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Genetics

Constitutive overexpression of a growth-regulated gene in transformed Chinese hamster and human cells

(*gro*/CHEF cells/tumor cells/transcriptional regulation)

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ABSTRACT **Comparison by subtractive hybridization of mRNAs revealed a moderately abundant message in highly tumorigenic CHEF/16 cells present at very low levels in closely related nontumorigenic CHEF/18 cells. After cloning and sequencing the corresponding cDNA, computer comparison showed closest homology with the human connective tissue-activating peptide III (CTAP III). The human tumor cell cDNA hybridizing with the Chinese hamster clone was isolated, sequenced, and found to have closer similarity to the Chinese hamster gene than to CTAP III. Thus, the cloned cDNAs from Chinese hamster and human cells represent a different gene, named *gro*. Studies of its transcriptional regulation have shown that expression is tightly regulated by growth status in normal Chinese hamster and human cells and relaxed in the tumorigenic cells so far examined.**

CHEF/18 and CHEF/16 are a pair of Chinese hamster fibroblastic cell lines derived from a single embryo (1). CHEF/18 cells, though immortal, are diploid and consistently nontumorigenic in nude mice, whereas CHEF/16 cells are diploid and highly tumorigenic, producing aneuploid tumors. We have used subtractive hybridization (2, 3) to investigate the molecular basis of tumorigenicity differences between these two cell lines.

We describe here the identification of a gene overexpressed in CHEF/16 cells compared with CHEF/18 cells. The sequence of the cloned cDNA from CHEF/16* shows considerable homology with a human platelet-derived protein, connective tissue-activating peptide III (CTAP III) (4). A related gene has been reported to be overexpressed in *src*-transformed chicken cells (5).

Using the Chinese hamster cDNA as probe, we have isolated a human gene from a human cDNA library made from tumor cells that overexpress the gene. The human gene* shows closer homology to the Chinese hamster gene than to human CTAP III or to any other member of this gene family. On this basis, it seems likely that these genes are not homologs of CTAP III but rather that both the hamster and human genes are different from CTAP III and are homologs of one another. These genes, initially called the group I genes, are now named *gro* (growth regulated).

The transcriptional regulation of *gro* is of particular interest. We have found that transcription is tightly regulated by the growth status in normal Chinese hamster and human cells. In tumorigenic CHEF/16 cells and in tumor-derived human T24 cells, however, growth regulation is relaxed and resulted in elevated constitutive expression levels. Thus, the *gro* gene resembles c-*myc* in growth regulation (6), showing tight control of mRNA levels in normal cells and relaxed control in tumor cells. As such, *gro* represents an additional member of the family of growth-regulated genes as well as shows sequence relatedness to the inflammatory response gene family.

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MATERIALS AND METHODS

Origin and Culture of Cell Lines. The origin and properties of the CHEF/18, CHEF/16, and 205-30 cell lines have been described (1). Cell line 205-30, a thioguanine-resistant derivative of CHEF/18, was the source of the poly(A)$^+$ RNA to be used as the normal partner in the subtractive hybridization. CHEF/16 cells are diploid but have lost their anchorage and epidermal growth factor requirements for growth and are tumorigenic in the nude mouse assay (1). T24 is a cell line derived from a human bladder carcinoma explant (7). FS-2 cells are diploid human fibroblasts (8); FSSV-27neoEJ and FSVK-27 are transformed strains derived from FS-2 (9).

Chinese hamster cells were grown and maintained in alpha minimal essential medium (α-MEM) supplemented with 10% fetal bovine serum [Hyclone (Logan, UT) or J.R. Scientific (Woodland, CA)]/2 mM glutamine/penicillin (100 units/ml)/streptomycin (100 μg/ml) in humidified 6.5% CO_2/93.5% air at 37°C and pH 7.3. All cell lines were found free of mycoplasma contamination by the Mycotech assay (GIBCO). Human cells were grown as above but supplemented with 2 g of glucose per liter, insulin at 10 μg/ml, and 1% human serum (GIBCO).

Preparation and Analysis of RNA and DNA. Cells were harvested in middle to late logarithmic phase. Total RNA was prepared by the guanidinium/CsCl method (10). Poly(A)$^+$ RNA was selected by two cycles of chromatography over oligo(dT)-cellulose (10). Genomic DNA was prepared as described (11). Small-scale plasmid preparations were prepared by the alkaline lysis method (10). Large-scale plasmid preparations were prepared by the NaCl/NaDodSO$_4$ lysis method (10) and purified through two CsCl/ethidium bromide centrifugations. RNA was electrophoresed on 1.2% agarose/formaldehyde gels and transferred to nitrocellulose. Southern and RNA transfer blots were performed as described (10) and screened with 10^7 cpm per filter of either CHEF/16 or T24 random primer (12) labeled cDNA representing the group 1 gene. DNA sequences were determined by the method of Maxam and Gilbert (13).

Preparation of Vector DNA. The cDNA cloning vector pSV2 was constructed in two steps from pSV2DHFR (where DHFR = dihydrofolate reductase) (14). First, the *Eco*RI site of pSV2DHFR was eliminated by cutting with *Eco*RI, blunt-ending with T4 polymerase, and ligating to form circles. In the second step, the DHFR insert was cut out by *Hin*dIII/*Bgl* II digestion. The plasmid was then blunt-ended, ligated to *Eco*RI linkers, cut with *Eco*RI, recircularized, and purified. The resulting vector possesses a unique *Eco*RI cloning site

Abbreviations: CTAP III, connective tissue-activating peptide III; CHX, cycloheximide; PBP, platelet basic protein.

*These sequences are being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession nos. J03560 and J03561).

between the simian virus 40 promoter/enhancer and the poly(A)/splice signals.

Preparation and Cloning of cDNA. First-strand cDNA synthesis from 10 μg of CHEF/16 or T24 poly(A)$^+$ RNA was carried out using avian myeloblastosis virus reverse transcriptase (15). Second-strand synthesis using RNase H and DNA polymerase was as described (16). The resulting double-stranded cDNA was blunt-ended, methylated at *Eco*RI sites, ligated to *Eco*RI linkers, cut with *Eco*RI, and purified on an A50M column (15). cDNAs >500 base pairs (bp) were ligated with an equimolar amount of *Eco*RI-cleaved alkaline phosphatase-treated pSV2 vector. Transformation of *Escherichia coli* strain MC1061 with the ligation mix gave 5×10^7 transformants per μg of closed circular vector.

Subtractive Hybridization. Cell lines 205-30 (CHEF/18) and CHEF/16 were used as the normal and tumorigenic pair. First-strand cDNA was synthesized (3) from 2 μg of CHEF/16 poly(A)$^+$ RNA. Hybridization was carried out in a 10-μl reaction volume containing 500 mM sodium phosphate (pH 6.8), 0.05% $NaDodSO_4$, 5 mM EDTA, the labeled CHEF/16 cDNA, and 10 μg of 205-30 poly(A)$^+$ RNA. The reaction mix was heated to 95°C for 2 min to denature the nucleic acids, rapidly cooled on ice, and then hybridized for 20–30 hr at 68°C (to 1500 R_0t). The hybridization mix was loaded on hydroxyapatite in 0.03 M sodium phosphate/0.05% $NaDodSO_4$ at 60°C. The single-stranded fraction was eluted with 0.12 M sodium phosphate/0.05% $NaDodSO_4$ at 60°C and concentrated on an Amicon 30 microconcentrator; this was followed by a second round using the same procedures. After two rounds of subtraction, 17.5% of the original labeled CHEF/16 single-stranded cDNA remained in the unhybridized fraction. When this cDNA was hybridized to CHEF/16 poly(A)$^+$ RNA to a R_0t of 3000, only 16% hybridized. Thus, the final subtracted probe represents 2.8% of the hybridizable sequences in total CHEF/16 cDNA.

Colony Hybridization. Colonies from the CHEF/16 cDNA library were replica-plated at 2000 colonies per 82-mm nitrocellulose filter, hybridized to 2.5×10^5 cpm of subtracted probe, washed, and autoradiographed. Positive colonies were replica-plated after growth in microtiter wells and rescreened with subtracted probe as well as with labeled 205-30 cDNA. Colonies from the T24 library were screened with 2×10^6 cpm of random primer-labeled cDNA representing the CHEF/16 group 1 gene.

Transcription Assays. Nuclei were isolated from $\approx 5 \times 10^7$ exponential cells and nuclear run-off transcription assays were performed as described (17) with some modifications.

Probes. The *fos* probe was the 1-kilobase (kb) *Pst* I/*Pvu* II fragment from PEBJ-2 (18). pA1 is chicken β-actin (19); pRAH 3.2 is mouse histone 3.2 (20); pMH2b is mouse histone 2b (20).

RESULTS

Subtractive Hybridization. Labeled first-strand CHEF/16 cDNA was hybridized with a large excess of 205-30 poly(A)$^+$ RNA. Nonhybridizing sequences recovered from a hydroxyapatite column were used to select clones from a CHEF/16 cDNA library prepared in the pSV2 expression plasmid. Of 263 initially positive colonies, 48 hybridized weakly or not detectably with 205-30 cDNA. Of these, 5 detected an mRNA that was greatly overexpressed in CHEF/16 (50-fold). These clones were shown to give essentially identical restriction fragment patterns when hybridized to Southern blots of restriction enzyme-digested genomic DNA from CHEF/16 cells. Only the investigation of this group of clones (group 1) is presented in this paper. Of the group 1 clones that were isolated, the two longest, CL22 and CL29, were selected for sequencing and characterization. Since their sequences and properties were indistinguishable, as shown below, both will be referred to as the group 1 clone and the *gro* gene.

Regulation of *gro* Gene Expression. The group 1 clone hybridized to a 1.1-kb band in CHEF/16 total RNA (Fig. 1*A*, lanes 1 and 2). In mRNA from CHEF/18 cells (lane 3), two hybridizing bands were seen, a stronger band at 1.2 kb and a weaker one at 0.9 kb. As described below, CHEF/18 cells express the 1.1-kb band after cycloheximide (CHX) pretreatment, and all three bands are expressed in serum-fed cells after serum starvation. The different length mRNAs may represent different transcription start sites or different genes. Mapping the Chinese hamster *gro* gene by *in situ* hybridization has revealed only a single site located on chromosome 1 (G. Stenman, personal communication). Thus, it is unlikely that the mRNA bands correspond to different genes.

A comparison of *gro* mRNA levels in CHEF/18 cells showed tight regulation by growth status. In cells harvested at 25% confluence (lane 3), a low level of hybridization was seen, representing about 2% of the steady-state level in exponential or stationary CHEF/16 cells. At higher confluence, however (lanes 4 and 5), the mRNA decreased and was undetectable in confluent CHEF/18 cells.

CHX pretreatment was used to determine whether ongoing protein synthesis was regulating transcription (21). We found that CHX pretreatment for 3 hr induced a 30-fold increase in *gro* mRNA levels of the 1.1-kb band in CHEF/16 and CHEF/18 cells (Fig. 1*B*). Several exposures were used for quantitation. Nuclear run-on transcription assays were performed with and without CHX to assess the role of transcription initiation in determining steady-state levels. In Fig. 1*C*, run-on transcripts were assayed with nuclei from (70%) confluent cells. In CHEF/16 the run-on rate was about 50-fold higher than in CHEF/18 for the *gro* gene (normalized to the controls). Histone and β-actin run-on rates were used for normalization. After CHX pretreatment (as in Fig. 1*B*), the run-on rates for both CHEF/18 and CHEF/16 were about 10-fold elevated (not shown).

Actinomycin D treatment of CHEF/16 cells was performed to assess *gro* mRNA stability (22) (Fig. 2). With actinomycin D alone, the mRNA was only decreased slightly in 4 hr. With actinomycin D and CHX, there was no measurable decrease. These results indicate high mRNA stability. In the same experiment (not shown), c-*myc* mRNA

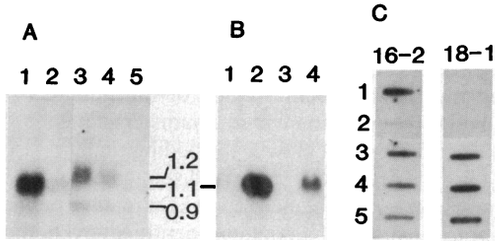

FIG. 1. RNA transfer blot and nuclear run-on analysis of CHEF *gro* mRNA. (*A*) RNA blots of total RNA were hybridized to ^{32}P-labeled CHEF *gro* probe, washed, and autoradiographed for 5 days. Lane 1, 2 μg of 16-2 per ml; lane 2, 0.2 μg of 16-2 per ml; lane 3, 20 μg of 18-1D-3 (25% confluent) per ml; lane 4, 20 μg of 18-1D-3 (50% confluent) per ml; lane 5, 20 μg of 18-1D-3 (confluent) per ml. Sizes are shown in kb. (*B*) Fifty percent confluent cells were refed with fresh medium with or without CHX at 10 μg/ml. Three hours later cells were harvested and total RNA was prepared. RNA blots were hybridized to ^{32}P-labeled CHEF *gro* probe, washed, and autoradiographed for 4 hr. Lane 1, 2 μg of 16-2 per ml; lane 2, 2 μg of 16-2 per ml and CHX; lane 3, 20 μg of 18-1D-3 per ml; lane 4, 20 μg of 18-1D-3 per ml and CHX. (*C*) Nuclear run-on. Ten micrograms of linearized denatured plasma DNA was loaded onto slots and hybridized to 5×10^6 cpm of ^{32}P-labeled nuclear run-on transcripts prepared from CHEF/16-2 or CHEF/18-1D-3. Autoradiography was performed for 3 days. Band 1, CL22 (group 1); band 2, pSV2 (cloning vector alone); band 3, pA1 (chicken β actin) (19); band 4, pRAH3.2 (mouse histone 3.2); band 5, pMH2b (mouse histone 2b) (20).

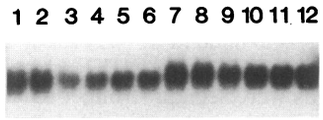

FIG. 2. Analysis of stability of CHEF group 1 message. Subconfluent CHEF/16-2 cells were refed with fresh medium containing 5 μg of actinomycin D per ml (lanes 1–6) or 5 μg of actinomycin D per ml and 10 μg of cycloheximide per ml (lanes 7–12). Total RNA was prepared at various times; 10 μg of RNA per ml was blotted and hybridized to ^{32}P-labeled CHEF *gro* probe. Autoradiography was for 15 hr. Lanes 1 and 7, 0 time; lanes 2 and 8, 15 min; lanes 3 and 9, 30 min; lanes 4 and 10, 1 hr; lanes 5 and 11, 2 hr; lanes 6 and 12, 4 hr.

showed expected lability, disappearing almost completely by 1 hr without CHX ($t_{1/2}$ = 30 min) and becoming somewhat stabilized in the presence of CHX ($t_{1/2} \approx$ 2 hr).

To assess *gro* gene regulation further, the expression of *gro* mRNA was determined during reinitiation of growth after serum starvation of CHEF/18 cells (Fig. 3). Subconfluent cells (50%) were maintained in medium with 0.5% serum for 40 hr. Medium with 10% serum was added and samples were harvested at time points from zero to 8 hr. By 30 min, a significant amount of *gro* mRNA appeared and peak values of all three bands were seen at 1 hr. By 2 hr the mRNA was down to the zero time level. In the same experiment *fos* mRNA peaked at 30 min and then declined as shown. *myc* mRNA peaked at 1 hr and then decreased slowly, still being detectable after 8 hr (not shown).

***gro* Gene Belongs to a Family of Inflammatory Response Peptides.** Two independent Chinese hamster clones were sequenced. Because no discrepancies were found, the reported sequence is probably correct. One human clone (see *Materials and Methods*) was sequenced, the majority of it (including all of the protein coding region) on both strands.

Chinese hamster clone 22 was found to be 928 nucleotides in length; clone 29 was ≈1070 nucleotides in length, extending 3 bases beyond clone 22 in the 5′ direction and ≈160 bases—including an ≈150-base poly(A) tail—beyond its 3′ terminus. Fig. 4 shows the protein coding region of the hamster and human group 1 nucleotide sequences aligned for maximum homology. The probable coding region was identified on the hamster cDNA by the position of the first ATG and by the homology of the signal peptide coding region to similar regions of other genes. The coding region of the human clone was determined by comparison with the hamster sequence. The two mRNAs are 65% identical over the coding region. Also shown in Fig. 4 are the predicted amino acid sequences of the hamster and human clones. Both encode ≈9 kDa mature peptides with ≈20 amino acid signal peptides, identified by homology to known signal peptide sequences (23).

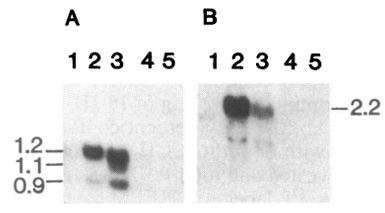

FIG. 3. Serum stimulation of CHEF *gro* message. Fifty percent confluent CHEF/18-1D-3 cells were serum-starved in medium containing 0.5% fetal calf serum for 40 hr, at which time they were refed with fresh medium containing 10% serum. RNA was prepared at various times and blotted. Hybridization was against ^{32}P-labeled CHEF *gro* probe (*A*) and ^{32}P-labeled *fos* probe (18) (*B*). Lanes 1, 0 time; lanes 2, 30 min; lanes 3, 1 hr; lanes 4, 2 hr; lanes 5, 4 hr. Sizes are shown in kb.

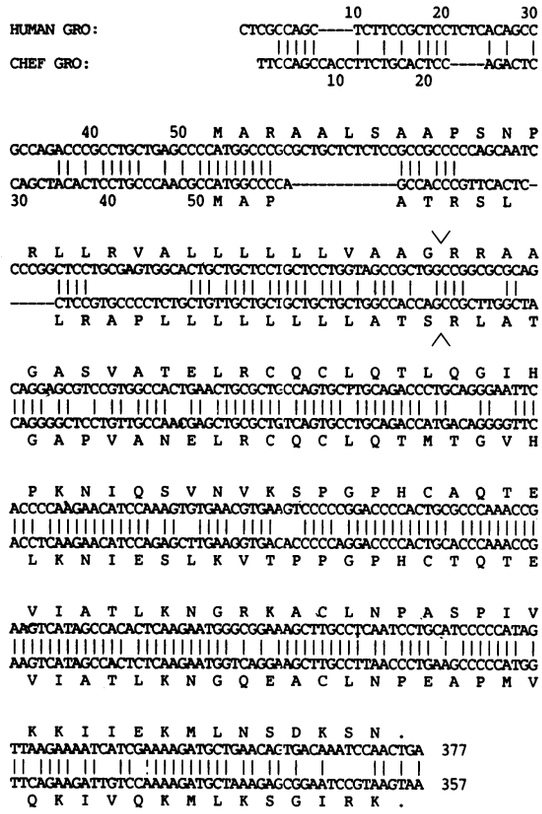

FIG. 4. Sequence and homology of the human and Chinese hamster group 1 (*gro*) mRNAs in the 5′ untranslated and protein coding regions. Predicted amino acid sequence is shown in the standard one-letter code. Carets (⋁, ⋀) indicate the likely peptidase cleavage sites as predicted by a published algorithm (23). Bars (|) between nucleotides indicate an exact match. Gaps are indicated by dashes (----). The amino acid single-letter code is located over the first nucleotide of the triplet code.

Fig. 5 shows the 3′ untranslated regions of the hamster and human mRNAs. As expected, the overall homology between the two sequences is much less in this region. There are local regions of homology: an 82% identical, 26-nucleotide region located ≈120 bases after the stop codon, a 100% identical, 13-nucleotide region located ≈300 bases further in the 3′ direction, and an 86% identical, 57-nucleotide region located another ≈40 bases 3′. Within the first two of these homologous regions are copies of the conserved octamer TTATTTAT found in the 3′ untranslated region of many inflammatory mediators (24). The chance occurrence of this octamer three times in the hamster 3′ region and three times (two overlapping) in the human 3′ region is highly unlikely. Embedded in this octamer is the pentamer ATTTA postulated to signal rapid mRNA degradation (22). This pentamer occurs a total of five times in the human and seven times in the hamster 3′ untranslated cDNA sequences.

The deduced amino acid sequences of the hamster and human group 1 mRNAs are very similar (Fig. 6 and Table 1). In addition, both presumptive proteins show extensive homology to a superfamily of proteins, including human platelet basic protein (PBP) (25), which is the precursor of both β-thromboglobulin (28) and CTAP III (4) (Fig. 4), human platelet factor IV (29), and γ-interferon-inducible peptide (30). Based on amino acid sequence, the *gro* gene is distinct from all of these genes and therefore represents an additional member of this superfamily. Recently, the cDNA cloning of a related gene (called 9E3) from chicken embryo fibroblasts was reported (5). Among the known members of this superfamily, 9E3 shows the most homology to *gro* (Table 1).

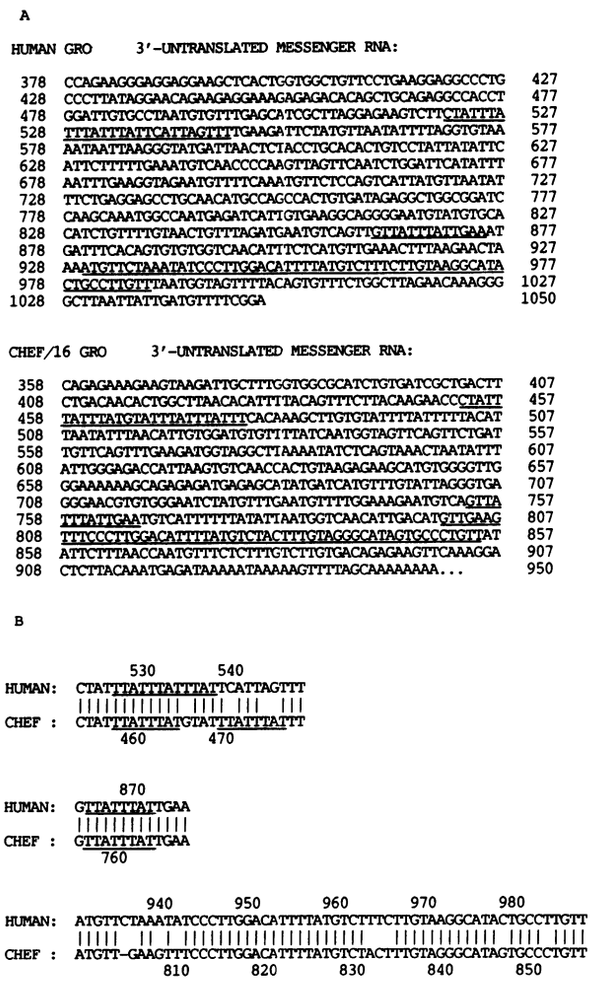

FIG. 5. (*A*) Nucleic acid sequences of the 3′ untranslated regions of the human and Chinese hamster group 1 (*gro*) mRNAs. Underlines denote the conserved regions detailed in *B*, the AATAAA poly(A) signal (CHEF sequence only), and the start of the poly(A) tail (CHEF sequence only). (*B*) Three regions of significant homology between the above sequences. Underlines indicate the TTATTTAT octamers present in the 3′ untranslated regions of many inflammatory response mRNAs (24). Bars (|) between nucleotides indicate exact matches. The dash (-) indicates a gap.

No other proteins in the National Biomedical Research Foundation data base showed significant homology to the predicted mature hamster or human *gro* peptides.

The conservation of the positioning of cysteine residues within this superfamily is particularly significant, suggesting that these peptides have highly conserved tertiary structures. The similarity in number and positioning of the α-helix and

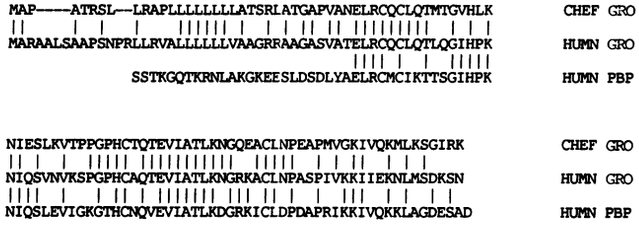

FIG. 6. Comparison of hamster and human predicted mature group 1 peptides to each other and to human platelet basic protein, the precursor for CTAP III and β-thromboglobulin (25). CHEF GRO, CHEF/16 *gro* amino acid sequence. HUMN GRO, human *gro* sequence. HUMN PBP, human platelet basic protein sequence. Bars (|) indicate exact matches.

Table 1. Protein sequence comparison of β-thromboglobulin superfamily

	CHEF gro	Human gro	Human PBP	Human PF4	Bovine PF4	Human γIP	Chicken 9E3
CHEF gro		63 (49)	37	30	24	26 (21)	44 (35)
Human gro			44 (41)	32	30	22 (20)	39 (32)
Human PBP				36	41	23	36
Human PF4					49	22	26
Bovine PF4						20	23
Human γIP							30 (24)
Length	101	107	94	70	88	98	103

Proteins were aligned using the program LOCAL (refs. 26, 27; gap penalty = 0.1). The number of exact matches in the optimal alignment is shown. Since the protein lengths differ, the number of exact matches in the conserved region located between the ninth amino acid in the NH_2 terminus direction from the Cys-Xaa-Cys motif and the COOH terminus is shown in parentheses. PF4, platelet factor 4; γIP, γ-interferon-inducible peptide.

β-turn forming regions (data not shown) reinforces this possibility. In β-thromboglobulin, the cysteines, numbered 1–4 starting with that nearest the NH_2 terminus, are known to disulfide bond 1 with 3 and 2 with 4 (28). Presumably this pattern is also conserved.

Expression of the *gro* Gene in Human Cells. The human cells tested in the following experiments resemble the CHEF cells described above in showing stringent growth-regulated expression of the *gro* gene in normal human cells and relaxed regulation in tumorigenic cells.

The RNA transfer blots in Fig. 7 show that the *gro* gene is growth regulated in normal human fibroblasts (FS-2) cells. Lanes 1 and 2 show FS-2 mRNA extracted from exponentially growing cells and from the same cells after a 3-hr exposure to CHX. Superinduction by CHX produced a 3-fold increase in expression level. In comparing untreated and CHX-treated tumor cells (T24) shown in lanes 3 and 4, the superinduced mRNA level was also about 3-fold elevated. However, the uninduced T24 mRNA level was about 10-fold above the uninduced FS-2 level. The steady-state mRNA levels of FS-2 cells (lane 1) were compared with oncogene-transformed derivatives of FS-2. In simian virus 40-transformed FS-2 cells (8, 9) transfected with pSV2neoEJ (lane 5) or infected with Kirsten murine sarcoma virus (lane 6), the steady-state levels of *gro* mRNA were elevated, comparable to the level seen in T24 (lane 3) (after normalization for differences in loading concentrations).

DISCUSSION

We have described the gene *gro* and presented preliminary evidence for its participation in two cellular processes. Our studies of mRNA expression levels have shown that the gene is tightly regulated transcriptionally in normal cells by growth status and that this regulation is relaxed in transformed cells. These results suggest that *gro* plays an important role in cellular growth control.

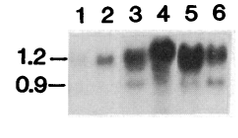

FIG. 7. RNA transfer analysis of human *gro* mRNA. RNA blots of 20 μg of total RNA were hybridized to ^{32}P-labeled human *gro* probe, washed, and autoradiographed. Lane 1, FS2; lane 2, FS2 and 10 μg of CHX per ml; lane 3, T24; lane 4, T24 and 10 μg of CHX per ml; lane 5, FSSV27 neoEJ; lane 6, FSVK27. Sizes are shown in kb.

Secondly, we found that the *gro* gene product shows considerable structural relatedness to CTAP III (4) and other members of a family of inflammatory response peptides. In addition, protein comparisons indicate strong similarities in three-dimensional structure based on positioning of four cysteines and on α-helix and β-turn predictions. Thus, on structural grounds, we would anticipate that *gro* plays some role in the inflammatory response. The related human protein CTAP III is platelet derived and reported to function as a paracrine stimulator of hyaluronic acid synthesis, plasminogen activator secretion, and DNA replication by synovial fibroblasts (4).

In unpublished experiments we examined the effects of *gro* overexpression by transfecting the Chinese hamster *gro* cDNA into CHEF/18 cells and using indirect selection for G418 resistance to recover transfectants. Neither CL22 nor CL29 cDNA in the pSV2 expression vector was found to confer any distinctive properties upon CHEF/18 cells when stably introduced by DNA-mediated gene transfer, although the transfected cDNA was transcribed at elevated levels (comparable to CHEF/16) and the mRNA was found to be associated with polysomes. The introduced gene did not induce hyaluronic acid synthesis or plasminogen activator production. Furthermore, in growth studies the transfectants did not acquire epidermal growth factor-independent or anchorage-independent growth, altered morphology, or tumor-forming ability.

Thus, the *gro* gene did not confer any of the distinctive CTAP III-associated properties on CHEF/18 cells. Experiments to assay paracrine activity in conditioned medium were also ineffective with CHEF/18 cells (unpublished) but may require a different target cell with suitable receptors to elicit a response. Nor did the transfected gene induce any properties typical of transformed fibroblasts. The *gro* gene may act in concert with other genes involved in cell growth and, if so, their characterization may be necessary to understand *gro* gene function.

The difference in transcription rates between CHEF/16 and CHEF/18 cells may result from the decreased activity of a labile transcriptional repressor in CHEF/16 cells, resulting from a mutation in the repressor binding site or in the repressor itself. Steady-state mRNA levels could be influenced further by differential mRNA stability, perhaps mediated by the ATTTA sequences (22). Lability of *gro* mRNA was seen in serum-stimulated CHEF/18 cells (Fig. 3) but not in exponentially growing CHEF/16 cells (Fig. 2).

The serum-stimulator experiment demonstrates early growth responsiveness but does not distinguish between cell-cycle control and a growth response evoked by starvation and refeeding (31). However, the sensitivity of mRNA expression to cell-cycle control was seen in experiments comparing the steady-state levels of *gro* mRNA in CHEF/18 cells harvested to low, moderate, and high confluence (Fig. 1).

The structural relatedness of the Chinese hamster and human *gro* genes is impressive. Fig. 4 shows that nucleotides are frequently conserved even in the third position of the coding triplet. Also, the signal peptide is highly conserved. Most remarkable is the nucleotide conservation in the 3′ noncoding region shown in Fig. 5. The first two sequences in Fig. 5*B* contain the TTATTTAT repeats (24). The third sequence is unique in the length of the conserved region, suggesting an important function.

Thus, the strong conservation of sequence as well as the tight regulation of gene expression both indicate the importance of the *gro* gene, despite the fact that no function has yet been identified.

It is likely that *gro* gene transcription is determined by a class of cis-acting signals and corresponding trans-acting factors that also exert tight control on the expression of other growth-regulated genes. Thus, further analysis of *gro* gene regulation should provide insights into normal growth regulation and its dysfunction in tumor cells. Finally, we suggest that the trans-acting factors that determine stringent transcriptional control of the *gro* gene may represent an example of tumor suppressor gene-encoded products.

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