Isolation and Characterization of a Novel Zinc-finger Protein with Transcriptional Repressor Activity*

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To identify genes that can repress the expression of growth regulatory molecules, a human fetal cDNA library was screened with a degenerate oligonucleotide that corresponds to the conserved stretch of 6 amino acids connecting successive zinc-finger regions in the Wilms’ tumor suppressor/Egr-1 family of DNA-binding proteins. One clone, designated zinc-finger protein 174 (ZNF174), corresponds to a putative transcription factor with three zinc fingers and a novel finger-associated domain, designated the SCAN box. The three Cys2-His2-type zinc fingers are positioned at the carboxyl terminus, while the 65-amino acid finger-associated SCAN box is located near the amino terminus. Chromosomal localization using somatic cell hybrid analysis and fluorescent in situ hybridization mapped the gene for ZNF174 to human chromosome 16p13. The 2.5-kilobase transcript from this gene is expressed in a variety of human organs, but most strongly in adult testis and ovary. Fusion of the upstream regulatory region of ZNF174 to the DNA-binding domain of GAL4 revealed that the gene could confer a repression function on the heterologous DNA-binding domain. ZNF174 selectively repressed reporter activity driven by the platelet-derived growth factor-β chain and transforming growth factor-β1 promoters and bound to DNA in a specific manner. This member of the C2H2-type zinc-finger family is a novel transcriptional repressor.

Cellular responsiveness to environmental signals is mediated, at least in part, by the induction or modulation of gene transcription. Relatively detailed models of basal and activated transcription have been generated in eukaryotes (reviewed by Tjian and Maniatis (1994)). Repression, an equally important aspect of transcriptional control, is not nearly as well characterized. Transcriptional repressors can inhibit gene expression by at least two general mechanisms. Passive repressors down-regulate the activity of transcription factors by competing for their DNA binding sites; alternatively, active repressors have an intrinsic repressing activity and directly inhibit transcription (reviewed by Cowell (1994) and Johnson (1995)). The regulation of growth factor gene expression can involve both types of repressive mechanisms. There is increasing evidence that members of the Cys2-His2 zinc-finger class of transcription factors are involved in the transcriptional repression of growth factor gene expression. The Wilms’ tumor suppressor gene (wt-1) encodes a zinc-finger DNA-binding protein, which can interact with the insulin-like growth factor type II promoter to repress expression of the gene during nephrogenesis (Drummond et al., 1992; Pritchard-Jones et al., 1990). Similarly, WT-1 can also repress expression driven by the transforming growth factor TGF-β1 (Dey et al., 1994), PDGF-A chain (Gashler et al., 1992), colony-stimulating factor-1 (Harlington et al., 1993) and the insulin-like growth factor I (IGF-I) receptor (Werner et al., 1994) promoters. Recent studies have revealed that WT-1 expression can be autoregulated through multiple potential WT-1 binding sites located in its promoter (Fraizer et al., 1994; Malik et al., 1994). The loss of function of the wt-1 gene product is thought to contribute to neoplastic transformation. Structural alteration of the wt-1 gene or its abnormal expression have been implicated in the continued proliferation of embryonic kidney blastemal cells seen in Wilms’ tumors. Mutations in the wt-1 gene have been detected in 5–10% of Wilms’ tumors (Haber et al., 1990; Gessler et al., 1990; Call et al., 1990; Huff et al., 1991).

Since the expression pattern of WT-1 is restricted to specific cell types (reviewed by Rauscher (1993)), we reasoned that there may be additional Cys2-His2 factors, which could play an important role in negative growth factor gene regulation. In this report, we have cloned and characterized a novel zinc-finger-containing gene, ZNF174, from a human fetal library. ZNF174 corresponds to a putative transcription factor with three zinc fingers and a novel finger-associated structural element. When the amino-terminal region of ZNF174 was fused to the DNA-binding domain of GAL4, the chimera repressed transcription of a reporter construct containing multiple GAL4 binding sites. Moreover, overexpression of full-length ZNF174 cDNA with a series of promoter-reporter constructs revealed that ZNF174 could repress expression driven by the human PDGF-B chain and TGF-β1, promoters, but not the PDGF-A chain, Egr-1, c-Fos, or c-jun promoters. ZNF174 repression of PDGF-B promoter activity was dose-dependent, and 5′-deletion analysis mapped the putative negative element to the proximal

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1 The abbreviations used are: TGF-β1, transforming growth factor-β1; ZNF174, zinc-finger protein 174; PDGF, platelet-derived growth factor; IGF, insulin-like growth factor; WT, Wilms’ tumor; CAT, chloramphenicol acetyltransferase (EC 2.3.1.28); TSC, tuberous sclerosis complex; GAL, galactosidase; TK, thymidine kinase; PCR, polymerase chain reaction; kb, kilobase; bp, base pair(s); BAEC, bovine aortic endothelial cell(s).
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promoter. DNase I footprint and gel shift analysis revealed that recombinant ZNF174 protein binds to an element near the transcriptional start site, indicating that ZNF174 repression may be mediated by direct interaction with DNA. These results suggest that ZNF174 may contribute to the negative regulation of certain growth factor genes.

EXPERIMENTAL PROCEDURES

RNA Isolation and Construction of a Human Fetal CDNA Library—Total RNA was extracted with guanidine isothiocyanate from each organ and purified by spinning through a cesium-chloride cushion (Sambrook et al., 1989). Poly(A⁺) RNA was prepared from 10- and 12-week-old human embryos according to standard procedures (Sambrook et al., 1989). Total RNA was isolated from human COS cells obtained under an approved Brigham and Women's Hospital Human Subjects Protocol for discarded human tissue. The techniques of Guber and Hoffman (1983) were used for cDNA synthesis. Briefly, oligo(dT) was used as a primer for first strand synthesis. Following second strand synthesis, NotI/EcoRI linkers were added. The library was size-selected for inserts greater than 1.0 kb, ligated to calf intestinal phosphatase-treated λgt 10 arms, and recombinant bacteriophage generated with high efficiency packaging extract. The library had a primary size of 2,750,000 recombinants with a vector background of less than 10%. Randomly chosen cDNA inserts ranged in size from 300 to 7000 bp. The partial cDNA clones were obtained from the amplified portion of the library.

cDNA Cloning—A region from the conserved “knuckle” section of the WT-3/Egr zinc fingers (TGEKPFCC) was used to generate a degenerate 24-bp oligonucleotide (5′-ACGGCAGAAGACACGTAAACGTT-3′). The oligonucleotide was used as a forward primer with a 3′ reverse primer (5′-CTTATGAATTCTCTTAAGG GACA-3′) from the λgt 10 phage arms. The human fetal library was used as a DNA source with the following polymerase chain reaction (PCR) protocol. Thirty PCR cycles were performed: denaturation at 92 °C for 30 s, annealing at 60 °C for 30 s, and extension for 1 min at 72 °C. The prominent 590-bp PCR product from a parallel set of six PCR reaction tubes was pooled and purified. The ends of the PCR product were repaired with the Klenow fragment of DNA polymerase and phosphorylated with T4 polynucleotide kinase. The fragment was subcloned into the HindIII site of the plasmid vector pBS (Stratagene, La Jolla, CA), according to standard procedures (Sambrook et al., 1989). A reverse primer from the 5′-end of the initial partial cDNA clone (5′-CTAAGGCACTGTAGTTGAATCCTTGTTAGTCACT-3′) was used with a 5′ forward λgt 10 primer (5′-AGCAAGTGTCATGGAGTCGGTTAA-3′) and the human fetal library was used to clone the 5′ portion of the cDNA. PCR conditions were: denaturation at 92 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min. A 1170-bp amplification product was subcloned into pBS, as described above, and designated ZNF174.

DNA Sequencing and Sequence Analysis—Nucleotide sequence was determined by the dideoxynucleotide chain termination procedure with modified Sequenase (U.S. Biochemical Corp.) and [α-32P]dATP using T4 polynucleotide kinase (SQ). After reaction, sequencing products were loaded on a sequencing gel and were visualized by autoradiography (vector Laboratories). Slides were evaluated with a Nikon fluorescence microscope. Forty-one metaphases were examined. Q (DAP) counterstaining and R banding (propidium iodide counterstaining) were used to confirm the identity of the chromosome. The ideogram demonstrates the localization of an ZNF174 genomic clone to the p13.3 region of chromosome 16 (Cherif et al., 1989; Fan et al., 1990). Metaphase chromosomes were prepared from 5-bromodeoxyuridine-synchronized lymphocyte cultures. The probe was biotinylated, hybridized to the chromosomes spread and detected by fluorescent conjugated avidin (Vector Laboratories). Slides were evaluated with a Nikon fluorescence microscope. Forty-one metaphases were examined. Q (DAP) counterstaining and R banding (propidium iodide counterstaining) were used to confirm the identity of the chromosome. The ideogram demonstrates the localization of an ZNF174 genomic clone to the p13.3 region of chromosome 16 (Cherif et al., 1989; Fan et al., 1990). Metaphase chromosomes were prepared from 5-bromodeoxyuridine-synchronized lymphocyte cultures. The probe was biotinylated, hybridized to the chromosomes spread and detected by fluorescent conjugated avidin.
added to the binding reaction and loaded onto a 5% polyacrylamide/Tris borate/EDTA gel and run at 200 V for 2 h at 20° C.

**RESULTS**

**Isolation of cDNA Clones**—A predicted peptide from the conserved “knuckle” region of the WT-1/Egr zinc fingers (TGEK-FPQC) was used to synthesize a degenerate 24-mer oligonucleotide. This probe was used in a PCR strategy to screen a human fetal cDNA library generated from 10–12-week embryos. The degenerate oligonucleotide was used as the forward primer with a 3'-9'-reverse primer derived from the bacteriophage arms. DNA sequence analysis of one of the amplification products revealed a novel zinc-finger-containing structure. The open reading frame was completed by generating a series of overlapping partial cDNA clones using standard anchored PCR, as well as by rescreening additional cDNA libraries. DNA sequence from at least two independent clones for each amplification product was obtained to eliminate PCR-generated sequence errors.

Sequence analysis of the cDNA clones revealed a single open reading frame of 1221 nucleotides (Fig. 1A). The open reading frame has a calculated relative molecular mass of 46,414 Da and an estimated isoelectric point of 10.1. A long 5’-untranslated region containing multiple translational stop codons flanks the open reading frame. The cDNA has a relatively short 3’-untranslated region with a consensus polyadenylation signal (AAUAAA) and poly(A) tail. A schematic representation of ZNF174 cDNA appears in Fig. 1B.

The predicted protein contains three tandemly repeated sequence motifs related to the zinc fingers of the WT-1/Egr-1/Sp1 or Drosophila Kruppel gene family of transcription factors (Fig. 2A). All three zinc fingers encoded by the cDNA of ZNF174 fit the consensus sequence for this type of zinc finger. The conserved amino acid “knuckle” (or H/C link) between zinc fingers, typified by the amino acid sequence TGE(R/K)P(F/Y)X, is also conserved in the deduced amino acid sequence. From these features it is reasonable to predict that ZNF174 could encode a DNA-binding protein with transcriptional regulatory properties.

Outside the putative zinc-finger DNA-binding domain, the predicted protein contains proportionately high numbers of serine (9.3%), glycine (8.9%), proline (8.6%), and glutamine (8.2%) residues (Fig. 1A). Outside the strikingly high content and stretches of these residues, characteristics of the other transcription factors in the WT-1/Egr/Sp1 family were not seen. Although amino acid sequence homology within these enriched regions is not generally observed among transcription factors, the high content of these amino acids in the non-zinc-finger segment of the predicted protein fits the motif of a number of known and putative transcription factors (Mitchell and Tjian, 1989). A single consensus phosphorylation site (Ser*/Thr*-Pro-X-Lys/Arg) for Cdc2 kinase was identified in the open reading frame (SLK, Fig. 1A). An adjacent region rich in basic amino acid residues (LKKSKGKSK) (Fig. 1A) suggests the existence...
of a nuclear localization signal within the protein.

Upstream Finger-associated SCAN Box—The deduced amino acid sequence contains a novel element upstream of the zinc-finger domain that consists of approximately 65 amino acids. This region, which we have designated the SCAN box, was identified by its homology with similar elements in several other zinc-finger transcription factors such as SRE-ZBP, CTfin-51, #18 cDNA, ZNF165, and 3c3 (Fig. 2B). The term "SCAN" box was derived from the first letters of the names of the four proteins initially found to contain this domain (SRE-ZBP, CT-fin-51, AW-1,2 Number 18 cDNA). The SCAN box is separated from the zinc-finger domains by amino acid regions that vary in length and do not share extensive sequence homology. The element is enriched in hydrophobic and negatively charged residues with the L(X)_nL motif at its core. This core is flanked by certain residues (e.g., A, E, L, M, H, C) that are frequently found in α-helices. This element also has a high proportion of glutamic acid residues, suggesting that it constitutes a negatively charged acidic domain, commonly found in transcription factors. When the SCAN box amino acid sequence is plotted as a helical wheel representation (Fig. 2C), there appears to be a higher proportion of hydrophobic residues on one side of the helix as compared to the opposite side, especially in the first five turns of the helix. This feature suggests that ZNF174 may have the ability to multimerize with either itself or other molecules with a similar domain.

Tissue Distribution of the ZNF174 Transcripts—ZNF174 cDNA was used as a probe to determine the expression pattern of the zinc-finger protein in a variety of human organs. Northern blot analysis defined a major transcript of approximately 2.5 kb, which is consistent with the size of the overlapping cDNA clones. The gene is widely expressed in many adult organs, with the highest levels found in ovary and testis. Interestingly, two minor transcripts of about 1.5 and 3 kb in size were also detected and found to be expressed at different levels in various tissues (Fig. 3). In contrast to low transcript levels detected in adult kidney, the ZNF174 gene is highly expressed...

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2 ZNF174 was originally named AW-1. The designation ZNF 174 was obtained from the HUGO Nomenclature Committee.
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FIG. 3. Expression pattern of the ZNF174 gene. Northern blots containing 2 µg of poly(A+) RNA/lane from 16 different human adult tissues (Clontech) were hybridized with an EcoRI fragment of ZNF174 that did not contain the zinc-finger region. Lanes 2-17 contain mRNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and peripheral blood leukocytes, respectively. The Northern blots were also hybridized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to normalize for loading differences.

in normal 18-20-week human fetal kidney specimens. The 2.5-kb transcript was detected in total RNA from a variety of individual cell types, including human umbilical vein endothelial cells, human foreskin fibroblasts, and HepG2 cells.

Chromosomal Localization of the ZNF174 Gene—The gene for the zinc-finger protein maps to a locus at 16p13.3. DNA isolated from 34 human-mouse somatic hybrid cell lines and parental controls was examined for the presence or absence of the human gene by Southern blotting techniques. On BamHI-digested DNA, the ZNF174 cDNA produced readily distinguishable patterns of restriction fragments (data not shown). The presence or absence of the human gene was tabulated against the presence or absence of human chromosomes in each hybrid (ZNF174/chromosome) for concordance (+/+ or −/−) and discordancy (−/+ or +/−). 0% discordance indicates a matched segregation of the DNA probe with a chromosome. The gene maps to human chromosome 16.

TABLE I Segregation of the ZNF174 gene with human chromosome 16 on BamHI-digested human-mouse cell hybrid DNA

| Chromosome | Concordant no. of hybrids (+/+) | Discordant no. of hybrids (−/−) | Discordance |
|------------|--------------------------------|---------------------------------|-------------|
| 1          | 7                               | 15                              | 4           |
| 2          | 6                               | 10                              | 7           |
| 3          | 9                               | 9                               | 4           |
| 4          | 9                               | 15                              | 3           |
| 5          | 9                               | 10                              | 4           |
| 6          | 8                               | 9                               | 5           |
| 7          | 10                              | 7                               | 2           |
| 8          | 9                               | 9                               | 4           |
| 9          | 10                              | 3                               | 2           |
| 10         | 10                              | 3                               | 2           |
| 11         | 8                               | 7                               | 2           |
| 12         | 11                              | 11                              | 2           |
| 13         | 8                               | 13                              | 5           |
| 14         | 9                               | 7                               | 4           |
| 15         | 8                               | 11                              | 4           |
| 16         | 13                              | 21                              | 0           |
| 17         | 9                               | 5                               | 2           |
| 18         | 9                               | 9                               | 4           |
| 19         | 5                               | 16                              | 8           |
| 20         | 10                              | 7                               | 3           |
| 21         | 12                              | 6                               | 1           |
| 22         | 9                               | 13                              | 4           |
| X          | 7                               | 9                               | 4           |

|              |                                |                                | %         |
|--------------|--------------------------------|--------------------------------|-----------|
|              | Concordance                      | Discordance                     |           |
| 1            | 7                               | 15                              | 4         |
| 2            | 6                               | 10                              | 7         |
| 3            | 9                               | 9                               | 4         |
| 4            | 9                               | 15                              | 3         |
| 5            | 9                               | 10                              | 4         |
| 6            | 8                               | 9                               | 5         |
| 7            | 10                              | 7                               | 2         |
| 8            | 9                               | 9                               | 4         |
| 9            | 10                              | 3                               | 2         |
| 10           | 10                              | 3                               | 2         |
| 11           | 8                               | 7                               | 2         |
| 12           | 11                              | 11                              | 2         |
| 13           | 8                               | 13                              | 5         |
| 14           | 9                               | 7                               | 4         |
| 15           | 8                               | 11                              | 4         |
| 16           | 13                              | 21                              | 0         |
| 17           | 9                               | 5                               | 2         |
| 18           | 9                               | 9                               | 4         |
| 19           | 5                               | 16                              | 8         |
| 20           | 10                              | 7                               | 3         |
| 21           | 12                              | 6                               | 1         |
| 22           | 9                               | 13                              | 4         |
| X            | 7                               | 9                               | 4         |

A. Williams and T. Collins, unpublished observations.

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TABLE I Segregation of the ZNF174 gene with human chromosome 16 on BamHI-digested human-mouse cell hybrid DNA

Data are compiled from Southern blots probed with ZNF174 cDNA. DNA obtained from 34 human-mouse somatic hybrid cell lines derived from 19 unrelated human cell lines and 4 mouse cell lines (Shows et al., 1982) digested with BamHI. The hybrids were characterized by karyotypic analysis and by mapped enzyme markers (Shows, 1983). A line in the table indicates a translocated chromosome with no intact chromosome present and is not tabulated for percent discordance. Scoring was determined by the presence (+) or absence (−) of human bands in the hybrids on the blots and was compared to the presence or absence of human chromosomes in each hybrid. A 0% discordance indicates a matched segregation of the DNA probe with a chromosome. The gene maps to human chromosome 16.

To map the ZNF174 gene more precisely, we performed fluorescent in situ hybridization to metaphase chromosomes. An idiogram indicates the distribution of fluorescent signal at 16p13.3 on both chromatids of a single chromosome 16 in 21 out of the 41 cells (51%) (Fig. 4A). Double signals also appeared once at 7q22 and twice at 16q12.13; however, the frequency of the appearance of these signals suggests false hybridization sites.

The ZNF174 gene was further localized to the polycystic kidney disease (PKD1) region of chromosome 16 by analysis of selected somatic cell hybrids. The breakpoints of two der(16) chromosomes isolated in rodent-human somatic cell hybrids (N-OH1, 23HA) approximate the telomeric and centromeric boundaries of the PKD1 region (Fig. 4C) (Germino et al., 1992). Southern blot analysis revealed that the gene for ZNF174 was contained within the breakpoint interval defined by these hybrids (Fig. 4B).

ZNF174 Encodes a Portable Transcriptional Repression Element—To assess whether ZNF174 can affect the transcriptional activity of other genes, the putative regulatory region of ZNF174 was fused to a homologous DNA-binding domain of the yeast transcriptional activator GAL4. The amino-terminal region of ZNF174 was amplified by PCR and cloned into the expression vector pSG-424, which encodes GAL4 amino acids 1–147. The resulting chimeras were tested for their ability to transactivate a reporter construct containing five GAL4 binding sites (Fig. 5, A). However, the chimera containing amino acids 1–320 suppressed transcription about 5-fold in this assay (Fig. 5, B). To determine whether ZNF174 encoded a repressor function, the GAL4-ZNF174 chimera was cotransfected with a GAL4 reporter with high basal expression suitable for examining transcriptional repression (pGAL4_TK-CAT). The GAL4-ZNF174 chimera containing ZNF174 amino acids 3–320 suppressed transcription about 5-fold in this assay (Fig. 5B). However, the chimera containing amino acids 3–128, which is the portion of the gene that corresponds to the SCAN box, was not able to suppress transcription. Repression was dependent on the presence of the GAL4 binding sites (Fig. 5, A and B). These observations demonstrate that ZNF174 contains a transcriptional repression element that is portable in the context of a homologous DNA-binding domain.

Certain zinc-finger transcription factors, such as Egr-1, have the capacity to regulate transcription in a bimodal manner (Gashler et al., 1993). To determine whether ZNF174 also encodes an activator function, the GAL4-ZNF174 chimera was
cotransfected with a reporter containing five GAL4 binding sites in front of the E1b minimal promoter (pGAL4-E1bCAT) (Fig. 5A). This construct, however, failed to stimulate the expression of the reporter gene (Fig. 5B). In contrast, a similarly constructed Egr-1 (amino acids 3–281) chimera (Gashler et al., 1993) stimulated transcription by approximately 100-fold. Thus, ZNF174 (amino acids 3–320) does not confer positive transcriptional activity on a heterologous DNA-binding domain.

Full-length ZNF174 Inhibits Reporter Gene Expression—To determine whether ZNF174 could repress the expression of authentic promoter-reporter gene constructs, full-length ZNF174 cDNA was inserted into an expression vector and introduced with a series of chimeric genes in a cotransfection protocol. The reporter genes included a series of growth factor promoters directed by the PDGF-B chain promoter in a specific manner and suppressed the activity of B chain reporter constructs.

ZNF174 Interacts with the PDGF-B Promoter and Represses Expression Driven by the PDGF-B Promoter—The preceding data indicate that ZNF174 has the capacity to modulate the expression of pathophysiologically relevant genes in the context of reporter constructs in transfected cells. Bovine aortic endothelial cells (BAEC) were used in cotransfection studies to determine whether overexpression of ZNF174 can repress PDGF-B promoter-reporter gene expression in cells in which the PDGF-B gene is expressed constitutively at high levels (Khachigian et al., 1994). To determine whether ZNF174 could affect expression driven by the PDGF-B promoter, a reporter construct bearing 1.3 kb of promoter sequence (6a-CAT) was used in a transient cotransfection setting with the ZNF174 expression construct. ZNF174 inhibited expression of 6a-CAT in a dose-dependent manner (Fig. 7). In contrast, the pcDNA backbone alone had no effect (Fig. 7). Additionally, a construct directing expression of a region of ZNF174 without the zinc-finger domain fused to an irrelevant DNA binding element (pGAL4 ZNF174 aa3–320) had no effect on expression of the PDGF-B chain promoter. This result indicates that the DNA-binding domain of ZNF174 is required for repression. Deletion derivatives of the B chain promoter (Khachigian et al., 1994) were used to map the ZNF174 binding site. Expression driven by the PDGF-B promoter deletion construct (d77-CAT), extending 82 bp upstream of the TATA box and defined previously as the minimal region required for basal expression in BAEC (Khachigian et al., 1994), was inhibited by ZNF174 in a concentration-dependent manner (Fig. 7). The ability of ZNF174 to repress expression driven by several reporter constructs including the minimal PDGF-B promoter and its structural similarity with a number of zinc-finger transcription factors suggests that ZNF174 repression of gene expression may be mediated by direct interaction with DNA elements within the core promoter.

To examine whether ZNF174 protein interacts directly with the PDGF-B promoter, DNase I footprint and gel shift assays were performed with a recombinant form of ZNF174. Since ZNF174 could suppress expression of d77-CAT as effectively as 6a-CAT (Fig. 7), a footprint probe encompassing the minimal promoter was used to allow visualization of bases protected from DNase I digestion. Bacterially expressed ZNF174 bound to a defined region in the PDGF-B promoter in a dose-dependent manner (Fig. 8A). To demonstrate the specificity of the interaction, another zinc-finger protein, Egr-2, failed to interact with this site (Fig. 8A).

In support of these observations, a single nucleoprotein complex was observed when a 32P-labeled oligonucleotide (B-prom) spanning the region bound by ZNF174 was used in electrophoretic mobility shift assay with the recombinant protein (Fig. 8B). The shift was abolished by the presence of a 50-fold molar excess of the unlabeled cognate (Fig. 8B), whereas 100-fold excess of an unrelated oligonucleotide, PEA-3, failed to compete (Fig. 8B). The region bound by ZNF174 spans the major transcriptional start site in the human PDGF-B gene (Rao et al., 1986). These findings suggest that ZNF174 may serve as a competition-type transcriptional repressor (reviewed by Levine and Manley (1989)). Thus, ZNF174 can interact with the PDGF-B chain promoter in a specific manner and suppress the activity of B chain reporter constructs.

DISCUSSION

This paper describes the isolation and characterization of a novel zinc-finger protein, ZNF174, located on human chromosome 16p13.3 with the apparent ability to repress expression driven by the promoters of a number of pathophysiologically relevant genes. ZNF174 was also found to have a number of interesting structural features. The primary sequence of the putative ZNF174 polypeptide revealed a potential single phos-
phorylation site and nuclear translocation sequence. The SPLK sequence (Fig. 1A) fits the consensus phosphorylation sequence recognized by Cdc2 kinase (Ser*/Thr*/Pro-X-Lys/Arg). Cdc2 is a highly conserved cell cycle-regulatory protein serine kinase that has been reported to phosphorylate nuclear transcription factors (reviewed by Hunter and Karin (1992)). Located near

**Fig. 5. Repression by GAL4-ZNF174 chimeras.** A, schematic representation of various plasmid constructs used to assess the function of upstream domains of ZNF174. B, CAT activities from cotransfections with GAL4-ZNF174 fusion constructs and promoter-CAT reporter plasmids. The left portion of the figure shows transfections with the GAL4x5 E1B CAT reporter, which contains five copies of the GAL4 binding site placed in front of the minimally active E1B promoter and CAT. The plasmid pE1B CAT does not contain GAL4 binding sites and is used as a control. As a positive control, a cotransfection was done with a known activator GAL4-Egr-1 (amino acids 3–281) (Gashler et al., 1993). The right portion of the figure depicts transfections with the reporter GAL4x5 TK-CAT, which is a pBL-CAT2-based plasmid with 5 copies of the GAL4 binding site placed in front of the basally active TK promoter. The histograms represent the mean of four replicative assays. The error bars indicate standard deviation from the mean. Where the error bars are not visible, this indicates that the standard deviation was smaller than the bar data point.
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FIG. 6. Effect of ZNF174 on the expression of various promoter-CAT constructs in COS cells. Fifteen μg of the various promoter-CAT constructs were transiently cotransfected into COS cells with 2 μg of ZNF174 expression construct (pcZNF174) or 2 μg of pcDNA using calcium phosphate technique. CAT activity was assessed as described under “Experimental Procedures.” Transcriptional repression is expressed as a percentage of the reporter activity obtained using the empty expression vector (pcDNA) alone. The values (% repression) represent the mean of between two and five separate experiments.

The ZNF174 gene contains a novel finger-associated element located upstream of the zinc-finger domain. This domain, or SCAN box, is also found in a number of other zinc-finger proteins containing similar domains. CTfin-51 is a mouse protein also containing seven tandemly repeated carboxyl-terminal zinc fingers, which has been found in both male and female gametogenesis (Noce et al., 1992) and has also been shown to be a strong transcriptional activator (Chowdhury et al., 1992). SRE-ZBP is a human transcription factor that binds to the c-fos serum response element. This protein also contains seven tandemly repeated carboxyl-terminal zinc fingers and is thought to function as a repressor of c-fos transcription (Attar and Gilman, 1992). ZNF165 is a zinc-finger gene located on chromosome 6p21, which is expressed specifically in the testis. The element was also identified in two novel partial cDNAs termed #18 cDNA (Pengue et al., 1993) and 3c3 (Calabro et al., 1995), both of which correspond to transcribed sequences from chromosome 3p21. The 3p region is of particular interest because of its frequent involvement in rearrangements and deletions associated with various human tumors, including lung and renal carcinomas (Naylor et al., 1987; Bergenhein et al., 1989). The conserved SCAN box does not correspond with previously reported modules linked to zinc-finger domains such as the 75-amino acid Kruppel-associated box (KRAB), which occurs in one-third of the Kruppel-type finger genes (Bellefroid et al., 1991; Rosati et al., 1991) and mediates transcriptional repression (Witzgall et al., 1994; Margolin et al., 1994), or the finger-associated box (FAX), which has been found in Xenopus finger proteins (Knochel et al., 1989). Although the function of the SCAN box has not yet been elucidated, the conservation of this module and its α-helical structure suggests that it may serve ZNF174 as a dimerization domain or a site that interacts with components of the transcriptional machinery resulting in the repression of gene expression.

The ability of ZNF174 to function as a transcriptional repressor is similar to the activities of other zinc-finger transcription factors such as Egr-1 and WT-1 (Madden et al., 1991; Gashler et al., 1993). Repression domains, like activation domains, have been demonstrated to function as independent modular elements in transcription factors (reviewed by Levine and Manley (1989) and Johnson (1995)). Only a small number of repression domains have been well characterized. These include the Drosophila proteins Kruppel (Licht et al., 1990; Zuo et al., 1991), Engrailed (Han et al., 1989; Jaynes and O'Farrell, 1991), and Even-Skipped (Han and Manley, 1993) and the mammalian...
DNA-binding proteins WT-1 (Madden et al., 1991), YY1/NF-E1 (Shi et al., 1991), and Kid-1 (Witzgall et al., 1994). Like ZNF174, these proteins can confer their repression function onto heterologous DNA-binding domains. The specific region(s) within ZNF174 that repress transcription of other genes is not yet clear, although the GAL4 chimera studies were able to show that the SCAN box alone can not confer repression. This implies that either the repression domain of ZNF174 is contained within another portion of the gene, or that for conformational reasons a more extensive region of the gene is required in order to confer repression. The suppression domain in Kruppel was mapped to an alanine-rich domain (Zuo et al., 1991), and that of Egr-1 was mapped to a 34-amino acid element (Gashler et al., 1993). ZNF174, however, does not contain an alanine-enriched element or a region that resembles the repression element of Egr-1 (Fig. IA). A small number of factors contain modular domains capable of regulating transcription both positively and negatively. These include Egr-1 (Gashler et al., 1993), WT-1 (Wang et al., 1993), Kruppel (Zuo et al., 1991), YY1/NF-E1 (Park et al., 1991), and the immediate-early proteins c-Fos and c-Jun (Abate et al., 1991). The inability of ZNF174 to activate transcription in the context of promoter-reporter constructs examined (Figs. 5B, 6, and 7) does not exclude the possibility that the zinc-finger protein has a positive regulatory function with other genes.

Gel retardation and DNase I footprint studies established the ability of the purified zinc-finger domain of ZNF174 to interact with promoter elements that appear in certain reporter constructs it could repress. These assays shed light on a possible mechanism with which ZNF174 suppressed expression driven by the PDGF-B promoter. That ZNF174 bound at the transcriptional start site of the PDGF-B gene (Rao et al., 1986) suggests that it may impair the assembly of various components of the general transcriptional machinery into a functional complex at the initiation site. Thus, ZNF174 could function as a competition-type transcriptional repressor (Levine and Manley, 1989) as recently observed with another zinc-finger protein (Werner et al., 1994). Transcriptional repression of the IGf-I receptor gene by WT-1 is thought to be mediated, at least in part, by direct interaction with the initiator site (Werner et al., 1994).

The gene encoding ZNF174 was mapped to human chromosome 16p13.3. Because two disease loci have been identified in this region near the distal end of the short arm of chromosome 16, tuberous sclerosis and polycystic kidney disease, it has been intensely studied and is known to be rich in transcribed sequences. Autosomal dominant polycystic kidney disease is a common genetic defect in humans that frequently results in renal failure (Gabow, 1993). The candidate gene for this disease occurs within a duplicated region on the chromosome and it encodes a large novel protein of unknown function (European Polycystic Kidney Disease Consortium, 1994). Tuberous sclerosis complex (TSC) is a dominantly inherited disorder that causes mental retardation, seizures and tumors in multiple organs (Gomez, 1988). Linkage studies have demonstrated that TSC has at least two possible genetic loci within the human genome with disease loci on chromosomes 9 (Fryer et al., 1987) and 16 (Kandt et al., 1992). A recently described locus, designated TSC-2, generates a transcript that is widely expressed, and its protein product, tuberin, has a region of homology to the GTPase-activating protein GAP3. Loss of heterozygosity for alleles at 16p has been observed in hamartomatous lesions seen in TSC patients (Green et al., 1994). Often, an alteration in one allele, such as a point mutation or small deletion, is followed by a more extensive loss of sequence from the homologous chromosome. If ZNF174 lies in close proximity to the TSC-2 gene, it may be lost in the second mutational event. While not the causative gene for TSC, the inclusion or exclusion of the ZNF174 gene in subsequent chromosomal deletions may account for some of the variation of symptoms and complications seen in the disorder.

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