Negative and Positive cis-Acting Elements in the Promoter of the Mouse Gene That Encodes the Serine/Glycine-rich Peptide Core of Secretory Granule Proteoglycans*

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The gene that encodes a proteoglycan peptide core rich in serine and glycine (SG-PG) is selectively expressed by hematopoietic cells that store in their cytoplasmic granules negatively charged proteoglycans bound ionically to numerous positively charged proteins. With deletion analysis, a negative transcription regulatory element was located between residues -250 and -180 of the 5' flanking region of the mouse SG-PG gene, and a positive regulatory element was located between residues -118 and -81. The negative regulatory element was predominantly active in fibroblasts that do not express the SG-PG gene whereas the positive regulatory element was predominantly active in hematopoietic cells that do express the SG-PG gene. Site-directed mutagenesis was used to demonstrate that the proximal element within the gene's atypical promoter resided between residues -40 and -20. As assessed by gel mobility shift analyses, the nuclei of rat basophilic leukemia-1 cells and rat-1 fibroblasts contain a number of trans-acting factors that interact with the positive and negative cis-acting regulatory elements of the SG-PG gene. Furthermore, some of these trans-acting factors appear to be different for the two cell types. These studies on cell types that do and do not express the SG-PG gene indicate that transcription of this proteoglycan peptide core gene is regulated constitutively by both positive and negative cis-acting elements located 5' of an atypical promoter.

Many cells of hematopoietic origin (including serosal mast cells, mucosal mast cells, basophils, natural killer cells, cytotoxic T lymphocytes, eosinophils, macrophages, and platelets) store a family of proteoglycans in a cytoplasmic granule compartment that is distinct from the plasma membrane-localized and extracellular matrix-localized families of proteoglycans (1). These intracellular proteoglycans have five to seven glycosaminoglycans attached O-glycosidically to a common 18,600-16,700 M, peptide core possessing a protease-resistant glycosaminoglycan attachment region that is a repeat of serine and glycine amino acids (2-13). Because these proteoglycans are bound by ionic linkage in the secretory granules of mouse and rat mast cells to positively charged endopeptidases and exopeptidases that are enzymatically active at neutral pH, it has been assumed that they prevent intragranular autolysis of the proteases. The proteoglycan/protease macromolecular complexes remain intact when they are exocytosed from activated mast cells (14-17), presumably attenuating diffusion of the proteases from inflammatory sites and facilitating concerted proteolysis of protein substrates.

cDNAs that encode a proteoglycan peptide core rich in serine and glycine (SG-PG)* have been isolated from rat (7-9), mouse (10), and human (11-13) cDNA libraries. These cDNAs encode 1.0-, 1.0-, and 1.3-kb transcripts in the mouse, rat, and human, respectively. The mouse SG-PG gene resides on chromosome 10, is ~15 kb in size, and consists of three exons (10). When rat-1 fibroblasts were stably transfected with the mouse genomic clone, λ-MG-PG1, two cell lines were obtained which expressed low levels of the 1.0-kb SG-PG mRNA (18). This finding indicated that λ-MG-PG1 contained the entire mouse SG-PG gene, including some of the regulatory elements within its promoter region. S1 nuclease mapping and primer extension analysis revealed that the primary transcription initiation site for this gene in mouse bone marrow-derived mast cells resides ~40 nucleotides upstream of the translation initiation site (18). Although no classical TATA box is present ~30 nucleotides upstream of the transcription initiation site in either the mouse or human gene, the 333 base pair (bp) nucleotide sequence 5' of the transcription initiation site of the mouse gene is nearly identical to the corresponding region of the human gene (19). Because of this high degree of conservation, it seemed likely that this 5'-flanking region contains cis-acting elements that regulate constitutive expression of the SG-PG gene in hematopoietic cells.

In the present study, we have linked the 504-bp 5'-flanking region of the mouse SG-PG gene to plasmid DNA that contains the structural sequences of the human growth hormone (hGH) reporter gene and have quantitated the amount of hGH produced by different cell types transfected with the resulting plasmid construct. With deletion analysis and site-directed mutagenesis, three motifs in the 5'-flanking region of the mouse SG-PG gene were identified which regulate its constitutive transcription. One of these elements suppresses transcription of the gene whereas the other two elements

* The abbreviations used are: SG-PG, serine/glycine-rich proteoglycan peptide core; bp, base pair(s); hGH, human growth hormone; RBL-1, rat basophilic leukemia-1; Hepes, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid.

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enhance its transcription. As indicated by gel mobility shift assays, hematopoietic cells that express the SG-PG gene possess trans-acting factors in their nuclei which recognize these elements, and a different profile of trans-acting factors is present in fibroblasts that do not express the SG-PG gene.

**EXPERIMENTAL PROCEDURES**

**Cell Lines**—Rat basophilic leukemia-1 (RBL-1) cells (line CRL-1378; American Tissue Culture Collection (ATCC), Rockville, MD) and mouse myelomonocytic WEHI-3 cells (line TIB-68; ATCC) are cell lines of hematopoietic origin which abundantly express the SG-PG gene. The cells (1 × 10⁶/dish) were then cultured in 10 ml of enriched medium, 0.2 ml of a 250 mM solution of calcium chloride, and 10% fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (GIBCO) at 37 °C in a humidified atmosphere of 6% CO₂. No matter which DNA construct was used in the transfection, 100 h later each culture dish contained ~4 × 10⁶ fibroblasts. Approximately 100 h after the transfection by either method, 0.1-ml samples of culture medium were removed, and the levels of hGH were determined with an immunoassay kit from Nichols Institute Diagnostics (San Juan Capistrano, CA). The absolute amounts of hGH in the culture media for each cell type is preferable to a comparison of the amounts of absorbed labeled anti-hGH antibody in the sandwich assays.

The results of the transfection assays were normalized for transfection efficiency using pXGH5. The amount of hGH produced by pXGH5-transfected cells was arbitrarily assigned a value of 1, and then the relative amount of hGH produced by each cell type transfected with the test plasmid was calculated as a ratio to that obtained with this control plasmid. To determine the promoter activity of various DNA constructs in dissimilar cell types transfected by different methods, a comparison of the relative amounts of hGH in the culture media for each cell type is preferable to a comparison of the absolute amounts of hGH (24). It has been reported in other studies (22, 24, 25) that the amount of growth hormone produced by cells transfected with different hGH constructs is related to the amount of hGH mRNA in the transfected cells. To confirm that the variation in the amount of hGH in the culture media of cell lines transfected with different constructs reflects a change in the level of hGH mRNA in the cells, total RNA was isolated from transfected RBL-1 cells and rat-1 fibroblasts. Blots containing total RNA (10 μg/sample) were then prepared and probed with a 32P-labeled 950-bp BglII/EcoRI fragment of the hGH cDNA present in pGH.H.

**SG-PG Protein Binding**—Nuclear extracts were prepared from RBL-1 cells and rat-1 fibroblasts by a modification of the procedure described by Dignam and co-workers (31). Each preparation of pelleted cells (10⁶) was washed once with 2.5 ml of ice-cold 10 mM Hepes (pH 7.9), 1.5 mM MgCl₂, 10 mM KCl, 1.0 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 0.1% leupeptin, 0.1% pepstatin, and 0.1% aprotonin. After a 10-min incubation at 4 °C in the same buffer, the cells were centrifuged for 3 min at 500 × g. The pellet was resuspended in 1.0 ml of ice-cold buffer, lysed in a Dounce homogenizer, and centrifuged at 4 °C for 10 min at 900 × g and then for 20 min at 16,000 × g. The supernatants were aspirated, and the pelleted nuclear proteins were resuspended in 60 μl of a buffer containing 0.25 M sucrose, 5 mM MgCl₂, 50 mM KCl, 50 mM Hepes (pH 7.9), 25% glycerol, 1.5 mM MgCl₂, 0.42 mM NaCl, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 0.2% Nonidet P-40, 0.1% leupeptin, 0.1% pepstatin, and 0.1% aprotonin. The pellets were homogenized again in a Dounce homogenizer, agitated gently for 3 min, and centrifuged at 4 °C for 30 min at 100,000 × g. The solubilized nuclear proteins in each supernatant were dialyzed at 4 °C for 5 h against a 50-fold excess volume of 20 mM Hepes (pH 7.9), 20% glycerol, 0.1 mM KCl, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 0.1% leupeptin, 0.1% pepstatin, and 0.1% aprotonin and then stored at −80 °C in this condition until the protein concentrations of each nuclear extract were determined by the Bradford method (32) using a Bio-Rad protein assay kit with bovine serum albumin as standard.

DNA/protein binding experiments were carried out with 620 μl of 10 mg/ml Tris buffer (pH 7.5) containing 4% glycerol, 1 mM EDTA, 0.5 mM dithiothreitol, 0.1 M NaCl, 4 μg of carrier poly(dI-dC) (Stratagene, La Jolla, CA), and 1 ng of a double-stranded ³²P-endlabeled DNA probe corresponding to residues −250 to −161, residues −81 to −81, or residues −40 to +24 of the mouse SG-PG gene (18). In the binding competition assays, 5 ng of the specific SG-PG-derived unlabeled oligonucleotide, 100 ng of unlabeled sonicated salmon sperm DNA, or 100 ng of a 64-mer unlabeled double-stranded oligonucleotide that binds NFI/CTF, SP1, API, and AP3 (Stratagene) was added to each reaction. After incubation at 25 °C for 30 min, gel mobility shift analyses were performed to detect the presence of specific DNA-binding proteins in the nuclear extracts. Samples were loaded onto a 5% nondenaturing polyacrylamide/ bisacrylamide gel (30:1, w/w) that had been equilibrated before use by treatment for 1 h at 100 mA. The gels were run at 100 mA at 4 °C until the bromophenol blue tracking dye ran approximately two-thirds the length of the gel. The gels were then dried under vacuum and autoradiographed generally for 16–24 h.

Two control mixing experiments were used to confirm that the 620-bp probe corresponding to residues −250 to −161 of the mouse SG-PG gene bound to distinct trans-acting factors in nuclear extracts of RBL-1 cells and rat-1 fibroblasts. In the first experiment, 5 × 10⁶ fibroblasts and 5 × 10⁶ rat-1 fibroblasts were cultured for 10 days, and then a nuclear extract of the pooled cells was prepared and analyzed in the gel mobility shift assay. In the second experiment, nuclear
blasts were transfected with constructs pPG(-190/+24)hGH and pPG(-118/+24)hGH, production of hGH was also enhanced when RBL-1 cells were transfected with construct pPG(-118/+24)hGH; production of hGH was enhanced -2.5-fold when RBL-1 cells were transfected with pPG(-504/+24)hGH. As shown in Table I, the relative amount of hGH present in the 4-day conditioned medium of transfected mouse WEHI-3 cells and RBL-1 cells was 20-18-fold higher than for transfected fibroblasts of the respective species. Therefore, the 504-bp region immediately upstream of the transcription initiation site of the mouse SG-PG gene contains cis-acting elements that preferentially enhance transcription of this gene in hematopoietic cells.

To locate more precisely these cis-acting elements, nine additional plasmid constructs were prepared which had progressive deletions of the 5'-flanking region of this mouse gene fused to the hGH gene in ppg. As shown in Fig. 1A, RBL-1 cells and rat-1 fibroblasts transfected with constructs pPG(-423/+24)hGH, pPG(-333/+24)hGH, and pPG(-250/+24)hGH produced amounts of hGH comparable to the corresponding cells transfected with pXGH5. The production of hGH was enhanced ~2.5-fold when RBL-1 cells were transfected with construct pPG(-190/+24)hGH; production of hGH was also enhanced when RBL-1 cells were transfected with the pPG(-118/+24)hGH construct. When rat-1 fibroblasts were transfected with constructs pPG(-190/+24)hGH and pPG(-118/+24)hGH, production of hGH increased 21-fold and 24-fold, respectively. Therefore, at least one cis-acting element resides between -250 and -190 which suppresses transcription of the SG-PG gene in cells, and this negative element is dominantly active in fibroblasts. Although RBL-1 cells and rat-1 fibroblasts transfected with constructs pPG(-81/+24)hGH, pPG(-63/+24)hGH, and pPG(-40/+24)hGH produced some hGH, the amount was substantially less than that produced by cells transfected with construct pPG(-118/+24)hGH. Thus, at least one cis-acting element in the nucleotide sequence -118 to -81 constitutively enhances transcription of the SG-PG gene in RBL-1 cells and fibroblasts. When normalized for the efficiency of transfection, RBL-1 cells transfected with construct pPG(-118/+24)hGH produced 2.7-fold (p < 0.05) more hGH than similarly transfected fibroblasts, indicating that the positive regulatory element between residues -118 and -81 is more dominantly active in RBL-1 cells than in fibroblasts.

To determine if the positive cis-acting element in residues -118 to -44 of the mouse SG-PG gene functions as an enhancer and to confirm that the negative cis-acting element resides upstream of the positive regulatory element, two 5' flanking regions of the gene were ligated into pSV40-hGH to create constructs pPG(-250/-44)SV40-hGH and pPG(-118/-44)SV40-hGH (Fig. 2). RBL-1 cells and rat-1 fibroblasts were then transfected with pSV40-hGH or with one of the two plasmid constructs, and the relative levels of hGH in the culture media were determined. Greater than 3-fold more hGH was detected in the culture medium of RBL-1 cells and rat-1 fibroblasts transfected with pPG(-118/-44)SV40-hGH relative to pSV40-hGH, indicating that the positive regulatory element in this 5'-flanking region of the gene functions as an enhancer. This regulatory element also induced rat-1 fibroblasts to produce ~3.5-fold more hGH when linked in the plasmid in its opposite orientation 2.6 kb upstream of the SV40 early promoter (Fig. 2). The finding that the level of hGH produced by fibroblasts transfected with pPG(-250/-44)SV40-hGH is approximately one-half that of fibroblasts transfected with pPG(-118/-44)SV40-hGH again indicates that there is a negative cis-acting element within

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**Table I**

| Plasmid construct | Mouse fibroblasts | Mouse WEHI-3 | Rat-1 fibroblasts | RBL-1 cells | WEHI-3/mouse fibroblasts | RBL-1/rat fibroblasts |
|-------------------|-----------------|-------------|-----------------|-------------|-------------------------|---------------------|
| pXGH5             | 1.0             | 1.0         | 1.0             | 1.0         | 1.0                     | 1.0                 |
| pSV40-hGH         | ND*             | ND*         | 0.29 ± 0.06     | ND*         | ND*                     | 1.6                 |
| pTKGH             | 0.30 ± 0.05     | 0.11 ± 0.04 | ND              | 0.72 ± 0.20 | 0.3                     | ND*                 |
| pPG(-504/+24)hGH  | 0.01 ± 0.02     | 0.20 ± 0.05 | 0.04 ± 0.01     | 0.72 ± 0.12 | 20                      | 18                  |

*Results are expressed as the mean ± SD of five to six experiments of 4 days duration, with each experiment performed on two replicate dishes of cells.

*ND, not determined.
cis-Acting Regulatory Elements

**Fig. 2.** Effect of two 5′-flanking regions of the mouse SG-PG gene on the ability to enhance and suppress hGH production in cells transfected with plasmid constructs that contain an enhancerless SV40 early promoter. The hatched lines (Δ) and the round-dot lines (○) represent the structural sequences of the hGH gene and SV40 early promoter, respectively, within the plasmid construct. The solid, bold horizontal lines (■) represent the specific parts of the 5′-flanking region of the mouse SG-PG gene that are inserted upstream of the SV40 promoter in pSV04-hGH. The numbers on the right are the hGH values obtained at 4 days relative to those cells transfected with the control plasmid, pSV04-hGH. The indicated hGH activities represent the mean ± S.D. values of data from five to six experiments of 4-days duration, with each experiment performed on two or three replicate dishes of cells.

| Nucleotide sequence | Mutation position | Relative expression of hGH* |
|---------------------|-------------------|-----------------------------|
| ACCCTTTTTCTAAAGGG   | None              | RBL-1 cells: 1.00 Rat-1 fibroblasts: 1.00 |
|                     | (native)          |                             |
| ACCCTTTTTCTAAAGGG   | -28 bp            | RBL-1 cells: 0.32 ± 0.04 Rat-1 fibroblasts: 0.38 ± 0.06 |
|                     | (mutated)         |                             |
| GCCCTTTTTCTAAAGGG   | -30 bp            | RBL-1 cells: 0.09 ± 0.02 Rat-1 fibroblasts: 0.17 ± 0.03 |
|                     | (mutated)         |                             |
| GCCCTTTTTCTAAAGGG   | -38 bp            | RBL-1 cells: 6.43 ± 0.02 Rat-1 fibroblasts: 0.69 ± 0.02 |
|                     | (mutated)         |                             |

*Results are expressed as the mean ± S.D. range of two experiments with each experiment performed on eight replicate dishes of cells.

**Table II**

Relative human growth hormone production by rat basophilic leukemia-1 cells and rat-1 fibroblasts transfected with constructs containing a normal and mutated proximal promoter region of the mouse SG-PG gene

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**Fig. 1.** A, effects of progressive deletion of the 5′-flanking region of the mouse SG-PG gene on its ability to direct hGH expression in transfected cells. The solid, bold horizontal lines (■) represent the various lengths of the 5′-flanking region of the mouse SG-PG gene ligated to pDGH, a plasmid that contains a promoterless hGH gene. The negative and positive numbers in the various constructs refer to the length of the nucleotide sequence that extends upstream and downstream, respectively, of the transcription initiation site of the mouse SG-PG gene. In each experiment, the amount of hGH was quantitated 4 days after transfection of RBL-1 cells and rat-1 fibroblasts with the specific plasmid construct. The numbers on the right are the hGH values obtained relative to the same population of cells transfected with the control plasmid construct, pXGH5. The indicated hGH activities represent the mean ± S.D. of data from 6 to 18 experiments. ND, not determined. B, gel analysis of hGH mRNA in RBL-1 cells and rat-1 fibroblasts transfected with different DNA constructs. RNA blots containing approximately equal amounts of total RNA (10 μg/sample) from RBL-1 cells and rat-1 fibroblasts transfected with pPG(--504/+24)hGH (lane 1), pPG(--333/+24)hGH (lane 2), pPG(--250/+24)hGH (lane 3), pPG(--196/+24)hGH (lane 4), pPG(--118/+24)hGH (lane 5), or pXGH5 (lane 6) were probed with a 32P-labeled hGH cDNA. The migration positions of 2.5-kb RNA, hGH mRNA, and β-actin mRNA are indicated on the right.
Protein/DNA Binding Analyses—Gel mobility shift assays were used to determine whether or not RBL-1 cells and rat-1 fibroblasts contain trans-acting factors in their nuclei which bind specifically to the three identified cis-acting regulatory elements in the 5'-flanking region of the mouse SG-PG gene. An 89-bp \( {^3P}\)-labeled DNA fragment containing the putative negative regulatory element and corresponding to residues -250 to -161 of the mouse SG-PG gene was gel electrophoresed before and after it had been incubated with the nuclear extracts from RBL-1 cells and rat-1 fibroblasts. As shown in Fig. 3 for one of four experiments, in the absence of nuclear extract, the radioactive probe migrated to its expected position at the bottom of the gel (lane 1). When the \( {^3P}\)-labeled probe was incubated with the nuclear extracts from either one of the two populations of cells before electrophoresis, it was selectively retained in the gel by a putative DNA-binding protein (designated B/F(-250/-161)-I (lanes 2 and 5). The binding of this \( {^3P}\)-labeled oligonucleotide to B/F(-250/-161)-I was specific because retention of the probe was diminished when a 5-fold excess of the same nonradioactive DNA fragment was included in the assay (lanes 3 and 6); retention was not diminished when a 100-fold excess of sonicated salmon sperm DNA (lanes 4 and 7) or the 64-mer oligonucleotide that binds NFl/CTF, SP1, AP1, and AP3 (data not shown) was included in the assay. Based on its differential mobility in this gel mobility shift assay, a second trans-acting factor (F(-250/-161) II) was detected only in the nuclear extracts of rat-1 fibroblasts. Because RBL-1 cells, but not fibroblasts, contain proteases in their secretory granules (one of which is a chymase active at neutral pH (33)), two control experiments were carried out to determine if the absence of F(-250/-161)-II in RBL-1 cells was a consequence of the isolation procedure used to obtain the nuclear DNA-binding proteins. When the nuclear extracts from RBL-1 cells and fibroblasts were mixed in different proportions before analysis, the level of F(-250/-161)-II detected in the gel mobility shift assay varied according to the amount of fibroblast-derived protein that was used in the assay (data not shown). Furthermore, the amounts of B/F(-250/-161)-I and F(-250/-161)-II detected in the nuclear extract of a pooled preparation of RBL-1 cells and fibroblasts were compatible with the results obtained with the extracts of the individual cell types (data not shown). Thus, the absence of F(-250/-161)-II in the nuclear extracts of RBL-1 cells was not a consequence of preferential proteolysis of this trans-acting factor in RBL-1 cells.

A 37-bp \( {^3P}\)-labeled DNA fragment containing the putative enhancer element and corresponding to residues -118 to -81 of the mouse SG-PG gene was gel electrophoresed before and after it had been incubated with the nuclear extracts from RBL-1 cells and rat-1 fibroblasts. As shown in Fig. 4 for one of three experiments, a retarded species (B(-118/-81)-I) was detected when the \( {^3P}\)-labeled oligonucleotide was incubated with RBL-1 cell-derived nuclear extracts (lane 2). B(-118/-81)-I possessed a mobility different from that of the retarded species F(-118/-81)-I, present in the nuclear extracts of rat-1 fibroblasts (lane 5). The ability to inhibit binding of the \( {^3P}\)-labeled oligonucleotide to B(-118/-81)-I and to F(-118/-81)-I with a 5-fold excess of the same nonradioactive DNA probe (lanes 3 and 6), but not with a 100-fold excess of sonicated salmon sperm DNA (lanes 4 and 7) or the 64-mer oligonucleotide that binds NFl/CTF, SP1, AP1, and AP3 (data not shown), indicated that these interactions were specific. Although an additional retarded species was observed in this gel mobility shift assay when either nuclear extract was used, its binding could not be inhibited by an excess of the specific nonradioactive oligonucleotide.

A 64-bp \( {^3P}\)-labeled DNA fragment containing the putative proximal promoter element and corresponding to residues -40 to +24 of the mouse SG-PG gene was also gel electrophoresed in the absence of nuclear extracts 1-7, the probe was incubated before electrophoresis with nuclear extracts from RBL-1 cells and rat-1 fibroblasts (Fib.), respectively. Competition assays were performed using 5 ng of the same nonradioactive DNA probe (lanes 3 and 6) or 100 ng of sonicated salmon sperm DNA (lanes 4 and 7). The probe, nonspecific (ns) bound probe, and the trans-acting factors present in fibroblasts (F(-118/-81)-I) and RBL-1 cells (B(-118/-81)-I) are indicated on the right.

FIG. 3. Detection of trans-acting factors in the nucleus of RBL-1 cells and rat-1 fibroblasts which bind cis-acting elements in the putative suppressor region of the 5'-flanking region of the mouse SG-PG gene. Gel mobility shift assays were performed with the diagrammatically depicted nucleotide sequence in the 5'-flanking region of the mouse SG-PG gene. In lane 1, 1 ng of the \( {^3P}\)-labeled DNA fragment (residues -250 to -161) was electrophoresed in the gel in the absence of nuclear extracts. In lanes 2-4 and lanes 5-7, the probe was incubated before electrophoresis with nuclear extracts from RBL-1 cells and rat-1 fibroblasts (Fib.), respectively. Competition assays were performed using 5 ng of the same nonradioactive DNA probe (lanes 3 and 6) or 100 ng of sonicated salmon sperm DNA (lanes 4 and 7). The probe and the trans-acting factors present in fibroblasts (F(-250/-161)-I and B/F(-250/-161)-I) and RBL-1 cells (B/F(-250/-161)-I) are indicated on the right.

FIG. 4. Detection of trans-acting factors in the nucleus of RBL-1 cells and rat-1 fibroblasts which bind cis-acting elements in the putative enhancer region of the 5'-flanking region of the mouse SG-PG gene. Gel mobility shift assays were performed with the diagrammatically depicted nucleotide sequence in the 5'-flanking region of the mouse SG-PG gene. In lane 1, 1 ng of the \( {^3P}\)-labeled DNA fragment (residues -118 to -81) was electrophoresed in the gel in the absence of nuclear extracts. In lanes 2-4 and lanes 5-7, the probe was incubated before electrophoresis with nuclear extracts from RBL-1 cells and rat-1 fibroblasts, respectively. Competition assays were performed using 5 ng of the same nonradioactive DNA probe (lanes 3 and 6) or 100 ng of sonicated salmon sperm DNA (lanes 4 and 7). The probe, nonspecific (ns) bound probe, and the trans-acting factors present in fibroblasts (F(-118/-81)-I) and RBL-1 cells (B(-118/-81)-I) are indicated on the right.
phoresed before and after it had been incubated with the nuclear extracts from RBL-1 cells and rat-1 fibroblasts. As shown in Fig. 5 for one of three experiments, when the \(^{32}\)P-labeled probe was incubated with the nuclear extracts from either one of the two populations of cells before electrophoresis, a new species, designated B/F(-40/+24)-II, migrated more slowly in the gel (lanes 2 and 7). The binding of this \(^{32}\)P-labeled oligonucleotide to B/F(-40/+24)-I could be competitively inhibited by a 5-fold excess of the same nonradioactive DNA probe (lanes 3 and 8) but not by a 100-fold excess of sonicated salmon sperm DNA or the oligonucleotide that binds NF1/CTF, SP1, AP1, and AP3 (data not shown). Additional distinct trans-acting factors, designated F\(_1\)(-40/+24)-II and B\(_1\)(-40/+24)-II, were detected in the nuclear extracts of rat-1 fibroblasts and RBL-1 cells, respectively. The binding of this 64-bp SG-PG-derived \(^{32}\)P-labeled probe to F\(_1\)(-40/+24)-II, B/F(-40/+24)-I, and B\(_1\)(-40/+24)-II was minimally diminished in the competition assay when nonradioactive DNA that had a mutated residue \(-28, -30, \) or \(-38\) was used in a 50-fold excess over the nonmutated \(^{32}\)P-labeled probe (Fig. 5).

**DISCUSSION**

In the present study, we have identified constitutive enhancer and suppressor cis-acting elements of the transcription initiation site of the mouse SG-PG gene and have delineated a TATA box equivalent. In addition, we have demonstrated that hematopoietic cells that express the SG-PG gene and fibroblasts that do not express the gene contain trans-acting factors in their nuclei which differentially bind to these cis-acting elements.

RBL-1 cells, mouse WEHI-3 cells, rat-1 fibroblasts, and mouse 3T3 fibroblasts were transiently transfected with plasmid constructs containing various lengths of the 504-bp 5'-flanking region of the mouse SG-PG gene linked to the hGH reporter gene to identify those regions of the gene which are important for its constitutive transcription. RBL-1 cells and mouse WEHI-3 cells were specifically chosen for investigation because they contain cytoplasmic granules and express large amounts of this proteoglycan peptide core transcript whereas no transcript is present in either fibroblast line (20). RBL-1 cells and rat-1 fibroblasts have been transfected in a similar manner with constructs that contain the hGH reporter gene for analysis of the promoter of the rat mast cell protease-II gene (24). The hGH transient expression system was chosen because it is at least 10-fold more sensitive than the chloramphenicol acetyltransferase system (22) or other systems that are based on the expression of \(\beta\)-galactosidase (34) and xanthine-guanine phosphoribosyltransferase (35). This increased sensitivity enables hGH levels to be measured after transfection with a very small amount of plasmid, thus avoiding potential problems of competition (22). The hGH transient expression system is well suited for use because the plasmid pXGH5 can be used as an internal positive control for normalizing the efficiency of transfection, thereby facilitating the interpretation of data from separate experiments. Abundant amounts of hGH mRNA were detected in rat-1 fibroblasts transfected with pPG(\(-118/+24\))hGH, pSV40-hGH, or pXGH5, and in RBL-1 cells transfected with pPG(\(-504/+24\))hGH, pPG(\(-118/+24\))hGH, pSV40-hGH, or pXGH5 (Fig. 1B). In contrast, lesser amounts of hGH mRNA were detected in RBL-1 cells and rat-1 fibroblasts transfected with pPG(\(-40/+24\))hGH, and no hGH mRNA was detected in RBL-1 cells transfected with p\(_0\)GH or in rat-1 fibroblasts transfected with pPG(\(-504/+24\))hGH or p\(_0\)GH. Because large amounts of hGH were detected in the culture media of RBL-1 cells and rat-1 fibroblasts that contained abundant levels of hGH mRNA and because lesser amounts of hGH were detected in the culture media of cells containing intermediate levels of hGH mRNA (Fig. 1), \(A\) and \(B\), transcription and translation of the hGH gene were related in both transfected cell types.

When the results of the transfections with pPG(\(-504/+24\))hGH were normalized to that obtained with a reference plasmid pXGH5, it was found that RBL-1 cells produced 18-fold more hGH than transfected rat-1 fibroblasts (Table I). Likewise, mouse WEHI-3 cells transfected with pPG(\(-504/+24\))hGH produced 20-fold more hGH than transfected mouse 3T3 fibroblasts (Table I). Based on these findings, we concluded that cis-acting regulatory elements are present in the 504-bp 5'-flanking region of the mouse SG-PG gene which preferentially enhance its constitutive transcription in hematopoietic cells or preferentially suppress its transcription in fibroblasts although small differences may be a result of other factors.

To locate more precisely these cis-acting elements, nine additional plasmid constructs were prepared which contained progressively less of the 504-bp 5'-flanking region of the mouse SG-PG gene. The transfection of RBL-1 cells and rat-1 fibroblasts with these constructs revealed that a cis-acting element that resides between residues \(-250\) and \(-190\) suppresses transcription of this gene and that this suppressor element is more dominantly active in rat-1 fibroblasts than in RBL-1 cells (Figs. 1 and 2). Conversely, an element that resides between residues \(-118\) and \(-81\) not only appears to be important for the positive constitutive transcription of this gene but also is dominantly active in RBL-1 cells. RBL-1 cells and fibroblasts produced substantially more hGH when transfected with the construct pPG(\(-118/-44\))SV40-hGH than with pSV40-hGH (Fig. 2). Typical of other enhancers, the enhancer activity of this positive regulatory element was not diminished by changing its orientation and its distance from
the SV40 early promoter in the plasmid (Fig. 2).

For most genes, transcription is initiated -30 bp downstream of the proximal end of the promoter, which usually is a TATA box. Because no hGH was detected when RBL-1 cells and rat-1 fibroblasts were transfected with pPG (-20/+24)hGH, but some hGH was produced by cells transfected with pPG (-40/+24)hGH, the proximal element of this promoter resides between residues -40 and -20 (Fig. 1). Inasmuch as no TATA box is present in this region (18, 19), we speculated that the TCTAAAA sequence at residues -31 to -25 might serve as an alternative element; therefore, residues -28, -30, or -38 were mutated. Based on the relative amount of hGH produced in the transfected cells (Table II), we concluded that the 5'-flanking region containing the TCTAAA sequence functions as a TATA box equivalent.

Transcription is regulated by trans-acting factors that bind to distinct cis-acting elements located in the 5'-flanking regions of genes, and these DNA-binding proteins can act in synergy to enhance transcription or in an opposing manner to suppress transcription (36). As assessed by gel mobility shift assays, RBL-1 cells and rat-1 fibroblasts contain a number of DNA-binding proteins in their nuclei which specifically bind the region of DNA which contains the suppressor cis-acting element (Fig. 3), the enhancer cis-acting element (Fig. 4), and the proximal element of the promoter region (Fig. 5). Based on their similar mobilities in the gel mobility shift assays, RBL-1 cells and rat-1 fibroblasts probably contain a common trans-acting factor (B/F (-250/-161)) that binds to the suppressor element and a common trans-acting factor (B/F (-40/+24)) that binds to the proximal promoter. In addition, distinct trans-acting factors appear to be present in each cell line (Figs. 3-5). Rat-1 fibroblasts have distinct trans-acting factors that bind to the suppressor element (F (-250/-161)), the enhancer element (F (-118/-81)), and the proximal promoter (B (-40/+24)).

We have reported previously that stably transfected rat-1 fibroblasts that have incorporated 10-20 copies of the mouse genomic clone λ-MG-PG1 into their genome constitutively express low levels of the 1.0-kb SG-PG transcript (18). Based on the transient transfections described here, we now conclude that one reason normal fibroblasts contain no SG-PG mRNA and transfected fibroblasts contain only limited amounts is the presence of a trans-acting factor in these mesenchymal cells which is very effective in suppressing transcription of this gene. A computer search using the “Dynamics” program (37) failed to reveal a conserved cis-acting element within residues -250 to -118 of the mouse and human SG-PG gene which is recognized by a known suppressor DNA-binding protein, suggesting that a novel cis-acting element is present in this region of the SG-PG gene. Although considerably more is known about enhancers than suppressors, negative cis-acting elements have been found for other genes with cell-specific expression. For example, a negative cis-acting element in the 5'-flanking region of the gene that encodes type II collagen is not dominantly active in chondrocytes that produce this specific collagen but is dominantly active in fibroblasts that produce type I and type III collagen rather than type II (38). Because fibroblasts are more effective than RBL-1 cells in their use of the element that resides between residues -250 and -190 to suppress transcription of the mouse SG-PG gene, the responsible trans-acting factor may be more abundant, selectively expressed, or post-translationally modified to be more active. As assessed by the gel mobility shift assays with the residues -250 to -161 probe (Fig. 3), the nuclear extracts of rat-1 fibroblasts contained at least one trans-acting factor that was not recognized in RBL-1 cells.

A conserved Ets domain of GGA1 exists within the enhancer region (residues -82 to -79) which would be predicted to be recognized by the family of DNA-binding proteins that include Erg, Elk, Ets-1, Ets-2, F4, and PU.1. Macrophages contain PU.1 (39), and therefore this DNA-binding protein may stimulate expression of the SG-PG transcript in mouse myelomonocytic WEHI-3 cells. Because human (40) and mouse (41) mast cells are related to monocytes in their lineage and because RBL-1 cells are a transformed rat mucosal mast cell (33), RBL-1 cells may also contain PU.1. Mouse 3T3 fibroblasts use a cis-acting element that resides within residues -118 to -81 to enhance transcription of the hGH gene once the upstream suppressor element is removed (Fig. 1). Mouse 3T3 fibroblasts contain Ets-1 and Ets-2 (42), and thus it is possible that one of these two DNA-binding proteins is the trans-acting factor that stimulates hGH production when fibroblasts are transfected with pPG (-118/+24).hGH.

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