BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **241,** 59–67 (1997)
ARTICLE NO. RC977772

A Bidirectional Promoter Connects the p14.5 Gene to the Gene for RNase P and RNase MRP Protein Subunit hPOP1[1]

Gerno Schmiedeknecht, Christa Büchler, and Gerd Schmitz[2]
Institute for Clinical Chemistry and Laboratory Medicine, University of Regensburg, D-93042 Regensburg, Germany

Received October 23, 1997

We have identified the functional promoter of the translational inhibitor p14.5, the human homologue to a rat perchloric acid-soluble protein (PSP), a mouse heat-responsive protein (Hrp12) and a goat tumor antigen (UK114). Sequence analysis revealed a GC-rich promoter with several consensus sequences for transcription factors, but no TATA- and CAAT-box. To confirm promoter activity, DNA fragments of the p14.5 5′-flanking region were ligated in front of the luciferase gene and were transfected into HeLa and HepG2 cells. A minimal promoter between nt −104 and nt +88 relative to the transcription start site was responsible for basal activity. Furthermore, we observed a head-to-head orientation of p14.5 to the gene for the protein subunit of RNase P and MRP ribonucleoproteins (hPOP1). Luciferase assays with fragments of the hPOP1 5′-flanking region revealed a minimal promoter between nt −20 and nt +98 relative to the start of transcription. These data indicate that the 102 bp region between p14.5 and hPOP1 can act as a bidirectional promoter. The p14.5-hPOP1-cluster was mapped to chromosome 8q22 using *in situ* hybridization technique. © 1997 Academic Press

Blood monocytes represent a relatively quiescent circulating precursor cell pool for tissue macrophages with a basal activity of protein synthesis. Functional heterogeneity of cells at various stages of differentiation is closely linked with changes in specific gene expression. Upon differentiation to tissue macrophages, cytokines, chemokines and metabolic stimuli rapidly induce cellular protein synthesis. The identification of new candidate genes involved in macrophage differentiation may help to elucidate the molecular mechanisms involved in this process and the accompanying functional changes.

In our previous study, we described the isolation and characterization of a human 14.5 kDa trichloroacetic acid-soluble translational inhibitor (p14.5), that is significantly upregulated upon cellular differentiation from monocytes to macrophages (1). The 137-amino acid protein is most abundant in the cytosolic fraction of hepatocytes, renal distal tubular cells, smooth muscle cells and macrophages and belongs to a family of evolutionary conserved small proteins. Recombinant p14.5 strongly inhibits *in vitro* protein synthesis in a rabbit reticulocyte lysate system similar to a homologous perchloric acid-soluble rat protein (PSP) (2). PSP strongly inhibits the initiation stage of cell-free protein synthesis in a manner different from RNase A. The biphasic kinetics and the disaggregation of polyribosomes by the rat protein are similar to the characteristic of a liver inhibitory protein isolated by Delaunay et al. (3) and to the heme-regulated eukaryotic initiation factor-2α kinase (4).

Recently, Samuel et al. (5) published a mouse homologous translational inhibitor (Hrp12), which shows features of a heat responsive protein. In comparison to common heat shock proteins like Hsp90 and Hsp70, which display a continous increase of mRNA after heat treatment, the Hrp12 message responds atypically. Although the steady-state level of the Hrp12 message increases after heat shock, a marked oscillatory pattern is superimposed on it. Interestingly, heat shock proteins have been shown to inhibit protein synthesis and DNA-proliferation (6, 7). Furthermore, protein synthesis is also necessary for cell proliferation (8). Samuel et al. described, that the amount of Hrp12 mRNA in highly proliferating hepatocytes observed after partial hepatectomy declines dramatically in comparison with sham-operated liver controls. Analogous

[1] The novel nucleotide sequences published here have been submitted to the EMBL sequence data bank and are available under accession number Y15082 and Y15083.

[2] To whom correspondence should be addressed. Institute for Clinical Chemistry and Laboratory Medicine, University of Regensburg, Franz-Josef-Strauß-Allee 11, D-93053 Regensburg, Germany. Fax: +49-941-9446202. E-mail: Gerd.Schmitz@klinik.uni-regensburg.de.

0006-291X/97 $25.00

phenomenona are visible with human p14.5, which displays low mRNA and protein expression in a variety of undifferentiated proliferating liver and kidney tumor cells and high expression in relative quiescent fully differentiated cells. Since it is known, that adult liver is a mitotically quiescent organ with most of the hepatocytes resting in the G_0-stage of cell cycle (9), p14.5 may play an important role in preventing cells (e.g. hepatocytes) from entering the cell cycle.

The goat homologous protein (UK114) was recently characterized as a hepatic perchloric acid-soluble protein (10). UK114 is expressed on the cell surface of several human tumor cell lines, whereas differentiated cells are negative for a plasma membrane signal (11). This suggests, that the expression of UK114 on the cell membrane might be an event related to neoplasmic transformation. Antibodies against UK114 mediate *in vitro* complement-dependent cytolysis of cancer cells expressing the UK114 protein on the cell membrane. Administration of the antibodies *in vivo* suppresses the growth of human colon cancer HT29 cells xenografted into *nu/nu* mice (11). Whether these findings may open new perspectives for immunotherapy of cancer is recently under intensive investigation through clinical studies in patients with metastatic cancer (12, 13).

In this paper, we report the isolation and characterization of the p14.5-promoter region. The functional characterization of the promoter will help to understand the mechanism governing the regulation of this translational inhibitor protein.

MATERIALS AND METHODS

Identification of the transcription start site. Rapid amplification of 5′-cDNA ends (5′-RACE)-ready cDNA prepared from human leukocytes poly(A)-rich RNA (Marathon-Ready™ cDNA, Clontech, Palo Alto, USA) was amplified following the recommendations of the manufacturer. Primary PCR reaction was conducted with the provided anchor primer and the gene specific primer GS6r (5′-GCCTATCTGTCCTGAAATGTAAATGG-3′) matching nt +165 to nt +190 with respect to the 5′-end of the p14.5-cDNA (1). An aliquot of the initial PCR reaction served as template for the secondary PCR reaction with the provided anchor primer and the primer GS3r (5′-GCGGTGCTGATCACCCTTCTGATC-3′) matching nt +88 to nt +111. PCR reactions were carried out in a Perkin Elmer 9600 thermocycler (Vaterstetten, FRG). The PCR-fragment was cloned in the *Sma* I-site of pUC18 and sequenced using fluorescently labeled primers (Cy5 AutoRead Sequencing Kit, Pharmacia, Freiburg, FRG) and an AL-Fexpress DNA sequencer (Pharmacia).

Cloning of the promoter. Five libraries of uncloned, adaptor ligated genomic DNA fragments, constructed with *Eco* RV (library 1), *Sca* I (library 2), *Dra* I (library 3), *Pvu* II (library 4) and *Ssp* I (library 5) (PromoterFinder DNA Walking Kit, Clontech) were amplified according to the recommendations of the manufacturer. Primary PCR reaction was performed with the provided adaptor primer 1 and the gene specific primer GS3r (see above). An aliquot of the initial PCR reaction served as template in a secondary PCR re action with the provided nested adaptor primer 2 and the primer Prom3 (5′-GCCTTCCCTCTTGCAGCCCCTTCAG-3′) matching nt +46 to nt +70 with respect to the 5′-end. The main PCR fragment from library 1 and 3 was cloned and sequenced.

Reporter gene constructs for p14.5. A genomic PCR fragment (p14.5-1) was amplified using primers with *Sac* I or *Kpn* I-sites at their ends for cloning into the luciferase expression vector pGL3-basic (Promega, Madison, USA). p14.5-1 was amplified using primers Lucif.1 (5′-GGGAGCTCGCCTTCCCTCTTGCAGCCCCTTCAGG-3′) and Lucif.2 (5′-GGGGTACCATCCCAATCACTAGGGCTCTGTTCAG-3′) and extended 716 bp from nt −628 to nt +88. The genomic fragment obtained from library 1 served as template for the PCR reaction. PCR conditions were as previously described (14). Shorter fragments were derived from p14.5-1 upon amplification using internal primers with *Sac* I or *Kpn* I-sites. Primers for p14.5-2 were: Lucif.1 and Lucif.3 (5′-GGGGTACCGCTCCAGCGCAGGGGACGAAGGAGATG-3′); p14.5-3: Lucif.1 and Lucif.4 (5′-GGGGTACCCTGCCAGACGCTGTGAGGAATCCCC-3′); p14.5-4: Lucif.1 and Lucif.5 (5′-GGGGTACCATGCGCCAGACCGGGAGCTTCCTCC-3′); p14.5-5: Lucif.1 and Lucif.6 (5′-GGGGTACCCCGCCCCCAGCGGGAGCTGTGGGG-3′). The PCR products were digested with *Sac* I and *Kpn* I and cloned into pGL3-basic.

Reporter gene constructs for hPOP1. A genomic PCR fragment (hPOP1-1) was amplified using primers with *Sac* I or *Kpn* I-sites at their ends. The fragment hPOP1-1 was amplified using the primer hPOPlucif.1 (5′-GGGAGCTCCTGCCAGACGCTGTGAGGAATCCCC-3′) matching nt +74 to nt +98 with respect to the transcription initiation site of hPOP1 (15) and hPOPlucif.2 (5′-GGGGTACCAATGGCCCCTGGGGCTTTCGCGG-3′) matching nt −228 to nt −250. PCR was performed using the Expand High Fidelity PCR System (Boehringer Mannheim, FRG) with genomic DNA as template. The fragment hPOP1-1 extended 348 bp from nt +98 to nt −250. Shorter fragments were derived from hPOP1-1 upon amplification using internal primers with *Sac* I or *Kpn* I-sites. Primers for hPOP1-2 were: hPOPlucif.1 and hPOPlucif.3 (5′-GGGGTACCGCCTTCCCTCTTGCAGCCCCTTCAGG-3′); hPOP-3: hPOPlucif.1 and hPOPlucif.4 (5′-GGGGTACCGGAAGCTCCCGGTCTGGCGCATGCG-3′); hPOP-4: hPOPlucif.1 and hPOPlucif.5 (5′-GGGGTACCATGCGCTCTCCAGCGCGCTCTCC-3′).

Transient transfection of HepG2 and HeLa cells. HepG2 or HeLa cells were cultured at a 10% CO_2 atmosphere at 37°C, in DMEM-medium (Biowhittaker, Verviers, Belgium) supplemented with 10% fetal calf serum (Pan Systems, Aidenbach, FRG). Cells at a density of 60 - 70% were transiently transfected with 7.5 μg of plasmid DNA using DOTAP-lipofection (Boehringer, Mannheim) as described by the manufacturer. The transfected cells were incubated for 40 h. In each experiment, the cells were transfected with a pGL3-basic and a pGL3-promoter plasmid, that served as negative and positive control, respectively. The pGL3-basic construct had no promoter or enhancer elements upstream of the luciferase gene. The pGL3-promoter plasmid contained the SV40 early promoter.

Luciferase assays. Transfected cells were harvested 40 h after transfection and lysed in 500 μl of reporter lysis buffer (Promega). Subsequent to centrifugation (5 min, 10,000 × g) of the lysates, 20 μl aliquots of the supernatants were mixed with 100 μl luciferase assay reagent containing luciferyl-CoA (Promega). The luciferase activity was measured in a LUMAT LB9501 (Berthold, Munich, FRG). Protein concentrations of the cell extracts were determined according to Smith et al. (16).

Slides preparation for fluorescence in situ hybridization (FISH). Lymphocytes isolated from human blood were cultured in α-minimal essential medium (MEM) supplemented with 10% fetal calf serum and phytohemagglutinin at 37 °C for 68-72 h. The lymphocyte cultures were treated with 5-bromo-2′-deoxyuridine (0.18 mg/ml) to synchronize the cells. The synchronized cells were washed three times with serum-free medium to release the block and recultured at 37 °C for 6 h in α-MEM with thymidine (2.5 μg/ml). Cells were harvested and slides were prepared by using standard procedures including hypotonic treatment, fix and air-dry.

In situ hybridization and FISH detection. The 2116 bp probe was biotinylated with dATP for 1 h at 15 °C using the BioNick labeling

system (Gibco BRL) (17). The procedure for FISH detection was performed according to Heng et al. (17) and Heng and Tsui (18). Briefly, slides were baked at 55 °C for 1 h, treated with RNase and denaturated in 70% formamide in 2× SSC for 2 min at 70 °C followed by dehydration with ethanol. Probes were denaturated at 75 °C for 5 min in a hybridization mix consisting of 50% formamide and 10% dextrane sulphate and loaded on the denaturated chromosomal slides. After over night hybridization, slides were washed and detected. FISH signals and the DAPI banding pattern was recorded separately by taking photos, and the assignment of FISH mapping data with chromosomal bands was achieved by superimposing FISH signals with DAPI bandet chromosomes (18).

RESULTS AND DISCUSSION

As a first step towards characterization of the promoter of the p14.5 gene we used 5′-RACE-PCR to determine the position of transcription initiation. Sequence analysis of the longest 5′-RACE-PCR product revealed a fragment of 167 bp in length. It was composed of the used anchor primer (38 bp) at the 5′-end and 110 bp of already known cDNA sequence at the 3′-end. The remaining 19 bp represented new 5′-cDNA sequence, revealing a transcription start site 94 bp upstream from the ATG codon. Interestingly, most of the cloned and sequenced 5′-RACE-PCR fragments revealed a transcription start site 75 bp upstream from the ATG codon. This is in accordance with the published length of the p14.5 cDNA and indicates, that the transcription process may be initiated at multiple locations.

Amplification of the provided promoter finder libraries from the PromoterFinder Kit revealed PCR products ranging in size from 700 bp (library 1) up to 3000 bp (library 4) (Fig.1A). The main fragments from library 1 and 3 with 716 bp (Fig.2A) and 2116 bp (accession number Y15082) in length, respectively, were sequenced. The 3′-end of the 2116 bp fragment was complete identical to the PCR product obtained from library 1, confirming the identity of the smaller fragment.

Within the 716 bp fragment putative transcription factor binding sites were detected using the FACTOR algorithm of the HUSAR package (DKFZ, Heidelberg, Germany) (Fig.2A). The 5′-flanking region of the p14.5 gene shows typical features for a group of housekeeping genes, exemplified by dihydrofolate reductase. These promoters lack TATA- and CAAT-boxes, are GC-rich, and contain multiple transcription start sites. Housekeeping genes are mostly constitutively expressed, and the GC-box binding protein SP1 appears to play a prominent regulatory role. In fact, the p14.5 promoter exhibits a 67% GC-rich sequence localized between nt −448 and nt +1 and contains no TATA-box. A CAAT-box was identified at nt −625, however this is not the expected position of a CAAT-box relative to the transcription start site. In addition to a GC-box at nt −425, a second conserved GC-box is located at nt −26.

In contrast to the observed promoter structure for a housekeeping gene, p14.5 mRNA expression is regulated in a tissue- and differentiation-dependent manner (1). The existence of putative binding sites for the ETS-family transcription factor PU.1 (nt −547, −327) could be the reason for upregulation of p14.5 upon differentiation from monocytes to macrophages (1). PU.1 is a candidate transcription factor for mediating macrophage-specific gene expression. The expression of genes encoding prointerleukin-1β, macrophage inflammatory protein-1α, CD11b, Fc gamma receptor-1b and the colony-stimulating factor-1 receptor appear to be regulated by this protein (19).

The high expression in hepatocytes (1) may be mediated by three binding sites for the liver-specific transcription factor LF-AI (nt −7, −341, −421) (20), which is required for the expression of a set of genes in hepatocytes (21).

In regard to the function of p14.5 as a translational inhibitor (1, 2) and its putative role in regulation of cell growth and differentiation (1, 5), two additional transcription factor binding sites for c-Myc at nt −80 and α-palindrome-binding protein (α-PAL) at nt −107 may be important. c-Myc appears to be a member of a transcriptional regulatory network responsible for controlling a set of genes whose functions impinge directly on the machinery of cell growth and proliferation. For example, eukaryotic initiation factor-2α (eIF2α) and eIF4e, two important proteins of the translational apparatus, are candidate c-Myc regulated genes (22). Binding sites for α-PAL, which was originally identified as a key transcription factor for the eIF2α-gene (23), are localized in numerous genes involved in translation, cellular proliferation, growth-responsive pathways and DNA replication/repair. α-PAL may be a transcription factor that links the transcriptional modulation of key metabolic genes to cell growth and differentiation (24). In addition to the transcription factor recognition sites mentioned above, two half-palindromic estrogen response elements (ERE) (nt +16, −319), an incomplete ERE (nt −174), a myc-associated zinc finger protein (MAZ) site (nt −271), an activator protein-2 (AP2) site (nt −346), a cyclic nucleotide regulatory element-binding protein (CREB)/activating transcription factor (ATF) site (nt −401), a GC-rich sequence binding factor (GCF) site (nt −426) and two CACCC-boxes (nt −430, −576) were identified.

Potential promoter activity was ascertained by designing fusion constructs of the 5′-flanking region of p14.5 linked to luciferase gene as a reporter in the pGL3-basic vector. Five different promoter-luciferase constructs were transiently transfected into HeLa and HepG2 cells and assayed for luciferase activity (Fig.3A). The general pattern of luciferase activity in these two cell types was similar, but transfection of HepG2 cells resulted in a significant higher level of promoter activity compared to HeLa cells. Only pro-

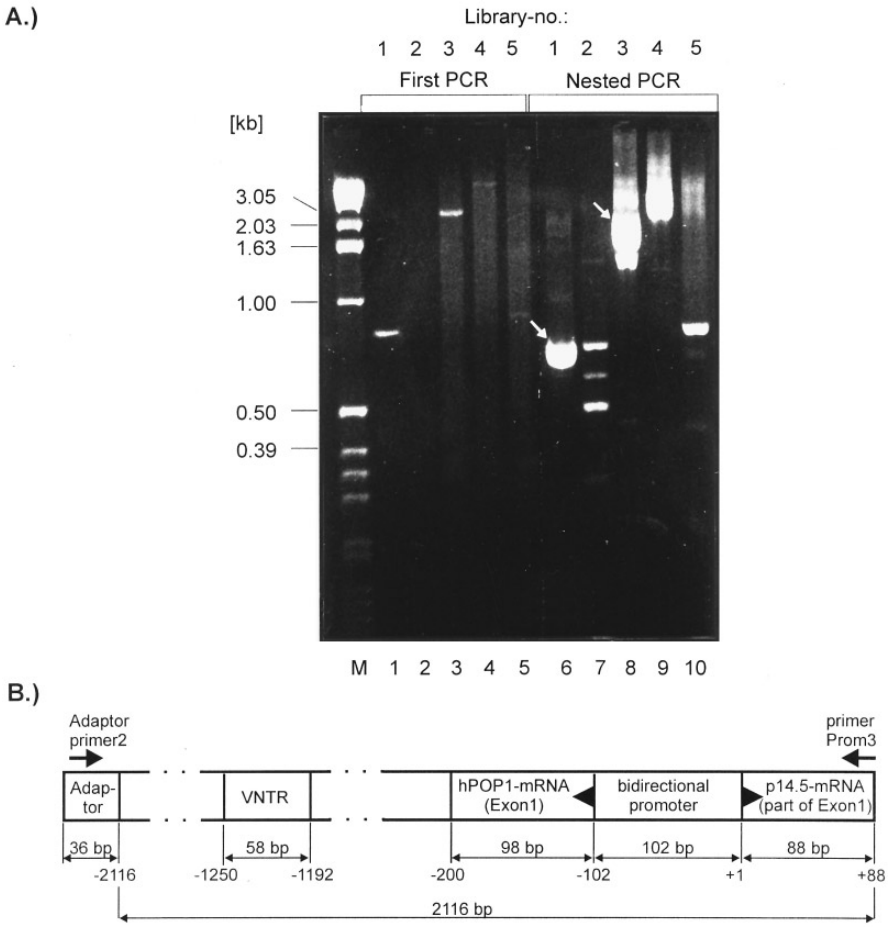

FIG. 1. (**A**) Agarose gel electrophoresis of the amplified genomic fragments of the p14.5 promoter region. Primary (lanes 1–5) and secondary (lanes 6–10) PCR was performed as described by the PromoterFinder™ DNA Walking Kit protocol. Lanes 1 and 6: *Eco* RV library, lanes 2 and 7: *Sca* I library, lanes 3 and 8: *Dra* I library, lanes 4 and 9: *Pvu* II library, lanes 5 and 10: *Ssp* I library. Lane M contains 0.5 μg 1 kb DNA-ladder (Gibco BRL). The sequenced fragments are indicated by arrowheads. (**B**) Schematic structure of the 2116 bp promoter fragment obtained from library 3. The genes for p14.5 and hPOP1 are found in a head-to-head orientation. A 102 bp intergenic region is sufficient for divergent transcription of both genes. Various numbers of tandem repeats (VNTR) are localized between nt −1192 and nt −1250.

moter plasmid p14.5-2 (−390/+88) exhibited a lower activity in HepG2. No activity was detected in the lysate of cells transfected with the promoterless pGL3-basic plasmid. Plasmid p14.5-1 (−628/+88) contained the whole 716 bp fragment obtained from library 1 and exhibited a 21-fold (HeLa) and 59-fold (HepG2) higher luciferase activity compared to cells transfected with pGL3-basic. Deletion of the proximal 238 bp of the 726 bp fragment (plasmid p14.5-2; −390/+88) increased promoter activity to the 165-fold (HeLa) and 122-fold (HepG2) activity. The presence of a repressor or silencer in the region between nt −716 and nt −390 is likely. A putative binding site for the transcription factor GCF (nt −426), which represses transcription in the epidermal growth factor receptor and β-actin promoter (25), may be responsible for the observed repression of transcription. The constructs p14.5-3 (−200/+88) and p14.5-4 (−104/+88) showed 70-fold (HeLa)/93-fold (HepG2) and 38-fold (HeLa)/88-fold (HepG2) luciferase activity, respectively. Very low promoter activity was detected when cells were transfected with construct p14.5-5 (−28/+88), suggesting that the sequence between nt −104 and nt +88 is necessary for basal transcription of the p14.5 gene. The disruption of the potential SP1 site (nt −26) in construct p14.5-5 may be the reason for the almost complete loss of luciferase activity.

The nucleotide sequence of the 2116 bp fragment obtained from library 3 was aligned with sequences from the EMBL-databases using the FASTA algorithm of the HUSAR package. Interestingly, nt −103 to nt −200 displayed a 100% homology to the 5′-end of the hPOP1-cDNA, encoding the protein subunit of the human RNase P and MRP ribonucleoproteins (15). RNase P endonucleolytically cleaves precursor tRNA molecules to remove the

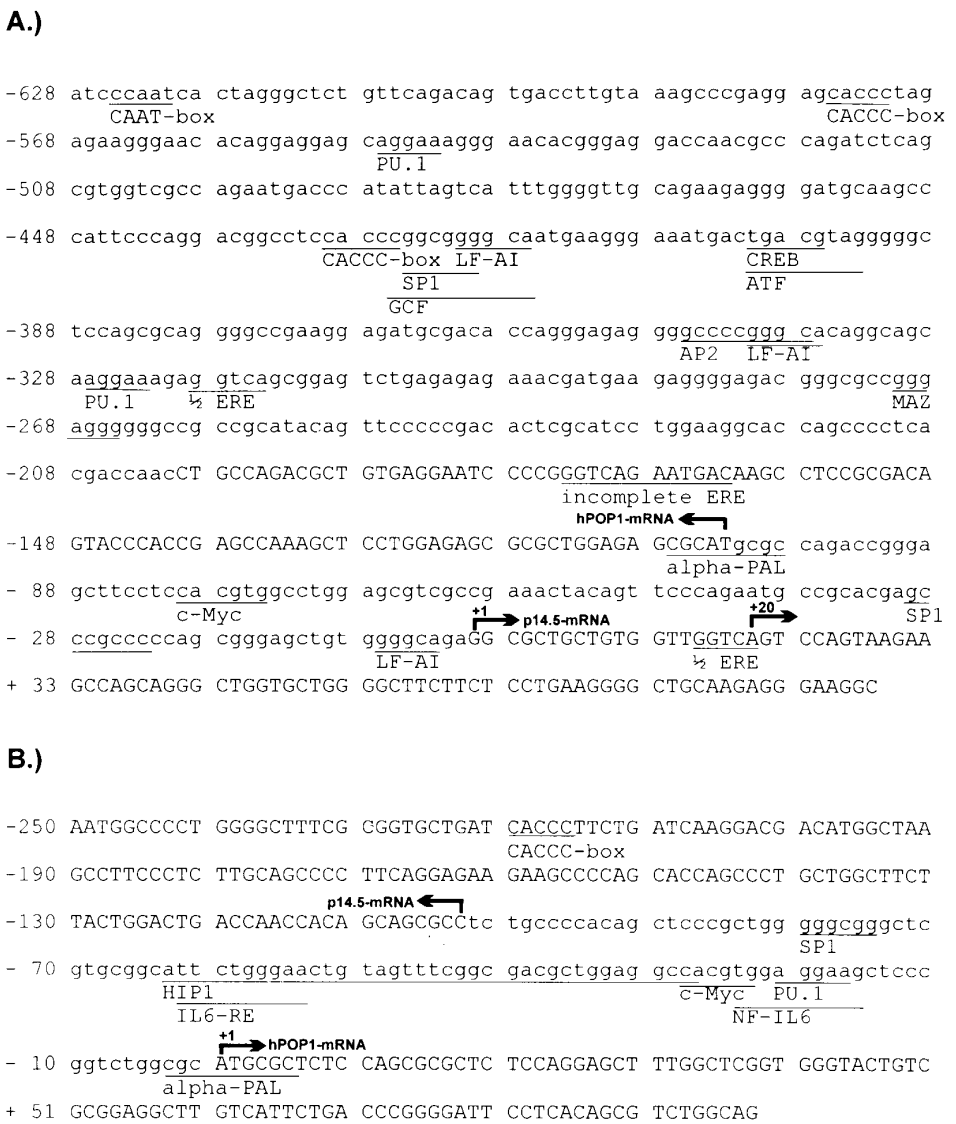

FIG. 2. Nucleotide sequence of the 716 bp human p14.5 promoter fragment (**A**) and a 348 bp hPOP1 promoter fragment (**B**). Nucleotide position +1 is assigned to the first of the transcription initiation sites, and negative numbers refer to 5′-flanking sequences. Exon 1 of p14.5 and hPOP1 is written in capital letters. Putative binding sites for transcription factors are underlined and indicated.

5′-leader sequences and generates the correct 5′-termini of the mature tRNAs (26). The eukaryotic RNase MRP was originally identified as an endonuclease able to cleave a RNA substrate derived from mitochondrial origin of DNA replication *in vitro* (27). In the yeast *S. cerevisiae*, RNase MRP cleaves the ribosomal RNA precursor to allow the subsequent formation of the 5′-end of the major 5.8S rRNA species (28).

The head-to-head orientation of p14.5 and hPOP1 in close proximity suggest, that both genes are transcribed divergently from a bidirectional promoter (Fig.1B). To check this hypothesis, the 5′-flanking region of hPOP1 was aligned for putative transcription factor binding sites (Fig.2B). Furthermore, four different promoter-luciferase fusions for measuring the promoter activity of the 5′-flanking region of the hPOP1 gene have been constructed (Fig.3B). Within the 348 bp fragment containing the first exon of hPOP1 and 250 bp of the hPOP1 5′-flanking region the following consensus sequences for transcription factors have been found: an α-PAL site (nt −3), a PU.1 site (nt −21), a nuclear factor interleukin-6 (NF-IL6) site (nt −24), a c-Myc site (nt −28), an interleukin-6 response element (IL6-RE) (nt −62), a housekeeping initiator protein 1 (HIP1) site (nt −63), a SP1 site (nt −80) and a CACCC-box (nt −220). Almost all transcription factor binding sites are located within the region between the p14.5 and hPOP1 transcription initiation sites, supporting the proposed function of this intergenic region as a bidirectional promoter.

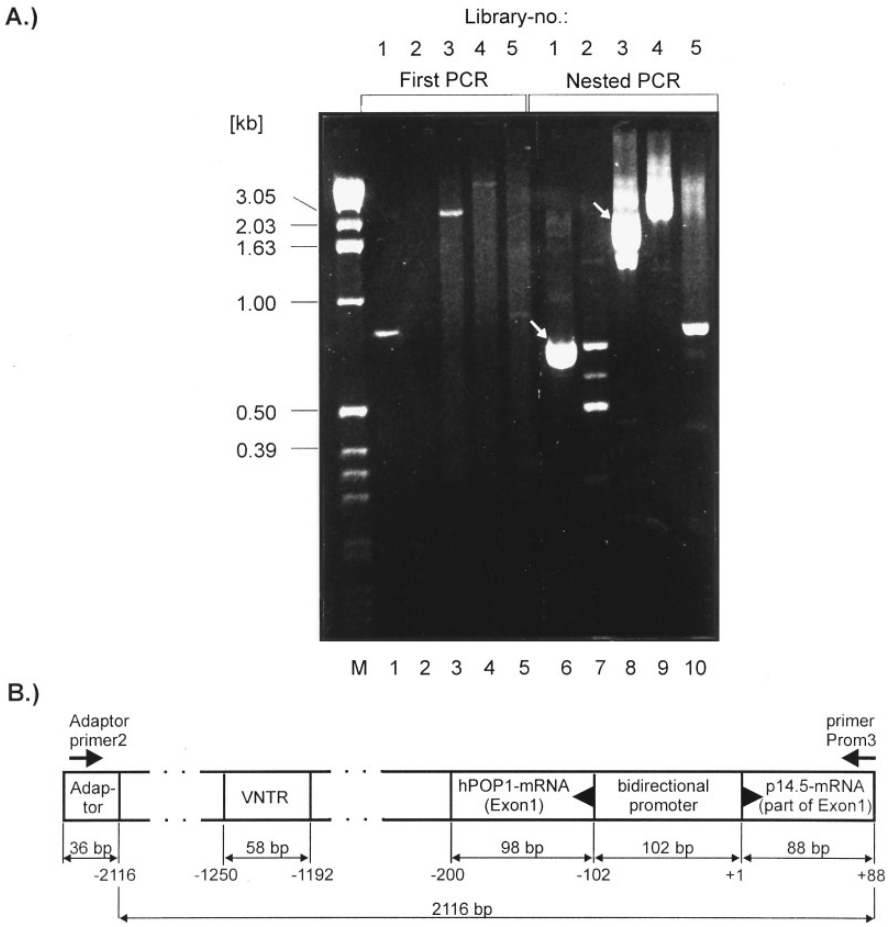

FIG. 3. Promoter activity of p14.5-luciferase gene fusions (**A**) and hPOP1-luciferase gene fusions (**B**) transiently transfected in HeLa cells (black bars) and HepG2 cells (white bars). The localization of the tested promoter fragments are shown to the *left*. Numbers indicate the relative positions with respect to the start of transcription. *Right*, luciferase activity is shown as x-fold value compared to cells transfected with a promoterless vector. Activity expressed by a pGL3-vector containing the SV40 early promoter is included as a positive control. Data represent the means and standard deviation of three independent ex periments, each performed in triplicate.

Measurement of the luciferase activity of the four hPOP1 promoter constructs revealed a higher promoter activity than observed for the p14.5 5′-flanking region (Fig.3). In contrast to the p14.5 promoter, the general pattern of hPOP1 promoter activity varied slightly among HeLa and HepG2 cells. The constructs hPOP-2, -3 and -4 exhibited similar activities in both tested cells with a moderate higher activity in HeLa cells. However, the activity for the largest construct hPOP-1 was significantly decreased in HepG2 cells. Luciferase activity for hPOP-1 (−250/+88) was 291-fold (HeLa) and 114-fold (HepG2) higher compared to cells transfected with a promoterless pGL3-basic. Deletion of the proximal 60 bp of the largest promoter fragment (plasmid hPOP-2; −190/+98) decreased promoter activity in HeLa cells (200-fold) and increased it in HepG2 (161-fold). The construct hPOP-3 (−20/+98) showed 197-fold (HeLa) and 116-fold (HepG2) activity, which were almost the same values as ob served for hPOP-2. A complete loss of promoter activity was detected when HeLa/HepG2 cells were transfected with construct hPOP-4 (+1/+98), suggesting that the DNA sequence between nt −20 and nt +98 is responsible for basal transcription of the hPOP1 gene. Disruption of a potential α-PAL site (nt −3) in construct hPOP-4 may be the reason for the loss of activity, because there is no other known transcription factor binding site in the region from nt −20 to nt +98. It may be suggested, that α-PAL could be a key transcription factor in regulation of hPOP1.

The significant promoter activity for both genes, originating from the same intergenic region, confirmed the existence of a new bidirectional promoter. Bidirectional promoters, although common in prokaryotes and viruses, are seemingly rare among higher eukaryotes. In verte brates, examples of genes sharing a bidirectional promoter include the α1 and α2 chains of type IV collagen (29), WIT-1 / WIT-2 of the Wilms tumor locus (30),

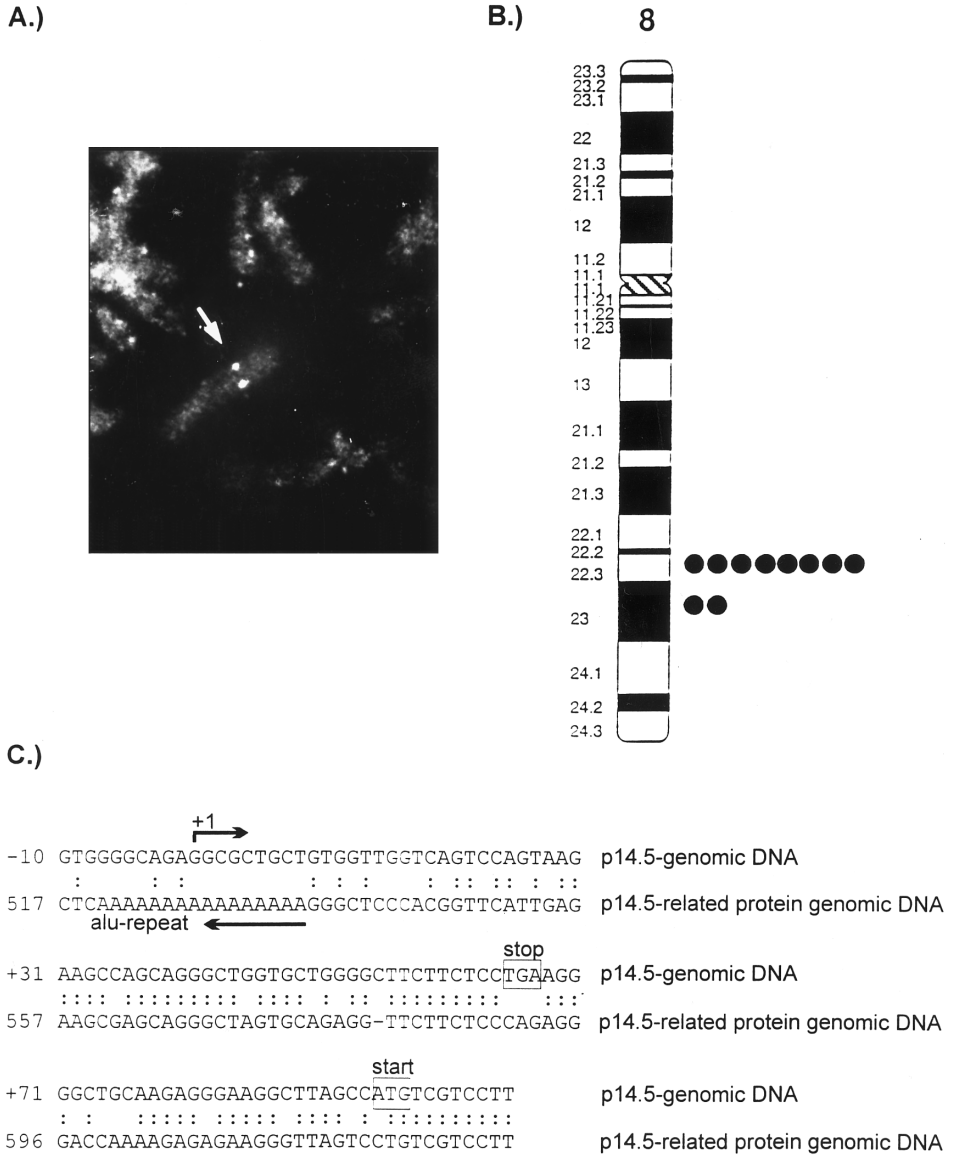

FIG. 4. FISH mapping of human p14.5 to chromosome 8q22. (**A**) Fluorescence detection of a biotinylated human p14.5 genomic probe (2116 bp promoter fragment) on a metaphase chromosome spread from human lymphocytes. (**B**) Diagrammatic representation showing the G-banding pattern for human chromosome 8 and the labeling distribution of the p14.5 probe. Each dot represents the double FISH signals detected on human chromosome 8. (**C**) Sequence comparison between p14.5-genomic DNA and the genomic DNA for the p14.5-related protein. Nucleotide exchanges in the p14.5 initiator codon and in a stop codon upstream of the p14.5 initiator codon are boxed. Nucleotide +1 in the p14.5 genomic sequence is assigned to the first transcription initiation site, and negative numbers refer to the 5′-flanking sequence. The start of an Alu-repeat in the genomic sequence for the p14.5-related protein is indicated by an arrowhead.

the proliferating cell nuclear antigen (PCNA) / unknown cDNA (31), the genes *Surf1* / *Surf2* of the mouse surfeit locus (32), dihydrofolate reductase / mismatch repair protein 1 (33), the transporter associated with antigen processing 1 (TAP1) / low molecular mass polypeptide 2 (LMP2) (34), histidyl-tRNA synthetase / unknown cDNA HO3 (35), the gene for ataxia telangiectasia (ATM) / unknown cDNA E14 (36) and the enzymes glutamine 5-phosphoribosylpyrophos phate amidotransferase (GPAT) / 5′-phosphoribosylaminoimidazole carboxylase + 5′-phos phoribosyl 4-(N-succinocarboxamide)-5-aminoimidazole synthetase (AIRC) (37). Bidirectio nal transcription units often encode proteins sharing very similar structural and functional characteristics. A comparison of the amino acid sequences predicted by the p14.5 and hPOP1 cDNAs revealed no sequence identity, indicating that the p14.5-hPOP1-cluster did not evolve by gene duplication as is likely for the α1 and α2 chains of type IV collagen (29). On the other hand, bidirectional transcription units also

encode proteins, that are different in structure but have similar biological functions as it is the case for the enzymes GPAT/AIRC involved in different steps of purine nucleotide synthesis (37) or the TAP1/LMP2 proteins important for peptide-antigen processing and transport to generate functional MHC-II-peptide complexes (34). In regard to the function of p14.5 as a translational inhibitor and the involvement of RNase P and MRP in processing of components of the translational apparatus, it is likely that the p14.5-hPOP1-cluster belongs to the second group of functionally but not structurally related proteins.

The genomic localization of the p14.5-hPOP1-cluster was identified by FISH on human metaphase chromosomes using the 2116 bp fragment as a probe (Fig.4A). The hybridization efficiency was 62% (among 100 checked mitotic figures, 62 of the showed signals were on one pair of the chromosomes). Since the DAPI banding was used to identify the specific chromosome, the assignment between signal from probe and the long arm of chromosome 8 was obtained. The detailed position was further determined based on the summary from 10 photos (Fig.4B). There was no additional locus picked by FISH detection under the conditions used, demonstrating that p14.5 is located at human chromosome 8, region q22. Analysis for other known genes localized in this region revealed, that 8q22 does not contain any genes with obvious functional or structural similarity to p14.5 or hPOP1.

Initial experiments to amplify the p14.5 promoter region by primers homologous to a sequence more upstream in the p14.5 cDNA (GS6r and GS3r) and the PromoterFinder library 3 generated a genomic fragment of 630 bp in length (accession number Y15083) displaying a high degree of similarity to the 5′-end of the p14.5 cDNA. An alignment between p14.5 genomic DNA and the genomic DNA from this putative p14.5-related protein indicates, that the DNA sequence for the p14.5-related protein revealed critical nucleotide exchanges (Fig.4C). In detail, there is an exchange in the p14.5 initiator codon (ATG→CTG) and an exchange in a 30 bp upstream from the p14.5 initiator codon localized stop codon (TGA→CAG). In contrary to the p14.5 genomic fragment, the genomic DNA encoding the p14.5-related protein contains an Alu-repeat instead of a functional promoter. According to these facts, it is likely, that the p14.5-related protein is a longer variant of p14.5 encoded from a independent gene. Homology-searches within the SwissProt-databases revealed the existence of two variants of the yeast *S.cerevisiae* homologous protein, supporting the hypothesis of two forms of p14.5 in human. In detail, there is a 13.9 kDa protein YER057c (chromosome V) and a 15.9 kDa protein YIL051c (chromosome IX).

The data presented here may contribute to the understanding of the transcriptional regulation of the genes that encode the translational inhibitor p14.5 and the RNase P and MRP protein subunit hPOP1. The relevance of the identified putative transcription factor binding sites, especially for the tissue- and differentiation-dependent regulation is currently investigated and experiments are in progress to clone the cDNA of the p14.5-related protein in order to clarify, whether it constitutes an additional translational inhibitor protein.

ACKNOWLEDGMENTS

This study was supported by the Deutsche Forschungsgemeinschaft (AN-11/6-1, project 1). We thank the SeeDNA Biotech Inc. (Ontario, Canada) for performing of FISH-mapping. The expert technical assistance of S. Potra is appreciated.

REFERENCES

1. Schmiedeknecht, G., Kerkhoff, C., Orsó, E., Stöhr, J., Aslanidis, C., Nagy, G. M., Knuechel, R., and Schmitz, G. (1996) *Eur. J. Biochem.* **242,** 339–351.
2. Oka, T., Tsuji, H., Noda, C., Sakai, K., Hong, Y.-m., Suzuki, I., Munoz, S., and Natori, Y., (1995) *J. Biol. Chem.* **270,** 30060–30067.
3. Delaunay, J., Ranu, R. S., Levin, D. H., Ernst, V., and London, I. M. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 2246–2268.
4. Chen, J. J., Crosby, J. S., and London, I. M. (1994) *Biochimie* **76,** 761–769.
5. Samuel, S. J., Tzung, S.-P., and Cohen, S. A. (1997) *Hepatology* **25,** 1213–1222.
6. Yost, H. J., and Lindquist, S. (1991) *Mol. Cell. Biol.* **11,** 1062–1068.
7. Mivechi, N. F., and Dewey, W. C. (1985) *Radiation Res.* **103,** 337–350.
8. Yost H. J., Petersen, R. B., and Lindquist, S. (1990) *in* Stress Proteins in Biology and Medicine (Morimoto, R., Tissieres, A., and Georgopoulos, C., Eds.) pp. 379–409, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
9. Fausto, N., and Weber, E. M. (1994) *in* The Liver: Biology and Pathobiology (Arias, I. M., Boyer, J. L., and Fausto, N., Eds.) 3rd ed., Raven, New York.
10. Ceciliani, F., Faotto, L., Negri, A., Colombo, I., Berra, B., Bartorelli, A., and Ronchi, S. (1996) *FEBS-Let.* **393,** 147–150.
11. Bartorelli, A., Bussolati, B., Millesimo, M., Gugliotta, P., and Bussolati, G. (1996) *Int. J. Oncol.* **8,** 543–548.
12. Mor, C., Gariboldi, A., Bissi, O., Concas Benevelli, D., Di Mattia, D., Gajani, M. R., Gilardoni, E., Liverani, A., Mancini, S., Santi, C., and Bartorelli, A. (1997) *J. Tumor Marker Oncol.* **12,** 29–37.
13. Bussolati, G., Geuna, M., Bussolati, B., Millesimo, M., Botta, M., and Bartorelli, A. (1997) *Int. J. Oncol.* **10,** 779–785.
14. Aslanidis, C., and De Jong, P. J. (1990) *Nucl. Acid. Res.* **18,** 6069–6074.
15. Lygerou, Z., Pluk, H., van Venrooij, W. J., and Séraphin, B. (1996) *EMBO J.* **15,** 5936–5948.
16. Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Klenk, D. C. (1985) *Analyt. Biochemistry* **150,** 76–85.
17. Heng, H. H. Q., Squire, J., and Tsui, L.-C. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 9509–9513.
18. Heng, H. H. Q., and Tsui, L.-C. (1993) *Chromosoma* **102,** 325–332.

19. Sweet, M., and Hume, D. A., (1996) *J. Leuk. Biol.* **60,** 8–26.
20. Ammendola, R., Gounari, F., Piaggio, G., De Simone, V., and Cortese, R. (1990) *Mol. Cell. Biol.* **10,** 387–390.
21. Ramji, D. P., Tadros, M. H., Hardon, E. M., and Cortese, R. (1991) *Nucl. Acid Res.* **19,** 1139–1145.
22. Grandori, C., and Eisenman, R. N. (1997) *Trends Biochem. Sci.* **22,** 177–181.
23. Jacob, W. F., Silverman, T. A., Cohen, R. B., and Safer, B. (1989) *J. Biol. Chem.* **264,** 20372–20384.
24. Efiok, B. J. S., Chiorini, J. A., and Safer, B. (1994) *J. Biol. Chem.* **269,** 18921–18930.
25. Kageyama, R., and Pastan, I. (1989) *Cell* **59,** 815–825.
26. Altman, S., Kirsebom, L., and Talbot, S., (1993) *FASEB J.* **7,** 7–14.
27. Clayton, D. A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4615–4617.
28. Lygerou, Z., Allmang, C., Tollervey, D., and Séraphin, B. (1996) *Science* **272,** 268–270.
29. Pöschl, E., Pollner, R., and Kühn, K. (1988) *EMBO J.* **7,** 2687–2695.
30. Huang, A., Campbell, C. E., Bonetta, L., McAndrews-Hill, M. S., Chilton-MacNeill, S., Coppes, M. J., Law, D. J., Feinberg, A. P., Yeger, H., and Williams, B. R. (1990) *Science* **250,** 991–994.
31. Rizzo, M. G., Ottavio, L., Travali, S., Chang, C.-D., Kaminska, B., and Baserga, R. (1990) *Exp. Cell Res.* **188,** 286–293.
32. Lennard, A. C., and Fried, M., (1991) *Mol. Cell. Biol.* **11,** 1281–1294.
33. Fujii, H., Shinya, E., and Shimada, T. (1992) *FEBS Let.* **314,** 33–36.
34. Wright, K. L., White, L. C., Kelly, A., Beck, S., Trowsdale, J., and Ting, J. P.-Y. (1995) *J. Exp. Med.* **181,** 1459–1471.
35. O'Hanlon, T. P., Raben, N., and Miller, F. W. (1995) *Biochem. Biophys. Res. Commun.* **210,** 556–566.
36. Byrd, P. J., Cooper, P. R., Stankovic, T., Kullar, H. S., Watts, G. D. J., Robinson, P. J., and Taylor, A. M. R. (1996) *Hum. Mol. Gen.* **5,** 1785–1791.
37. Chen, S., Nagy, P. L., and Zalkin, H. (1997) *Nucleic Acid Res.* **25,** 1809–1816.