The Human Histidine Decarboxylase Promoter Is Regulated by Gastrin and Phorbol 12-Myristate 13-Acetate through a Downstream cis-Acting Element

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Transcriptional regulation of the human histidine decarboxylase (HDC) gene by gastrin and the phorbol ester phorbol 12-myristate 13-acetate (PMA) was studied using transient transfection of human HDC promoter-luciferase constructs in a human gastric carcinoma cell line (AGS-B) that expresses the human cholecystokinin-B/gastrin receptor. The transcriptional activity of the human HDC promoter was stimulated 3- to 4-fold by gastrin and 13-fold by PMA, effects that could be blocked by down-regulation or antagonism of protein kinase C. 5'- and 3'-deletion analysis demonstrated that the sequence responsible for gastrin- and PMA-stimulated transactivation (gastrin response element (GAS-RE)) was located in a region (+2 to +24) downstream of the transcriptional start site (+1) in the human HDC promoter and contained a palindrome (5'-CCCTTTAAAATAAAGGG-3'). When ligated upstream of the herpes simplex virus 1 thymidine kinase promoter, a single copy of the GAS-RE was sufficient to confer responsiveness to gastrin and PMA. Electrophoretic mobility shift assays with specific competitors and factor-specific antibody supershifts showed that the labeled GAS-RE bound a novel nuclear factor(s). In addition, both gastrin and PMA increased binding of this factor to the GAS-RE. Hence, the palindromic GAS-RE site is sufficient to explain the gastrin/PMA responsiveness of the human HDC promoter and appears to bind a novel transcription factor.

Histamine is a chemical mediator that serves a variety of biologic roles, including allergic responses, inflammation, neurotransmission in the central nervous system, and gastric acid secretion (1). The formation of histamine from its precursor, L-histidine, is catalyzed in a single step by the enzyme histidine decarboxylase (HDC) (2). HDC is expressed in a variety of adult cell types, including mast cells/basophils, skin, platelets, histaminergic neurons in the brain, and enterochromaffin-like cells of the gastric corpus (1). In addition to its role in allergic responses, HDC and histamine have been implicated in the modulation of cell growth and/or differentiation. Kahlson et al. (3) initially showed high levels of HDC activity and histamine formation in the rat during late fetal life. Inhibition of HDC at this time leads to arrest of fetal growth (4), suggesting that HDC is essential for fetal development. In addition, HDC activity is significantly elevated during liver regeneration after partial hepatectomy (5, 6), in the tissues of healing skin wounds (5), in experimental tumors (7–9), and in the repair process after reperfusion injury of the ischemic bowel (10). The activation of HDC during stages of growth and regeneration suggested that HDC gene expression may be regulated through growth factor pathways.

The possible regulation of HDC through a protein kinase C (PKC) pathway was initially raised by studies showing that a single application to mouse skin of the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA), a known activator of PKC, could result in a rapid increase in HDC activity (11). HDC mRNA was also shown to be increased in rat basophilic leukemia (RBL-2H3) (12), mouse mastocytoma (P-815) (13), and human basophilic leukemia (KU-812-F) (14) cells after TPA treatment. These latter studies suggested that the TPA response was due to changes in the rate of transcription rather than in HDC mRNA stability, but they did not address the mechanism of transcriptional control.

In the gastrointestinal tract, HDC is present at high levels in the enterochromaffin-like cells of the corpus of the stomach, where HDC activity is closely correlated with the level of the circulating peptide hormone gastrin (15). In the rat stomach, gastrin appears to regulate acid secretion through stimulation of histamine release and HDC enzymatic activity. In most species, including humans, gastrin-induced acid secretion can be completely inhibited by histamine-2 receptor antagonists such as cimetidine and ranitidine, indicating that histamine represents the final common pathway for stimulated acid secretion (16). Gastrin also appears to regulate growth in the gastric corpus. Studies in both rats and humans have shown that conditions that lead to higher levels of gastrin in the circulation are associated with hypertrophy of the gastric mucosa and hyperplasia of enterochromaffin-like cells (17). In addition, long-standing hypergastrinemia in rats can lead to the development of gastric carcinoids. Gastrin has also been...
shown to play a role in the growth of a number of tumors and tumor cell lines (18), although the mechanism for growth stimulation by gastrin in this setting is unclear.

Studies from several groups, utilizing Northern blot and in situ hybridization analyses, have now clearly shown that hypergastrinemic states are associated with elevated HDC mRNA levels in rat gastric enterochromaffin-like cells (19–21). Gastrin activates HDC gene expression through binding to the CCK-B/gastrin receptor, a member of the superfamily of G protein-coupled receptors with seven transmembrane domains. The cloned human CCK-B/gastrin receptor has been shown to transduce cellular signals in response to both gastrin-17 and CCK-8, with an increase in free cytosolic calcium and in the level of inositol 1,4,5-triphosphate, suggesting that the CCK-B/gastrin receptor is coupled with a signaling pathway activating phosphoinositidase hydrolysis (22, 23). The activation of PKC-related pathways by gastrin has been less well studied, although one report demonstrated induction of PKC translocation and increased DNA synthesis by gastrin (24).

To study transcriptional regulation of HDC by gastrin, we recently developed the AGS-B cell line, a human gastric cancer cell line expressing the recombinant human CCK-B/gastrin receptor (21). Initial studies from our laboratory demonstrated that gastrin stimulates the rat HDC promoter in transiently transfected AGS-B cells through a PKC-related pathway (21). In addition, these studies showed that the rat HDC promoter is strongly activated in AGS-B cells by phorbol 12-myristate 13-acetate (PMA), a known activator of PKC. The goal of the present study was to extend these initial observations and to define the cis-regulatory elements of the human HDC promoter critical for responsiveness to gastrin and PMA. Through deletion analysis of the HDC promoter, we have identified a 23-nucleotide cis-acting DNA element downstream of the transcriptional start site that is sufficient to mediate gastrin and PMA responsiveness. The response element contains a palindrome and appears to bind a novel transcription factor(s) that can be induced by gastrin and PMA.

**EXPERIMENTAL PROCEDURES**

**Cloning of the Human HDC 5′-Flanking Region and Construction of Reporter Genes—**A human genomic library (human placenta; CLONTECH, Palo Alto, CA) was screened with a synthetic 50-mer oligonucleotide derived from the proximal 5′-noncoding region of the human HDC cDNA (25, 26). Of 500,000 plaques examined, three positive recombinants were identified and plaque-purified. A 4-kilobase BamHI restriction fragment positive on Southern blot analysis was isolated from plaque-purified phage DNA, subcloned into plasmid Bluescript II KS(+) (Stratagene), and sequenced by the chain termination method (Sequenase, U. S. Biochemical Corp.).

A human HDC promoter fragment, containing −1.8 kb of 5′-flanking DNA and 126 nucleotides of the noncoding first exon, was amplified by polymerase chain reaction (PCR) using primers containing BamHI restriction sites and ligated into the promoterless pX2 vector containing the firefly luciferase gene (27). A series of human HDC promoter 5′-deletion constructs (hHDC-luciferase) were constructed (125, 75, 59, and 47 nucleotides upstream of the start site as well as 126 nucleotides of the noncoding first exon) and subjected to PCR amplification of segments from the human HDC promoter utilizing the 1800-nucleotide promoter template. The 5′-deletion PCR primers were designed with the identity of the PCR products. 

**Poly(A)† RNA (5 μg) isolated from plain AGS-B cells, AGS-B cells transfected with the 1.8-kb hHDC-luciferase construct, and human corpus for 12 h at 30°C in 1× NaCl, 167 mM Hepes (pH 7.5), and 0.33 mM EDTA (pH 8.0). Transcripts were extended for 2 h at 37°C using avian myeloblastosis virus reverse transcriptase (Perkin-Elmer) as described previously (22, 23). The extended products were subjected to electrophoresis on a 7% urea, 6% polyacrylamide gel, and the sizes of the extended products were determined by comparison with the sequencing ladder derived from the same oligonucleotide used for primer extension.

To confirm the results obtained by primer extension analysis, anchored polymerase chain reaction was carried out as described previously (28). Briefly, first-strand cDNA was synthesized from 1 μg of mRNA from different sources as described above using the cDNA Cycle-it (Invitrogen) containing a specific primer (5′-CTCTCCCTCTCGTATC-3′) that is complementary to nucleotides +175 to +156 downstream of the transcriptional start site. After removing the excess primer, 2 μl of the first-strand cDNA was used in a PCR by Perkin-Elmer amplification followed by electrophoresis on a 7% polyacrylamide gel, and the sizes of the extended products were determined by comparison with the sequencing ladder derived from the same oligonucleotide used for primer extension.

Eletrophoretic Mobility Shift Assays (EMSAs)—Nuclear extracts
from AGS-B cells were prepared by Nonidet P-40 detergent lysis and 0.5 M NaCl extraction as described by Schreiber et al. (29). Nuclear extracts from different cell lines for EMSA tissue distribution studies were a kind gift of Dr. Anil K. Rustgi. Protein concentrations were determined by a colorimetric method (Bio-Rad protein assay). EMSAs were performed by incubating the extracts with the 4-fmol of double-stranded oligonucleotide probe (40,000 cpm) end labeled with [α-32P]dCTP (Amersham Corp.) by Klenow DNA polymerase (New England Bio Labs Inc.) in a 20-μl binding reaction mixture containing 25 mM Hepes (pH 7.9), 100 mM KCl, 5 mM MgCl2, 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol, 0.1 μg of poly(dI-dC), and 10 μg of nuclear extracts. The wild-type, double-stranded synthetic probe used in this study was 5′-CCCTTTAG-AACGACCTAC-3′ and was polycytidylylated gel-purified. Competition experiments were carried out by preincubating the nuclear extracts with a 100-fold excess of unlabeled competitor oligonucleotide prior to addition of the probe. After incubation at room temperature for 10 min, samples were loaded onto a 4% nondenaturing polyacrylamide gel and electrophoresed in 0.5 × Tris borate/EDTA at 10 V cm. The gels were dried and exposed to Kodak X-AR film for 2–12 h at room temperature. Where appropriate, autoradiographs were analyzed by scanning densitometry.

Competitor Oligonucleotides and Antibodies Used in EMSAs—The competitor AP1 oligonucleotide corresponds to the AP1-binding site found in the human collagenase promoter (30). The CRE oligonucleotide corresponds to the cyclic AMP response element (CRE) present in the rat somatostatin gene (31). The SRE oligonucleotide corresponds to the serum response element (SRE) found in the human c-fos promoter (32). The AP2 oligonucleotide corresponds to the AP2-binding site found in the human metallothionein-Ia gene (33). The SP1 and NF-κB oligonucleotides correspond to the binding sites found in the simian virus 40 early promoter and the human immunoglobulin κ light chain gene, respectively (34, 35). The sense strands of the annealed oligonucleotides were as follows: AP1, 5′-CTAGTGATGACGCTCCTCTCTCATCCACGATA-3′; CRE, 5′-GATTGGCGAGTCGTCACTAGGCATGCT-3′; CRE2, 5′-TGATGTCATATTAGGACATCA-3′; and NF-κB, 5′-GATGC-AGGGAGCATTCTAC-3′. The AP2 oligonucleotide corresponds to the AP2-binding site found in the human metalbumin II Agene (33). The SP1 antibody was a generous gift of Anil K. Rustgi.

RESULTS

Creation of Human HDC-Luciferase Constructs and Analysis of the Transcriptional Start Site—The 5′-flanking sequence of the human HDC gene was isolated from a human EMBL-3 T7/SP6 genomic library using a synthetic 50-mer oligonucleotide probe corresponding to the proximal 5′-noncoding region of the human HDC cDNA. Three genomic DNA clones were isolated after screening 500,000 separate phage plaques. A 6-kb BamHI restriction fragment was subcloned from one of the phage isolates and sequenced. 1258 nucleotides of the 5′-flanking sequence and 150 nucleotides of exon I from the human HDC gene are shown in Fig. 1. The sequence of our clone is similar to that previously reported (36), except for three nucleotide differences: C instead of G at –916, two C nucleotides instead of three at –820 and –821, and six C nucleotides instead of five at –558 to –563. 1800 base pairs of the human HDC promoter were amplified using polymerase chain reaction and ligated into pXP2, a promoterless vector containing the firefly luciferase gene.

The human HDC-luciferase construct was transiently transfected into a human gastric cancer cell line (AGS-B), and the start site was analyzed from poly(A)+ RNA prepared from these transfected AGS-B cells. Primer extension was performed using a 26-mer oligonucleotide complementary to the human HDC noncoding sequence in exon I (5′-CTCGTCTCCCTTCA-CAGATGGCACG-3′) and poly(A)+ RNA from human corpus, transfected AGS-B cells, and untransfected AGS-B cells. Extension products of the same size were detected in poly(A)+ RNA from transfected AGS-B cells and human corpus, but not in untransfected AGS-B cells (Fig. 2), indicating that the labeled products were derived from the transfected plasmid and that transcription was being initiated from the authentic human HDC promoter. Among the extension products, the predominant band of 126 nucleotides indicated that the major transcriptional start site was located 139 bases upstream of the cap site.

To confirm the results obtained by primer extension analysis, anchored PCR was carried out using poly(A)+ RNA from KU-812-F cells, human corpus, and untransfected AGS-B cells using the strategy outlined in Fig. 3A. PCR products of the expected size (142 bp) were seen in KU-812-F human basophilic leukemia cells and human corpus, but not in untrans-
The labeled 26-mer oligonucleotide primer was annealed to poly(A)
site was determined as described under “Experimental Procedures.”

The AGS-B cell line has been stably transfected with the hu-
transfection studies with the AGS-B gastric cancer cell line.

The 1.8-kb hHDC-luciferase construct could also be stim-
phorbol ester PMA. Dose-response studies indicated that a slight increase in hHDC promoter activity could be seen with 10 \( \times \) 10\(^{-6} \) M PMA, with a maximal 13-fold increase seen with 10 \( \times \) 10\(^{-7} \) M PMA (Fig. 4C). Time course studies using a submaximal dose (10 \( \times \) 10\(^{-9} \) M) of PMA also showed that the first increase (2.5-fold) occurred after 2 h, with a maximal increase seen at 8–10 h, followed by a slight decrease at 10 h and then a gradual plateau (Fig. 4D).

The relationship of the protein kinase C-related pathway to

CCK-B/gastrin receptor) AGS cells (data not shown). In addi-
carry out with an antisense primer (+37 to +18), followed by addition of a

**Fig. 2. Primer extension analysis.** The transcriptional initiation

**Fig. 3. Anchored PCR.** A, a schematic diagram showing the overall

The transcriptional initiation site was determined as described under “Experimental Procedures.” The labeled 26-mer oligonucleotide primer was annealed to poly(A)-RNA from AGS-B cells transfected with 1.8-kb hHDC-luciferase (lane 1), human corpus (lane 2), and untransfected AGS-B cells (lane 3). Extended products were run on a 7 M urea, 6% polyacrylamide gel. Sequencing ladders of the human HDC gene obtained using the same oligonucleotide as a sequencing primer are also shown. The arrow indicates the position of the major extension product (seen in lanes 1 and 2, but not in lane 3) and the asterisk indicates the position of the major extension product in the sequencing ladder.

Based on these results, the transcriptional start site was identified as the adenine nucleotide located 20 bases upstream of the 5′-end of the previously reported cDNA (25), which differs somewhat from a previous report (36). No definitive TATA box was observed in the –25 to –30 region of the human HDC promoter. A TATA-like sequence was present farther upstream in the –48 to –54 region (Fig. 1). Additional 5′- and 3′-deletion studies suggested that this TATA-like region of the gene may be functioning as a promoter element (see below and Fig. 5).

The Human HDC Promoter Is Responsive to Gastrin and Phorbol Esters in AGS-B Cells—To study the effect of gastrin stimulation on the activity of the human HDC promoter, the 1.8-kb hHDC-luciferase construct was employed in transient transfection studies with the AGS-B gastric cancer cell line. The AGS-B cell line has been stably transfected with the human CCK-B/gastrin receptor cDNA and expresses functional receptors as previously assessed by Northern blot analysis, binding studies, and measurements of intracellular calcium (21). AGS-B cells were transiently transfected with the 1.8-kb hHDC-luciferase construct and stimulated with various concentrations of gastrin for 24 h. This dose-response analysis showed that a slight increase in luciferase activity could be detected first at 10\(^{-9} \) M, while maximal (3-4-fold) stimulation occurred at 10\(^{-7} \) M with a slight decrease seen at higher concentrations (Fig. 4A). An identical dose-response relationship was observed with CCK-B (data not shown). Time course studies with continuous gastrin stimulation showed that a 2-fold increase in promoter activity could be observed after 4 h, and maximal elevation was achieved after 12–24 h of gastrin exposure (Fig. 4B). No stimulation by gastrin was observed with an enhancerless heterologous promoter (pT81-luciferase) or with the hHDC-luciferase construct in plain (lacking the
plus 126 bp of noncoding first exon were generated using synthetic oligonucleotide primers (Fig. 1 and Table I) and polymerase chain reaction. Transfection studies showed that using constructs as small as −59 hHDC-luciferase resulted in essentially full basal luciferase activity, along with intact gastrin and PMA responses (Fig. 6A). However, when 12 additional nucleotides were removed, resulting in the −47 hHDC-luciferase construct, basal promoter activity was completely destroyed, and thus, gastrin and PMA responses could not be assessed (Fig. 6A).

Deletions from the 3′-end of the human HDC promoter were then carried out, starting with the −125 hHDC-luciferase construct, which contained 126 bp of noncoding first exon. Deletion of nucleotides from +126 to +20 reduced basal luciferase activity by 60%, but did not impair gastrin or PMA responsiveness (Fig. 6B). However, further deletions down to +14 had a minimal effect on basal promoter activity, but essentially destroyed the gastrin response. This deletion (+20 to +14) also reduced the PMA response by 50%; interestingly, the −125 to +3 promoter fragment was quite active (indicating that the AT-rich sequence between +3 and +20 was not essential for basal promoter activity) and retained a significant response to PMA (Fig. 6B), suggesting that there may be an additional basal promoter element(s) in this region of the promoter. Thus, taken together, the 5′- and 3′-deletion studies indicate that the gastrin response element (GAS-RE) is located between −58 and +20 within the human HDC 5′-flanking region.

The +2 to +24 Region of the Human HDC Gene Is Sufficient to Permit Response to Gastrin and PMA—To further localize the cis-acting DNA element mediating responsiveness to gastrin and PMA and to demonstrate that the element acted as an enhancer, a heterologous (thymidine kinase) promoter system was utilized. Various fragments of human HDC 5′-flanking DNA were ligated upstream of the enhancerless thymidine kinase promoter in the luciferase-containing plasmid pT81. These pT81 constructs were then used in transfection studies of AGS-B cells to assess responsiveness to gastrin and PMA stimulation. Initially, a large segment (−47 to +126) of 5′-flanking and untranslated DNA from the human HDC promoter was ligated into pT81. This −47 to +126 sequence resulted in a 10-fold increase in basal luciferase activity as well as significant gastrin (2-fold) and PMA (8-fold) responses (Fig. 7A).

Removal of 90 nucleotides from the 3′-end and 22 nucleotides to +20 reduced basal luciferase activity (Fig. 7B). Further deletions in pT81 resulted in no loss of basal activity or of gastrin and PMA responsiveness. An additional deletion from −25 to −6, which removed a putative (GGCGG) SP1 site, resulted in a significant reduction of basal activity, but again, no change in gastrin or PMA responsiveness. Further deletions in pT81 resulted in the identification of a minimal 23-nucleotide element (+2 to +24) that increased basal thymidine kinase promoter activity 4-fold and was sufficient to confer essentially full gastrin and PMA responses, and this element was labeled the GAS-RE. The GAS-RE sequence includes a palindrome (5′-CTCTTTAATAAAGGG-3′) at the 5′-end. Removal of a single nucleotide (C) from the 5′-end of the GAS-RE to yield the −3 to +36 hHDC-pT81-luciferase construct resulted in a significant (40%) decrease in gastrin and PMA responsiveness. Removal of seven nucleotides from the 3′-end of the GAS-RE to yield the +2 to +17 hHDC-pT81 construct resulted in the complete loss of gastrin and PMA responsiveness (Fig. 7A). Hence, these studies defined the GAS-RE as the minimal sequence (+2 to +24) required to mediate both gastrin and PMA responses.

A Distinct Nuclear Factor Binds to the +2 to +24 Sequence and Is Induced by Gastrin and Phorbol Esters—To characterize the nuclear factors that bind to the GAS-RE and that may therefore mediate gastrin and PMA transcriptional responses, we performed EMSAs. These assays utilized as probe α-32P-labeled, double-stranded oligonucleotides that represented the sequence (+2 to +24) of the GAS-RE shown in Fig. 7B and nuclear extracts prepared from AGS-B cells as described under "Experimental Procedures". Fig. 8 demonstrates that the
The antibody to SP1 was used because of the GC-rich nature of the 3'-half of the GAS-RE. Antibodies to c-fos, c-jun, CREB, and SP1 had no effect on complex formation (Fig. 9A), while these same antibodies either blocked binding (anti-c-Fos and anti-c-jun) or generated supershifts (anti-CREB and anti-SP1) when using labeled oligonucleotides probes representing known AP1-, CREB-, and SP1-binding sites (Fig. 9B). We sought to identify qualitative and/or quantitative differences in DNA-protein complex formation in response to gastrin or PMA stimulation. Nuclear extracts were prepared prior to and after addition of gastrin (10^{-8} M) or PMA (10^{-8} M) to AGS-B cells cultivated in serum-free medium (Ultraculture, BioWhittaker, Inc.) for 48 h. A significant increase in complex formation was observed 30 and 120 min after addition of gastrin or PMA compared with that observed with nuclear extracts from unstimulated AGS-B cells (Fig. 10A). Scanning densitometry revealed that gastrin stimulation increased binding to the GAS-RE by ~3-fold, while PMA stimulation resulted in an ~4-fold increase in binding (Fig. 10B). Finally, to characterize the tissue distribution of the GAS-RE-binding protein, we carried out EMSAs using nuclear extracts made from cancer cell lines derived from a variety of human tissues and cell types. As shown in Fig. 11, the GAS-RE-binding protein appeared to be a widely distributed transcription factor that was present in most cell types.

### DISCUSSION

This study demonstrates that the 5'-flanking region of the human HDC gene is active and can be regulated by gastrin and the phorbol ester PMA in AGS-B cells. In a previous study, we showed that the rat HDC promoter is regulated by gastrin primarily through a PKC-related pathway [21]. In addition, the effect of gastrin on the rat HDC promoter is additive when combined with cAMP, but not additive when combined with PMA [21]. In the present study, which represents the first functional characterization of the human HDC promoter, we provide further evidence that gastrin and PMA work through a single mechanism to regulate HDC transcription. In our AGS-B cell culture system, both gastrin and PMA were able to stimulate the human HDC promoter in a similar fashion, although PMA gave somewhat stronger (~13-fold) and faster (~2 h) responses compared with gastrin (3-4-fold and ~4 h, respectively). Both gastrin and PMA responses by the human HDC promoter could be blocked completely by PMA depletion and H-7 blockade, again implicating a protein kinase C-related pathway. Finally, deletions of the human HDC promoter sequence that affected responsiveness to gastrin tended to affect responses to phorbol ester in a similar fashion. The PMA response appeared slightly more complex since only part of the PMA response could be reconstituted by the GAS-RE. In addition, our 3'-deletion studies clearly indicated that additional PMA response element(s) were located farther upstream.

### TABLE I

| Oligonucleotides | Sequences (5' → 3') |
|------------------|---------------------|
| -125 to -108, sense | CGGCTGTGTCTTGAGGCACT |
| -73 to -60, sense | CGGCTGTGTCTTGAGGCACT |
| -59 to -42, sense | CGGCTGTGTCTTGAGGCACT |
| -47 to -30, sense | CGGCTGTGTCTTGAGGCACT |
| +126 to +109, antisense | CGGCTGTGTCTTGAGGCACT |
| +36 to +19, antisense | CGGCTGTGTCTTGAGGCACT |
| +20 to +3, antisense | CGGCTGTGTCTTGAGGCACT |
| +14 to -4, antisense | CGGCTGTGTCTTGAGGCACT |
| +3 to -15, antisense | CGGCTGTGTCTTGAGGCACT |

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### TABLE I

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| -59 to -42, sense | CGGCTGTGTCTTGAGGCACT |
| -47 to -30, sense | CGGCTGTGTCTTGAGGCACT |
| +126 to +109, antisense | CGGCTGTGTCTTGAGGCACT |
| +36 to +19, antisense | CGGCTGTGTCTTGAGGCACT |
| +20 to +3, antisense | CGGCTGTGTCTTGAGGCACT |
| +14 to -4, antisense | CGGCTGTGTCTTGAGGCACT |
| +3 to -15, antisense | CGGCTGTGTCTTGAGGCACT |
Overall, our deletion analysis demonstrates that the predominant gastrin and PMA response elements map to the same region of the promoter. In the AGS-B cell culture system, we have shown initially that gastrin and PMA responses could be mapped by 5'- and 3'-deletions to a region containing the basal promoter elements, spanning 58 nucleotides of 5'-flanking DNA and 36 nucleotides of the untranslated first exon. The human HDC promoter does not contain a canonical TATA box around the −30 region, as has previously been noted (36); however, it does contain a proximal SP1 site (−25GGGCGG−20) as well as a TATA-like element farther upstream (−55TTAAT−TAA−47). Deletion of this upstream TATA-like element resulted in the complete loss of promoter activity, suggesting that it may function in some fashion in the assembly of basal transcription factors. Further mapping of the gastrin and PMA response elements, carried out in the heterologous thymidine kinase promoter system, revealed that the responsible element is located downstream of the transcriptional start site of the human HDC gene. This element was further localized to a
23-bp region, spanning +2 to +24 and including a palindromic sequence (5'-CCCTTTAAATTAAGGG-3'), that we have designated the GAS-RE. Our 3'-deletion studies (Fig. 6B) also suggested that the 3'-end of the GAS-RE may actually be closer to +20 since deletion to +20 did not markedly impair gastrin or PMA responses.

Two pieces of evidence strongly suggest that the GAS-RE functions as an enhancer-like response element rather than as a basal promoter element. First, our 3'-deletion studies showed that the −125 to +3 hHDC-luciferase construct, which lacks the GAS-RE, still has significant basal activity (Fig. 6B), indicating that the GAS-RE is not essential for promoter activity. Second, placement of the +2 to +24 sequence upstream of a heterologous minimal promoter increased basal activity 4-fold and was also sufficient to confer responsiveness to gastrin and PMA, consistent with an enhancer-like response element. In addition, the GAS-RE appears sufficient to act as a binding site for a novel nuclear factor(s).

The activation of HDC by both gastrin and PMA suggests a general pathway for growth stimulation. The phorbol ester...
PKC. Other PKC response elements include the SRE of the mediate induction responses to TPA and other activators of but the TRE is only one of several ester response element or TRE is the binding site for AP1, (41) as well as members of the CREM/CREB family that recognition sites (40). However, the CRE has been shown to bind a region that contained several potential SP1- and AP2-binding sites. A symmetry or palindromic nature of our GAS-RE sequence is consistent with its being a transcription factor-binding site. A palindromic sequence often implies a binding site for a transcription factor, which is part of a network whose activity will be required to characterize the intracellular signal transduction pathways downstream of the CCK-B/gastrin receptor and PKC that are regulating this factor. In conclusion, we believe that the GAS-RE-binding protein (s) represents a novel transcription factor, which is part of a network whose activity is stimulated after activation of PKC.

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