

Function of AP-1 in Transcription of the Telomerase Reverse Transcriptase Gene (*TERT*) in Human and Mouse Cells

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The transcriptional regulation of the human telomerase catalytic subunit (*hTERT*) plays a critical role in telomerase activity. Approximately 200 bp of the proximal core promoter is responsible for basic *hTERT* expression; however, the function of the distal regulatory elements remains unclear. The transcription factor activator protein 1 (AP-1) is involved in cellular proliferation, differentiation, carcinogenesis, and apoptosis and is expressed broadly in both cancer and normal cells. There are several putative AP-1 sites in the *hTERT* promoter, but their functions are unknown. The present study examined the regulatory role of AP-1 in *hTERT* gene transcription. Overexpression of AP-1 leads to transcriptional suppression of *hTERT* in cancer cells. The combination of c-Fos and c-Jun or c-Fos and JunD strongly suppresses *hTERT* promoter activity in transient-expression analyses. The *hTERT* promoter region between –2000 and –378 is responsible for this function. Gel shift and supershift analyses, as well as ChIP, show binding of JunD and c-Jun on two putative AP-1 sites within this region. Mutations in the AP-1 binding sites rescued suppressions caused by AP-1, suggesting this is a direct regulation of the *hTERT* promoter. In contrast, there was no effect on *mTERT* expression or *mTERT* promoter activity by AP-1 overexpression in mouse fibroblasts. The species-specific function of AP-1 in *TERT* expression may in part help explain the difference in telomerase activity between normal human and mouse cells.

Telomeres are essential for protecting chromosomal ends against end-end fusions or degradation (14). The progressive shortening of telomeres with each cell division leads to cellular replicative senescence; thus, telomeres are thought to be a molecular timing mechanism (42). Telomerase, a ribonucleoprotein complex that extends telomeres, resets this molecular clock, and leads to cellular immortality (5, 6, 30). Therefore, the expression of telomerase is tightly controlled in normal human cells. Detectable telomerase activity is observed only in some highly regenerative tissues such as hematopoietic progenitor, intestinal crypt, skin basal layer, cervical keratinocyte, endometrium, and germ line cells; however, 75 to 85% of cancer cells show telomerase activity (9, 13, 16, 21, 23, 25, 37, 43). The structural RNA component of human telomerase (*hTR* or *hTERC*) contains an 11-bp sequence complementary to the telomeric single-stranded overhang that functions as a template for the synthesis of telomeric DNA (TTAGGG)_n directly onto the ends of chromosomes. The other core component is the enzymatic reverse transcriptase catalytic protein subunit (*hTERT*) (11, 28, 29). Although *hTR* is expressed ubiquitously, many studies have found that *hTERT* is expressed only in telomerase-positive cells and tissues (19, 20, 24, 39). Expression of *hTERT* is regulated mainly at the transcriptional level, and it is believed that the proximal 180 bp of the *hTERT* promoter is important for maintaining basal transcriptional

activity and that c-Myc/Max and Sp-1 are the main activators of the *hTERT* core promoter (8, 17, 26, 38).

In contrast to *hTERT*, mouse telomerase (*mTERT*) is activated in many normal tissues, including lung, intestine, liver, and muscle tissues, and cultured mouse cells spontaneously re-express telomerase with a high frequency; however, human cells rarely re-express telomerase spontaneously (12, 28). The causes of this difference in telomerase suppression between normal human and mouse cells are unknown. The proximal 225 bp of the *mTERT* promoter is the core promoter that maintains basal transcriptional activity (32). It contains two E boxes and three GC boxes, similar to the *hTERT* core promoter (Fig. 1A). In contrast, the 5' flanking sequence of the core promoter is quite different in humans and mice (Fig. 1B). It is likely that some transcriptional repressors work specifically on the *hTERT* promoter to achieve more strict suppression in human cells.

The transcription factor activator protein 1 (AP-1) is mainly a heterodimeric complex of the Jun (c-Jun, JunB, or JunD) and Fos (c-Fos, FosB, Fra-1, or Fra-2) family proteins. The AP-1 complex binds to the palindromic DNA sequence 5'-TGAC/GTCA-3'. The activation of AP-1 is involved in cellular proliferation, apoptosis, differentiation, and carcinogenesis (36). In the present study, we examined the effect of AP-1 on human and mouse *TERT* expression. We found that AP-1 binds to the *hTERT* promoter and acts as potent suppressor; however, *mTERT* expression is not affected by AP-1.

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MATERIALS AND METHODS

Cell culture. HeLa cells and NIH 3T3 cells were cultured at 37°C under 5% CO₂ in a 4:1 mixture of Dulbecco's modified Eagle's medium and medium 199

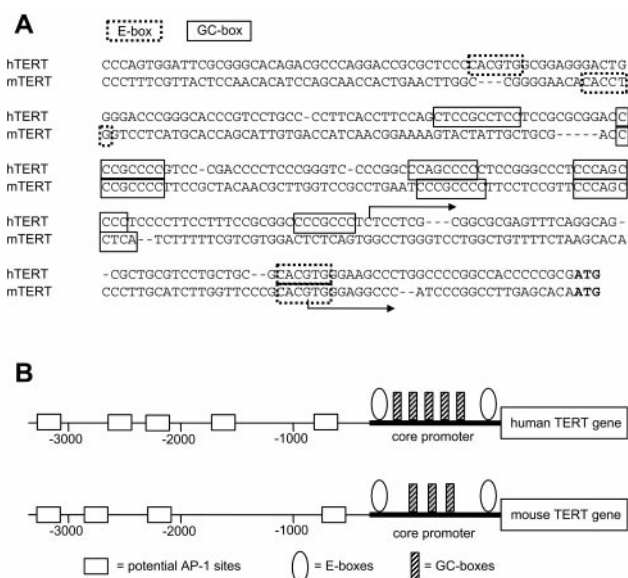


FIG. 1. Comparison of the *hTERT* and *mTERT* promoters. (A) Core promoter sequences of *hTERT* (GenBank accession number AB016767) and *mTERT* (GenBank accession number AF121949). Approximately 250 bp of the 5' flanking sequence of each gene is shown. The transcription start sites are shown by arrows. The translation-initiating ATG codons are in bold. E boxes are boxed by dashed lines. GC boxes are boxed by solid lines. (B) Schematic diagram of the *hTERT* and *mTERT* promoters. The core promoters of *hTERT* and *mTERT* are similar, but the distal promoter sequences are not. The distributions of potential AP-1 element are also different.

supplemented with 10% cosmic calf serum (HyClone) and 50 μ g/ml of gentamicin (Sigma).

Telomeric repeat amplification protocol (TRAP) assay. Telomerase activity was measured by the TRAP assay using the TRAP-eze Telomerase Detection system (Intergen) according to the manufacturer's protocol.

RNA PCR analysis. Expression of *hTERT* and *mTERT* mRNAs was analyzed by reverse transcription (RT)-PCR and real-time RT-PCR as described previously (33, 39). Briefly, total RNA was isolated from the cells using an RNeasy Mini Kit (QIAGEN) according to the manufacturer's protocol. cDNA was synthesized from 2 μ g of RNA using the Superscript III First-Strand Synthesis System (Invitrogen) with random primers. Two-microliter aliquots of cDNA were used for PCR amplification carried out using *Taq* DNA polymerase (Invitrogen) or a LightCycler DNA Master SYBR Green I Kit (Roche). The primer pairs 5'-CGGAAGAGTGTCTGGAGCAA-3' (LT5) and 5'-GGATGAACCGGAGTCTGGA-3' (LT6) for *hTERT* and 5'-ATGGCGTTCTGATGATG-3' (2674S) and 5'-TTCAACCGCAAGACCGACAG-3' (2931AS) for *mTERT* were used for amplification. PCR used 30 s at 94°C, 30 s at 60°C, and 30 s 72°C for *hTERT* and 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C for *mTERT*, with 28 cycles for conventional PCR and 40 cycles for real-time PCR. The efficiency of cDNA synthesis from each sample was estimated by PCR for human glyceraldehyde-3-phosphate dehydrogenase or mouse β -actin as described previously (33, 39).

Western blot analysis. Nuclear extracts were prepared from HeLa and NIH 3T3 cells as previously described (34). First, 20 μ g of protein extract was electrophoresed on a 20% sodium dodecyl sulfate (SDS)-polyacrylamide gel and then transferred to polyvinylidene difluoride membranes. Filters were incubated with specific antibodies against c-Jun (sc-45 or sc-45-G; Santa Cruz Biotechnology), JunB (sc-73 or sc-73-G; Santa Cruz Biotechnology), JunD (sc-74 or sc-74-G; Santa Cruz Biotechnology), c-Fos (k-25, Santa Cruz Biotechnology), or Sp-1 (sc-59-G; Santa Cruz Biotechnology), followed by reaction with horseradish peroxidase-linked anti-immunoglobulin G. Immunoreactive bands were visualized using the ECL detection system (Amersham Bioscience, Piscataway, NJ) in accordance with the manufacturer's suggestions.

Plasmid construction. The structures of the *hTERT* and *mTERT* promoter-luciferase constructs are shown in Fig. 1B. Various lengths of DNA upstream of

the initiating ATG codon of each gene were PCR amplified and inserted into the promoterless and enhancerless luciferase reporter pGL3-basic (Promega) as described before (38). The *mTERT* promoter vector used as a PCR template was a kind gift from Fuyuki Ishikawa (Kyoto University, Kyoto, Japan). In these reporter constructs, the transcriptional start site was designated nucleotide +1. The pGL3-2000AP-1MT reporters have mutations in the putative AP-1 site(s) at nucleotide -1655 and/or -718 introduced by PCR-based site-specific mutagenesis. c-Jun, JunB, JunD, and c-Fos expression vectors were a kind gift from M. Yaniv (Institut Pasteur, Paris, France) (15).

Transient-expression assay. HeLa cells incubated in 24-well dishes for 24 h were transfected with 0.1 μ g of reporter plasmid and 0.2 μ g of effector plasmids using PolyFect (QIAGEN) according to the manufacturer's protocol. Cells were harvested 36 h after transfection, and cell extracts were subjected to a luciferase assay using the Dual Luciferase Reporter Assay System (Promega), in which *Renilla* luciferase plasmids were cotransfected as controls to standardize the transcription efficiency. All experiments were performed at least three times for each plasmid, and the relative luciferase activity reported here is the average of the three resultant values.

Gel shift assay. Nuclear extracts were prepared from HeLa cells as previously described (34). Five micrograms of protein was incubated with 1 μ g of poly(dI-dC) in the presence or absence of a 100-fold molar excess of unlabeled competitor DNAs on ice for 20 min in a 20- μ l reaction volume containing 10% glycerol, 25 mM HEPES (pH 7.9), 50 mM KCl, 0.5 mM phenylmethylsulfonyl fluoride, and 1 mM dithiothreitol. For supershift assay, specific antibodies against transcription factors were preincubated with nuclear extracts at 4°C for 60 min. After incubation, 20,000 cpm of ³²P-end-labeled oligonucleotide probes was added and the reaction mixture was incubated at room temperature for an additional 30 min. The DNA-protein complexes were then separated from free probes by electrophoresis on a 5% polyacrylamide gel. The gel was dried and subjected to autoradiography. For competition assays, consensus oligonucleotides for AP-1 (5'-CGCTTGATGAGTCAGCCGGAA-3'), Oct1 (5'-GATCGAAGTACCGCCCGCGGC-3'), and Sp-1 (5'-ATTCGATCGGGGCGGGGCGAGC-3') and a mutant oligonucleotide for AP-1 (5'-CGCTTGACCAGTTTGGCCGAA-3') were used as competitors. The antibodies against c-Jun (sc-45), JunB (sc-73), and JunD (sc-74) were purchased from Santa Cruz Biotechnology.

Chromatin immunoprecipitation (ChIP) assays. ChIP assays were performed using the ChIP Assay Kit (Upstate, Lake Placid, NY) according to the manufacturer's protocol. Briefly, HeLa cells incubated in 10-cm-diameter dishes for 24 h were cross-linked by treatment with formaldehyde (final concentration, 1%) for 10 min at room temperature. After washing with phosphate-buffered saline, cells were pelleted and resuspended in SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl [pH 8.1], 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride). The lysates were then subjected to sonication to reduce the DNA length to between 500 and 1,000 bp, diluted with dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl [pH 8.1], 167 mM NaCl), and precleared by incubation with a salmon sperm DNA-protein A agarose 50% slurry for 60 min at 4°C. The supernatant was incubated with anti-c-Jun, anti-c-Fos, anti-JunD, or anti-SP-1 antibodies at 4°C overnight. Immunocomplexes were collected with a salmon sperm DNA-protein A agarose 50% slurry and eluted after extensive washings, and cross-linkage was reversed by heating at 65°C, followed by treatment with 40- μ g/ml proteinase K at 45°C for 60 min. DNA was recovered by phenol-chloroform-ethanol precipitation and used as a template for PCR to amplify the region including the AP-1 site at -1655 in the *hTERT* promoter. The primer pairs 5'-TCAGATGATCCACCTGCCTCTG C-3' (-1855) and 5'-TGCAGAGAAGCCAACATAGAAAACACA-3' (-1451) for the first PCR and 5'-GAGGTAGGAAGCTCACCCCACTCA-3' (-1754) and 5'-CCGGCATTCAATGAAGATTGCTG-3' (-1562) for the second PCR were used for amplification. The position of each primer is shown as sequence numbers corresponding to the 5' nucleotides. Each PCR used 30 s at 94°C, 30 s at 58°C, and 30 s 72°C for 20 cycles. The PCR products were electrophoresed on a 7% polyacrylamide gel and stained with SYBR Gold (Molecular Probes, Eugene, OR).

RESULTS

Effect of AP-1 on *TERT* transcription and telomerase activity in HeLa cells and NIH 3T3 cells. To examine the effect of AP-1 on *TERT* expression in human and mouse cells, HeLa cells (human cervical cancer cell line) and NIH 3T3 cells (immortalized mouse fibroblasts) were transfected with a c-Jun or c-Fos expression vector or a blank vector, and RT-PCR for

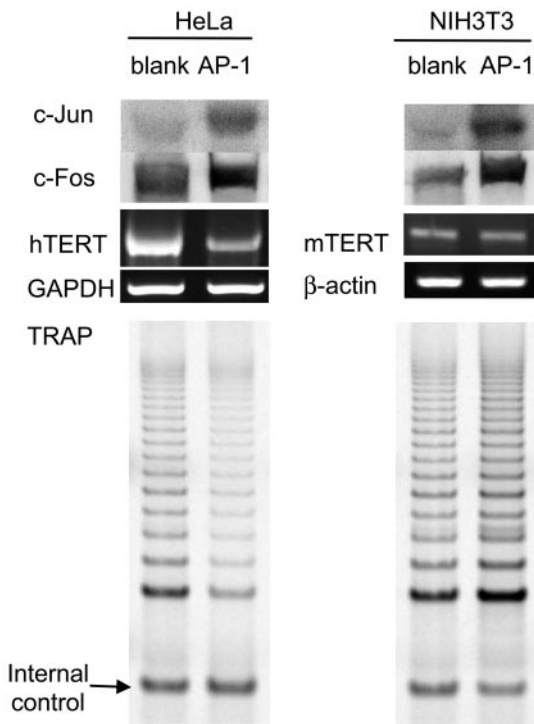


FIG. 2. Effect of AP-1 on *TERT* transcription and telomerase activity in HeLa and NIH 3T3 cells. c-Jun, c-Fos, or blank expression vectors were transfected into HeLa and NIH 3T3 cells. Western blotting analyses for the c-Jun and c-Fos proteins, RT-PCRs for *TERT* and *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) or β -actin, and TRAP assays were performed 48 h after transfection.

TERT and a TRAP assay were performed 48 h after transfection. The expression levels of the c-Jun and c-Fos proteins were confirmed by Western blotting analysis (Fig. 2). In HeLa cells, the expression of *hTERT* was significantly suppressed when c-Jun or c-Fos was overexpressed. In addition to suppression of *hTERT* mRNA, telomerase activity was also suppressed. We next performed quantitative analysis of the expression of *hTERT* mRNA using real-time PCR using the $2^{-\Delta\Delta CT}$ method (27). The expression of *hTERT* mRNA calibrated by expression of *GAPDH* when AP-1 is overexpressed was 1/50 of the control cells (data not shown). In contrast, expression of *mTERT* in NIH 3T3 cells was not affected by overexpression of AP-1. Expression of *mTERT* mRNA calibrated by expression of mouse β -actin showed 1.1-fold activation compared with the control using quantitative real-time PCR (data not shown). In addition, telomerase activity was not affected by overexpression of AP-1 in NIH 3T3 cells. These findings suggest that *TERT* expression and telomerase activity are suppressed by AP-1 in human cells but not in mouse cells.

Effect of AP-1 on the *TERT* promoter in HeLa cells and NIH 3T3 cells. In order to determine the AP-1 targets in *hTERT* and the *hTERT* promoter, luciferase assays were performed with reporter plasmids of various lengths (Fig. 3A). The longest reporter, pGL3-3300, contains 3.3 kb of the 5' flanking sequence of the *hTERT* gene. A computer-assisted homology search revealed five degenerate AP-1 binding sequences, at -3232, -2501, -2273, -1655, and -718 (the transcription

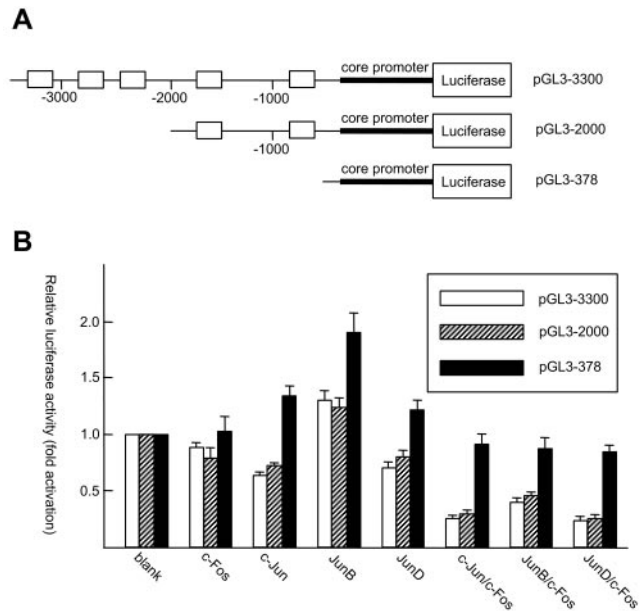


FIG. 3. Transient-expression assays to examine the role of AP-1 in the *hTERT* promoter. (A) Schematic diagrams of reporter plasmids. The 3.3-kb and 5' truncated fragments of the *hTERT* promoter, upstream of the initiating ATG, were inserted into the luciferase reporter vector in the sense orientation. The name of each reporter construct was assigned according to the nucleotide at the 5' end of the inserted promoter sequence. The open squares indicate potential AP-1-responsive sites on the *hTERT* promoter. (B) AP-1 induction assays. Jun family and/or c-Fos expression vectors were cotransfected with various lengths of the *hTERT* promoter plasmids into HeLa cells. Blank vectors in which cDNA sequences were deleted were used as a control. The luciferase activity in control samples was normalized to 1.0. Error bars indicate standard deviations.

start site was designated +1); pGL3-2000 includes two of these sites, and pGL3-378 did not contain any putative AP-1 binding sites. These reporters were cotransfected with an expression vector(s) of AP-1 components (Fig. 3B). Overexpression of c-Fos/c-Jun and c-Fos/JunD results in an 80% reduction of the *hTERT* promoter activity in both pGL3-3300 and pGL3-2000. In spite of pGL3-2000 lacking three of the five putative AP-1 binding sites compared with pGL3-3300, the degree of suppression was not significantly different in these two reporters. In contrast, the promoter activity was not significantly affected in pGL3-378, suggesting that the AP-1-responsive regions exist in the -2000 to -378 region of the *hTERT* promoter. Furthermore, the effects of overexpression of the single factors c-Fos, c-Jun, and JunD were not as strong (30 to ~50% reduction), suggesting both Jun and Fos family proteins are needed for repression of the *hTERT* promoter. We also analyzed the effect of AP-1 overexpression on the *mTERT* promoter using reporters with 3.3-kb (pGL3-m3300) and 1-kb (pGL3-m1500) 5' flanking sequences of the *mTERT* gene (Fig. 4A). There are putative AP-1 binding sequences at -3247, -2833, -2197, and -457 of the *mTERT* promoter. Overexpression of an AP-1 component(s) did not affect the promoter activity of the *mTERT* promoter (Fig. 4B).

AP-1 directly binds to the *hTERT* promoter. Two putative AP-1 binding sites reside at -1655 and -718 of the *hTERT* promoter (Fig. 5A). We numbered them 1 and 2, respectively,

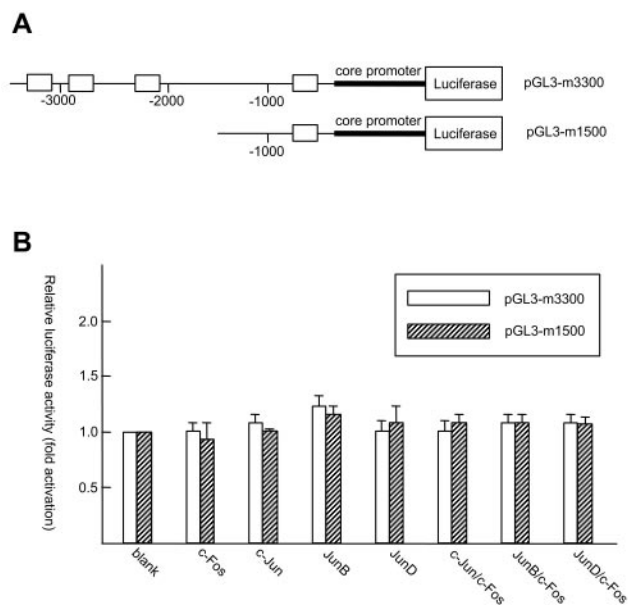


FIG. 4. Transient-expression assays to examine the role of AP-1 in the *mTERT* promoter. (A) *mTERT* promoter fragments of 3.3 and 1.5 kb were inserted into the same luciferase reporter vectors as the *hTERT* promoter reporters. The open squares indicate potential AP-1-responsive sites in the *mTERT* promoter. (B) AP-1 induction assays. Jun family and/or c-Fos expression vectors were cotransfected with *mTERT* promoter reporters into NIH 3T3 cells. Blank vectors in which cDNA sequences were deleted were used as a control. The luciferase activity in control samples was normalized to 1.0. Error bars indicate standard deviations.

and performed gel shift analysis to examine the actual binding of AP-1 to the *hTERT* promoter. A significantly retarded band was observed with both probes 1 and 2. This band was completely competed by a homologous competitor, as well as by AP-1 consensus oligonucleotides, but not by an AP-1 site mutated oligonucleotide or unrelated oligonucleotides such as an Sp-1 consensus motif (Fig. 5B). The intensity of the retarded band was much stronger with probe 1, suggesting stronger binding of AP-1 at this site. The band was supershifted by the addition of c-Jun and JunD antibodies, while a JunB antibody failed to supershift the band. The supershifted band was much stronger in the JunD antibody lane versus in the c-Jun lane. We next performed the same analysis using the three putative AP-1 binding sites at -3232 , -2501 , and -2273 as probes, and no retardation was observed (data not shown). Taken together, these findings suggest that AP-1 can directly bind to the *hTERT* promoter through AP-1 binding sites at positions -1655 and -718 , with position -1655 being stronger and associated with JunD. Next, we performed a ChIP analysis to investigate AP-1 binding to the *hTERT* promoter in HeLa cells. DNA was extracted after cross-linking treatment and incubated with c-Jun, JunB, and JunD antibodies and an unrelated control antibody (anti-SP-1 antibody). The protein-DNA complexes were then immunoprecipitated, and cross-linking was reversed, followed by PCR amplification targeting the AP-1 binding site at -1655 of the *hTERT* promoter. The efficiency of immunoprecipitation was confirmed by Western blotting analysis. The immunoprecipitant obtained with the

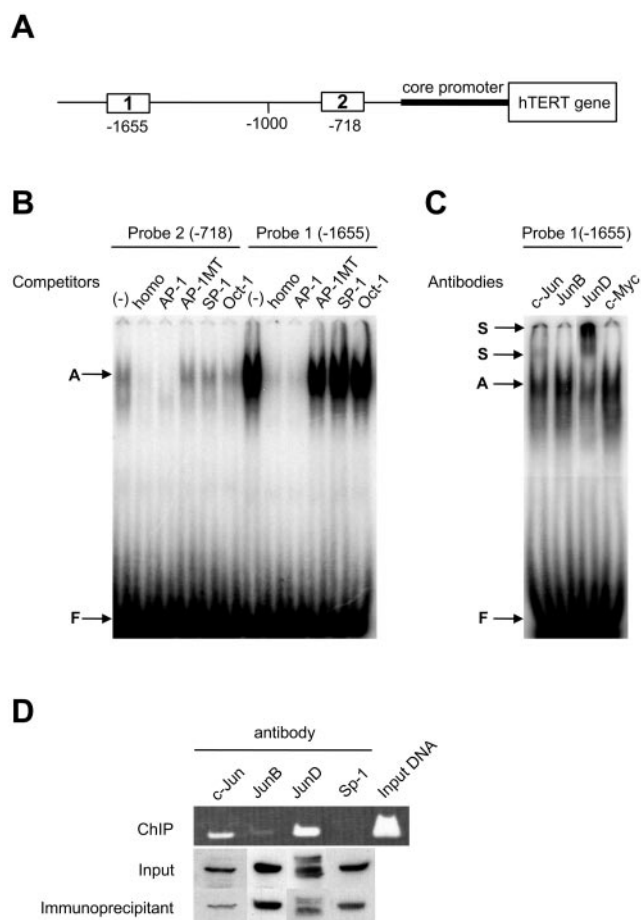


FIG. 5. Direct bindings of AP-1 proteins to the *hTERT* promoter. (A) Putative AP-1 binding sites on the 2-kb *hTERT* promoter. The distal site was numbered 1, and the proximal site was numbered 2. (B) Gel shift assays of putative AP-1 binding sites. Arrow A, AP-1-specific binding complex; arrow F, free probe; homo, homologous competitor; AP-1, AP-1 consensus motif; AP-1MT, mutated AP-1 consensus motif; SP-1, SP-1 consensus motif; Oct-1, Oct-1 consensus motif. (-), no competitor. (C) Supershift assay using specific antibodies against Jun family proteins. Arrow S, supershifted complex. (D) ChIP assay to verify the binding of AP-1 to the *hTERT* promoter in vivo using HeLa cells. Immunoprecipitants obtained with each antibody (rabbit immunoglobulin G against c-Jun, JunB, JunD, and SP-1) were amplified by nested PCR surrounding the AP-1 binding site at -1655 of *hTERT*. The immunoprecipitant and the same number of cells used in the ChIP assay (input) were also subjected to Western blot analysis using goat antibodies to confirm the efficiency of immunoprecipitation.

JunD antibody generated the strongest PCR band. That obtained with the c-Jun antibody generated a weaker band; however, the JunB band was very faint (Fig. 5D). This result is consistent with the results of the supershift analysis, suggesting that AP-1 directly associates with the *hTERT* promoter in vivo, but that the binding efficiency may be different for each protein.

AP-1 site mutational analysis of the *hTERT* promoter. Finally, to examine the site specificity of *hTERT* transcriptional suppression by AP-1, we introduced site-specific mutations into the AP-1 binding sites of the pGL3-2000 reporter plasmid and performed luciferase assays. The distal AP-1 site was mu-

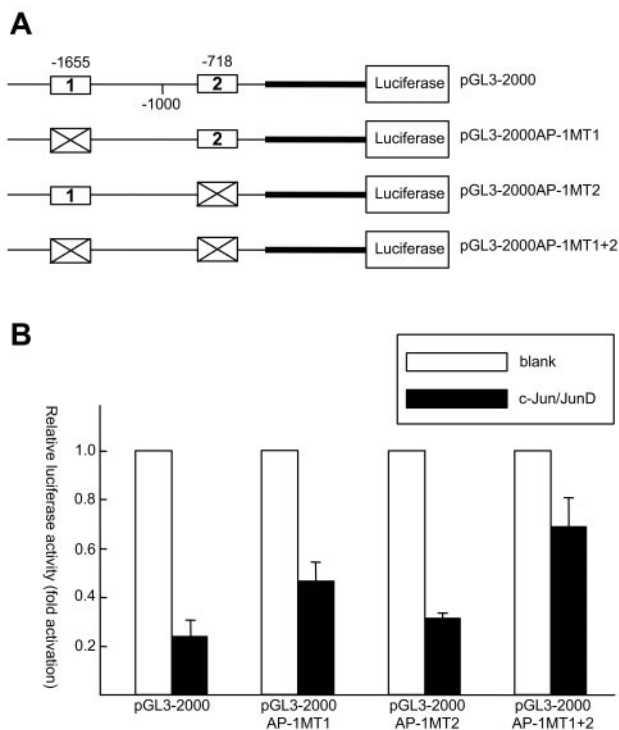


FIG. 6. AP-1 site mutational analysis of the *hTERT* promoter. (A) Schematic diagrams of the luciferase reporter plasmids used in these assays. Mutated AP-1 sites are indicated as crossed boxes. (B) c-Fos and JunD expression vectors were cotransfected with reporter plasmid pGL3-2000 with or without mutations in the AP-1 binding site(s). Blank vectors were used as a control. The luciferase activity in control samples was normalized to 1.0. Error bars indicate standard deviations.

tated in pGL-2000AP-1MT1 (5'-TGACTAA-3' → 5'-AAGG TAA-3') (Fig. 6A). The proximal one was mutated in pGL3-2000AP-1MT2 (5'-TGATTAA-3' → 5'-AAGGTAA-3'). Both sites were mutated in pGL3-2000AP-1MT1 + 2. These reporter plasmids were cotransfected with c-Fos/JunD expression plasmids or blank vectors. The *hTERT* promoter activity was suppressed by 75% in the pGL3-2000 wild-type reporter compared with blank vector-transfected controls (Fig. 6B). The mutation at the -1655 AP-1 site reduced this suppression by about half, while the mutation at -718 had minimal effects. The mutations at both AP-1 sites eliminated a large part of the suppressive effect caused by AP-1 (about 70%), suggesting that suppression of the *hTERT* promoter by AP-1 is primarily but not exclusively dependent on these AP-1 sites.

DISCUSSION

To our knowledge, this is the first attempt to examine the effect of AP-1 on *hTERT* and *mTERT* gene expression. The present results show a suppression of endogenous telomerase activity and *hTERT* expression by AP-1 and direct binding of JunD and c-Jun to the degenerate AP-1 sites at *hTERT* promoter positions -1655 and -718. In contrast, mouse telomerase and *mTERT* expression was not affected by AP-1. The sequences of the distal promoters of *hTERT* and *mTERT* are

quite different, and the -1655 and -718 sites are missing in the *mTERT* promoter.

The transcription factor AP-1 works on a large number of cellular events and consists of a mixture of the dimers of the Jun and Fos family or other proteins. Historically, AP-1 has been characterized as a transcriptional activator, but several reports have recently defined AP-1 also as a transcriptional repressor (7, 35). AP-1 is also known to work in both cellular differentiation and proliferation (3, 36). Most of the positive regulators of the *hTERT* gene, such as c-Myc or Sp-1, are also positive regulators of proliferation and are ubiquitously expressed in both normal and cancer cells (although the expression amount is usually larger in cancer cells than in normal cells) (26). It is difficult to explain the reason why *hTERT* gene expression is strictly suppressed in most normal cells, even though they proliferate rapidly. Some *hTERT* negative regulatory genomic loci have been reported (22, 31, 41), but the specific genes involved have not been defined. Furthermore, there are several reports which indicate that epigenetic regulations such as DNA methylation or histone deacetylation of the *hTERT* promoter may be involved in suppressing telomerase in normal cells, but the mechanism of telomerase suppression is not understood (10, 40). AP-1 may also work as a novel *hTERT* suppressor in cellular differentiation and proliferation. Since AP-1 is expressed in both cancer and normal cells, it may be a constitutive transcriptional repressor of the *hTERT* gene. It is reasonable to propose that some transcriptional repressors may work on distal promoters, because the amount of *hTERT* mRNA is extremely low even in strongly telomerase-positive cells (44). Interestingly, the transcriptional activity of the *hTERT* core promoter is as strong as the cytomegalovirus promoter, so negative regulation of *hTERT* must be important (38).

While it has been reported that transactivation of the c-Jun NH₂-terminal kinase (JNK) activates *hTERT* transcription, the mechanism is not known (2). JNK phosphorylates serines 63 and 73 of c-Jun, resulting in an increase in the ability to bind CBP/P300 and activate transcription (4). Phosphorylation of c-Jun plays a critical role in transcriptional activation by AP-1; however, the relationship between transcriptional suppression by AP-1 and JNK is unknown. A previous report also indicates that a 1,375-bp length of the *hTERT* promoter reporter is activated by a JNK activator, suggesting that the AP-1 binding site at -1655, which has strong AP-1 binding activity, is not essential for the transcriptional activation of *hTERT* by JNK (2). Furthermore, overexpression of JunD, as well as c-Jun, suppressed *hTERT* transcription. Taken together, transcriptional suppression by direct binding of AP-1 and activation by JNK (via an indirect pathway) could occur independently and simultaneously. c-Myc, one of the most important activators of the *hTERT* core promoter, is known to be up-regulated by JNK (1, 18). It is possible that up-regulated c-Myc works on the core promoter of *hTERT* and activates it. The combination of such up-regulation and suppression caused by direct binding of AP-1 to the *hTERT* promoter may decide the transcriptional state of the *hTERT* gene.

In our present studies, mutations in AP-1 binding sites of the *hTERT* promoter rescued about 70% of the transcriptional suppression by AP-1. The effects of mutations in reporter assays were stronger at the distal site (-1655) versus the proxi-

mal site (-718), and the binding ability of AP-1 in gel shift assays was also much greater at the distal site. These data suggest that suppression by AP-1 is site specific to a certain degree. The minor residual AP-1 repression still present when both sites are mutated might be due to some weak AP-1 binding activity in other regions of the *hTERT* promoter or some indirect effects caused by AP-1 overexpression.

While a computer-assisted homology search revealed some putative AP-1 sites on the mouse *TERT* promoter, we observed no significant effect on *mTERT* transcription or promoter activity by AP-1 overexpression. It is known that *mTERT* transcription is suppressed during cellular differentiation similarly to *hTERT*, but the degree of suppression is weaker than that of *hTERT* (12, 32). The core promoters of *hTERT* and *mTERT*, which are similar but not identical to each other, are responsible for the basic transcriptional regulation of each gene. The difference in the regulation of each *TERT* gene may depend on species-specific functions of each distal promoter. AP-1 is only one potential candidate that would explain the difference in the transcriptional suppression of *TERT* between human and mouse cells.

In summary, we demonstrated that AP-1 suppresses telomerase through transcriptional suppression of the *hTERT* promoter in human cells; however, mouse telomerase and *mTERT* are not affected by AP-1. Suppression of *hTERT* is a direct effect of AP-1 mainly associated with c-Jun, JunD, and c-Fos. These findings provide important insights into the molecular mechanisms of telomerase regulation in human and mouse cells.

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