Am. J. Hum. Genet. 42:726–734, 1988

Resolution of a Missense Mutant in Human Genomic DNA by Denaturing Gradient Gel Electrophoresis and Direct Sequencing Using In Vitro DNA Amplification: $HPRT_{Munich}$

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Summary

The combination of denaturing gradient gel electrophoresis (DGGE) and in vitro DNA amplification has allowed us to (1) localize a DNA mutation to a given 100-bp region of the human genome and (2) rapidly sequence the DNA without cloning. DGGE showed that a mutation had occurred, but the technique revealed little about the nature or position of that mutation. The region of the genome containing the mutation was amplified by the polymerase chain-reaction technique, providing DNA of sufficient quality and quantity for direct sequencing. Amplification was performed with a ^{32}P end-labeled primer that allowed direct Maxam-Gilbert sequencing of the amplified product without cloning. $HPRT_{Munich}$ was found to contain a single-base-pair substitution, a C-to-A transversion at base-pair position 397. We report the generation of a 169-bp, wild-type DNA probe that encompasses most of exon 3 of the human hypoxanthine guanine phosphoribosyltransferase (HPRT) gene and contains a low-temperature melting domain of ~100 bp. $HPRT_{Munich}$, an HPRT mutant isolated from a patient with gout, has a single amino acid substitution; the corresponding DNA sequence alteration must lie within the low-temperature melting domain of exon 3. We report the separation of $HPRT_{Munich}$ from the wild-type sequence using DGGE. In addition to base-pair substitutions, DGGE is also sensitive to the methylation state of the molecule. The cDNA for HPRT was cloned into a vector and propagated in *Escherichia coli* dam^+ and dam^- strains; thus, methylated and unmethylated HPRT cDNA was obtained. A single *dam* methylation site exists within exon 3, and we have separated the methylated and unmethylated sequences by DGGE. To rapidly sequence $HPRT_{Munich}$, we used the polymerase chain reaction. Two 20-bp oligonucleotide primers complementary to the intron sequence immediately 5′ and 3′ to exon 3 were hybridized in solution to wild-type or $HPRT_{Munich}$ genomic DNA, and the primed template was extended with T_4 DNA polymerase. The process was repeated 30 times, yielding ~150 ng of amplified product starting from 5 μg of genomic DNA. A combination of DGGE and polymerase chain reaction should permit rapid identification and sequencing of base-pair-substitution mutants in human genomic DNA. Both techniques are expected to play a significant role in the analysis of base-pair mutations and in the latters' relationship to human disease.

Introduction

Denaturing gradient gel electrophoresis (DGGE) can demonstrate that a mutation has occurred in genomic DNA, but the technique does not provide precise information about the nature or location of the mutation. This information can be revealed by sequencing, which, until recently, involved construction of genomic libraries and screening for the desired clone. The polymerase chain reaction (PCR) permits rapid in vitro amplification of a given region of the genome. The DNA produced by PCR is often of sufficient quality and quantity for direct DNA sequencing. We have identified a human hypoxanthine guanine phosphoribosyltransferase (HPRT) mutant by using DGGE, amplified the region of the genome contain-

Received May 27, 1987; revision received November 9, 1987.

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0002-9297/88/4205-0008$02.00

ing the mutation, and directly sequenced the amplified region to determine the precise nature of the mutation.

DGGE can separate DNA sequences differing by a single nucleotide substitution (Fischer and Lerman 1983; Lerman et al. 1984, 1986; Myers and Maniatis 1986). The technique utilizes an increasing concentration of denaturant in a polyacrylamide gel. When the DNA has migrated through the gel to a certain concentration of denaturant, the molecule undergoes an abrupt transition from the totally helical state to a partially melted state. Importantly, the point in the denaturing gel at which this transition occurs is *extremely* sequence dependent. Although DGGE can identify a mutant, it does not reveal the nature or exact location of the mutation.

DGGE has been used to resolve single-base-pair substitutions in the β-globin gene in total human genomic DNA (Myers et al. 1985*c*), as well as to identify polymorphic sites within a 1.2-kb region of chromosome 20 (Noll and Collins 1987). In both cases, a radiolabeled wild-type probe was hybridized in solution to a mutant locus, thus forming a heteroduplex containing a mismatched base. This heteroduplex was then resolved on a denaturing gradient gel. We have employed this method to resolve a base-pair mutant at the human HPRT locus.

The HPRT gene spans ~44 kb in genomic DNA and is split into nine exons (see Stout and Caskey 1985 for review). We have examined exon 3 of HPRT because this exon (1) contains the largest continuous portion of the coding frame (28%) and thus offers a sizable region for detection of mutations and (2) is reported to code for both catalytic sites of the enzyme (Wilson et al. 1983) (and mutations within this region may impair the function of the enzyme such that a patient suffers Lesch-Nyhan syndrome or gouty arthritis). To discover whether any mutant sequences can be separated from the wild-type sequence by using DGGE, we examined the melting behavior of $HPRT_{Munich}$, an HPRT mutant isolated from a patient with gout. $HPRT_{Munich}$ has a serine-to-arginine transition at amino acid 103 (Wilson and Kelley 1984); this mutant has not been characterized at the DNA-sequence level. From the position of the amino acid substitution, we know that the DNA mutation must be located near the 3′ end of exon 3 as shown in figure 1. We report the resolution of this mutant from the wild-type sequence and discuss the advantages and limitations of this technique.

To quickly determine the nature of the mutation in $HPRT_{Munich}$, we used the PCR (Saiki et al. 1985; Mullis and Faloona 1987), which permits a rapid synthesis of a particular sequence directly from genomic DNA. Two oligonucleotide primers flanking the desired region of DNA are hybridized in solution to genomic DNA, a DNA polymerase is added, and synthesis occurs. The cycle of denaturation, hybridization, and polmerization is repeated 20–30 times, resulting in an exponential increase of blunt-ended DNA fragments delimited by the primers; a 220,000-fold increase in a 110-bp fragment of the human β-globin locus has been reported (Saiki et al. 1985). The quantity and quality of amplified DNA is such that direct sequencing of the amplified product is possible (McMahon et al. 1987; Wrischnik et al. 1987).

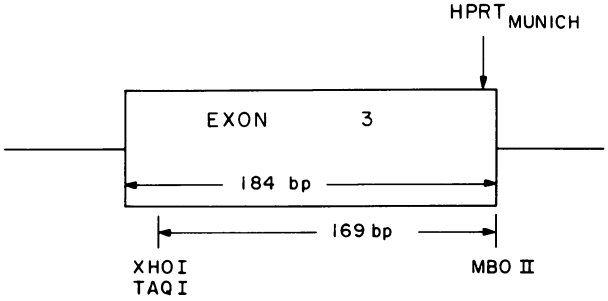

Figure 1 Diagram showing the size of HPRT exon 3, relevant restriction-enzyme sites, and the location of the DNA base-pair substitution in $HPRT_{Munich}$, an HPRT mutant isolated from a patient with gout.

We have used two 20-bp oligonucleotide primers complementary to the intron sequence immediately 5′ and 3′ to HPRT exon 3, such that the entire coding region of exon 3 was amplified, producing a 224-bp fragment. Amplification was performed using T_4 DNA polymerase (Keohavong et al. 1988) with a single radiolabeled primer so that the amplified material could be directly sequenced by the Maxam-Gilbert method. A single-base-pair substitution, a C-to-A transversion at base-pair position 397, was found.

Material and Methods

DGGE

TK6 is a diploid human male lymphoblast line that is $HPRT^+$ (Skopek et al. 1978). $HPRT_{Munich}$, a human male lymphoblast line isolated from a patient with gout, was a gift from James M. Wilson (Massachusetts General Hospital, Boston). GM1416B, a hu-

man lymphoblast cell line containing four X chromosomes, was obtained from the Human Genetic Mutant Cell Repository in Camden, NJ. RJK853, a human male lymphoblast Lesch-Nyhan cell line that is totally deleted for HPRT, was a gift from C. T. Caskey (Baylor College of Medicine, Houston). Cell lines were grown in RPMI 1640 with 10% horse serum (GIBCO, Grand Island, NY) in a 5% CO_2–humidified atmosphere.

High-molecular-weight genomic DNA was isolated essentially as described by Blin and Stafford (1976). Plasmid pHPT30, bearing the cDNA for human HPRT, was a gift from C. T. Caskey. The vector was propagated in *Escherichia coli* stain HB101, a *dam*$^+$ strain. Plasmid DNA was isolated using an alkaline lysis method (Maniatis et al. 1982) and purified by CsCl centrifugation. This plasmid cDNA was used as a source of wild-type *dam*$^+$ HPRT sequence.

Human genomic DNA was digested with a 2.5-fold excess of *Mbo*II and *Taq*I (see fi . 1) according to manufacturer's specifications (New England Biolabs, Beverly, MA). The 5′ site may be cut by either *Xho*I or *Taq*I. Genomic DNA was digested with *Taq*I, because *Xho*I may be inhibited by the 5-methyl cytosine present in the genomic DNA; *Taq*I is not inhibited by 5-methyl cytosine (Kessler et al. 1985). Plasmid DNA containing the cDNA for HPRT was also digested with *Mbo*II and *Taq*I.

The *Pst*I fragment of vector pHPT30, which contains the entire coding region of HPRT, was (1) subcloned in both orientations in M13mp18, (2) propagated in JM110, a *dam*$^-$*dcm*$^-$ *E. coli* strain, and (3) used to isolate both double-strand (ds) and single-strand (ss) DNA (Messing 1983). ss M13 DNA with the (−) strand of HPRT was used as a template to synthesize the radiolabeled (+) strand, which was used as a probe. ds DNA with HPRT was used as a source of unmethylated cDNA. Growth of M13 template was done in JM110 to eliminate methylation-induced mobility differences on the denaturing gradient gels; a single *dam* methylation site exists in exon 3. We found that using a *dam*$^-$ bacterial strain to prepare the phage template simplified later analysis of denaturing gradient gels.

A 2.5-fold molar excess of 15-mer primer (New England Biolabs) was hybridized to the ss M13 template. The radiolabeled (+) strand was generating by adding 30 U Klenow fragment (5 U/μl; New England Biolabs), 53 nM ss primed template, 50 mM NaCl, 10 mM $MgCl_2$, 10 mM dithiothreitol, 30 mM Tris (pH 7.5), 4 μM ^{32}P dATP (800 Ci/mmol; Amersham, Arlington Heights, IL), and 50 μM each dCTP, dGTP, and dTTP in a reaction volume of 60 μl. After incubation for 12 h at 30 C, the polymerase was inactivated by phenol extraction and the DNA was ethanol precipitated. The DNA was then digested with *Mbo*II and *Xho*I, ethanol precipitated, resuspended in water, and electrophoresed in a parallel denaturing gradient gel. The double-strand exon 3 fragment was located by autoradiography and cut from the wet denaturing gradient gel; the DNA was recovered by electroelution, ethanol precipitated, and resuspended in water.

Purification of the ds probe on a parallel denaturing gradient gel was necessary to obtain a homogeneous probe preparation. If a normal polyacrylamide gel was used to purify the ds exon 3 fragment, this DNA gave several bands when analyzed on a denaturing gradient gel.

The gel-purified, ds 169 fragment was hybridized to an excess of ss M13 bearing the (+) strand of HPRT; this ss M13 DNA is complementary to the nonradiolabeled strand of exon 3. After a 10-fold molar excess of the ss M13 was added and the NaCl concentration was adjusted to 300 mM, the solution was boiled for 4 min and incubated at 65 C for 1 h. The DNA was ethanol precipitated, resuspended in water, and run on a conventional polyacrylamide gel. The ss exon 3 fragment was visualized in the wet gel by autoradiography and was isolated as described above.

Twenty micrograms of *Mbo*II/*Taq*I-digested genomic DNA was resuspended in 30 μl of 300 mM NaCl, 30 mM Tris (pH 8), 1 mM EDTA with 1.7×10^4 cpm of radiolabeled ss probe (1.2×10^8 copies). The mixture was boiled for 4 min and placed at 65 C for 18 h. One hundred nanograms of ss M13 DNA containing the (−) strand of HPRT was added, and hybridization was continued another hour; this DNA is complementary to the ss probe and retains most of the unbound probe at the origin of the gel. The DNA was ethanol precipitated, washed twice with −20 C 75% ethanol, and resuspended in 8 μl of water and 1.6 μl of loading buffer. One thousand counts per minute of ss probe was also hybridized to a molar excess of *Taq*I/*Mbo*II-digested plasmid containing the cDNA for HPRT.

A denaturing gradient gel was used to purify the ds probe and to resolve mutants from the wild-type DNA sequence. A 1-mm thick, 12.5% polyacrylamide gel (37.5% polyacrylamide/1% bis) with

either a 5%–30% or a 10%–35% denaturing gradient was used (100% denaturant = 7 M urea/40% formamide). Use of a 5%–30% gel gave better separation of the unbound ss probe from the wild-type sequence; otherwise the behavior of the DNA in both gels is identical (data not shown). Denaturant was prepared fresh, and the gradient was formed with a Hoeffer gravity-flow gradient maker. The gels were run submerged at 60 C at 150 V (7.5 V/cm) in TAE buffer (40 mM Tris, 20 mM NaOAc, 1 mM EDTA [pH 8.2]); the 5%–30% gel was run for 5.5 h, and the 10%–35% gel was run for 4 h. If DNA was to be recovered from the gel, it was not fixed; otherwise the gel was fixed for 2 h in 40% methanol/5% glycerol, dried, and then exposed to Kodak XAR-5 film for 2 days at −70 C with DuPont Cronex Lightning Plus® intensifying screen.

PCR

Oligonucleotides were synthesized by Synthetic Genetics (San Diego) and were purified by gel electrophoresis. The sequence of the downstream primer was CATAT ATTAA ATATA CTCAC, and the sequence of the upstream primer was TCCTG ATTTT ATTTC TGTAG. The downstream primer was end-labeled using the reagents in the BRL 5′ terminus–labeling kit (Bethesda Research Laboratories, Bethesda, MD). The primer was labeled with ^{32}P γ-ATP (6,000 Ci/mmol; New England Nuclear, Boston) to a final specific activity of $\sim 3.6 \times 10^5$ cpm/pmol. Approximately 140 pmol of radiolabeled primer and 160 pmol of nonradiolabeled primer were added to the PCR reaction mix to give a final primer concentration of 3 μM.

dNTPs were obtained in powder form (Sigma, St. Louis), dissolved in water, and adjusted to pH 7.5 with NaOH. A buffer containing 100 mM Tris (pH 8.0), 50 mM $MgCl_2$ was obtained from New England Biolabs. T_4 DNA polymerase, Lot 21, 1 U/μl, was from New England Biolabs.

The 100-μl reaction mixture contained 5 μg genomic DNA, 3 μM of each primer, 1.5 mM of each dNTP, 10 mM Tris (pH 8.0), 5 mM $MgCl_2$. The denaturation time for the first three cycles was 3 min; the remainder of the denaturations were performed for 1 min. The cycle was (1) denaturation in a boiling water bath, (2) hybridization of primers to template for 45 s at room temperature, followed by a 15-s incubation in a 37 C water bath, (3) addition of 1 U T_4 DNA polymerase followed by brief vortexing, and (4) DNA polymerization for 4 min in a 37 C water bath. The cycle of denaturation, hybridization, and polymerization was repeated 30 times.

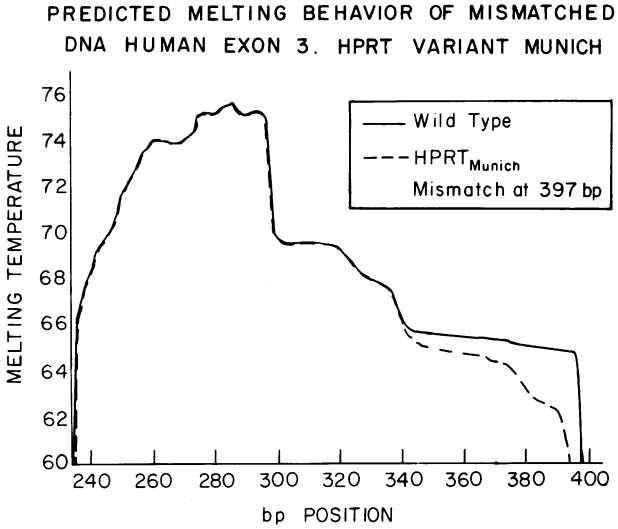

Figure 2 Calculated melting map of the wild-type homoduplex and the wild-type:$HPRT_{Munich}$ heteroduplex. The melting map represents the temperature at which each base pair is in 50:50 equilibrium between the melted and helical state. The effect of a mismatch was approximated by placing the Gotoh-Tagashira value at the mismatch (base-pair position 397) to zero and by reducing the values of each neighbor by 25 (L. Lerman, personal communication). The melting algorithm was provided by L. Lerman.

After 30 cycles of amplification the reaction mix was extracted with phenol, ethanol precipitated, dissolved in water, and loaded onto an 8% polyacrylamide gel. The desired 224-bp amplified product was located by autoradiography, electroeluted from the gel, and sequenced by the Maxam-Gilbert method (Maxam and Gilbert 1980).

Results and Discussion

DGGE can detect mutations only in the lower-temperature melting domains, so it is important to determine what portion of the molecule becomes single stranded when the DNA melts. This can be determined by use of a computer algorithm, developed by L. Lerman, that predicts DNA melting behavior solely from the base-pair sequence. The calculated melting map of the wild-type homoduplex (see fig. 2) shows a low-temperature melting domain of ~100 bp, from base-pair position 300 to base-pair position 400. Thus, we expect to be able to resolve mutations in ~100 bp of the 169-bp probe; these 100 bp represent 15% of the coding region of HPRT. Mutations

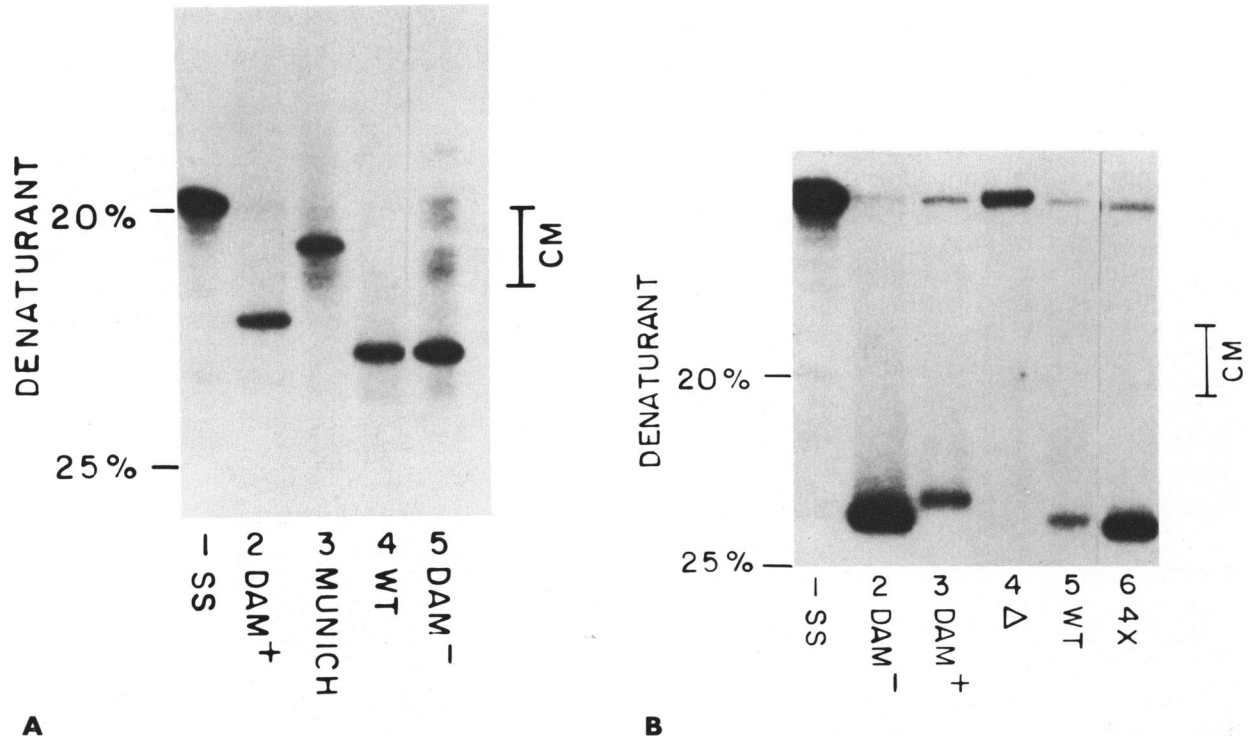

Figure 3 Autoradiograms of the denaturing gradient gels. Twenty micrograms of genomic DNA was digested with *Taq*I and *Mbo*II and then hybridized to an HPRT exon 3, wild-type, ss, 169-bp probe. *A*, 10%–35% denaturant. Lane 1: SS, 10^3 cpm ss probe; lane 2: DAM$^+$, 10^3 cpm ss probe hybridized to a molar excess of *Taq*I/*Mbo*II-digested HPRT cDNA propagated in a *dam*$^+$ *E. coli* strain; lane 3: MUNICH, $HPRT_{Munich}$ genomic DNA (lymphoblast line); lane 4: WT, TK6 genomic DNA (wild-type lymphoblast line); lane 5: DAM$^-$, 10^3 cpm ss probe hybridized to HPRT cDNA propagated in a *dam*$^-$ strain. *B*, 5%–30% denaturant. Lane 1: SS, 10^3 cpm ss probe; lane 2: DAM$^-$, 10^3 cpm ss probe hybridized to HPRT cDNA propagated in a *dam*$^-$ strain; lane 3: DAM$^+$, 10^3 cpm ss probe hybridized to HPRT cDNA propagated in a *dam*$^+$ strain; lane 4: Δ, RJK853 genomic DNA (HPRT deletion lymphoblast line); lane 5: WT, TK6 genomic DNA (wild-type lymphoblast cell line); lane 6: 4X, GM1416B genomic DNA (lymphoblast line with four X chromosomes). The behavior of the DNA is identical in both the 5%–30% and 10%–35% denaturing gradient gels. The DNA undergoes an abrupt transition to the partially melted state at ~23% denaturant (data not shown). The electrophoresis time for the 5%–30% gel must be extended to allow the DNA to reach a concentration of 23% denaturant. This results in a greater separation of the unbound ss probe from the wild-type sequence than occurs with the 10%–35% gel (3*A*), yielding gels that are easier to interpret. ss, dam$^+$, dam$^-$, and wild-type samples are on both gels as internal controls.

occurring from base-pair position 234 to base-pair position 300 will lie in the high-temperature melting domain of the molecule and should not be separated from the wild-type sequence by DGGE.

The calculated melting map of the heteroduplex formed by hybridization of the wild-type probe to $HPRT_{Munich}$ is given in figure 2. The effect of a mismatch can be approximated by a modification of the melting algorithm (L. Lerman, personal communication; see legend to fig. 2). The melting algorithm predicts that the mismatched heteroduplex will melt at a lower temperature than the perfectly base-paired homoduplex.

Autoradiograms of the denaturing gradient gels are shown in figure 3*A* and 3*B*. The wild-type DNA shows a band at ~23% denaturant (WT; fig. 3*A*, lane 4, and fig. 3*B*, lane 5). The cell line deleted for HPRT (Δ; fig. 3*B*, lane 4) shows no signal, while the cell line with four X chromosomes (4X; fig. 3*B*, lane 6) shows a strong band; this cell line is expected to show a more intense signal than does the wild type because HPRT is X linked. $HPRT_{Munich}$ is displaced ~1.2 cm from the wild-type band (MUNICH; fig. 3*A*, lane 3). Later sequencing of $HPRT_{Munich}$ revealed that the DGGE shift was due to a single-base-pair substitution.

Denaturing gradient gels are sensitive to the methylation state of the molecule. Therefore, a methylated molecule could appear to be a mutant. In mammalian DNA, the only methylated base is 5-methyl cytosine,

which is found almost exclusively at the sequence CpG (Wyatt 1951; Vanyushin et al. 1970). However, the region of exon 3 that we are examining has no CpG sequences, and thus no 5-methyl cytosine residues are expected to confound our results.

To investigate the effect of methylation, we have propagated the cDNA for HPRT in both *dam*$^+$ and *dam*$^-$ bacterial strains, and the effect of bacterial methylation can be seen in the denaturing gradient gel. A single *dam* site exists at base-pair position 357 in exon 3; *dam* methylase will produce an N^6-methyl adenine residue in the sequence GATC (Hattman et al. 1978). The presence of a single 6-methyl adenine destabilizes the molecule (DAM^+; fig. 3*A*, lane 2, and fig. 3*B*, lane 3) so that it melts at a lower temperature than does the unmethylated sequence (DAM^-, fig. 3*A*, lane 5, and fig. 3*B*, lane 2). The molecule is actually hemimethylated, since unmethylated ss probe was hybridized to fully methylated cDNA.

Although four HPRT pseudogenes exist (Patel et al. 1984), there is no pseudogene interference in this system. Pseudogenes might have created undesirable background problems in the denaturing gradient gels. A cell line that is totally deleted for HPRT shows no signal when hybridized to ss exon 3 probe and resolved on a denaturing gradient gel (Δ; fig. 3*B*, lane 4); the HPRT deletion line also shows no signal at the wild-type position when the DNA is run on a conventional polyacrylamide gel (data not shown). No pseudogene cross-hybridizing species can be seen. Apparently these pseudogenes do not have restriction sites such that a fragment of ~169 bp is produced when genomic DNA is digested with *Mbo*II and *Taq*I.

The 169-bp probe appears to be well suited for work with DGGE because it has a distinct, small, high-temperature melting domain. A run of six consecutive guanines near the 5′ end of the molecule helps form the high-temperature melting domain, in effect forming a small GC-rich clamp (for discussion of GC clamps, see Myers et al. 1985*a*, 1985*b*). We expect to be able to detect nearly all base-pair-substitution mutations that occur in the 100-bp low-temperature melting domain of the molecule. These 100 bp represent ~15% of the coding region of HPRT. DGGE may be well suited for analyzing other loci possessing long low-temperature melting domains adjacent to a short higher-temperature melting domain.

To sequence $HPRT_{Munich}$, we used PCR to generate a sufficient quantity of template for direct sequencing. We have made one important modification of the PCR, namely, the substitution of T_4 DNA polymerase (Keohavong et al. 1988) for the Klenow fragment that was employed in the original technique. The error rate for the Klenow fragment is reported to be 10^{-4}–10^{-6} error/bp, while the error rate for T_4 DNA polymerase is reported to be 10^{-6}–10^{-8} error/bp (Loeb and Kunkel 1982). DNA amplified with T_4 DNA polymerase may contain fewer polymerase-induced errors than does DNA amplified with Klenow fragment. DNA amplified

Figure 4 Ethidium bromide–stained polyacrylamide gel with 1/10 of PCR reaction mixture after 30 cycles of amplification. Visible is a discrete band ~224 bp in length, which is the expected size of the amplification product. Lane 1: TK6 genomic DNA (wild type); lane 2: $HPRT_{Munich}$ genomic DNA; lane 3: molecular-weight marker, pBR322/*Msp*I.

with the Klenow fragment has been cloned and sequenced (Scharf et al. 1986); in one case the frequency of nucleotide misincorporation was $<1.5 \times 10^{-3}$ error/bp, and in another case the error rate was $\sim 1.7 \times 10^{-3}$ error/bp.

After 30 cycles of amplification, an ethidium bromide–stained gel (fig. 4) shows a distinct band ~224 bp in length, which is the expected size of the amplified exon 3 product; the exon itself is 184 bp and each primer is 20 bp. After 30 cycles of amplification, ~150 ng of exon 3 was obtained, which represents $\sim 6 \times 10^{11}$ copies. Five micrograms of genomic DNA contains $\sim 7 \times 10^5$ copies of HPRT, so 30 cycles of amplification produced an 8.7×10^5–fold increase; this represents an average efficiency of ~58%/cycle ($[1 + X]^n = Y$; X = efficiency, n = cycles, Y = amplification; see Saiki et al. 1985 for discussion of efficiency).

A single radiolabeled primer was used during the amplification so that the amplified product could be directly sequenced by the Maxam-Gilbert method. Sequence analysis of the amplified product showed that $HPRT_{Munich}$ contained a single-base-pair substitution, a CG-to-AT transversion at base-pair position 397 (see fig. 5). This base-pair substitution is consistent with the amino acid substitution reported for $HPRT_{Munich}$, a serine-to-arginine substitution at amino acid 103 (Wilson and Kelley 1984).

DGGE provides a method to localize base-pair mutations to a region of DNA, and the PCR can be used to generate sufficient DNA for direct sequencing. We are applying both techniques to the study of human mutagenesis both in vivo and in vitro (Thilly 1985; Cariello and Thilly 1986). DGGE and PCR may have

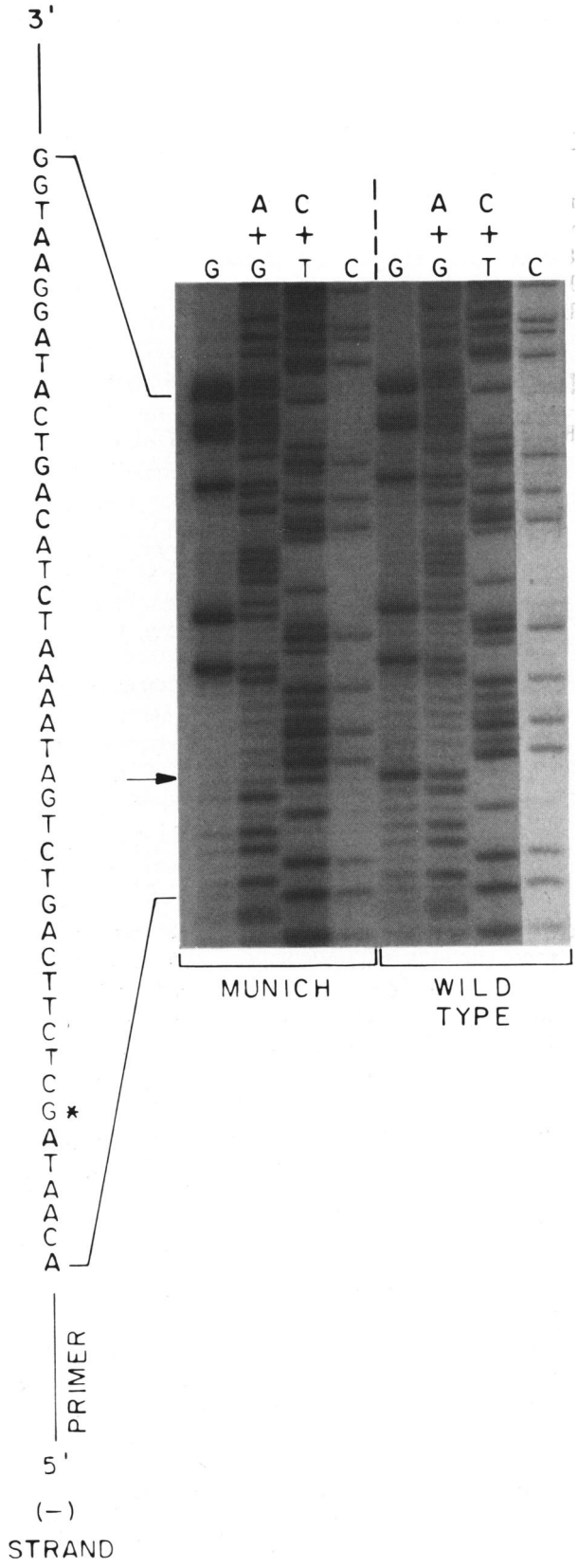

Figure 5 Direct sequencing of $HPRT_{Munich}$ and wild-type DNA amplified by PCR. DNA amplification was performed for 30 cycles by using one end-labeled primer. After electrophoresis of the amplification mixture on an 8% polyacrylamide gel, the 224-bp band was located by autoradiography, excised, and electroeluted from the gel. The DNA was chemically cleaved by the method of Maxam and Gilbert (1980) and loaded onto an 8% polyacrylamide, 8 M urea sequencing gel; the gel was dried and exposed to Kodak XAR-5 film with a DuPont Cronex Lightning Plus® intensifying screen at −70 C for 48 h. The first four lanes show the sequence of the (−) strand of $HPRT_{Munich}$, and the next four lanes correspond to the sequence of the wild type; the sequence of the wild-type (−) strand is given. A single-base-pair substitution in $HPRT_{Munich}$ was found, a G-to-T transversion that corresponds to a C-to-A transversion at base-pair position 397 of the (+) strand. The arrow indicates the position of the mutation in the sequencing gel, and the asterisk indicates the location of the mutation in the wild-type sequence.

many applications in the analysis of mutation and human disease.

Acknowledgements

The authors wish to thank L. Lerman for the use of his melting algorithm and for helpful discussions. This work was supported by Department of Energy grant DE-FG02-86ER60449 and National Institute of Environmental Health Sciences grants NIH-5-P01-ES00597, NIH-5-P30-ES02109, NIH-5-P01-ES01640, and NIH-5-P01-ES03926.

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