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Differential Utilization of Calcitonin Gene Regulatory DNA Sequences in Cultured Lines of Medullary Thyroid Carcinoma and Small-Cell Lung Carcinoma

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Regulation of expression of the human calcitonin gene was found to differ between two tumor lines of different tissue origin, medullary thyroid carcinoma (TT line) and small-cell lung carcinoma (DMS53 line). Distal 5′ DNA elements between −750 and −2000 exhibited a stronger basal activity in DMS53 than in TT cells, whereas proximal DNA sequences between −132 and −252 mediated a dramatic cyclic AMP response in TT but not DMS53 cells.

Investigating the regulation of genes expressed in cells of separate embryological origin is critical for understanding the differential gene control that accompanies cell differentiation. Peptide hormone genes such as the calcitonin (CT) gene offer an excellent opportunity to study this problem. In general, these genes are most highly expressed in specific endocrine glands and their tumors. However, cells secreting these hormones are found in other tissues, and on occasion, nonendocrine tumors express these genes so-called ectopically (for reviews, see references 4 and 9).

The cell-specific regulation of CT gene expression is complex. Alternative RNA splicing generates predominantly CT mRNA in thyroid C cells and calcitonin gene-related peptide (CGRP) mRNA in the nervous system (1, 18). In addition, CT and CGRP immunoreactivity has been identified in discrete endocrine cells such as those scattered throughout the respiratory mucosa (15, 19). In this study, we examined, by transient gene transfer experiments, the *cis*-acting DNA elements mediating basal and induced CT gene expression in two CT-producing human tumors of different embryological derivation: the medullary thyroid carcinoma (MTC) line TT, of thyroid C cell origin (20), and the small-cell lung carcinoma (SCLC) line DMS53, of bronchial

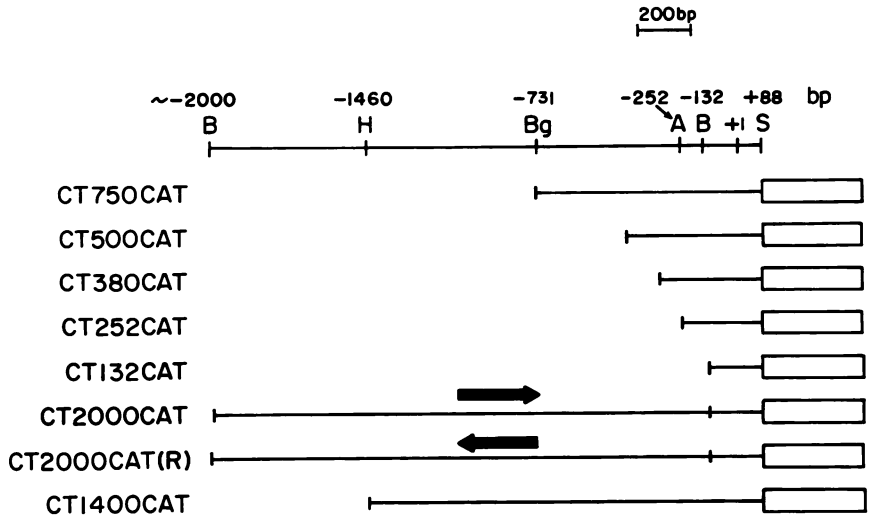

FIG. 1. Diagram of CT gene constructs. The 5′ end of the CT gene is shown at the top. The transcription start site is indicated by +1, and sites for the restriction enzymes used in construction of some of the CT-CAT plasmids are shown (A, *Aat*II; B, *Bam*HI; Bg, *Bgl*II; H, *Hin*dIII; S, *Sau*3AI). CT-CAT plasmids contain various 5′-flanking-region fragments and the first 91 bp of the coding region of the CT gene inserted in front of the CAT gene (boxed area) as shown. The 1.9-kbp *Bam*HI fragment was inserted in both orientations, as indicated by the arrows [pCT2000CAT, forward; pCT2000CAT(R), reverse].

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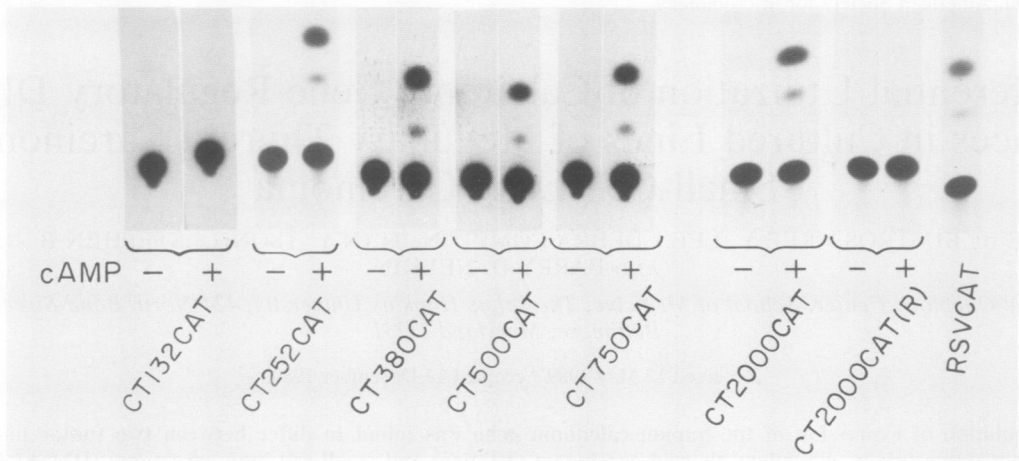

FIG. 2. Transient expression of the CT-CAT constructs in TT cells. A representative experiment is shown. CAT activity was quantitated by liquid scintillation counting of regions of thin-layer chromatography plates containing acetylated chloramphenicol (see Table 1). Plasmid concentrations were carefully verified by spectrophotometry and by visualization of ethidium bromide-stained agarose gels. CAT activity was normalized to cell number and to pRSVCAT, an external control included in each experiment. In key experiments, pRSV β-galactosidase was cotransfected with the CT-CAT plasmids as an internal control, and CAT activity was normalized to β-galactosidase levels determined as described previously (21).

epithelial origin (7, 25, 29). Previous studies from our laboratory have shown that in both cell lines, the 5′-flanking region of the CT gene is hypomethylated and in a so-called open chromatin configuration favorable for expression (11). We now find that CT gene expression in these two cell lines is mediated by differential utilization of 5′-flanking DNA sequences.

CT gene transcriptional elements in the TT line of MTC. Plasmid pCT750CAT (Fig. 1), containing human CT gene 5′ sequences from a *Bgl*II site at −731 to a *Sau*3AI site at +88 in exon 1, was derived by subcloning this region from cosmid coshCT2, containing the entire human CT gene (30), into pUC18. A *Sal*I fragment from pCATB′ (2), containing the chloramphenicol acetyltransferase (CAT) gene and simian virus 40 splicing and polyadenylation sites, was then ligated via *Sal*I linkers downstream from the CT gene sequences.

pCT750CAT was first transfected into the TT cell line of human MTC, characterized by us and others (8, 10, 20, 23). These cells produce high basal levels of CT and CGRP mRNAs (24). We have also demonstrated that treatment of these cells with cyclic AMP (cAMP) derivatives results in an 8- to 10-fold transcriptional stimulation of the endogenous CT gene (10). Transfection (10^7 cells per ml of 280 mM dextrose–10 mM KCl–1.5 mM KH_2PO_4–5 mM $MgCl_2$ [pH 7.5]) was by electroporation (26), using a Gene Pulser apparatus (Bio-Rad Laboratories) (450 V, 25 μF) and 50 μg of cesium chloride gradient-purified supercoiled plasmid. Cells were then plated in growth medium with or without 1 mM dibutyryl cAMP; 48 h later, assays of CAT activity were conducted essentially as described by Gorman et al. (13).

In parallel with transfection with pCT750CAT, the cells were transfected with pRSVCAT (14), a control plasmid containing the Rous sarcoma virus promoter-enhancer, which exhibits a strong activity in most eucaryotic cell types. Surprisingly, the basal level of expression of pCT750CAT was low and equal to only 6.2 ± 2.5% that of pRSVCAT (Fig. 2 and Table 1). However, when the cells were treated with 1 mM dibutyryl cAMP for 48 h, there was a 19 ± 3.3-fold induction of pCT750CAT activity, to a level approximately equal that of pRSVCAT (Fig. 2 and Table 1). Neither pRSVCAT (14) nor pCATB′, a promoterless CAT plasmid (2), had increased expression in response to cAMP (data not shown).

To delineate the DNA sequences responsible for cAMP induction, deletion mutants of pCT750CAT (pCT500CAT and pCT380CAT) were obtained by partial digestion of pCT750CAT with *Sau*3AI and insertion of a *Bam*HI kanamycin resistance gene cartridge derived from pUC4-KIXX (Pharmacia, Inc.). After selection for kanamycin resistance and mapping of the cartridge insertion site, portions of the 5′ CT gene region were excised along with the kanamycin resistance gene by use of appropriate restriction enzymes. pCT252CAT and pCT132CAT were obtained by using the *Aat*II restriction site at −252 and the *Bam*HI restriction site at −132, respectively (Fig. 1). The basal activities of all of these deletion mutants were comparable with that of pCT750CAT and ranged from 5.7 to 11.3% of the pRSVCAT level (Fig. 2 and Table 1). Similarly, the cAMP responses for pCT252CAT, pCT380CAT, and pCT500CAT were equal to that of pCT750CAT and ranged from a 19.5- to a 27.3-fold increase (Fig. 2 and Table 1). By contrast, pCT132CAT was only minimally stimulated by cAMP (2.1 ± 0.5-fold increase) (Fig. 2 and Table 1), suggesting that cAMP response elements (CREs) (27) residing between nucleotides −132 and −252 are highly active in TT cells.

TABLE 1. Quantification of transient expression assays in TT cells[a]

Plasmid	Determination (mean ± SE)	
	Basal activity (% of pRSVCAT value)	Fold increase in response to cAMP
pCT132CAT	11.3 ± 5.3	2.1 ± 0.5
pCT252CAT	9 ± 3.9	23.5 ± 11.5
pCT380CAT	9.7 ± 1.8	27.3 ± 5.8
pCT500CAT	5.7 ± 0.7	19.5 ± 2
pCT750CAT	6.2 ± 2.5	19 ± 3.3
pCT2000CAT	9.9 ± 5	22 ± 8
pCT2000CAT(R)	8.4 ± 5.3	1.5 ± 0

[a] Shown are data from the assays described in the legend to Fig. 2. Values are means of at least three independent experiments done in duplicate for each construct.

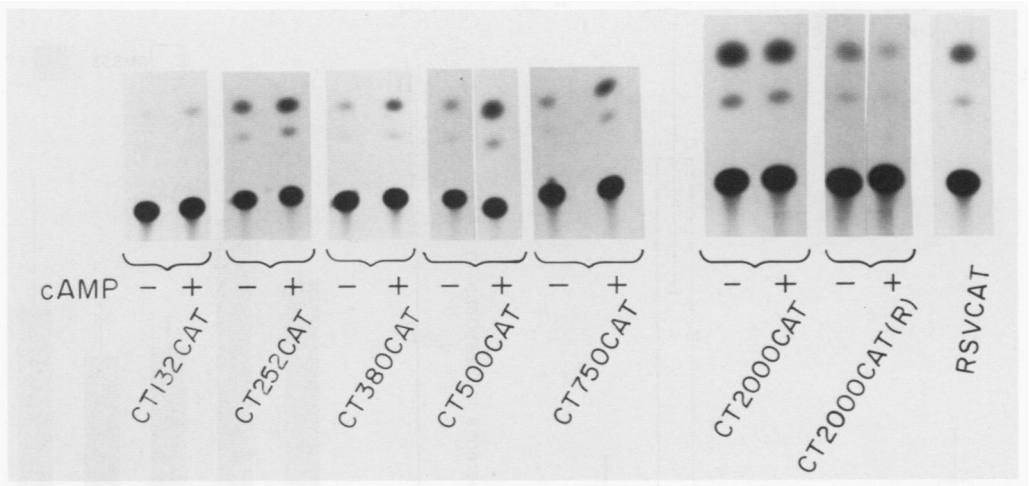

FIG. 3. Transient expression of CT-CAT constructs in DMS53 cells. A representative experiment is shown. Quantitation of the data is given in Table 2.

The results also suggested that the first 750 base pairs (bp) of the 5′ CT gene region do not include basal enhancer elements for TT cells. We therefore sought to determine whether DNA sequences located further upstream confer the high basal CT gene expression present in these cells. pCT2000CAT, a plasmid containing a larger 5′ CT gene region, was made by replacing the DNA fragment from *Bam*HI at −132 to *Bgl*II at −731 in pCT750CAT (Fig. 1) with a 1.9-kbp *Bam*HI fragment (Fig. 1) from the human CT gene-containing cosmid coshCT2 (30). The 1.9-kbp *Bam*HI fragment was inserted in both the forward and reverse [pCT2000CAT and pCT2000CAT(R)] orientations. The basal activity of pCT2000CAT (9.9 ± 5% that of pRSVCAT) was only slightly higher than that of pCT750CAT (6.2 ± 2.5%) (Fig. 2 and Table 1), thus ruling out the presence, within 2,000 bp from the transcription start site, of strong basal enhancer activity for the CT gene in TT cells. pCT2000CAT mediated a dramatic response to cAMP, comparable to that of pCT750CAT (22 ± 8-fold increase), whereas pCT2000CAT(R) did not (Fig. 2 and Table 1), suggesting that for optimal activity, the CREs located between −132 and −252 need to be correctly positioned with respect to promoter sequences located within the first 132 bp of the 5′-flanking region of the CT gene.

CT gene transcriptional elements in the DMS53 line of SCLC. DMS53 is an unusual line of SCLC that produces levels of CT peptide and CT mRNA comparable to those produced by the MTC cells discussed above (7, 25, 29). However, the two cell types regulate the posttranscriptional and posttranslational processing of the CT gene differently. DMS53 cells produce predominantly high-molecular-weight species of immunoreactive CT peptide (7) and a much higher ratio of CT to CGRP mRNA than do TT cells (12, 24). In this study, we first ascertained whether these cells express the CT gene from the same promoter and transcriptional start site as do the TT cells. An antisense oligonucleotide primer complementary to exon 1 (+90 to +110) was annealed to either TT or DMS53 RNA and extended by reverse transcriptase. One single transcriptional start site at position +1 of the CT gene (18) was detected in both cell types (data not shown).

We next tested the basal expression for the CT-CAT plasmids transfected into DMS53 cells. The activity was generally higher than in TT cells whether normalized to pRSVCAT (Fig. 3 and 4A; Table 2) or to expression of a cotransfected internal control plasmid, pRSV β-galactosidase (data not shown). Transfection efficiencies were comparable in the two cell types, as judged by expression of the β-galactosidase construct and a similar cell survival of 25 to 35% after electroporation. In DMS53 cells, unlike in TT cells, an increase in basal activity was seen between plasmids pCT132CAT (16 ± 5.3% of pRSVCAT activity) and pCT252CAT (54 ± 15% of pRSVCAT activity). However, the most striking difference between the cell types was that in DMS53 cells, distal DNA sequences located between 750 and 2,000 bp mediated a dramatic increase in basal activity compared with proximal regions (15.3 ± 4.7% of pRSVCAT activity for pCT750CAT versus 148 ± 39% of pRSVCAT activity for pCT2000CAT) (Fig. 3 and 4A; Table 2). In an attempt to further localize the distal sequences, pCT1400-CAT was made by replacing the *Bam*HI (−132)-to-*Bgl*II (−731) fragment in pCT750CAT with the *Bam*HI (−132)-to-*Hin*dIII (−1460) fragment from cosmid coshCT2 (30). pCT1400CAT exhibited a basal activity as high as that of pCT2000CAT (Table 2).

These data suggest that proximal (−132 to −252) and particularly distal (−750 to −2000) transcriptional elements mediate basal transcription more efficiently in DMS53 than in TT cells. We analyzed further the relationship between

TABLE 2. Quantification of transient expression assays in DMS53 cells[a]

Plasmid	Determination (mean ± SE)	
	Basal activity (% of pRSVCAT value)	Fold increase in response to cAMP
pCT132CAT	16 ± 5.3	2 ± 0.7
pCT252CAT	54 ± 15	2.5 ± 0.8
pCT380CAT	25 ± 4.3	3.5 ± 0.8
pCT500CAT	30 ± 8.7	3.3 ± 0
pCT750CAT	15.3 ± 4.7	3.3 ± 0.2
pCT1400CAT	152 ± 62	ND
pCT2000CAT	148 ± 39	2.6 ± 1
pCT2000CAT(R)	41 ± 20.7	2 ± 0.8

[a] Data from the assays shown in Fig. 3 were generated and analyzed as described in the legend to Fig. 2 and footnote to Table 1. An additional CT-CAT plasmid, pCT1400CAT, not used in the assays of TT cells, was included. ND, Not determined.

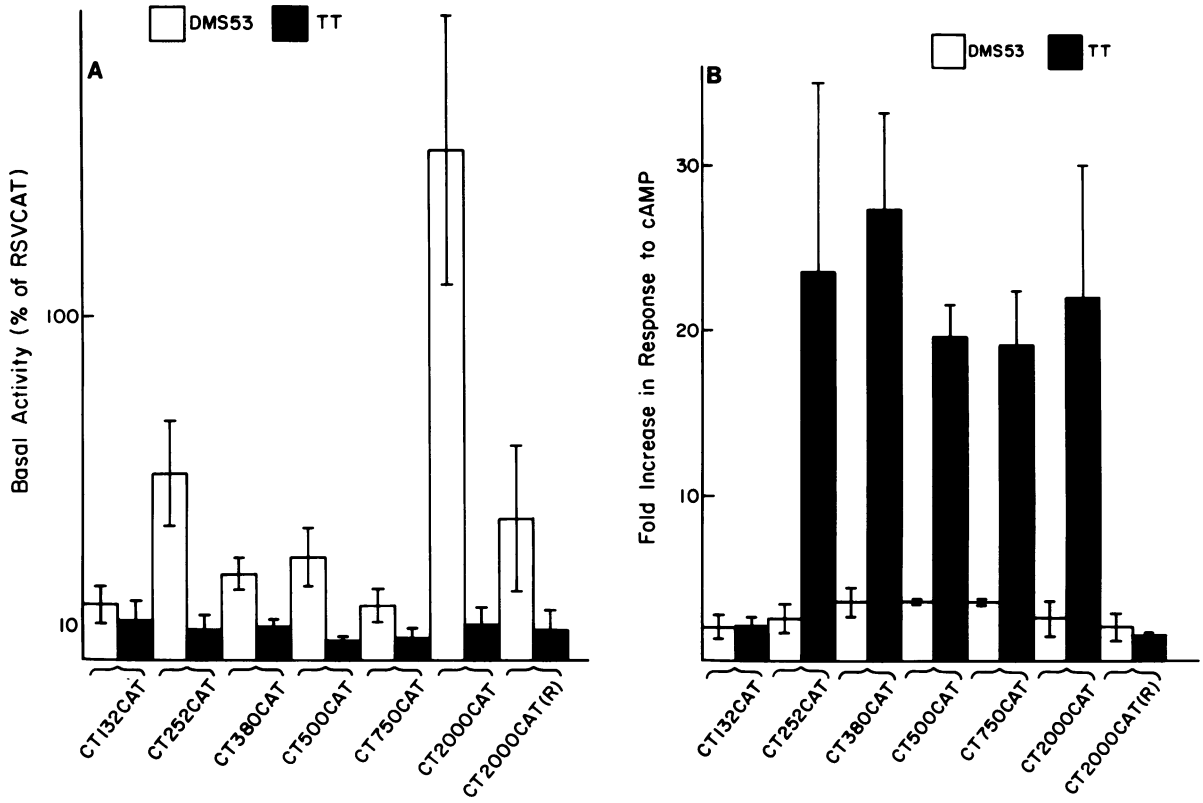

FIG. 4. Comparison of the basal activity (A) and cAMP response (B) in TT and DMS53 cells. Shown is a graphic summary of the data presented in Fig. 2 and 3 and Tables 1 and 2.

proximal and distal elements in DMS53 cells as follows. The *Bam*HI-to-*Aat*II (−132 to −252) and *Bgl*II-to-*Hin*dIII (−731 to −1460) CT fragments were separately excised from pCT2000CAT and inserted into the multiple cloning site of pBLCAT2, a CAT plasmid containing the herpes simplex virus thymidine kinase gene promoter (22). These constructs increased thymidine kinase expression by 1.7 ± 0.4- and 17 ± 7-fold, respectively (Fig. 5), suggesting that although both elements contribute to enhancing CT gene expression in DMS53 cells, the distal element is the more powerful. Potential interaction between the proximal and distal elements was studied by selectively deleting the proximal element (−132 to −429) from pCT2000CAT. This resulted in a marked decrease in activity (to 13.7 ± 2.4% that of pCT2000CAT) (Fig. 5), strongly suggesting a cooperative interaction between the two elements, perhaps involving DNA looping and DNA-binding protein contact. In turn, these two elements appear to be orientation dependent, or at least to require a correct positioning with respect to CT promoter sequences, since the plasmid containing the 1.9-kbp *Bam*HI fragment in the reverse orientation, pCT2000-CAT(R), had 30% of the activity of pCT2000CAT (41 ± 20.7% versus 148 ± 39% of pRSVCAT activity) (Fig. 3 and Table 2).

In addition to the differential use of basal *cis*-acting DNA elements, TT and DMS53 cells differed by the magnitude of their cAMP responses. The cAMP response of each of the CT-CAT constructs in DMS53 cells ranged only from a 2- to 3.5-fold increase (Fig. 3 and Table 2). No increase in cAMP response was observed between nucleotides −132 and −252, as seen in the TT cells (Fig. 4B). This result suggests that the CREs located in this region, which mediate a 20- to 30-fold increase in transcriptional activity in response to cAMP in TT cells, are less active in DMS53 cells or that the higher basal activity in the lung carcinoma cells is not further

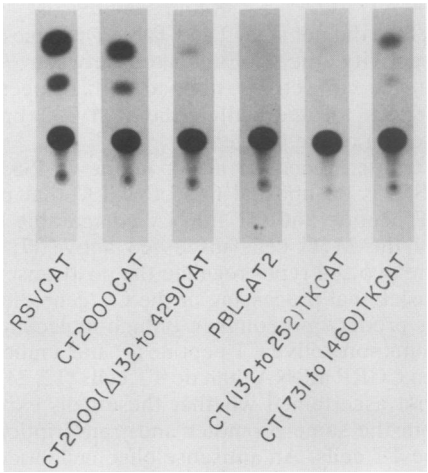

FIG. 5. Analysis of proximal and distal DNA elements in DMS53 cells. A representative experiment is shown. The means and standard errors of at least three independent experiments done in duplicate were as follows: the CAT activity of CT2000(Δ132 to 429)CAT normalized to pRSVCAT activity was 13.7 ± 2.4% that of CT2000CAT; the ratios of CT(132 to 252)TKCAT and CT(731 to 1460)TKCAT to pBLCAT2 were 1.7 ± 0.4- and 17 ± 7-fold, respectively.

inducible. This difference in cAMP induction of the CT gene constructs between the TT and DMS53 cell lines paralleled induction of the endogenous CT gene, as judged by Northern (RNA) analysis (data not shown) of endogenous mRNA in control and cAMP-treated cells (eightfold increase for TT cells and twofold increase for DMS53 cells).

In conclusion, these data show that the transcriptional activity of the human CT gene is regulated by differential utilization of 5′ DNA sequences in two human tumor cell lines of different embryological origin, MTC and SCLC. This may reflect quantitative or qualitative differences in the *trans*-acting factors that interact with these DNA sequences. Thus, proteins interacting with proximal CREs in the 5′ region of the CT gene (−132 to −252) may be more abundant or more active in TT than in DMS53 cells. By contrast, cellular factors recognizing proximal (−132 to −252) and distal (−750 to −1400) elements in the 5′-flanking region of the CT gene may be more abundant or more active in the basal state in DMS53 than in TT cells. Alternatively, TT cells may contain negative factors interacting with the distal region. It is interesting that both cell types use the proximal (−132 to −252) CT gene region for different functions: in TT cells it mediates a dramatic cAMP response, whereas in DMS53 cells it serves as a modest basal enhancer. Delineation of the exact DNA sequences mediating these two functions is currently being pursued.

The diversity of *cis*-acting DNA elements utilized by MTC and SCLC lines may reflect the separate embryological origins of these tumor cells. Thus, by maintaining high levels of CT gene transcription through constant activation of signal transduction pathways such as those mediated by cAMP, TT cells could be mimicking the normal function of their parent thyroid C cells, programmed to respond acutely to physiological regulators such as calcium or gastrin (3). DMS53 cells, of bronchial epithelial origin, may similarly reflect the function of their parent cells, which may express the CT gene predominantly in a constitutive fashion. Recently, a few eucaryotic genes in which different DNA sequences control expression in different cell types have been reported. These include the human apolipoprotein AI (28), rat phosphoenolpyruvate carboxykinase (5), and mouse alpha-fetoprotein (16) genes. The human CT gene may represent an additional example in which the differential utilization of DNA sequences contributes to a diversity of patterns of cell-specific and developmental expression.

We and others (6) have sequenced the first 1,460 bp of the 5′ region of the CT gene and identified several consensus binding sites for known transcription factors, including AP-2 (17) and CRE-binding protein (27). Deletion mutagenesis studies and DNase I footprinting analysis are under way to demonstrate the importance of these and other DNA sequences for basal and induced CT gene expression in TT and DMS53 cells. Isolation of the *trans*-acting factors mediating CT gene expression in MTC and SCLC should provide valuable tools for understanding the differential expression of polypeptide hormone genes between normal cells of diverse tissue origins and between tumors arising from these different sites.

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LITERATURE CITED

1. **Amara, S. G., V. Jonas, M. G. Rosenfeld, E. S. Ong, and R. M. Evans.** 1982. Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. Nature (London) **298**:240–244.
2. **Andrisani, O. M., T. E. Hayes, B. Roos, and J. E. Dixon.** 1987. Identification of the promoter sequences involved in the cell specific expression of the rat somatostatin gene. Nucleic Acids Res. **15**:5715–5728.
3. **Austin, L. A., and H. Heath.** 1981. Calcitonin physiology and pathophysiology. N. Engl. J. Med. **304**:269–275.
4. **Baylin, S. B., and G. Mendelsohn.** 1980. Ectopic (inappropriate) hormone production by tumors: mechanisms involved and the biological and clinical implications. Endocr. Rev. **1**:45–77.
5. **Benvenisty, N., H. Nechushtan, H. Cohen, and L. Reshef.** 1989. Separate cis-regulatory elements confer expression of phosphoenolpyruvate carboxykinase (GTP) gene in different cell lines. Proc. Natl. Acad. Sci. USA **86**:1118–1122.
6. **Broad, P. M., A. J. Symes, R. V. Thakker, and R. K. Craig.** 1989. Structure and methylation of the human calcitonin/α-CGRP gene. Nucleic Acids Res. **17**:6999–7011.
7. **Cate, C. C., O. S. Pettengill, and G. D. Sorenson.** 1986. Biosynthesis of procalcitonin in small cell carcinoma of the lung. Cancer Res. **46**:812–818.
8. **Cote, G. J., and R. F. Gagel.** 1986. Dexamethasone differentially affects the levels of calcitonin and calcitonin gene-related peptide mRNA's expressed in a human medullary thyroid carcinoma cell line. J. Biol. Chem. **261**:15524–15528.
9. **de Bustros, A., and S. B. Baylin.** 1985. Hormone production by tumours: biological and clinical aspects. Clin. Endocrinol. Metab. **14**:221–256.
10. **de Bustros, A., S. B. Baylin, M. A. Levine, and B. D. Nelkin.** 1986. Cyclic AMP and phorbol esters separately induce growth inhibition, calcitonin secretion, and calcitonin gene transcription in cultured human medullary thyroid carcinoma. J. Biol. Chem. **261**:8036–8041.
11. **de Bustros, A., B. D. Nelkin, A. Silverman, G. Ehrlich, B. Poiesz, and S. B. Baylin.** 1988. The short arm of chromosome 11 is a "hot spot" for hypermethylation in human neoplasia. Proc. Natl. Acad. Sci. USA **85**:5693–5697.
12. **Edbrooke, M. R., D. Parker, J. H. McVey, J. H. Riley, G. D. Sorenson, O. S. Pettengill, and R. K. Craig.** 1986. Expression of the human calcitonin/CGRP gene in lung and thyroid carcinoma. EMBO J. **4**:715–724.
13. **Gorman, C. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2**:1044–1051.
14. **Gorman, C. M., G. T. Merlino, M. C. Willingham, I. Pastan, and B. H. Howard.** 1982. The Rous sarcoma virus long terminal repeat is a strong promoter when introduced into a variety of eukaryotic cells by DNA-mediated transfection. Proc. Natl. Acad. Sci. USA **79**:6777–6781.
15. **Gosney, J. R., and M. C. J. Sissons.** 1985. Widespread distribution of broncho pulmonary endocrine cells immunoreactive for calcitonin in the lung of the normal adult rat. Thorax **40**: 194–198.
16. **Hammer, R. E., R. Krumlary, S. A. Camper, R. L. Brinster, and S. M. Tilghman.** 1987. Diversity of alpha-fetoprotein gene expression in mice is generated by a combination of separate enhancer elements. Science **235**:53–58.
17. **Imagawa, M., R. Chiu, and M. Karin.** 1987. Transcription factor AP-2 mediates induction by two different signal-transduction pathways: protein kinase C and cAMP. Cell **51**:251–260.
18. **Jonas, V., C. R. Lin, E. Kawashima, D. Semon, L. W. Swanson, J.-J. Mermod, R. M. Evans, and M. G. Rosenfeld.** 1985. Alternative RNA processing events in human calcitonin/calcitonin gene-related peptide gene expression. Proc. Natl. Acad. Sci. USA **82**:1994–1998.
19. **Lauweryns, J. M., and L. V. Ranst.** 1987. Calcitonin gene-related peptide immunoreactivity in rat lung: light and electron

microscopic study. Thorax **42**:183–189.

20. **Leong, S. S., J. S. Horoszewicz, K. Shimaoka, M. Friedman, E. Kawinski, M. J. Song, R. Zeigel, T. M. Chu, S. B. Baylin, and E. A. Mirand.** 1981. A new cell line for study of human medullary thyroid carcinoma, p. 95–108. *In* M. Andreoli, F. Monaco, and J. Robbins (ed.), Advances in thyroid neoplasia. Field Educational Italia, Rome, Italy.
21. **Lim, K., and C.-B. Chae.** 1989. A simple assay of DNA transfection by incubation of the cells in culture dishes with substrates for beta-galactosidase. Biotechniques **7**:576–579.
22. **Luckow, B., and G. Schütz.** 1987. CAT constructions with multiple unique restriction sites for the functional analysis of eucaryotic promoters and regulatory elements. Nucleic Acids Res. **15**:5490.
23. **Nakagawa, T., B. D. Nelkin, S. B. Baylin, and A. de Bustros.** 1988. Transcriptional and post-transcriptional modulation of calcitonin gene expression by sodium n-butyrate in cultured human medullary thyroid carcinoma. Cancer Res. **48**:2096–2100.
24. **Nelkin, B. D., K. I. Rosenfeld, A. de Bustros, S. S. Leong, B. A. Roos, and S. B. Baylin.** 1984. Structure and function of a gene encoding human calcitonin and calcitonin gene related peptide. Biochem. Biophys. Res. Commun. **123**:648–655.
25. **Pettengill, O. S., G. D. Sorenson, D. H. Wurster-Hill, T. J. Curphey, W. W. Noll, C. C. Cate, and L. H. Maurer.** 1980. Isolation and growth characteristics of continuous cell lines from small cell carcinoma of the lung. Cancer **45**:906–918.
26. **Potter, H., L. Weir, and P. Leder.** 1984. Enhancer-dependent expression of human K immunoglobulin genes introduced into mouse pre-B lymphocytes by electroporation. Proc. Natl. Acad. Sci. USA **81**:7161–7165.
27. **Roesler, W. J., G. R. Vandenbark, and R. W. Hanson.** 1988. Cyclic AMP and the induction of eucaryotic gene transcription. J. Biol. Chem. **263**:9063–9066.
28. **Sastry, K. N., U. Seedorf, and S. K. Karathanasis.** 1988. Different *cis*-acting DNA elements control expression of the human apolipoprotein AI gene in different cell types. Mol. Cell. Biol. **8**:605–614.
29. **Sorenson, G. D., O. S. Pettengill, T. Brinck-Johnson, C. C. Cate, and L. H. Maurer.** 1981. Hormone production by cultures of small cell carcinoma of the lung. Cancer **47**:1289–1296.
30. **Steenbergh, P. H., J. W. M. Hoppener, J. Zandberg, W. J. M. Van De Ven, H. S. Jansz, and C. J. M. Lips.** 1984. Calcitonin gene related peptide coding sequence is conserved in the human genome and is expressed in medullary thyroid carcinoma. J. Clin. Endocrinol. Metab. **59**:358–360.