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Sara Peleg

Gilbert J. Cote

Ronald V. Abruzzese

Robert F. Gagel

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Transcriptional Regulation of the Human Calcitonin Gene: A Progress Report

Sara Peleg,* Gilbert J. Cote,* Ronald V. Abruzzese,* and Robert F. Gagel*

We have applied DNA transfer techniques to study the transcriptional regulation of the calcitonin (CT) gene in a C-cell line (TT) derived from a human medullary thyroid carcinoma. TT cells were transfected with a fusion gene containing the CT gene promoter and 5′-flanking DNA attached to the promoter-less growth hormone gene (reporter). We quantitated the reporter gene product to monitor transcriptional activation by the CT promoter and deletion mutants of the 5′-flanking DNA. We found that the proximal CT promoter which includes the DNA sequence from +1 to −129 bp upstream from the CT transcription start site did not induce transcription in C-cells or in NIH 3T3 cells. The attachment of additional 5′-flanking DNA, extending up to −1460 bp enhanced transcription up to twelvefold in TT cells but had no effect on transcription in 3T3 cells. Deletion of a sequence located at −1290 to −820 bp on the CT 5′-flanking DNA abolished the transcription of the reporter gene. Attachment of the DNA sequence located between −1333 to −731 to the fusion gene, containing the CT promoter (+1 to −129) and the reporter gene, restored transcription of the reporter gene in TT cells. We conclude that an enhancer of CT transcription, which is active in C-cells but not in 3T3 cells, is located between −1290 and −820 of the CT 5′-flanking DNA. (Henry Ford Hosp Med J 1989;37:194-7)

Transcription of the calcitonin (CT) gene is extensive in thyroid C-cells, neuronal cells, and small numbers of cells in normal lung, gut, and pancreas (1-3). Expression of the CT gene is also common in neoplastic cells derived from these tissues (4-8). In cell culture systems the transcription of the CT gene appears to be constitutive in C-cells derived from rat or human medullary thyroid carcinoma (MTC) (9,10). This basal transcriptional activity can be upregulated by glucocorticoids (11), cAMP (12), and phorbol esters (12,13) or down-regulated by vitamin D_3 (14). However, little is known about the factors which maintain basal transcription of the CT gene and the nature of their interaction with the other modulators of CT gene expression.

We have recently described the employment of DNA-mediated transfection techniques in the study of cis-regulatory elements in the CT promoter and 5′-flanking DNA (15). We reported that production of growth hormone (GH) by a fusion gene containing the metallothionein promoter, fused to the GH gene, was augmented by the attachment of 1400 bp of the CT 5′-flanking DNA to the metallothionein promoter. As a consequence of that addition, the fusion gene also acquired responsiveness to cAMP and phorbol esters. The sequence analysis of the 1400 bp fragment revealed that it contained multiple elements which could potentially function as binding sites for cAMP response element binding (CREB) protein (16,17), AP1 (18) and AP2 (19), which require cAMP or phorbol esters for their transcriptional activity. In the present study we used a series of deletion mutants of the CT 5′-flanking DNA to further map functional cis-elements within this 5′-flanking DNA.

Methods

Cell culture

Cells (TT) from a C-cell line derived from human MTC were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum. NIH 3T3 cells were grown in DMEM supplemented with 10% fetal bovine serum (11,20).

Transfection

Twenty-four hours prior to transfection, cells were plated in 35 mm dishes at a density of 3×10^5/dish (TT) or 10^5/dish (3T3) in DMEM and 10% fetal bovine serum. Transfection (8 μg plasmid DNA per dish) was by the DEAE dextran method followed by brief treatment (1 minute) with 10% DMSO. Medium samples or cell extracts were collected five days after transfection. GH production by the reporter gene was measured by a two-site immunoradiometric assay as described by the manufacturer (Nichols Institute, San Juan Capistrano, CA).

DNA cloning

Plasmid pCT GH was prepared by subcloning of a BamHI-MboI fragment encoding −129 to +91 of the CT gene into the

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*Laboratory of Molecular and Cellular Endocrinology, Baylor College of Medicine and Veterans Administration Medical Center, Houston, TX.
Address correspondence to Dr. Gagel, Laboratory of Molecular and Cellular Endocrinology (111E), Veterans Affairs Medical Center, 2002 Holcombe Blvd, Houston, TX 77030.

BAMHI site located at the transcription start site of the GH gene. Plasmid pCT GH-1 was prepared by a subcloning of the DNA fragment encoding −1460 to −129 of the CT 5′-flanking DNA into HindIII-BamHI sites of pCT GH. The plasmid pCT GH-2 was prepared by deletion of the DNA between −1290 to −820 of pCT GH-1 by restriction digestion with BspMI. The plasmid pCT GH-3 was prepared by a deletion of the DNA sequence between −820 to −429 of pCT GH-2, by a double digestion with the restriction enzymes BspMI and ApaI. Plasmid pCT GH-7 was prepared by isolation of HindIII to BglII fragment between −1460 to −731 of pCT GH-1, ligation of the fragment into HindIII-BamHI sites of pCT GH and subsequent deletion of a HindIII-MluI fragment located at position −1460 to −1333.

Results

We have recently reported that the attachment of a DNA fragment located at −1460 to −129 of the CT 5′-flanking DNA to the metallothionein promoter/GH fusion gene induced a sixfold increase in the basal transcriptional activity of the fusion gene (15). This enhancement was observed in TT cells but not in 3T3 cells. We wished to determine if similar results would be obtained when the CT promoter replaced the metallothionein promoter and, therefore, constructed plasmid pCT GH which contains the sequence between position −129 to +91 of the CT gene fused to the GH gene. We also constructed plasmid pCT GH-1 which contains an additional fragment of CT 5′-flanking DNA, including the sequence from −1460 to +91 (Fig 1A). The transfection of pCT GH resulted in the production of low levels of GH (0.5 ng/mL) by TT cells and by 3T3 cells (Fig 1B). Transfection of pCT GH-1 into TT cells induced a twelvefold increase in GH production by TT cells, whereas the level of GH production was unchanged in the similarly treated NIH 3T3 cells (Fig 1B). We concluded that there are DNA elements between the −1460 to −129 bp of the CT 5′-flanking DNA which enhance transcription in TT but not in 3T3 cells. To narrow the region in which the enhanced sequence(s) are located, we prepared plasmids pCT GH-2 and pCT GH-3 (Fig 1A). The transcriptional activity induced by these constructs was tested in TT and 3T3 cells. We found that removal of the sequence between −1290 to −820 of the CT 5′-flanking DNA (pCT GH-2) was sufficient to abolish the transcriptional activity of the fusion gene (Fig 1B). We next wished to define whether the attachment of the DNA sequence between −1290 to −820 to plasmid pCT GH would be sufficient to enhance transcription. A new construct (pCT GH-7) was therefore prepared (Fig 2A), which produced identical levels of GH as pCT GH-1 (Fig 2B). Neither pCT GH-1 nor pCT GH-7 had any significant transcriptional activity in 3T3 cells (Fig 2B). The lack of transcription in 3T3 cells was not due to inefficient transfection or to the instability of GH protein in these cells, because a control plasmid, containing the metallothionein promoter fused to the GH gene, produced 12 ng/mL GH in the same experimental conditions (data not shown).

We examined the transcriptional activity of the "enhancer"-containing constructs in several cell-lines which do not produce CT (CV1, HeLa, HIT, C6 [not shown]) and found them inactive. We, therefore, concluded that an enhancer of transcription is located between −1290 to −820 of the CT 5′-flanking DNA. The activity of this enhancer appears to be exclusive to CT-secreting cells.

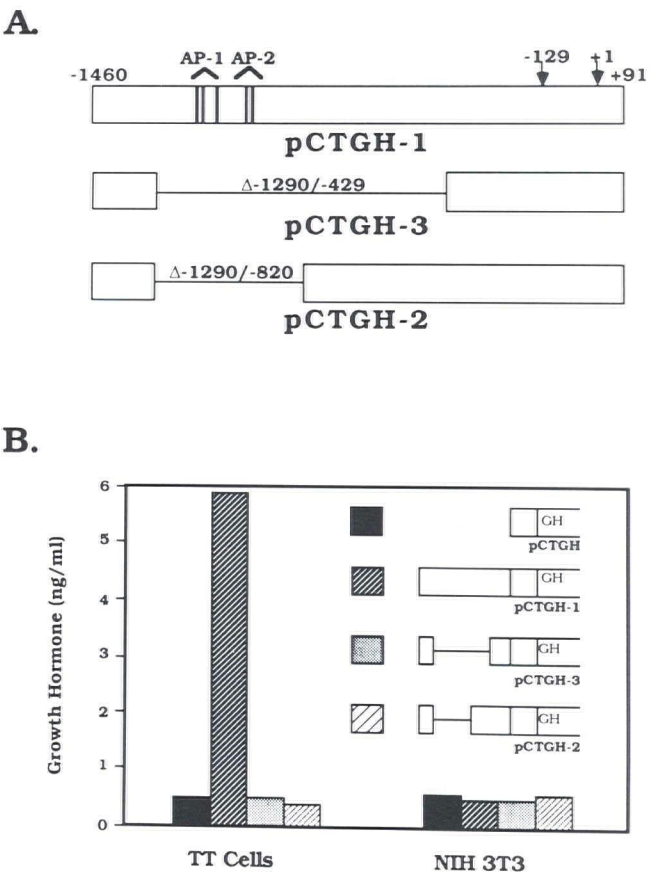
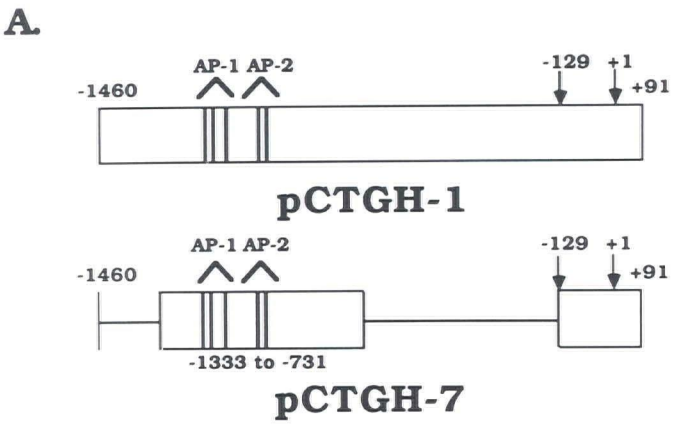


Fig 1—Mapping a cis-regulatory element of transcription in the calcitonin (CT) 5′-flanking DNA: We prepared deletion mutants from the "wild-type" plasmid (pCT GH-1), which contains the CT 5′-flanking DNA fused to the GH gene. Panel A shows the position of the deletion in pCT GH-2 and pCT GH-3 in respect to pCT GH-1. Panel B shows the results of a transfection experiment in which 8 μg of either plasmid were introduced into TT cells or NIH 3T3 cells. Media were collected five days later and GH levels were determined by radioimmunoassay. Shown are the average values (ng/mL) of samples obtained from three to five separate dishes.

Discussion

A region located within the CT 5′-flanking DNA appears to function as an enhancer of transcription in TT cells and has been partially mapped to between −1290 to −820 bp upstream from the transcription start site of the CT gene. Enhancement of transcription by this region could occur through an interaction of a known transcription factor (21,22) with a specific DNA sequence located within that region. Examination of the sequence included within −1290 to −820 reveals that it contains three



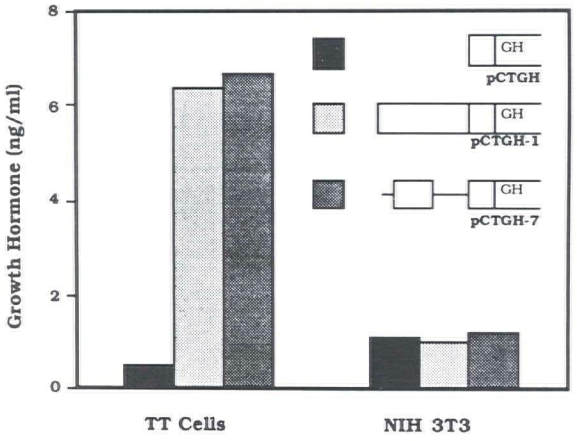


Fig 2—The cis-element within the calcitonin (CT) 5′-flanking DNA operates as an enhancer of transcription: The DNA fragment, between −1333 to −731 was attached to the transcriptionally-inactive pCT GH to produce the new plasmid pCT GH-7. The position of the added sequence in respect to the unmutated plasmid pCT GH-1 is illustrated in panel A. Panel B shows the results of the transfection experiment, performed and analyzed as described in the legend to Fig 1 and in the text. Shown are the average values (ng/mL) of samples obtained from three to five separate dishes.

potential activator protein 1 (AP1) binding sites (18) and three potential AP2 binding sites (19). The transcription factors binding to these DNA motifs may act independently or in concert to augment transcription of the CT gene. However, we think it unlikely that the action of AP1 and/or AP2 at that region is responsible for the results described here. When constructs containing the putative AP1 and AP2 sites were transfected into HeLa cells, which are known to express both AP1 and AP2 (23,24), they failed to enhance transcription. We believe, therefore, that the possibility exists that another previously undescribed DNA motif is located between −1290 and −820. Additional studies are required to prove this point. de Bustros et al (25) have recently presented data suggesting that this same region of the CT 5′-flanking DNA is active as an enhancer of transcription in a lung carcinoma cell line which expresses the CT gene. These findings raise the possibility that utilization of the enhancer located between −1290 and −820 may result from ectopic expression (or function) of a transcription factor in both the transformed C-cells and in the lung carcinoma cells. Alternatively, this transcription factor may be normally functional in C-cells but aberrantly produced and function in the lung carcinoma cell line.

Acknowledgments

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References

1. Rosenfeld MG, Amara SG, Evans RM. Alternative RNA processing: Determining neuronal phenotype. Science 1984;225:1315-20.

2. Pettersson M, Ahren B, Bottcher G, Sundler F. Calcitonin gene-related peptide: Occurence in pancreatic islets in the mouse and the rat and inhibition of insulin secretion in the mouse. Endocrinology 1986;119:865-9.

3. Nylen ES, Linnoila RI, Snider RH, Tabassian AR, Becker KL. Comparative studies of hamster calcitonin from pulmonary endocrine cells in vitro. Peptides 1987;8:977-82.

4. Edbrooke MR, Parker D, McVey JH, et al. Expression of the human calcitonin/CGRP gene in lung and thyroid carcinoma. EMBO J 1985;4:715-24.

5. Morris HR, Panico M, Etienne T, Tippins J, Girgis SI, MacIntyre I. Isolation and characterization of human calcitonin gene-related peptide. Nature 1984;308:746-8.

6. Steenbergh PH, Hoppener JWM, Zandberg J, Van de Ven WJM, Jansz HS, Lips CJM. Calcitonin gene related peptide coding sequence is conserved in the human genome and is expressed in medullary thyroid carcinoma. J Clin Endocrinol Metab 1984;59:358-60.

7. Nelkin BD, Rosenfeld KI, de Bustros A, Leong SS, Roos BA, Baylin SB. Structure and expression of a gene encoding human calcitonin gene related peptide. Biochem Biophys Res Commun 1984;123:648-55.

8. Oberg K, Eriksson B, Lundqvist M. Neuroendocrine tumors of the upper gastrointestinal tract and pancreas. Acta Chir Scand 1988;541(suppl):76-85.

9. Cote GJ, Gould JA, Huang SCE, Gagel RF. Studies of short-term secretion of peptides produced by alternative RNA processing. Mol Cell Endocrinol 1987;53:211-9.

10. Gagel RJ, Zeytinoglu FN, Voelkel EF, Tashjian AH Jr. Establishment of a calcitonin-producing rat medullary thyroid carcinoma cell line. II. Secretory studies of the tumor and cells in culture. Endocrinology 1980;107:516-23.

11. Cote CJ, Gagel RF. Dexamethasone differentially affects the levels of calcitonin and calcitonin gene-related peptide mRNAs expressed in a human medullary thyroid carcinoma cell line. J Biol Chem 1986;261:15524-8.

12. de Bustros A, Baylin SB, Levine MA, Nelkin BD. Cyclic AMP and phorbol esters separately induce growth inhibition, calcitonin secretion, and calcitonin gene transcription in cultured human medullary thyroid carcinoma. J Biol Chem 1986;261:8036-41.

13. de Bustros A, Baylin SB, Berger CL, Roos BA, Leongg SS, Nelkin BD. Phorbol esters increase calcitonin gene transcription and decrease c-myc mRNA levels in cultured human medullary thyroid carcinoma. J Biol Chem 1985;260:98-104.

14. Cote GJ, Rogers DG, Huang ESC, Gagel RF. The effect of 1,25-dihydroxyvitamin D3 treatment on calcitonin and calcitonin gene-related peptide mRNA levels in cultured human thyroid C-cells. Biochem Biophys Res Commun 1987;149:239-43.

15. Cote GJ, Abruzzese RV, Lips CJM, Gagel RF. Transfection of calcitonin gene regulatory elements into a cell culture model of the C-cell. J Bone Min Res 1990 (in press).

16. Montminy MR, Sevarino KA, Wagner JA, Mandel G, Goodman RH. Identification of a cyclic-AMP-responsive element within the rat somatostatin gene. Proc Natl Acad Sci USA 1986;83:6682-6.

17. Yamamoto KK, Gonzalez GA, Biggs WH III, Montminy MR. Phosphorylation-induced binding and transcriptional efficacy of nuclear factor CREB. Nature 1988;334:494-8.

18. Lee W, Mitchell P, Tjian R. Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. Cell 1987;49:741-52.

19. Imagawa M, Chiu R, Karin M. Transcription factor AP-2 mediates induction by two different signal-transduction pathways: Protein kinase C and cAMP. Cell 1987;51:251-60.

20. Cote GJ, Gagel RF. The decision to produce calcitonin and calcitonin gene-related peptide in the C-cell can be regulated by glucocorticoids. In: Cohn DV, ed. Calcium regulation and bone metabolism, basic and clinical aspects. Amsterdam: Excerpta Medica 1987;9:76-81.

21. Dynan WS, Tjian R. Control of eukaryotic messenger RNA synthesis by sequence-specific DNA-binding proteins. Nature 1985;316:774-8.

22. Serfling E, Jasin M, Schaffner W. Enhancers and eukaryotic gene transcription. Trends Genet 1985;1:224-30.

23. Angel P, Allegretto EA, Okino ST, et al. Oncogene jun encodes a sequence-specific trans-activator similar to AP-1. Nature 1988;332:166-71.

24. Williams T, Admon A, Luscher B, Tjian R. Cloning and expression of AP-2, a cell-type specific transcription factor that activates inducible enhancer elements. Gene and Development 1988;2:1557-69.

25. de Bustros A, Lee RY, Baylin SB, Nelkin BD. Characterization of cell-specific basal and inducible enhancers for the calcitonin gene (Abstract). Program of the Annual Meeting of the Endocrine Society, 1989:427.