Cloning of a Growth Arrest-Specific and Transforming Growth Factor β -Regulated Gene, TI 1, from an Epithelial Cell Line

BENGT KALLIN,[†] RAINER DE MARTIN, THURE ETZOLD, VINCENZO SORRENTINO, AND LENNART PHILIPSON*

European Molecular Biology Laboratory, Postfach 10.2209, D-6900 Heidelberg, Germany

Received 10 May 1991/Accepted 18 July 1991

By cDNA cloning and differential screening, five genes that are regulated by transforming growth factor β (TGF β) in mink lung epithelial cells were identified. A novel membrane protein gene, TI 1, was identified which was downregulated by TGF β and serum in quiescent cells. In actively growing cells, the TI 1 gene is rapidly and transiently induced by TGF β , and it is overexpressed in the presence of protein synthesis inhibitors. It appears to be related to a family of transmembrane glycoproteins that are expressed on lymphocytes and tumor cells. The four other genes were all induced by TGF β and correspond to the genes of collagen α type I, fibronectin, plasminogen activator inhibitor 1, and the monocyte chemotactic cell-activating factor (JE gene) previously shown to be TGF β regulated.

Cell growth is believed to be controlled by a complex balance of stimulatory and inhibitory factors. Among factors with dual effects, transforming growth factor β (TGF β) constitutes a family of pleiotropic cytokines with physiological importance. Three different forms of TGF β have been identified in mammals, and homologs have been found in evolutionarily distant species (for a review, see reference 27).

TGF β is a strong inhibitor of proliferation of most epithelial, endothelial, and lymphoid cells, whereas it stimulates growth of mesenchymally derived cells, probably through induction of platelet-derived growth factor (4, 23). In addition to its role in cell proliferation, TGF β has been implicated in early embryo development, immunomodulation, stimulation of angiogenesis, and wound healing (41, 51; for a review, see reference 31). TGF β obviously regulates expression of many genes, including those coding for extracellular matrix proteins, proteases, protease inhibitors, and acute phase-proteins (22, 25, 29). More directly, TGF β can also induce expression of some serum-induced early genes; however, the expression pattern is not always related to growth control (36, 46, 49).

Reduction of myc gene expression and posttranslational modification of the retinoblastoma gene product have both been proposed as possible ways for TGF β to exert its growth-inhibiting effect (21, 37, 38; for a review, see reference 30), although these events are not observed in all cell lines, indicating that TGF β may act through several different pathways in inhibiting cell growth (40).

Although little is known about the mechanism of growth arrest by TGF β , it is well documented that microinjection of mRNA from arrested cells into growing cells can induce growth arrest, suggesting that growth arrest may be mediated by specific mRNA species expressed during arrest (24, 35). One such cDNA has recently been cloned (32). Entry into quiescence or the G₀ phase is furthermore accompanied by the expression of a complex set of genes. From growtharrested mouse fibroblasts, six genes, termed growth-arrestspecific (gas) genes 1 to 6, were isolated whose mRNAs accumulated when cells exit from the cell cycle (44). Another, apparently nonoverlapping, set of five genes termed gadd genes induced after exposure of cells to UV irradiation also proved to be specifically expressed in the G_0 phase (12).

To better understand the mechanism of the TGF β response, we isolated and characterized TGF β -regulated genes in growth-arrested mink lung epithelial cells.

MATERIALS AND METHODS

Tissue culture. The TGFB-sensitive mink epithelial cell line CCL64 (21) was maintained in Dulbecco modified Eagle's medium with 10% fetal calf serum (FCS), penicillin, and streptomycin. The cells were passaged twice weekly by trypsinization and reseeded at a 10-fold dilution. For induction of quiescence and preparation of RNA, cultures were grown in 24- by 24-cm plates (Nunc, Roskilde, Denmark). After 3 days, the confluent cultures were shifted to fresh medium with 0.5% FCS and incubated for 2 additional days. Cells harvested at this point are termed arrested. TGFBtreated cells were obtained by further incubation for 24 h in the presence of human TGF β 1 (1 ng/ml; R & D Systems, Minneapolis, Minn.). Serum-stimulated cells were obtained by exposure of arrested cells to 10% FCS for 4 h. DNA synthesis was scored by incorporation of bromodeoxyuridine for 6 h and detecting the incorporated nucleoside by a monoclonal antibody (Partec, Arlesheim, Switzerland) as previously described (26). Cycloheximide treatment of arrested cells was performed by treatment with cycloheximide (10 μ g/ml), TGF β 1 (1 ng/ml), or a combination of the two.

Preparation of RNA and Northern (RNA) blots. For harvest of cells, the medium was aspirated and the cells were washed twice in phosphate-buffered saline, lysed in guanidine thiocyanate, and loaded onto a cushion of 4 ml of 5.7 M CsCl-1 mM EDTA in SW40 tubes (6). Total cellular RNA was pelleted by centrifugation at 33,000 rpm for 18 h; the pellet was resuspended in 10 mM Tris HCl (pH 7.5)-1 mM EDTA-0.5% sodium dodecyl sulfate, extracted with phenol once, and precipitated with ethanol. Poly(A) mRNA was prepared by passage over oligo(dT)-cellulose twice, and RNA was resuspended at a concentration of around 1 mg/ml in sterile distilled water. For Northern blot analyses, 10-μg aliquots of

^{*} Corresponding author.

[†] Present address: Department of Bacteriology, Karolinska Institutet, 104 01 Stockholm, Sweden.



FIG. 1. Northern blot hybridization analysis of RNA from CCL64 cells harvested daily for 1 to 5 days (d) after passage as indicated in panel A. On day 3, the cultures were shifted to medium with 0.5% FCS, leading to arrest of DNA synthesis. After 2 days in low serum, the cells were fed with fresh medium containing 10% FCS, and cells were harvested 1, 2, 4, or 6 h later, as indicated in panel B. The blots were probed with the cDNA clone for the TI 1 gene and with the JE gene as a control. C corresponds to a mink cDNA clone hybridizing with an 800-bp mRNA, the expression of which was only moderately affected by cell growth or TGF β exposure.

total cellular RNA were denatured with formamide and formaldehyde and then loaded onto 1.4% agarose gels. The RNA was transferred onto GeneScreen Plus hybridization membranes (Du Pont, Wilmington, Del.), and hybridization was performed as recommended by the supplier.

Construction and screening of cDNA libraries. cDNA was synthesized from 2.5 μ g of poly(A)⁺ mRNA from TGF β treated cells, using an oligo(dT) primer as previously described (14, 15). The cDNA was adapted with EcoRI linkers and subcloned into lambda gt10 arms (Stratagene, La Jolla, Calif.). The library contained about 4.5×10^5 recombinants and had an average insert size of about 1 kb (library I). Before screening, the library was amplified in Escherichia coli C600hfl. Screening was performed in E. coli C600. A second library was prepared with a directional cloning strategy (15) and cloned in plasmid pUEX (47). This second library had about 2.5×10^5 recombinants and an average insert size of 1.4 kb. A third library was prepared in lambda gt10 from CCL64 cells that after 36 h of culture were treated with TGF β for 2 h in the presence of 10% FCS. This library contained about 10^5 recombinants, and about 2×10^4 phages were used for screening.

For screening, an aliquot of the amplified library I was plated at a density of 800 PFU/150-mm petri dish. A total of 10,000 plaques were screened. Two lifts were made from each petri dish, using nylon membranes (Duralon; Stratagene). The first lift from each plate was probed with a single-stranded cDNA probe from serum-stimulated CCL64 cells; the second lift was probed with cDNA from TGF β -



1 3 6 12 24 h

0

FIG. 3. Northern blot analysis of the effect of TGF β on expression of the TI 1 gene in growing cells in the presence of 10% FCS (see Materials and Methods). Cells were harvested at the indicated hour after addition of TGF β . The JE, PAI 1, and C clones were included as controls.

treated cells. Plaques that specifically hybridized with the cDNA probe from TGF β -treated cells were isolated and rescreened twice. Inserts from purified lambda phages were excised with *Eco*RI and subcloned into the Bluescript pKS M13+ vector. Purified subcloned inserts were labeled (11) and used for cross-hybridization of all phages isolated and for probing of Northern blots with RNA from TGF β -treated, arrested, and serum-stimulated CCL64 cells. Clones that specifically hybridized with RNA from TGF β -treated cells were sequenced from both termini.

Subcloning, DNA sequencing, and sequence comparison. Dideoxy DNA sequencing was performed with use of a Sequenase kit (U.S. Biochemical, Cleveland, Ohio) or an automated sequencing protocol (20). The TI 1 clone was subcloned at seven unique sites in the 5' half of the insert. The 3' half of the clone was sequenced by using walking primers. Sequence analysis and comparison were performed with the University of Wisconsin Genetics Computer Group sequence analysis software package (9, 34).

Primer extension. A total of 10^5 cpm of 32 P-labeled primer complementary to positions 230 to 276 of TI 1 was annealed to 5 µg of poly(A) RNA from CCL64 cells in 40 mM piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES; pH 6.4)-0.4 M NaCl-1 mM EDTA-80% formamide for 16 h at 37°C, precipitated with ethanol, and resuspended in 20 µl of reverse transcription buffer. The annealed primer was extended at 42°C for 1 h with RNase H⁻ mouse mammary tumor virus reverse transcriptase (Bethesda Research Laboratories, Bethesda, Md.). After RNase treatment, phenolchloroform extraction, and ethanol precipitation, one-third of the reaction was analyzed on a 6% acrylamide-urea gel

6

12h

T C C/T

JE

1

0 T C C/T

3



TCC/TTCC/T

FIG. 2. Northern blot analysis of the effect of TGF β on expression of the TI 1 gene in arrested CCL64 cells. Cells were harvested at the indicated hour following addition of TGF β (1 ng/ml). The JE, PAI 1, and C clones were included as controls.

JE

TI1

MOL. CELL. BIOL.

10	30	50	70
AGAGTGGGCCGGCACA	GCACAAGAAGGAGGAGAAGGAAG	AGAGGGCAAGCTTGTGCCAA J	TCCCGACAATGGCGAAAGAT
			MAKD
90	110	130	150
GACTCCTCTGTTCGTT	GCTTCCAGGCCTGCTGATTTTT	GGAAATGTGATTGTTGGTATG	TGCGGCATCGCCCTGACCGC
DSSVRC	FQGLLIF	GNVIVGM	CGIALTA
170	190	210	230
AGAGTGCATCTTCTTC		CCCATTGCTTGAAGCCACCG	CAACGATGACATCIACGGGG
ECIFF	A S D A B S P I	FLLEXID	N D D I I G X
250	270	290	310
CAGCCTGGATTGGCAT	GTTTGICGGCATCTGCCTCTTCT	GTCTGTCCGTTCTAGGCATTO	TAGGCATCATGAAGTCCAAC
A W I G M	FVGICLFC	LSVLGIV	/ G I M K S N
330	350	370	390
AGGAAAATICTICIGG	V F T L M F T	GTATATGGCTTTGAAGTGGC	R C T T A A T
K K I D D A		VI GFEV X	
410	430	450 Kpn i	470
ACAACGAGACTTCTTC	ACGCCCAACCTCTTCCTGAAGCA	GATGCTGGAGAGGTACCAAAI	CANTAGCCCTCCAAACAATG
QRDFF	TPNLFLKQ	MLERYQN	N S P P N N D
490	510	530	550
ATGACCAATGGAAAAA	TAATGGAGTCACCAAGACTTGGG	ACAGACICATECTCCAGGAC	
DQWKN	NGVIKIWD		I C C G V N G
570	590	610	630
CCGTCAGACTGGCAGA	GATACACATCTGCCTTCCGGACT	GCGAATAATGATGCCGACTA	CCCTGGCCTCGTCAGTGCTG
PSDWQF	YTSAF RT	ANNDADY	PWPRQCC
		Sime	1
650	670	690	• 710
TGTGATGAACAGTCTG	EAAAGAACCTCTCAATGTGGAGGG	CTCCAACCTAGGAGTGCCCG	GTACTATCACAAAGAGGGGT
VMNSL	K E P L N V E A	CKLGVPG	YYHKEGC
730	750	770	780
730 CCTATCA CTCATCTC	750 1500 (1500 (1500 (1500 (1500 (1500 (1500 (1500 (1500 (1500 (1500 (1500 (1500 (1500 (1500 (1500 (1500 (1500 (1500	0000000770007770028	/ 50 PTTCCCNTTCTCTCCTCCACA
YELIS	G P M N R H A W	GVAWFG	FAILCWT
	~ .		
810	Kpan I 830	850	870
810 TTTTGGGTTCTCCTG	Kpn I 830 SGTACCATGTTCTACTGGAGCAGA	850 Attgaatattaagaacaaag	870 FGTCACCACCACCATCTTC
810 TTTTGGGTTCTCCTGG F W V L L G	Kpnl 830 SGTACCATGTTCTACTGGAGCAGA G T M F Y W S R	850 Attgaatattaagaacaaag I e y	870 IGTCACCACCACCATCTTC
810 TTTTGGGTTCTCCTGG F W V L L G	Kpni 830 SGTACCATGITCTACTGGAGCAGA G T M F Y W S R 910	850 ATTCRATATTRAGRACAAAG I E Y 930	870 FGTCACCACCACCATCTTC 950 April
810 TTTTGGGTTCTCCTGG F W V L L 0 890 CTCCAGTTGACTCTGG	Kmi 830 DOTACCATOTICTACTOGAGCAGA T M F Y W S R 910 DOCCCOGTOCTOGAAGCCAGCTCT	850 ATTGAATATTAAGAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT	870 IGTCACCACCACCATCTTC 950 Apal 2000000000000000000000000000000000000
810 TTTTGGGTTCTCCTGG F W V L L G 890 CTCCAGTTGACTCTGG	Kpn I 830 NOTACCATGTTCTACTGGAGCAGA 5 T M F Y W S R 910 NGCCCGGTGCTGCAAGCCAGCTCT	850 АТТСАЛТАТТАЛСАЛАС I E Y 930 ССТОСТАСЛАСССАЛСАЛАТ	870 RGTCACCACCACATCTTC 950 April 2009066660CCTGTGCCTC
810 TTTTGGTTCTCCTGG F W V L L C 890 CTCCAGTTGACTCTGG 970	Kpn I 830 BGTACCATGTTCTACTGGAGCAGA C T M F Y W S R 910 BGCCCGGTGCTGCAAGCCAGCTCT 990	850 ATTGAATATTAAGAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010	870 rgtcaccaccacaccatcttc 950 ^{Apal} ccccccgcggggccctgtgcctc 1030
810 TTTTGGGTTCTCCTGG F W V L L C 890 CTCCAGTTGACTCTGG 970 TTACTCCAACTGCCGJ	Kps1 830 SGTACCATGTTCTACTGGAGCAGA SGCCCGGAGCAGA 2 T M F W S 910 SGCCCGGTGCTGCGAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCTGT 990 SGGTAGAGGTGTACCCCTGGGCCCCGGGCCCCCGGGCCCCCCGGCCCCCCGGCCCCCC	850 ATTGRATATTARGARCAARG I E Y 930 CCTGGTAGAGCCARCGACAT 1010 GTAGCATCTCAAAATTCTCA	870 FGTCACCACCACACCATCTTC 950 Apal 3000 1030 CTAGGGTTTTCAGTCTGGTCT
810 TTTTGGGTTCTCCTGG F W V L L C 890 CTCCAGTTGACTCGC 970 TTACTCCAACTGCCG	Kpn I 830 SOTACCATGTTCTACTGGAGCAGA 5 T M F Y W S R 910 SGCCCGGTGCTGCTAGCAGCCAGCTCT 990 AGGTAGAGGTGTACCCCTGGGCTC	850 ATTCANTATTANGANCANAG I E Y 930 CCTGGTAGAGCCANCGACAT 1010 GTAGCATCTCANAATTCTCAN	870 FGTCACCACCACATCTTC 950 Apal SCCGCGGGGGGCCCTGTGCCTC 1030 CTACGGTTTTCAGTCTGGTCT
810 TTTTGGTTCTCCTGC F W V L L C 890 CTCCAGTTGACTCTGC 970 TTACTCCAACTGCCGU	Kpn I 830 SGTACCATGTTCTACTGGAGCAGA SGTACCAGGTGTTACTGGAGCAGCAGAGAGG 5 T M F Y W S R 910 SGCCCGGTGCTGGAAGCAGGCCAGCTCT 910 SGCCCGGTGCTGCCAGCCCCCGCCCCCGGCCCCCGCGCCCCCGCGCCCCCC	850 ATTGAATATTAAGAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAATTCTCA 1090	870 RGTCACCACCACATCTTC 950 April SCCGCGGGGGGCCCTGTGCCTC 1030 CTAGGGTTTTCAGTCTGGTCT 1110
810 TTTTGGGTTCTCCTGG F W V L L C 890 CTCCAGTTGACTCTGC 970 TTACTCCAACTGCCG 1050 CGGGTACTGCCAACATT	Kps1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCAGAGCAGAGCAGA G T M F Y W S R 910 SGCCCGGTGCTGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	850 ATTGRATATTARGARCAARG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCRAAATTCTCA 1090 ACTTTGGAAAGTCAATAATT	870 RGTCACCACCACCATCTTC 950 Apal 2000000000000000000000000000000000000
810 TTTTGGGTTCTCCTGG F W V L L C 890 CTCCAGTTGACTCTGC 970 TTACTCCAACTGCCGJ 1050 CGGGTACTGCAACATT	Kps1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCAGTGTTCTACTGGAGCAGAGCAG 2 T M F W S R 910 SGCCCGGTGCTGCTGCTGCAGCAGCAGCCAGCTGT 990 SGGTAGAGGTGTACCCCTGGGCTGC 1070 TTTTATAGCCAGTAGAAAGGAAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	850 ATTCRATATTAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATT 1170	870 RGTCACCACCACACCATCTTC 950 Apal SCCSCGGGGGGGCCCTGTGCCTC 1030 CTAGGGTTTCAGTCTGGTCT 1110 ACTTCTTCTATCCCTGCCTAT 1190
810 TTTTGGGTTCTCCTGG F W V L L C 890 CTCCAGTTGACTCTGC 970 TTACTCCAACTGCCGG 1050 CGGGTACTGCAACATT 1130 TTTTAATTGGGGGC	Kpn I 830 SOTACCATGITCIACTGGAGCAGE SOTACCATGITCIACTGGAGCAGE ST M F Y W S R 910 SGCCCGGTGCTGCTGCAGCCAGCCCCT 990 SGTAGAGGTGTACCCCTGGGCTC 1070 TTTTATAGCCAGTAGGAAGGGAG 1150 1150 1150	850 ATTCANATATTANGAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATT 1170 GTTATCTCAGCAAGCCAAGT	870 RGTCACCACCACATCTTC 950 Apal 950 Conservation 1030 CTAGGGTTTTCAGTCTGGTCT 1110 ACTTCTTCTATCCCTGCCTAT 1190 TTGTATTGCACAGCAAGGTT
810 TTTTGGGTCTCCTGG F W V L L G 890 CTCCAGTTGACTCTGC 970 TTACTCCAACTGCCG 1050 CGGGTACTGCCAACATT 1130 TTTTAATTTGAGGGCC	Kps I 830 SGTACCATGTTCTACTGGAGCAGA SGTACCAGGTGTTCCTACTGGAGCAGAGCAGA G T M F Y W S R 910 SGCCCCGGTGCTGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	850 ATTGAATATTAAGAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATT 1170 GTTATCTCAGCAAGCCAAGT	870 RETCACCACCACACCATCTTC 950 April 950 CAPRIL 950 April 950 CAPRIL 1030 CTAGGGTTTTCAGTCTGGTCT 1110 ACTTCTTCTATCCCTGCCTAT 1190 CTGTATTTGCACAGCAAGGTT
810 TTTTGGGTTCTCCTGG F W V L L C G 890 CTCCAGTTGACTCTGC 970 TTACTCCAACTGCCGG 1050 CGGGTACTGCAACATT 1130 TTTTAATTTGAGGGCJ 1210	Kps 1 830 SGTACCATGTTCTACTGGAGCAGA SGCCCGGAGCAGAGCAGAGCAGAGCAGAGCAGAGCAGCAGC	850 ATTGRATATTARGARCAARG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 CGTAGCATCTCAARATTCTCA 1090 ACTTTGRAARGTCAATAATT 1170 CGTTATCTCAGCAAGCCAAGT 1250	870 IGTCACCACCACACCATCTTC 950 Apal 950 CTAGGGGGGGCCCTGTGCCTC 1030 CTAGGGTTTTCAGTCTGGCTCT 1110 ACTTCTTCTATCCCTGCCTAT 1190 CTGTATTTGCACAGCAAGGTT 1270
810 TTTTGGGTTCTCCTGG F W V L L C G 890 CTCCAGTTGACTCTGG 970 TTACTCCAACTGCCGJ 1050 CGGGTACTGCCAACATT 1130 TTTTAATTTGAGGGCJ 1210 GCGTGCATTTCTCCCCT	Kps 1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGAGAGCAGCAGAGAGCAGCAGCAGCAGCAGCAGC	850 ATTCANTATTAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATT 1170 STTATCTCAGCAAGCCAAGT 1250 GGCTTTCATTCTTCCAATC	870 IGTCACCACCACACCATCTTC 950 Apal 950 CTACGCGCGCGGCGCCCCGTGCCCTC 1030 CTACGCTTTCTAGTCTGGTCT 1110 ACTTCTTCTATCCCCTGCCTAT 1190 CTGTATTGCACAGCTAGAAATTAA
610 TTTTGGGTTCTCCTGG F W V L L G 890 CTCCAGTTGACTCTGG 970 TTACTCCAACTGCCGI 1050 CGGGTACTGCCAACATG 1130 TTTTAATTTGAGGGGCJ 1210 GCGTGCATTCCCCC	Kps 1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCAGTCTACTGGAGCAGAGCAGA SGTACCAGTCTTACTGGAGCAGCAGAGAGCAGAGCAGCAGCAGCAGCAGCAGCAG	850 ATTCANATATTAAGAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATT 1170 GTTATCTCAGCAAGCCAAGT 1250 GGCTTTCATTCTTCAATC	870 RGTCACCACCACACCATCTTC 950 Apal 950 Apal 950 CAPACITOR 1030 CTAGGGTTTTCAGTCTGGTCT 1110 ACTTCTTCTATCCCTGCCTAT 1190 CTGTATTTGCACAGCAAGGTT 1270 TTGCCCCAGGTGAGAGAATTAA
610 TTTTGGGTTCTCCTGC F W V L L C 890 CTCCAGTTGACTCTCG 970 TTACTCCAACTGCCG 1050 CGGGTACTGCCAACTTG 1130 TTTTAATTTGAGGGCJ 1210 GCGTGCATTTCTCCCCT	Kps1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGAGCAGA SGTACCATGTTCTACTGGAGCAGCAGCAGAGCAGCAGCAGCAGCAGCAGCAGCAGC	850 ATTCRATATTAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 STAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATT 1170 SGTTATCTCAGCAAGCCAAGT 1250 SGCTTTCATTCTCCAATC	870 INGTCACCACCACACCATCTTC 950 Apal 950 CTAGGGTTTTCAGTCTGGCCTC 1030 CTAGGGTTTTCAGTCTGGCCTAT 1110 ACTTCTTCTATCCCTGCCTAT 1190 CTGTATTTGCACAGCAAGGTT 1270 ITGCCCAGGTAGAGAATTAA 1350
810 TTTTGGGTTCTCCCGG F W V L L C C 890 CTCCAGTTGACTCTGC 970 TTACTCCAACTGCCGJ 1050 CGGGTACTGCAACTTC 1130 TTTTAATTTGAGGGCJ 1210 GCGTGCATTTCTCCCCT 1290 GGAAAAATTGCTGAC	Kps 1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGAGCAGA S T M F Y W S R 910 SGCCCGGTGCTGGAGCAGCCAGCCCT 990 AGGTAGAGGTGTCCCCTGGGCTC 1070 TTTATAGCCAGTAGGAAGCAGCAGCCGTGCTGGAGGAGCAGCCTGGAGGAGAAGCAGCAGCAGTAGGAAAGGACA 1150 NANARGACTTCCACAAGAAACGGAG 1150 NANARGACTTCCACAAGAACCTGCAAJ 1230 IGCTTTCTGAAAGAGACTTGCCAAJ 1310 GAGAGATCTTTGGCCTTTGTTCTJ 1310	850 ATTCRATATTAAGAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAAATTCTCAA 1090 ACTTTGGAAAGTCAATAATT 1170 GTTATCTCAGCAAGCCAAGT 1250 GGCTTTCATTCTTCCAATC 1330 TGGGTGGCTTCCATCTACACA	870 IGTCACCACCACACCATCTTC 950 Apal 950 CTGCGCGGGGGGCCCTGTGCCTC 1030 CTAGGGTTTTCAGTCTGGTCT 1110 ACTTCTTCTATCCCTGCCTAT 1190 CTGTATTTGCACAGCAAGGTT 1270 ITGCCCAGGTGAGAGAATTAA 1350 GATTCAAGTTGATTCCGTTGA
610 TTTTGGGTTCTCCTGG F W V L L G 890 CTCCAGTTGACTCTGG 970 TTACTCCAACTGCCGI 1050 CGGGTACTGCCAACATT 1130 TTTTAATTTGAGGGCJ 1210 GCGTGCATTTCTCCCT 1290 GGANAMATTGCTGAG	Km I 830 SGTACCATGTTCTACTGGAGCAGF SGTACCAGTCTTACTGGAGCAGF SGTACCAGTCTTACTGGAGCAGCAGF 910 SGCCCGGTGCTGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	850 ATTCANTATTAAGAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATT 1170 CTTTATCTCAGCAAGCCAAGT 1250 GGCTTTCATCTTCCAATC 1330 TGGTGGCTTCCATCTACACA 1410	870 INTERCERCEACACCATETTE 950 April 950 CONCERCISCO 1030 CTACGGTTTTCAGTETGGTET 1110 ACTTETTETATECETGGETAT 1190 CTGTATTTGCACAGCAAGGTT 1270 ITGCCCAGGTGAGAGAATTAA 1350 GATTCAAGTTGATTCCGTTGA 1430
610 TTTTGGGTTCTCCTGG F W V L L G 890 CTCCAGTTGACTCTGG 970 TTACTCCAACTGCCGA 1050 CGGGTACTGCCAACATT 1130 TTTTAATTTGAGGGGCI 1210 GCGTGCATTTTCTCCCCI 1290 GGAAAAAATTGCTGGA 1370 CTGGCCACTCTAGAAA	Kps1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGAGCAGA SGTACCATGTTCTACTGGAGCAGAGCAGA 910 SGCCCGGTGCTGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	850 ATTCRATATTAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATT 1170 GTTATCTCAGCAAGCCAAGT 1250 GGCTTTCATCTTCTCAATC 1330 ITGGTGGCTTCCATCTACACA 1410 CCCACCTTGGTGATTAATAGTG	870 INGTCACCACCACACCATCTTC 950 Apal 2000000000000000000000000000000000000
810 TTTTGGGTTCTCTCGG F W V L L C G 890 CTCCAGTTGACTCTGG 970 TTACTCCAACTGCCGJ 1050 CGGGTACTGCAACATT 1130 TTTTAATTTGAGGGCJ 1210 GCGTGCATTTCTCCCT 1290 GGAAAAAATTGCTGAA	Kps1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGA ST M F W S 910 SGCCCGGTGCTGCTGCAGCAGCCAGCTCT 990 AGGTAGAGGTGTACCCCTGGGCTCT 1070 TTTATAGCCAGTAGGAAGCAAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	850 ATTCRATATTAAGAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATTC 1170 GTTATCTCAGCAAGCCAAGT 1250 GGCTTTCATTCTTCCAATC 1330 TGGTGGCTTCCATCTACACA 1410 CCCAACCTTGAGTTAATAGTG	870 INGTCACCACCACACCATCTTC 950 Apal 950 CTACGOGGOGOCCCTGTGCCTC 1030 CTACGOTTTCAGTCTGGTCT 1110 ACTTCTTCTATCCCTGCCTAT 1190 CTGTATTTGCACAGCAAGGTT 1270 ITGCCCAGGTGAGAGAATTAA 1350 GATTCAAGTTGATTCCGTTGA 1430 GTTGAAACTTCCTCTCAGACA
810 TTTTGGGTTCTCCTGG F W V L L G 890 CTCCAGTTGACTCTGG 970 TTACTCCAACTGCCGA 1050 CGGGTACTGCCAACATT 1130 TTTTAATTTGAGGGCJ 1210 GCGTGCATTCTCCCT 1290 GGANAMATTGCTGAC 1370 CTGGCCATCTTAGAAC	Km I 830 SGTACCATGTTCTACTGGAGCAGF SGTACCATGTTCTACTGGAGCAGF SGTACCATGTTCTACTGGAGCAGCAGF 910 SGCCCGGTGCTGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGTAGGAGACTGCAGCAGTAGGAAAAGGGAC 1070 TTTTATAGCCAGTAGGAAAAGGGAA 1150 NANAAGACTTCCGACAAGAAAAGGGAA 1230 TGCTTTCTGAAAGGCCTTGCTACT 1310 CAAGAAACTTTGTCCTTCAGAAACACGT 1390 CCATTTTGTTCTTCAGAACAACGT 1470	850 ATTCANTATTAAGAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAATTCTCA 1090 ACTTTGGAAGTCAATAATT 1170 NTTATCTCAGCAAGCCAAGT 1250 NGGCTTTCATCTTCCAAGT 1330 NGGGGGCTTCCATCTACACA 1410 NCCAACCTTGAGTTAATAGTG 1490	870 INTERCERCERCEACACENTETE 950 April 950 CONTROLOGICAL 1030 CTAGGGTTTTCAGTETGGTET 1110 ACTTETTETATECETGGETAT 1190 CTGTATTGCACAGGTAGAGAATTAA 1270 ITGCCCAGGTGAGAGAATTAA 1350 GATTGAAACTTCCTCTCAGACA 1510
610 TTTTGGGTTCTCCTGG F W V L L G 890 CTCCAGTTGACTCTGG 970 TTACTCCAACTGCCGACTGCCGAC 1050 CGGGTACTGCCAACTGCCGAC 1130 TTTTAATTTGAGGGCG 1210 GCGTGCATTTCTCCCCT 1290 GGAAAAAATTGCTGAC 1370 CTGGCCATCTTAGAAC	Kps1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGA 910 SGCCCGGTGCTGCTACTAGAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	850 ATTCRATATTAACAAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATT 1170 CTTATCTCAGCAAGCCAAGT 1250 GGCTTTCATCTTCCAATC 1330 ICGGTGGCTTCCATCTACACA 1410 CCCAACCTTGAGTTAATAGTG 1490 CATTAATCCTTTTACTGTCA	870 INGTERCERCERCERCERCERCERCERCERCERCERCERCERCE
810 TTTTGGGTTCTCCTGC F W V L L C 890 CTCCAGTTGACTCTCG 970 TTACTCCAACTGCCACTGCCG 1050 CGGGTACTGCCACTGCCACATT 1130 TTTTAATTTGAGGGCI 1210 GCGTGCATTTCTCCCCI 1290 GGANAMATTGCTGAC 1370 CTGGCCACTCTTAGAAC	Kps1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGA 910 SGCCCGGTGCTGGTGCTGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	850 ATTCRATATTAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 CTAGCATCTCAAAATTCTCA 1090 ACTTTGCAAAGTCAATAATTC 1090 ACTTTCCAGCAAGCCAAGT 1250 GGCTTTCATCTTCCAACCAA 1250 GGCTTTCATCTTCCAACCAA 1330 CCCAACCTTCGAGTTAATAGTG 2410 CCCAACCTTCGAGTTAATAGTG 2410 CCCAACCTTCGAGTTAATAGTG	870 INGTCACCACCACACCATCTTC 950 Apal 950 1030 CTAGGGTITTCAGTCTGGCTCT 1110 ACTTCTTCTATCCCTGCCTAT 1190 CTGTATTTGCACAGCAAGGTT 1270 ITGCCCAGGTGAGAGAATTAA 1350 GATTCAAGTTGATTCCGTTGA 1430 GTTGAAACTTCCTCTCAGACA 1510 AATATTTCCTCTCTGAACTATG
810 TTTTGGGTTCTCCTGA F W V L L C 890 CTCCAGTTGACTCTGC 970 TTACTCCAACTGCCGA 1050 CGGGTACTGCAACATT 1130 TTTTAATTTGAGGGCJ 1210 GCGTGCATTCTCCCT 1290 GGANAMAATTGCTGAC 1370 CTGGCCACGGTGACCCGA	Kps1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGA 910 SGCCCGGTGCTGGGCTCGGCCCC 990 AGGTAGAGGTGTACCAGGTAGGAAGCAGCTCC 1070 TTTTATAGCCAGTAGGAAGCAGAACCAGCTC 1150 NANARGACTTCCACAMGAACCTGGCTTGCAAM 1150 NANARGACTTCCACAMGAACCTGC 1230 IGCTTTCTGAAMAGAGACTTGCAAM 1310 CAGAGATCTTTGGTCTTCAGAACAACGT 1470 CGTGTTTTAMAGCTCCCCTTCTCCC 1550	850 ATTCRATATTAAGAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAAATTCTCAA 1090 ACTTTGGAAAGTCAATAATT 1170 GTTATCTCAGCAAGCCAAGT 1250 NGGCTTTCATTCTTCCAATC 1330 NTGGTGGCTTCCATCTACACA 1410 CCCAACCTTGAGTTAATAGTG 1490 CATTAATTCCTTTTACTGTCA 1570	870 INGTCACCACCACACCATCTTC 950 Apal 950 CTGCCCGGGGGGCCCCGTGCCCTC 1030 CTGCGGTTTTCAGTCTGGTCT 1110 ACTTCTTCTATCCCTGCCTAT 1270 ITGCCCAGGTGAGAGAATTAA 1350 GATTCAAGTTGATCCGTTGA 1430 GTTGAAACTTCCTCTCAGACA 1510 AATATTTCTCTCTCTGAACTATG 1590
610 TTTTGGGTTCTCCTGG F W V L L G 890 CTCCAGTTGACTCTGG 970 TTACTCCAACTGCCGAC 1050 CGGGTACTGCCAACTGCCGA 1050 CGGGTACTGCCAACTGCCGA 1210 GCGTGCATTTTCTCCCT 1290 GGAAAAATTGCTGAC 1370 CTGGCCAGCGTGACCCGA 1450 TGGCCAGGTGACCCGG 1530 TTTCCATTTGTGGTC	Kps1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGA 910 SGCCCGGTGCTGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	850 ATTGRATATTAAGAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATT 1170 CTTATCTCAGCAAGCCAAGT 1250 CGCTTCCATCTTCCAATC 1330 TCGTGGCGCTTCCATCTACACA 1410 CCCAACCTTGGGTTAATAGTG 1490 ATTAATTCCTTTTACTGTCA	870 INGTERCERCERCERCEATETTC 950 Apal 2000000000000000000000000000000000000
810 TTTTGGGTTCTCCTGG F W V L L G 890 CTCCAGTTGACTCTGG 970 TTACTCCAACTGCCAACTG CGGGTACTGCCAACTG 1050 CGGGTACTGCCAACTTG 1130 TTTTAATTTGAGGGC 1210 GCGTGCATTTCTCCCC 1290 GGAAAAAATTGCTGAC 1370 CTGGCCAGGTGACCCG 1530 TTTCCATTGGTGGTC	Kps1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGAGCAGA SGTACCATGTTCTACTGGAGCAGAGCAGA 910 SGCCCGGTGCTGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	850 ATTCRATATTAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 STAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATT 1170 SGTTATCTCAGCAAGCCAAGT 1250 SGGCTTCATTCTCAAAAT 1250 SGGCTTCAATCTACACAA 1330 NCCAACCTTGAGTTAATAGTG CATTAATTCCTTGACACACA 1410 NCCAACCTTGAGTTAATAGTG CATTAATTCCTTGACACACA 1570 NTATTGCTAGTCCTTATAAAA	870 INGTERCERCERCERCERCERTETE 950 Apal 950 CTACCONSTITUENT 1030 CTACGOTITICAGTORGETET 1110 ACTTOTTETATECCTOCOCCTAT 1190 CTGTATTTGCACAGCANGGTT 1270 ITGCCCAGGTGAGAGAATTAN 1350 GATTCANGTTGCATCCGTTGA 1430 GTTGANACTTCCTCTCAGACA 1510 ANTATTTCTCTCTGAACTATG 1590 TANAGATGCCTTTANATATCG
810 TTTTGGGTTCCTCG F W V L L G 890 CTCCAGTTGACTCTGG 970 TTACTCCAACTGCCG 1050 CGGGTACTGCAACATT 1130 TTTTAATTTGAGGGCJ 1210 GCGTGCATTCTCCCT 1290 GGAAAAAATTGCTGAC 1370 CTGGCCAGGTGACCCG 1530 TTTCCATTGTGGTC 1610	Kps1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGA 910 SGCCCGGTGCTGGGAGCAGAGCAGCCAGCTCT 990 AGGTAGAGGTGTACCCCTGGGCTC 1070 TTTATAGCCAGTAGGAAGCAGAACCAGCTCT 1230 IGCTTTCTGAAGAAGCAAGCATGCCTTGGCATGTCTT 1310 CAAGAACTTTCTCAGAAGCAACGT 1470 CGTGTTTTTAAGCCTCCCTTCTCCC 1550 CTGAAAGAAAATCTTTACAAACTCTA 1630	850 ATTCRATATTAAGAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATT 1170 GGTTATCTCAGCAAGCCAAGT 1250 GGCTTTCATCTTCTAAAAT 1250 GGCTTTCATCTATCAACAA 1410 CCCAACCTTGAGTTAATAGTG 1490 CATTAATTCCTTTACTGTCA 1570 MATTTGTCTAGTCTTATAAAA 1650	870 INGTCACCACCACACATCITC 950 Apal 950 1030 TTAGGGTTTTCAGTCTGGTCT 1110 ACTTCTTCTATCCCTGGTCTAT 1190 CTGTATTTGCACAGCAAGGTA 1270 ITGCCCAGGTGAGAGAATTAA 1350 GATTCAAGTTGATCCGTTGA 1430 GTTGAAACTTCCTCTCAGACA 1510 AATATTTCCTCTCTGAACTATG 1590 TAAAGTGGCTTTAAATATG 1670
610 TTTTGGGTTCTCCTGG F W V L L G 890 CTCCAGTTGACTCTGG 970 TTACTCCAACTGCCGG 1050 CGGGTACTGCCAACTGCCGG 1050 CGGGTACTGCCAACTGCCGG 1130 TTTTAATTTGAGGGCG 1210 GCGTGCATTTCTCCGGG 1370 CTGGCCATCTTAGAAG 1450 TGGCCAGGTGACCCGG 1530 TTTCCATTTGTGGTC 1610 TCATTTTCTCGGGGG	Km I 830 SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGA 910 SGCCCGGTGCTGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	850 ATTCRATATTAACAAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATT 1170 CTTATCTCAGCAAGCCAAGT 1250 CGCTTCCATCTTCCAATC 1330 TCGTGGCTTCCATCTACACA 1410 CCCAACCTTGGGTTAATAGTG 1490 ATTAATTCCTTTACTGTCA 1570 ATTATTGCCAGCTTCCGCCTCAGG	870 RGTCACCACCACACCATCTTC 950 Apil 2000000000000000000000000000000000000
610 TTTTGGGTTCTCCTGG F W V L L G 890 CTCCAGTTGACTCTCG 970 TTACTCCAACTGCCGAC 1050 CGGGTACTGCAACATT 1130 TTTTAATTTGAGGGCG 1210 GCGTGCATTTCTCCCC 1290 GGAAAAAATTGCTGAC 1370 CTGGCCAGGGTGACCCGG 1530 TTTCCATTGGGCC 1610 TCCAGGGGGGGGGGGGGCC 1630	Kps1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGAGCAGA SGTACCATGTTCTACTGGAGCAGAGCAGAGCAGAGCAGAG	850 ATTCRATATTAACAAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 STAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATT 1170 SGTTATCTCAGCAAGCCAAGT 1250 SGGCTTCCATCTACACAA 1250 SGGCTTCCATCTACACAA 1410 NCCAACCTTGAGTTAATAGTG 2ATTAATTCCTTGTCAGTTAATAGTG 2ATTAATTCGTCTAGTCATAAAA 1650 MCCCACGCCTCGGCCTCAGG 1730	870 INGTERCERCERCERCEATECTIC 950 Apal 950 000 1030 CTAGGGITITECAGICIGGECTE 1110 ACTTETTECTATECETGECTAT 1190 CTGTATTTGERCEAGEAAGGTT 1270 ITGECCAGGITAGAGAATTAA 1350 GATTEAAGTTGATTCCGTTGA 1430 GTTGAAACTTECTCTCAGACA 1510 AATATTTECTCTCTGAACTATEG 1590 TAAAGATGGECTTTAAATATEG 1670 TCATGATECCAGGGTCCTGGG 1750
610 TTTTGGGTTCTCCTGG F W V L L C 890 CTCCAGTTGACTCTGG 970 TTACTCCAACTGCCGG 1050 CGGGTACTGCAACATG 1130 TTTTAATTTGAGGGCJ 1210 GCGTGCATTTCTCCCCG 1290 GGANAMAATTGCTGAC 1370 CTGGCCAGGGTGACCCG 1530 TTTCCATTTGTGGTCC 1610 TTCATTTCTCCGGGG 1690 ATCCGCCCCCCCTC	Kps1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGA 910 SGCCCGGTGCTGCGAGCAGCCAGCTCT 990 AGGTAGAGGTGTACCCCTGGGCTCT 1070 TTTATAGCCAGTAGGAAGCAGAACCAGCTCT 1230 IGCTTTCTGAAAGAACCTGCCTTGCTCT 1310 CAGAGAACTTTCGCCTTGTTCTT 1390 CCATTTTGTTCTTCAGAACAACGT 1470 CGGTGTTTTAAGCACCCCCTTGTCCAGA 1630 CGCCTGGGTGGGCTCAGCAGGGAGCAC 1710 GGGCTCTCTGTCTGTCTCTGTCCAGCAGCAGCACCAC 1710	850 ATTCRATATTAAGAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATTC 1170 GGTTATCTCAGCAAGCCAAGT 1250 GGCTTTCATTCTTCTAAAAT 1250 GGCTTTCATCTTTCAAACAA 1410 CCCAACCTTGGGTAAATAGTG 1490 CATTAATTCGTCTAGTCTTATAAA 1650 MGCCACTGCCTTCCGCTCAGG 1730 TGGTGCCTTCCTTCTCTCTC	870 INGTCACCACCACACACATCITC 950 Apal CCCCCCGGGGGGCCCCGTGCCTC 1030 CTAGGGTTTCAGTCTGGTCT 1110 ACTTCTTCTATCCCTGCCTAT 1270 ITGCCCAGGTGAGAGAAGGTT 1270 ITGCCCAGGTGAGAGAATTAA 1350 GATTCAAGTTCATTCCTCTCAGACA 1510 AATATTTCTCTCTCTGAACTATG 1590 TAAAGTGGCTTTTAAATATG 1670 TCATGATCTCCAGGGTCCTGGG 1750 TGCCTGCCTCTCTACCTACT
610 TTTTGGGTTCTCCTGG F W V L L G 890 CTCCAGTTGACTCTGG 970 TTACTCCAACTGCCGG 1050 CGGGTACTGCCAACTGCCGG 1130 TTTTTAATTTGAGGGCG 1210 GCGTGCATTTCTCCCT 1290 GGBANAMATTGCTGAG 1370 CTGGCCAGGTGACCCG 1370 CTGGCCAGGTGACCCG 1530 TTTCCATTTGTGGTC 1610 TCCATTGCGGCACCGCATC	Km1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGAGCAGA SGTACCATGTTCTACTGGAGCAGCAGAGCAGAGCAGAGAGAG	850 ATTCRATATTAACAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATT 1170 CTTATCTCAGCAAGCCAAGT 1250 CGCTTCCATCTACACAA 1330 CCCAACCTTGGTTAATAGTG 1410 CCCAACCTTGGTTAATAGTG 1410 CCCAACCTTGGTTAATAGTG 1570 MACCACTGCCTTCGGCTCAGG 1730 CTCCTTCCCTTCTCTCTCTCC	870 NGTCACCACCACACCATCTTC 950 Apil 2000000000000000000000000000000000000
810 TTTTGGGTTCTCCTGG F W V L L G 890 CTCCAGTTGACTCTCG 970 TTACTCCAACTGCCGAC 1050 CGGGTACTGCCAACTGCCGA 1050 CGGGTACTGCCAACTGCCGA 1130 TTTTAATTTGAGGGCG 1210 GCGTGCATTTTCTCCCT 1290 GGAAAAAATTGCTGAC 1370 CTGGCCAGGGTGACCCG 1530 TTTCCATTTGCGGCG 1610 TTCATTTCTCGGGGCACCCG 1690 ATCGAGTCCCCGCATC 1770	Kps1 830 SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGA SGTACCATGTTCTACTGGAGCAGA 910 SGCCCGGTGCTGCTGCAGCAGCAGCAGCAGC 990 AGGTAGAGGTGTACCCCTGGGCTCT 1070 TTTATAGCCAGTAGGAGAAGCAGCAGCAGCAGCAGCAGCAGAAGAAAGA	850 ATTCRATATTAACAACAAAG I E Y 930 CCTGGTAGAGCCAACGACAT 1010 GTAGCATCTCAAAATTCTCA 1090 ACTTTGGAAAGTCAATAATT 1170 GTTATCTCAGCAAGCCAAGT 1250 GGCTTTCATTCTCCAATCAAAT 1250 GGCTTCCATCAACACA 1330 CCCAACCTTGGGTGATAATAGTG 24490 247TAATTCGTCAGCTACACAA 1570 ATATTTGTCTAGTCATTATAAA 1650 MGCCACTGCCTTCCGCTCAGG 1730 CTGCTTCCCTTCCTCTCTCC	870 NGTCACCACCACACCATCTTC 950 Apal 2000000000000000000000000000000000000

FIG. 5. DNA and predicted protein sequence of the TI 1 cDNA clone. The positions of the triple polyadenylation signals are indicated by a horizontal bar. Some of the restriction sites used for subcloning are indicated.

with a sequencing reaction, using the same oligonucleotide and the TI 1 clone as a marker.

melting-point agarose gel, cloned into SmaI-cut pKSM13+, and sequenced.

Cloning of the 5' end of TI 1. The product of the primer extension was dC tailed with terminal transferase (Boehringer, Mannheim, Germany) and amplified by 35 cycles of a polymerase chain reaction (94°C for 1 min; 50°C for 1.5 min; 72°C for 1.5 min), using an oligonucleotide complementary to positions 69 to 86 of the TI 1 sequence and oligo(dG) as primers. After filling in and kinasing, DNA of approximately 100 bp was separated from the primers on a 1.5% lowNucleotide sequence accession number. The sequence of TI 1 cDNA has been submitted to the EMBL/GenBank/DDBJ data banks under accession number M64428.

RESULTS

Genes specifically expressed in growth-arrested and TGF β -treated cells. For the isolation of TGF β -regulated genes,



FIG. 6. Primer extension analysis of TI 1 RNA. E, extended primer (see arrow); P, unextended primer which has migrated out of the gel; SEQ, sequence of TI 1 with the same primer (ACGT).

cDNA library I was prepared in lambda gt10. The RNA was from mink lung epithelial cells that had first been arrested by exposure of confluent cultures to low serum for 48 h and then exposed to TGF β at 1 ng/ml for 24 h in the same medium. In the TGF_β-treated cells, 0.1% of the cells incorporated bromodeoxyuridine into DNA, whereas the corresponding figure for arrested cells was around 1% (not shown). This library was differentially screened with a total cDNA probe from TGF_β-treated cells and a probe from serum-stimulated cells. By differential screening of 10,000 plaques, 32 clones that preferentially hybridized with cDNA from TGFβtreated cells were recovered. Following subcloning into the Bluescript pKS M13+ plasmid and cross-hybridization, purified cDNA inserts were used to probe Northern blots of RNA from arrested, serum-stimulated, and TGF_β-treated cells. cDNA that specifically hybridized with RNA from arrested or TGFB-treated cells was further examined by DNA sequence analysis and additional Northern blot analyses.

Seven cross-hybridizing clones detected a 5-kb mRNA expressed at high levels in TGF_β-treated cells. Upon DNA sequencing, one of them was found to be similar to human fibronectin mRNA. Another clone with a similar expression pattern was similar to collagen α type I mRNA. The fibronectin and collagen clones were not studied further since it has been amply demonstrated that TGFB induces both collagen and fibronectin mRNA (for references, see reference 27). Likewise, two clones were isolated which upon sequencing were found to be identical to the plasminogen activator inhibitor 1 (PAI 1) gene (25) and the JE gene (42), respectively. The former clone was isolated by screening the third library prepared from RNA of rapidly growing CCL64 cells that had been treated for 2 h with TGF β . Both RNAs were induced by TGF β and used as internal controls in Northern blot analysis.

Finally, two cross-hybridizing clones hybridized with a 1.8-kb mRNA in arrested cells. The larger of these clones (TI 1), 1.4 kb in length, was used as a probe in subsequent Northern blot experiments.

Expression pattern of the TGF\beta-regulated TI 1 gene. To determine how the TI 1 gene was affected by growth condition and TGF β treatment, three different experiments were performed.

In the first type of experiment, the effect of serum starvation was analyzed. RNA was harvested daily for 5 days after reseeding of the cells. After day 3, the cultures were confluent and the cells were shifted to fresh medium containing 0.5% FCS. After day 5, the cultures were shifted back to fresh medium with 10% FCS, and cells were harvested after 1, 2, 4, and 6 h. The relatively abundant 1.8-kb mRNA detected by the TI 1 probe accumulated as the cells became confluent and remained at high levels after the shift to low serum. Addition of serum after 2 days in low serum resulted in a rapid decrease of RNA to a basal level which remained constant for the next 6 h (Fig. 1). A parallel blot (Fig. 1) probed with the JE clone as a control identified a 0.8-kb mRNA which was detectable at low levels during establishment of confluence. The shift to 10% FCS led to a sharp increase of mRNA which peaked after only 1 h and then slowly declined.

In a second experiment, TGF β was added to the cultures after 2 days in low serum (day 5), and the cells were harvested 1, 3, 6, 12, and 24 h later. Addition of TGF β led to a gradual reduction of the TI 1 mRNA, reaching a steadystate level after 12 h (Fig. 2). In contrast, the JE mRNA, present at low levels in control cells, remained unchanged for the first hours in TGF β . This mRNA increased in abundance between 3 and 6 h and remained unchanged thereafter (Fig. 2). The PAI 1 clone, which was isolated from a cDNA library prepared from mRNA of actively growing TGF β -treated cells, identified a 3.5-kb mRNA that reached its highest abundance after only 1 h of TGF β exposure and then declined within 24 h to the level before addition of TGF β (Fig. 2).

To establish whether the effect of TGF β observed in Fig. 2 was associated with the quiescence of the cells, a third experiment was performed with actively growing cells. TGF β was added 36 h after passage, and the cells were harvested at various times thereafter. Figure 3 shows that the TI 1 mRNA which was inhibited by TGF β in arrested cells (Fig. 2) was transiently induced by TGF β in growing cells, and maximal expression was observed 3 to 6 h after addition. After 24 h with TGF β , the level of TI 1 expression had returned to the low levels observed before addition (Fig. 3). Expression of the JE mRNA was higher than in arrested cells (Fig. 1), with maximal induction after 1 h followed by a gradual decline (Fig. 3). The effect of TGF β on the PAI 1 mRNA in growing cells followed the same time course as in arrested cells.

To determine whether the effect of TGF β on expression of the TI 1 gene is dependent on ongoing protein synthesis, arrested cells were treated in the absence and presence of cycloheximide at 10 µg/ml (Fig. 4). The downregulation of TI 1 expression in arrested cells was partially blocked by the addition of cycloheximide (Fig. 4). The JE mRNA was induced by cycloheximide alone, without addition of TGF β as previously demonstrated (42).

Sequence comparison of the TGF β -regulated genes. The original TI 1 (1.4 kb) clone subcloned into Bluescript was sequenced from both termini, as were the subfragments generated by cleavage with KpnI, SmaI, and ApaI (Fig. 5).

TI1 Tapa-1 Co-029 Sm23 Me491 Cd53 Cd37 R2 Rds	A K - G - T - A - A S A K F	D D V A C C C C C C C C C C C C C	SGGTGSSAK	S V G I G I G I G I G I G I G I G I G I G		ССССГГУК	F I L V L I T L	Q G K Y K S K F K Y K Y K Y K Y A Q			I FVTVFVFV FVVFVFVLL	GFFLLFFFM	N N N N N N N	V F I L L L L L L L L L L L L L L L L L L		GwwLCwFFV	M L L L A I V I L					T L L I L F L F	A E G V A G A G A G F F G F S I		I I A V I I V L	FWWYWWF	FVR V L L L L L L	S H V - I I A I	D (D 1 S 1 H 1 D 1 D 1 E 1			LTQYGGFFR	Y I N		Y-KVHVLM	- L - F L N				46 49 43 42 41 44 46 46 46 50
TI1 Tapa-1 Co-029 Sm23 Me491 Cd53 Cd37 R2 Rds	EA SE GDI IN - Q SH	T D P A D V N L Q P L A T S F V	N G H A S F S P	DI NS KT L V I N	O I F S W G L L S L	YYYQS - QRI	G V V A L - I M G	A A G I A P L P G A V G	W P D I V N K Y V	I I A V V V L		F A V A I G V	V V V V V V V S V F	GGGGGGGGN	I C A V A I V F S I F A V S L	L H I I T T A	FMMLFMMMG			VFFFFFFFF	ם 	G G G G G G G G G G G G G G G G G G G			I A A A A S A A A A	M I I I C I L V K	K SE K E E K E E E E	N S N N N L V K	R O R V Y K R R W			L G L Y I M G G L	A T L M T L L K		I T I F I I G A L	L G F F L M F A	MLLLLLV	F L L L L L L L L L L L L L L L L L L L	I L V L L L I	96 99 93 92 91 90 95 93 100
TI1 Tapa-1 Co-029 Sm23 Me491 Cd53 Cd53 Cd37 R2 Rds	V Y L I L M L F L I F F	G F L L I A L A L A I A N V	E E OE E E O O I	V V V V V V V V V V V V V V V V V V V		C G A A G G V	I I I I I A A	T A G W L G A G L L L L L L L	A F A V F S Y C	TVVVVTFF	Q R N K K F Y F Y F N L L	D D D D D Q A G R	F	F		N	LQKRKKQKT	F 1 S 1 I 1 L 1 L 1 L 1	L K D S D S M E F K Y		M V V I F V L M L	L K N D N A R G K	EF ET NNC US	R F L M F L V V G M	Q Y Y T R 	NDEGQTETI	N S A N T A D S T E E K	P L - I I Y	P L L H Q R Y		N D A V A A - E H G (N T	D V T K N S T S D	Q D G P Y N S T	N H D I E S F H F H F H F H F H F H F H		NNK – NNT – C	G N H S A S F	V 1 F (I 1 T 1 A L (T K Q T A K E Q K	146 143 138 131 132 131 139 136 141
TI1 Tapa-1 Co-029 Sm23 Me491 Cd53 Cd37 R2 Rds	K T E A E F S A E S C A K T	WC V N L U W U U U U U U U U U U		L F I M I V L		QTESDFQQE	DLFFFLLVF			0000000000	V-SSLAA- AA- W-N-]G]G]G]G]G]G]G	P T A - Y T P F F		W T W W W	QTG-ETFTF	R S III	Y - I - I - I - I - I - I - I - I - I -		A 	F S - R M S	R 7 			N EVD	D 3 A H F 5		Y				- - - Y E R			- - A E N	- - - T E V	- - N D		184 163 140 163 180 183 183
TI1 Tapa-1 Co-029 Sm23 Me491 Cd53 Cd37 R2 Rds	 S T S L R Y				 G F	W	P			C HKR-R-P	V M G H C		S L D S L R	L C Y C A P			L N D V V - H N Y	N N K P T S S R Q	V H Q A V G A L T L T		C S C K G A C G N	K G Q - I S A N S		G					- - - - T	V 1 I 1 E 1 S 1 E 1 E 1	P G G K E K E R E R E R E R E R	Y N Q L A K H P N	Y V T I V L L	H I F Y H I Y V V		66999994 1000 1000 1000 1000 1000 1000 100	000000000	Y H I V V Y A R	E Q S S E A Q E A	219 191 195 173 192 171 231 217 240
TI1 Tapa-1 Co-029 Sm23 Me491 Cd53 Cd37 R2 Rds	L I K I F I V F K I K A G L K V A L	S (D I G I G I Q I L I			N FOR SAKRHAQS		ALLUFLLM	W (Y 1 V 1 L 1 G 1 N		A G I A A G V L G			F I F I I V				WILFVILIE	TMEOREEEE		VEGSGGEGI		LIVVVSTVA	GLFIFFLLG	TM SM ACAL SI SI		YLLLLLLLL		RGQQSQNHA	I I I I L V L	E I GI KI DI HI	Y - SK N KY S G T H E V	- S - EYSVDS	- V - NEQYYN	Y - V I V I T I P I						259 239 239 239 239 217 237 218 280 260 290

FIG. 7. Sequence alignment of the p28 protein encoded by the TI 1 cDNA clone and the family of related transmembrane glycoproteins. Co-029, colon tumor antigen (48); Me491, melanoma-associated antigen (16); Sm23, antigen from *S. mansoni* (52); CD37 (8), CD53 (2), R2 (13), and Tapa-1 (33), leukocyte antigens; Rds, protein encoded by a gene responsible for the retinal degeneration slow phenotype in mice (50). Amino acid identities and conservative substitutions are boxed.

An open reading frame extending 380 bp into the 5' end was identified, and the 3' end carried a 47-bp poly(A) tail. Two larger clones were isolated from a cDNA library in plasmid pUEX from TGFβ-treated cells. The larger of these clones was completely sequenced, and about 400 bases were sequenced for the shorter clone. The sequence of the fulllength clone of TI 1 (1,807 bp) is shown in Fig. 5. An open reading frame starts at base 69 and ends at base 848, followed by 951 bp in the 3' untranslated region and then a poly(A) tail. Since the initiator methionine at base 69 is not preceded by a stop codon, we analyzed whether we had obtained a full-length TI 1 clone. Primer extension using an oligonucleotide complementary to bases 230 to 276 of TI 1 was carried out. As shown in Fig. 6, the extension gives one major band corresponding to the first nucleotide in TI 1. In addition, the sequence of the 5' end was confirmed by cloning the product of the primer extension by PCR and sequencing (data not shown).

The open reading frame codes for a hypothetical polypeptide of 260 amino acids with a molecular weight of ca. 28,500 (referred to as p28). The predicted p28 protein has an unusually balanced amino acid composition but has a relatively high (5%) cysteine content, including two cysteine doublets. Near the C terminus and at residues 60 to 100, clusters of hydrophobic amino acids are found. Charged residues are dispersed over the N-terminal 60 amino acids and residues 101 to 229. A potential site for N-linked glycosylation is found at residues 130 to 132.

Structural relationship between the hypothetical protein encoded by the TI 1 cDNA and a family of transmembrane glycoproteins. The sequence of the putative protein (designated p28) encoded by the TI 1 mRNA was compared with entries in the EMBL (release 26) and PIR (release 27) sequence libraries with the program FASTA (34). The search revealed a family of related transmembrane proteins, among them the membrane-bound glycoprotein Me491, which is



FIG. 8. Plot of amino acid sequence number versus membrane buried-helix parameter for each residue (see reference 28 for method) of TI 1 (solid line) and the average values of the other members (excluding Rds) of the glycoprotein family (dashed line). Bars indicate positions of gaps introduced into the sequences in the alignment.

expressed in carcinomas and particularly in the early stages of melanomas (16), the colon-associated tumor antigen Co-029 (48), the Schistosoma mansoni antigen Sm23 (52), and the leukocyte antigens CD53 (2), CD37 (8), R2 (13), and Tapa-1 (33). A more sensitive sequence comparison method (Profilesearch of the University of Wisconsin Genetics Computer Group program package) using only conserved regions in the alignment of the protein family detected a more distant homology to the bovine, murine, and rat Rds (retinal degradation slow) proteins (50). This homology has not been demonstrated previously. As shown in Fig. 7, identical and similar residues are clustered in four regions. Further analysis by the sensitive sequence comparison method (3) confirmed the significance of the relation of the individual family members (data not shown).

Similarities were striking when we compared plots of buried-helix parameter (28) between TI 1 and other members of the glycoprotein family, showing strict conservation of all four putative transmembrane domains (Fig. 8). The potential N-linked glycosylation site in TI 1 is conserved in the same region of Co-029, Me491, CD53, and R2.

DISCUSSION

Several cytokines, such as interferons, TGF β , and tumor necrosis factor α , are antiproliferative for some cell types. Each cytokine regulates several induced and suppressed genes, of which only a small fraction have been isolated. It remains unclear whether any of the known cytokine-regulated genes relate to growth suppression. However, a subset of these genes, or other similar, not yet identified genes, may play an important role in negative growth regulation. The antiproliferative action of alpha interferon in B-cell lines is blocked by the expression of the Epstein-Barr virus immortalizing gene EBNA 2 (1). This function of EBNA 2 seems to be mediated through its ability to block the induction of interferon-induced genes at the transcriptional level (17a). Because of the inherent difficulty in specifically cloning genes that act in an antiproliferative fashion, the mechanism of growth arrest is largely unknown. Decreased expression of c-myc is, however, related to the antiproliferative effect of interferon (19), and recently it has been demonstrated that TGF β -induced growth arrest may be associated with a moderate reduction of c-myc expression (37). A posttranslational effect on the retinoblastoma protein has also been proposed (21, 38). During the preparation of this report, Howe et al. (17) showed that TGF β induces G₁ arrest in CCL64 cells and that TGF β blocks the phosphorylation of the mink homolog of yeast p34cdc2. Again, a posttranslational control may be involved.

The intention of this work was to isolate genes that are associated with the induction of growth arrest by TGF β in an epithelial cell line. Three of the genes identified, those for fibronectin, collagen, and PAI 1, are known to be upregulated by TGF β in human lung fibroblasts (27). However, in these cells, maximal induction of PAI 1 is observed after 10 h and expression remains high after 2 days (25). In the mink cell homolog, a transient induction is observed (Fig. 2 and 3). It is, however, still unresolved whether matrix proteins and protease inhibitors play a role in growth regulation.

The JE cDNA clone isolated contained several small open reading frames but showed an overall homology of 75% with the human monocyte chemotactic and activating factor (JE) gene (43), which is transiently expressed following cytokine stimulation (42). Compared with the human sequence, the mink JE clone had two inserted sequences and thus appears to represent an incompletely processed JE mRNA. Our JE clone had a structure surprisingly similar to that of a human gamma interferon-induced gene called gamma 1 (10), which is 98% homologous to the JE cDNA clone. The hypothetical mink protein was 83% similar and 72% identical over 80 residues to the human protein. Thus, the gamma 1 cDNA is probably an unspliced precursor of the JE transcript (45). The JE gene is obviously regulated by many factors, not all able to stimulate cell growth. Its expression may, however, relate to the chemotactic properties of TGF β (39, 51).

The regulation of expression of the TI 1 clone is interesting. It is induced 3 to 6 h after addition of TGFB in growing cells, corresponding to the intermediate time of induction of growth arrest in CCL64 cells by TGF β (Fig. 3). Its expression is lower in actively growing cells than in quiescent cells, in which its expression is downregulated by serum stimulation (Fig. 1). Expression of the TI 1 gene is also downregulated by TGFB in quiescent cells (Fig. 2). Previously isolated genes that are negatively regulated by TGF β include those encoding extracellular proteases such as transin, urokinase, elastase, and collagenase but also genes such as the proliferin gene and c-myc (18, 30). These genes have a common regulatory element, and inhibition of the transin gene seems to be mediated by a fos-binding sequence (18). The TI 1 gene therefore seems to be similar to the recently described mouse gas genes (44), which accumulate in quiescent cells and are rapidly downregulated by serum. All but one of the gas genes are regulated by a posttranscriptional mechanism (7). None of the gas genes are expressed in epithelial cells, and thus it appears that TI 1 is a candidate for an epithelial gas-like gene. The TI 1 open reading frame shows homology with eight members of a family of transmembrane glycoproteins expressed on leukocytes and several types of tumor cells. Although very little is known about their function, the Me491 antigen might possibly serve as a rapid-growthinhibitory gene (16). Moreover, Tapa-1, expressed on hematopoietic, neuroectodermal, and mesenchymal cells, is the target of an antiproliferative monoclonal antibody (33). The expression of CD37 is high in resting B cells but is rapidly downregulated following induction of mitosis and differentiation with phorbol esters (5). Clearly, at least some members of the TI 1-related gene family are expressed at growth arrest. Given the similarity between these proteins, they may have similar functions. Further experiments are required to evaluate a possible causative role of the TI 1 protein in growth regulation.

ACKNOWLEDGMENTS

We thank Keith Stanley and Melanie Price for continuous expert advice and discussion, Claudia Winter and Alexandra Charlesworth for technical, and Nelly van der Jagt-González for secretarial assistance. The final part of this work was carried out at the Department of Bacteriology, Karolinska Institutet, Stockholm, Sweden. We thank Alexander von Gabain for providing laboratory space and support.

Bengt Kallin was supported by a fellowship from the Swedish Cancer Society.

REFERENCES

- 1. Åman, P., and A. von Gabain. 1990. An Epstein-Barr virus immortalization associated gene segment interferes specifically with the IFN-induced anti-proliferative response in human B-lymphoid cell lines. EMBO J. 9:147–152.
- Angelisová, P., C. Vicek, I. Stefanová, M. Lipoldová, and V. Horejsí. 1990. The human leucocyte surface antigen CD53 is a protein structurally similar to the CD37 and MRC OX-44 antigens. Immunogenetics 32:281–285.
- 3. Argos, P. 1987. A sensitive procedure to compare amino acid sequences. J. Mol. Biol. 193:385-396.
- 4. Battegay, E. J., E. W. Raines, R. A. Seifert, D. F. Bowen-Pope,

and R. Ross. 1990. TGF-beta induces bimodal proliferation of connective-tissue cells via complex control of an autocrine PDGF loop. Cell 63:515–524.

- 5. Carlsson, M., T. H. Tötterman, P. Matsson, and K. Nilsson. 1988. Cell cycle progression of B-chronic lymphocytic leukemia cells induced to differentiate by TPA. Blood 71:415-421.
- Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294– 5299.
- Ciccarelli, C., L. Philipson, and V. Sorrentino. 1990. Regulation of expression of growth arrest-specific genes in mouse fibroblasts. Mol. Cell. Biol. 10:1525–1529.
- Classon, B. J., A. F. Williams, A. C. Willis, B. Seed, and I. Stamenkovic. 1989. The primary structure of the human leukocyte antigen CD37, a species homologue of the rat MRC OX-44 antigen. J. Exp. Med. 169:1497–1502.
- 9. Devereux, J., P. Haeberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. 12:387–395.
- Fan, X. D., G. R. Stark, and B. R. Bloom. 1989. Molecular cloning of a gene selectively induced by gamma interferon from human macrophage cell line U937. Mol. Cell. Biol. 9:1922–1928.
- 11. Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132:6–13.
- Fornace, A. J., D. W. Nebert, M. C. Hollander, J. D. Luethy, M. Papathanasiou, J. Fargnoll, and N. I. Holbrook. 1989. Mammalian genes coordinately regulated by growth arrest signals and DNA-damaging agents. Mol. Cell. Biol. 9:4196–4203.
- Gaugitsch, H. W., E. Hofer, N. E. Huber, E. Schnabl, and T. Baumruker. 1991. A new superfamily of lymphoid and melanoma cell proteins with extensive homology to *Schistosoma mansoni* antigen Sm23. Eur. J. Immunol. 21:377–383.
- 14. Gubler, U., and B. J. Hoffman. 1983. A simple and very efficient method for generating cDNA libraries. Gene 25:263-269.
- Haymerle, H., J. Herz, G. M. Bressan, R. Frank, and K. K. Stanley. 1986. Efficient construction of cDNA libraries in plasmid expression vectors using an adaptor strategy. Nucleic Acids Res. 14:8615–8624.
- Hotta, H., A. H. Ross, K. Huebener, M. Isobe, S. Wendeborn, M. V. Chao, R. P. Ricciardi, Y. Tsujimoto, C. M. Croce, and H. Koprowski. 1988. Molecular cloning and characterization of an antigen associated with early stages of melanoma tumor progression. Cancer Res. 48:2955–2962.
- 17. Howe, P. H., G. Draetta, and E. B. Leof. 1991. Transforming growth factor β 1 inhibition of p34^{cdc2} phosphorylation and histone H1 kinase activity is associated with G1/S-phase growth arrest. Mol. Cell. Biol. 11:1185–1194.
- 17a.Kanda, K., P. Åman, A. von Gabain, and B. Kallin. Unpublished data.
- Kerr, L. D., D. B. Miller, and L. M. Matrisian. 1990. TGFβ1 inhibition of transin/stromelysin gene expression is mediated through a *fos* binding sequence. Cell 61:267-278.
- 19. Kimchi, A. 1987. Autocrine interferon and the suppression of the c-myc nuclear oncogene. Interferon 8:85-110.
- Kristensen, T., H. Voss, C. Schwager, J. Stegemann, B. Sproat, and W. Ansorge. 1988. T7 DNA polymerase in automated dideoxy sequencing. Nucleic Acids Res. 16:3487–3496.
- Laiho, M., J. A. de Caprio, J. W. Ludlow, D. M. Livingston, and J. Massagué. 1990. Growth inhibition by TGFβ linked to suppression of retinoblastoma protein phosphorylation. Cell 62: 175–185.
- Laiho, M., O. Saksela, and J. Keski-Oja. 1987. Transforming growth factor-β induction of type 1 plasminogen activator inhibitor. J. Biol. Chem. 262:17467-17474.
- 23. Leof, E. B., J. A. Proper, A. S. Goustin, G. D. Shipley, P. E. DiCorleto, and H. L. Moses. 1986. Induction of c-sis mRNA and activity similar to platelet-derived growth factor by transforming growth factor-beta: a proposed model for indirect mitogenesis involving autocrine activity. Proc. Natl. Acad. Sci. USA 83:2453-2457.
- 24. Lumpkin, C. K., Jr., J. K. McClung, O. M. Pereira-Smith, and

J. R. Smith. 1986. Existence of high abundance antiproliferative mRNA in senescent human diploid fibroblasts. Science **232**:393–395.

- 25. Lund, L. R., A. Riccio, P. A. Andreasen, L. S. Nielsen, P. Kristensen, M. Laiho, O. Saksela, F. Blasi, and K. Dano. 1987. Transforming growth factor-β is a strong and fast acting positive regulator of the level of type-1 plasminogen activator inhibitor mRNA in WI-38 human lung fibroblasts. EMBO J. 6:1281–1286.
- Manfioletti, G., M. E. Ruaro, G. Del Sal, L. Philipson, and C. Schneider. 1990. A growth arrest-specific (gas) gene codes for a membrane protein. Mol. Cell. Biol. 10:2924–2930.
- 27. Massagué, J. 1990. The transforming growth factor- β family. Annu. Rev. Cell Biol. 6:597–641.
- Mohana-Rao, J. K., and P. Argos. 1986. A conformational preference parameter to predict helices in integral membrane proteins. Biochim. Biophys. Acta 869:197-214.
- 29. Morrone, G., R. Cortese, and V. Sorrentino. 1989. Post-transcriptional control of negative acute phase genes by transforming growth factor beta. EMBO J. 8:3767-3771.
- Moses, H. L., E. Y. Yang, and J. A. Pietenpol. 1990. TGFβ stimulation and inhibition of cell proliferation: new mechanistic insights. Cell 63:245-247.
- Nielsen-Hamilton, M. 1990. Transforming growth factor-β and its actions on cellular growth and differentiation. Curr. Top. Dev. Biol. 24:95–136.
- 32. Nuell, M. J., D. A. Stewart, L. Walker, V. Friedman, C. M. Wood, G. A. Owens, J. R. Smith, E. L. Schneider, R. Dell'Orco, C. K. Lumpkin, D. A. Banner, and J. K. McClung. 1991. Prohibitin, an evolutionarily conserved intracellular protein that blocks DNA synthesis in normal fibroblasts and HeLa cells. Mol. Cell. Biol. 11:1372-1381.
- Oren, R., S. Takahashi, C. Doss, R. Levy, and S. Levy. 1990. Tapa-1, the target of an antiproliferative antibody, defines a new family of transmembrane proteins. Mol. Cell. Biol. 10:4007– 4015.
- Pearson, W. R., and D. J. Lipman. 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA 85:2444-2448.
- Pepperkok, R., M. Zanetti, R. King, D. Delia, W. Ansorge, L. Philipson, and C. Schneider. 1988. Automatic microinjection system facilitates detection of growth inhibitory mRNA. Proc. Natl. Acad. Sci. USA 85:6748–6752.
- 36. Pertovaara, L., L. Sistonen, T. J. Bos, P. K. Vogt, J. Keski-Oja, and K. Alitalo. 1989. Enhanced *jun* gene expression is an early genomic response to transforming growth factor β stimulation. Mol. Cell Biol. 9:1255-1262.
- Pietenpol, J. A., J. T. Holt, R. W. Stein, and H. L. Moses. 1990. TGFβ1 suppression of c-myc gene transcription: role in inhibition of keratinocyte proliferation. Proc. Natl. Acad. Sci. USA 87:3758-3762.
- 38. Pietenpol, J. A., R. W. Stein, E. Moran, P. Yaciuk, R. Schlegel, R. M. Lyons, M. R. Pittelkow, K. Münger, P. M. Howley, and H. L. Moses. 1990. TGF-β1 inhibition of c-myc transcription and growth in keratinocytes is abrogated by viral transforming

proteins with pRB binding domains. Cell 61:777-785.

- Postlethwaite, A. E., J. Keski-Oja, H. L. Moses, and A. H. Kang. 1987. Stimulation and chemotactic migration of human fibroblasts by transforming growth factor beta. J. Exp. Med. 165: 251-256.
- 40. Roberts, A. B., S.-J. Kim, and M. B. Sporn. 1991. Is there a common pathway mediating growth inhibition by TGF- β and the retinoblastoma gene product? Cancer Cells 3:19–21.
- 41. Roberts, A. B., M. B. Sporn, R. K. Assoian, J. M. Smith, N. S. Roche, L. M. Wakefield, V. I. Heine, L. A. Liotta, V. Falanga, J. H. Kehrl, and A. S. Fauci. 1986. Transforming growth factor type-β: rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro*. Proc. Natl. Acad. Sci. USA 83:4167-4171.
- 42. Rollins, B. J., E. D. Morrison, and C. D. Stiles. 1988. Cloning and expression of JE, a gene inducible by platelet-derived growth factor and whose product has cytokine-like properties. Proc. Natl. Acad. Sci. USA 85:3738–3742.
- Rollins, B. J., P. Stier, T. Ernst, and G. G. Wong. 1989. The human homolog of the JE gene encodes a monocyte secretory protein. Mol. Cell. Biol. 9:4687–4695.
- Schneider, C., R. M. King, and L. Philipson. 1988. Genes specifically expressed at growth arrest of mammalian cells. Cell 54:787-793.
- 45. Schwarz, E. M., X. Fan, B. Kallin, V. Sorrentino, and B. R. Bloom. Unpublished data.
- 46. Sorrentino, V., and S. Bandyopadhyay. 1989. TGFβ inhibits G0/S-phase transition in primary fibroblasts. Loss of response to the antigrowth effect of TGFβ is observed after immortalization. Oncogene 4:569-574.
- 47. Stanley, K. K. 1989. Techniques in molecular and cell biology. A laboratory manual. Version 6. EMBL, Heidelberg, Germany.
- Szala, S., Y. Kasai, Z. Steplewski, U. Rodeck, H. Koprowski, and A. J. Linnenbach. 1990. Molecular cloning of cDNA for the human tumor-associated antigen Co-029 and identification of related transmembrane antigens. Proc. Natl. Acad. Sci. USA 87:6833-6837.
- 49. Takehara, K., E. C. LeRoy, and G. R. Grotendorst. 1987. TGF-beta inhibition of endothelial cell proliferation: alteration of EGF binding and EGF-induced growth-regulatory (competence) gene expression. Cell **49**:415–422.
- Travis, G. H., M. B. Brennan, P. E. Danielson, C. A. Kozak, and J. G. Sutcliffe. 1989. Identification of a photoreceptor-specific mRNA encoded by the gene responsible for retinal degeneration slow (rds). Nature (London) 338:70-73.
- 51. Wahl, S. M., D. A. Hunt, L. M. Wakefield, N. McCartney-Francis, L. M. Wahl, A. B. Roberts, and M. B. Sporn. 1987. Transforming growth factor type β induces monocyte chemotaxis and growth factor production. Proc. Natl. Acad. Sci. USA 84:5788-5792.
- Wright, M. D., K. J. Henkle, and G. F. Mitchell. 1990. An immunogenic M_r 23,000 integral membrane protein of *Schisto*soma mansoni worms that closely resembles a human tumorassociated antigen. J. Immunol. 144:3195-3200.