putative negative element spanning from −570 to −388. Various amounts of pTZ19R plasmid DNA were added to ensure that the total amount of DNA transfected was the same. This experiment could predict the presence of a repressor factor if the competitor DNA enhanced CAT expression levels. Cotransfection studies showed that an augmentation in the concentration of competitor DNA from 0 to 10 μg caused a sixfold increase in hHPRT promoter activity (Fig. 6). This increase in CAT activity was specific for the pPP11CATA construct, since no increase in CAT activity driven by the ADA promoter was seen when pXN.8CAT was the indicator plasmid (Fig. 7). Similarly, when plasmid pPP10CATA, which contains the hHPRT core promoter element (−233 to −122), was used as the indicator plasmid in the competition experiment, no significant change in CAT expression levels was seen. Thus, these experiments suggest that a *trans*-acting repressor factor(s) binds specifically to a negative element located between −570 to −388 in the hHPRT promoter.

DISCUSSION

A functional characterization of the 5′-regulatory region of the human *HPRT* gene was conducted. Our previous work on human *HPRT* gene regulation suggested that a negative element was located upstream of position −245 relative to the translation initiation site (28). These preliminary data were largely obtained by gene transfer experiments aimed at measuring the frequency of stable $HPRT^+$ transformants.

In this study, using a transient expression assay, we identified two regulatory regions in the 5′-upstream se-

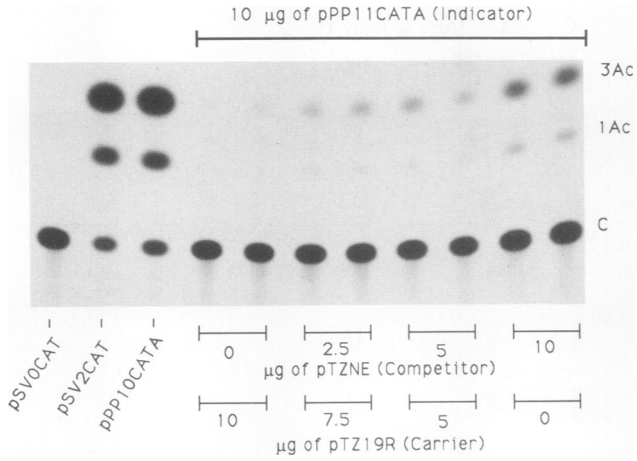

FIG. 6. In vivo competition assay with the hHPRT negative element indicating the presence of a *trans*-acting repressor factor(s). RJK88 cells were transfected with a mixture of an indicator plasmid, pPP11CATA (−1681 to −122), and increasing amounts of a competitor plasmid, pTZNE, and cell extracts were assayed for CAT activity as described in Materials and Methods. The total amount of DNA was kept the same by adjusting the amount of pTZ19R carrier DNA added. The competitor plasmid contains the sequence from −570 to −388 of the hHPRT 5′-flanking region ligated into pTZ19R. pPP10CATA served as a positive control. The autoradiogram is a representative example of three separate experiments performed in duplicate. The positions of the substrate chloramphenicol (C) and its acetylated products (1Ac and 3Ac) are indicated.

quence of the human *HPRT* gene. Our analyses revealed that *cis*-acting elements required to promote transcription are contained within a 97-bp fragment spanning from −219 to −122. This region contains four copies of the sequence GGGCGG, as previously described (27). One of these sequences (5′-GGGGCGGGGC-3′, −204 to −195) is homologous to the decanucleotide consensus sequence for a high-affinity binding site of transcription factor Sp1 (16). A second region, located between −570 and −388, was found to repress the activities of the hHPRT promoter and the promoters of the *ADA* and *DHFR* genes. This region appeared to function in an orientation-independent but position-dependent fashion. Deletion analysis of the murine HPRT promoter has suggested that the sequence spanning from −570 to −424 relative to the translation initiation site could function as a repressor element (23). We believe that this sequence in mice contains a similar negative element, but comparable functional analyses have not been conducted. So far, no specific sequence elements reported to be involved in the regulation of mammalian gene expression have been found in the HPRT negative element. Additional studies, including linker-scanning mutagenesis, are required to define the minimal structure of the hHPRT negative element.

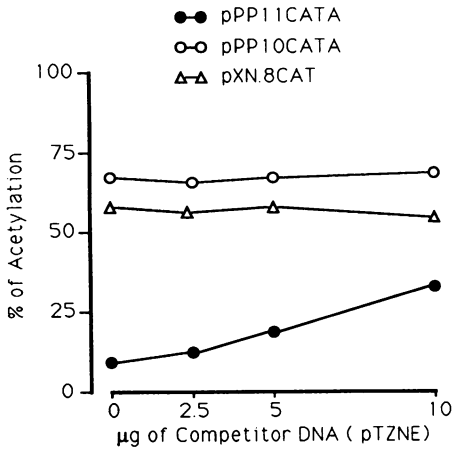

FIG. 7. Expression by RJK88 cells of a *trans*-acting repressor factor which selectively binds to the hHPRT negative element. Ten micrograms of the indicator plasmid pPP11CATA (−1681 to −122), pPP10CATA (−233 to −122), or pXN.8CAT (ADA promoter) was cotransfected with 0, 2.5, 5, or 10 μg of competitor pTZNE DNA, which contains the repressor sequence. The total amount of cotransfected DNA was adjusted to a final concentration of 10 μg by including appropriate amounts of pTZ19R carrier DNA. Cell extracts were assayed for CAT activity as described in Materials and Methods. The results for each datum point are the means of three independent experiments.

A variety of negative elements have been characterized. Some of these appear to function to repress the activity of only the homologous promoter, such as the repressor element of the chicken δ-1 crystallin gene (3). Several others have been reported to repress heterologous promoters. These include sequences found in the 5′-flanking regions of chicken lysozyme (2), chicken vimentin (8), rat insulin (18), rat growth hormone (19), and human gastrin (32) genes. The hHPRT negative element does not appear to be strictly promoter specific, since both the ADA and the DHFR promoters were also repressed by the sequence from −570 to −388. Furthermore, this element is orientation independent, since it could function in either orientation, and is likely position dependent, since it was not able to suppress activity when it was located 600 bp upstream of the core promoter of the ADA gene, regardless of its orientation (pXN.8NEA and pXN.8NEB).

DNA competition studies strongly suggest that the activity of the hHPRT promoter in RJK88 cells depends on a *trans*-acting repressor(s) which binds to its negative element. Transfection with an excess of a competitor plasmid, pTZNE, containing the hHPRT negative element stimulated the activity of indicator plasmid pPP11CATA, relative to cotransfection with the same amount of pTZ19R. However, cotransfection of the same competitor DNA with pXN.8CAT or with pPP10CATA containing an hHPRT truncated promoter did not affect the activity of the corresponding promoters. These results indicate that the negative element is unlikely to inhibit the hHPRT promoter by depleting a positively acting transcription factor. Furthermore, since the negative element does not function when it is located at a distance from the core promoter of the ADA gene, we propose that the repression by the negative element is dependent on its proximity to the promoter sequences and may involve interference with the binding or function of an activator protein(s). The in vivo role of the negative element will be investigated by generating transgenic mice harboring appropriate reporter plasmids. Analysis of reporter gene expression in various tissues in these mice will enable us to understand the in vivo role of this sequence.

Bidirectional activity of promoters has been previously reported for viral, procaryotic, and eucaryotic genes. The simian virus 40 promoter drives the expression of divergent flanking genes, and this bidirectional function is conserved when the promoter is located outside of its native context (11, 24). The hamster 3-hydroxy-3-methylglutaryl coenzyme A reductase promoter has bidirectional activity, but it is regulated by steroids only when it is located in the normal

orientation (1). Divergent transcripts originating from the DHFR promoters have also been described (6, 7, 9, 25). Linton et al. cloned and sequenced cDNAs whose mRNAs were transcribed from the divergent DHFR major and minor promoters (20). At present, relatively little information is available to determine the biological significance of the activity of the hHPRT promoter in the reverse orientation. RNase protection studies with HeLa cell mRNA designed to determine whether a transcript is expressed from the opposite strand in these cells have failed to detect such a transcript (data not shown; 15). In this study, the bidirectionality of the hHPRT promoter was demonstrated by its ability to direct high levels of transcription of the CAT gene, regardless of the orientation. This finding is opposed to the finding that the hHPRT promoter is fivefold more active in the normal orientation than in the reverse orientation (15). The reason for this discrepancy is not clear; the only apparent difference between these studies is the backbone of the vector. Our CAT reporter vectors were derived from pBR322, while those of Johnson and Friedmann (15) were derived from pBluescript (Stratagene).

In summary, our findings demonstrate that the expression of the hHPRT promoter is controlled by a combination of both positive and negative regulatory elements located within its 5′-flanking region. The presence of a negative element immediately upstream of the core promoter suggests that this array may act to regulate the expression pattern of this gene. We are now attempting to identify the *trans*-acting factors that interact with the negative element. We are conducting detailed mutational analyses and DNase I footprinting assays to further define the sequences necessary for the negative activity, which can be used to isolate the gene(s) coding for the repressor protein.

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