Characterization of a cell-specific modulatory element in the murine ornithine decarboxylase promoter

Jorma J. PALVIMO, Maija PARTANEN and Olli A. JÄNNE*

Institute of Biomedicine, Department of Physiology, University of Helsinki, P.O. Box 9, FIN-00014 Helsinki, Finland

The promoter of the murine ornithine decarboxylase (ODC) gene contains, adjacent to the TATA box, a cAMP response element (CRE)-like motif that interacts with specific nuclear proteins. Here we examine the role of this CRE-like element (CREL) in ODC promoter activation in proliferating cells. Mutations that abolished binding of nuclear proteins to CREL influenced only marginally the cAMP induction of the reporter constructs driven by 1.6 kb of the ODC promoter. Instead, these mutations altered the basal promoter function in a cell-specific manner, in that they reduced the promoter activity in CV-1 cells, but increased it in NIH/3T3, CHO and HeLa cells. Thus, depending on the cell type, the CREL motif is able to confer either repression or activation on ODC gene transcription. In contrast with 1.6 kb promoter constructs, the same mutations in the context of a shorter sequence (proximal 133 nt) reduced the promoter strength in all cell types studied. The ability of the CREL element to attenuate transcription seems to be connected

with the function of some upstream regulatory elements. Differences in nuclear proteins binding to CREL, as studied by electrophoretic mobility shift assays (EMSAs), did not explain the findings on cell-type specificity in transcriptional activation, as mutations in CREL abrogated formation of specific CREL–protein complexes in all cell lines examined. The protein complexes interacting with CREL were not recognized by antibodies specific for CRE-binding proteins CREB-1 and CREB-2, or activating transcription factors ATF-1, ATF-2 and ATF-3. EMSA experiments also demonstrated co-operative interactions between the CREL motif-binding proteins and other nuclear proteins, such as Sp1, interacting with CG-rich sequences of the promoter. In conclusion, the proximal ODC promoter contains a well-conserved regulatory element, which is clearly different from the CRE/ATF element. This motif acts in concert with other distal and proximal elements in a complex cell-specific manner.

INTRODUCTION

Ornithine decarboxylase (ODC) is the first and key regulatory enzyme in the biosynthesis of polyamines, molecules indispensable for both normal and malignant cell proliferation [1–3]. In most tissues ODC levels are low, and an increase in the enzyme activity is one of earliest events associated with cell proliferation. The ODC gene may be considered as an immediate early gene. Catalytically active ODC is necessary for progression into the S phase of the cell cycle, and specific inhibitors of the enzyme or polyamine depletion arrest cells in the G1 phase [2,3]. ODC activity is highly regulated in response to a large number of stimuli affecting cell growth, including growth factors, steroid hormones, tumour promoters and cAMP-elevating agents [1–10]. The regulation is complex and seems to occur at multiple levels, including the rate of synthesis and degradation of the enzyme protein, along with the stability and translational efficiency of mRNA. An intriguing feature in this regulation is that exerted by ODC antizyme, which not only inhibits the enzyme activity but also accelerates the rate of degradation of the enzyme protein [11]. Induction of ODC gene expression or activation of the ODC promoter in response to various stimuli involving different transcription factors has also been reported [12–17].

The ODC gene is well conserved, both in the protein-coding region and in potential transcriptional regulatory sequences. The human, mouse and rat promoters share 82 % nucleotide sequence identity from transcription start site to nt –148, and are only

slightly less conserved over the first 380 nt [18–23]. There are several potential regulatory elements for *trans*-acting factor sites within the first 380 nt of the murine promoter, such as a TATA box at –31, a cAMP response element (CRE)-like element at –48, a putative CAAT box at –81, and four or five Sp1-binding sites [19]. We have previously shown that the murine ODC promoter possesses strong basal activity, up to 80 % of that obtained with a vector driven by the Rous sarcoma virus promoter [24]. Interestingly, the first 200 nt of the murine promoter exhibited approx. 50 % of the activity of a 1.6 kb construct, indicating that the proximal promoter contains elements important for transcriptional activation. ODC promoter activity is stimulated by agents elevating intracellular cAMP concentration, and footprinting analyses have revealed that a CRE-like element (CREL), encompassing the region –59/–39, interacts with nuclear protein(s) in a specific manner [24]. In the present study we have characterized further the role of this CREL element in basal and cAMP-induced transcription of the murine ODC gene. We have also delineated protein–DNA complexes formed by nuclear extracts from various cell lines within the proximal region of the promoter.

MATERIALS AND METHODS

Plasmids and chemicals

Plasmids pSV- β -galactosidase (pSV- β -gal), pGL3control and pGL3basic, luciferase assay reagent, and purified Sp1 protein

Abbreviations used: ATF, activating transcription factor; 8Br-cAMP, 8-bromo-cAMP; CRE, cAMP response element; CREB, CRE-binding protein; CREL, CRE-like element; EMSA, electrophoretic mobility-shift assay; ODC, ornithine decarboxylase.

* To whom correspondence should be addressed.

Figure 1 Mouse ODC promoter sequence from -184 to $+16$

The G residues that are changed to T in CREL mutant MU ($-55/-47/-44$) are shown below the sequence. Missing nucleotides in mutant DE ($-59/-39$) are denoted by a horizontal line with arrowheads. The five oligonucleotides used in competition analysis are depicted with filled bars, and the positions of the first and last nucleotides are indicated by numbers on the right. The CREL motif is marked by bold letters, the TATA-box is boxed and the Sp1 consensus sequence is underlined.

were purchased from Promega (Madison, WI, U.S.A.). WT-1 expression vector pCMV-WT18A was a gift from Dr. Frank J. Rauscher III (The Wistar Institute, Philadelphia, PA, U.S.A.). Expression plasmid pSG5hRb was obtained from Dr. Marikki Laiho (University of Helsinki, Helsinki, Finland). 8-Bromo-cAMP (8Br-cAMP) and *o*-nitrophenyl- β -D-galactopyranoside were from Sigma (St. Louis, MO, U.S.A.). Restriction endonucleases, DNA-modifying enzymes and poly(dI-dC)·poly(dI-dC) were purchased from Pharmacia LKB Biotechnology (Uppsala, Sweden). [γ - 32 P]ATP was from Amersham (Bucks., U.K.) and [3 H]acetyl-CoA from New England Nuclear (Boston, MA, U.S.A.). Antibodies (supershift grade) for CRE-binding protein-1 (CREB-1), CREB-2, activating transcription factor-1 (ATF-1), ATF-2 and ATF-3 were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, U.S.A.).

Preparation of nuclear extracts

Nuclear extracts were prepared from cultured cells by the method of Hurst et al. [25] as described in detail [26], frozen in liquid nitrogen and stored at -70°C . Protein concentration was measured with Protein assay reagent (Bio-Rad Laboratories, Richmond, CA, U.S.A.).

Electrophoretic mobility-shift assay (EMSA)

EMSA experiments were carried out as previously described [24,27] with the amounts of nuclear extract indicated in the Figure legends. The binding reactions consisted of a 10 min preincubation at 4°C , followed by the addition of ^{32}P -labelled wild-type or mutated *Ava*I-*Xba*I fragments of the ODC promoter corresponding to the region $-136/+16$ relative to the transcription start site. After 30 min at 22°C , reaction mixtures were loaded onto a 4% (w/v) non-denaturing polyacrylamide gel (29:1) which was prerun at 220 V for 30 min. Electrophoresis was performed in 22.5 mM Tris base/22.5 mM boric acid/0.5 mM EDTA for 3 h at 22°C , the gel was dried and subjected to autoradiography for 2–16 h at -70°C . For competition studies, nuclear proteins were mixed with a 100-fold molar excess of appropriate non-radioactive double-stranded oligonucleotides just before addition of the radioactive probe. In experiments with antibodies, nuclear extracts were preincubated with either

immune IgG ($3\ \mu\text{l}$ per $20\ \mu\text{l}$) or normal IgG for 16 h at 4°C before addition of the DNA probe.

Plasmid constructions and mutagenesis

pODCcat($-1658/+16$)M was made from pODCcat($-1658/+16$) [24] by mutating each of the three G residues at -55 , -47 and -44 of CREL to T (Figure 1). pODCcat($-1658/+16$)D is pODCcat($-1658/+16$) in which the nucleotides from -59 to -39 are deleted. The mutations were made by PCR with the following 5'-primers: M-primer, 5'-CTCCCGGCCGGAACCGATCGCGGCTGGTTTGAGCTGGTGCTTCTCCATTACTACGTGTCG-3'; D-primer, 5'-CTCCCGGCCGGAACCGATCGCGGCTGGTTTGAGCTGCTCGGCGTAT-3'. The 3'-primer 5'-GCTCTCTAGAATGCATCCCG-3' was from the polylinker region of pODCcat($-1658/+16$). The fragments were assembled into pODCcat($-1658/+16$) as *Eag*I-*Xba*I fragments. Luciferase reporter vectors, pODCluc($-133/+16$), pODCluc($-133/+16$)M and pODCluc($-133/+16$)D, were constructed from *Sma*I-*Bgl*II fragments corresponding to the indicated sequences of the wild-type and mutated pODCcat constructs respectively and inserted into the polylinker region of pGL3basic vector. Nucleotide sequences of all constructs were verified by sequencing double-stranded DNA with reagents purchased from Pharmacia LKB Biotechnology [28].

Cell cultures and transfections

CV-1, HeLa, NIH/3T3 and CHO cells were obtained from American Type Culture Collection (ATCC, Rockville, MD, U.S.A.). S115 cells were from Dr. Pirkko Härkönen (University of Turku, Turku, Finland). Cells were maintained in Dulbecco's minimal essential medium containing penicillin (25 i.u./ml) and streptomycin (25 i.u./ml). CV-1 and HeLa cells were cultured in the presence of 10% (v/v) fetal calf serum, and S115 cells in 5% fetal calf serum plus 10 nM testosterone. The medium for NIH/3T3 cells contained 10% calf serum. Transient transfections were performed with the calcium phosphate precipitation method as previously described [24,29]. Briefly, 10^6 cells were plated onto a 10 cm dish 24 h before addition of the precipitate containing $7\ \mu\text{g}$ of ODC promoter reporter plasmid and $7\ \mu\text{g}$ of β -galactosidase expression plasmid (pSV- β -gal), which was used as an internal control for transfection efficiency. Chloramphenicol acetyltransferase and β -galactosidase assays were performed as described previously [24,29], and protein concentration of cell extracts was measured with a Bio-Rad protein assay reagent. For assay of luciferase activity, cells were harvested and lysed in 0.1 M potassium phosphate, pH 7.8, containing 1 mM dithiothreitol, and the enzyme activity was determined with Promega's luciferase assay reagent. In short, $100\ \mu\text{l}$ of the reagent was injected into $10\ \mu\text{l}$ of cell extract (on a 96-well plate), mixed for 2 s and subsequently measured for 10 s with a Labsystems Luminoskan RT reader (Labsystems, Helsinki, Finland).

RESULTS

Proteins binding to the proximal ODC promoter

We have previously demonstrated that the region from -59 to -39 relative to the transcription start site ($-59/-39$) of the murine ODC gene promoter, which encompasses a DNA motif resembling the consensus CRE (here termed CREL), interacts with a ubiquitous 70 kDa protein that is different from the well-characterized CREB [24]. In the present work we have extended

Figure 2 Nuclear proteins interacting with the wild-type (WT) ODC promoter region $-136/+16$ and the corresponding mutated promoter sequences

(a) 32 P-labelled probes were incubated with nuclear proteins extracted from CV-1 cells (lanes 1–4) and S115 cells (lanes 5–7). Nuclear protein extract ($5 \mu\text{g}$) was used in each assay except lane 1 ($1.5 \mu\text{g}$). The letters A, B, C, D and E denote the specific DNA–protein complexes formed with wild-type CREL. (b) Competition (COMP.) of DNA–protein complexes formed with the wild-type ODC promoter fragment $-136/+16$ as a probe. Competing oligomers are indicated at the bottom of the autoradiogram. The specific DNA–protein complexes are denoted by letters A–E. The asterisk denotes a complex that was not efficiently competed for by a 100-fold excess of unlabelled probe. Nuclear extracts ($5 \mu\text{g}$ of protein) were from CV-1 cells. Competing oligomers were present in a 100-fold molar excess. (c) Binding of recombinant Sp1 protein (4 ng) to the ODC promoter region $-136/+16$ in the absence (lane 1) and in the presence (lanes 2 and 3) of indicated competitor oligomers.

these studies and examined proteins interacting with the murine ODC promoter covering the sequence from -136 to $+16$, along with some mutated forms of this region. The CREL motif was

Figure 3 Nuclear proteins interacting with the murine ODC promoter region $-62/-33$ in the presence of various CREB and ATF antibodies

A 32 P-labelled 30-mer oligonucleotide corresponding to region $-62/-33$ of the murine ODC promoter was used as the probe. The antibodies against the following transcription factors were included in the preincubation (16 h at 4°C): lane 1, no IgG; lane 2, normal IgG; lane 3, CREB-1; lane 4, CREB-2; lane 5, ATF-1; lane 6, ATF-2; lane 7, ATF-3. The specific complexes B and C are indicated on the right and the asterisk denotes a non-specific complex.

either deleted or mutated according to our previous DNase I footprinting and methylation interference data [24]. In one mutated probe (MU) each of the three G residues located at -55 , -47 and -44 was changed to T (Figure 1). In another mutant probe (DE) the region $-59/-39$ was deleted. Figure 2(a) shows (lanes 2 and 5) that nuclear extracts from CV-1 (monkey kidney) cells and S115 (mouse mammary tumour) cells formed six major protein–DNA complexes with the wild-type probe. The three slowly migrating complexes, depicted by A, B and C, were totally absent when DE oligomer was used as a probe (Figure 2a, lanes 4 and 7). With the MU probe, complex A was undetectable and intensities of complexes B and C were markedly reduced (Figure 2a, lanes 3 and 6). Similar complexes were observed with nuclear proteins from HeLa, NIH/3T3 and CHO cells (results not shown). Complexes A, B and C were thus derived from interactions between nuclear proteins and CREL.

Complex A seemed to arise from co-operative interactions between several nuclear proteins, as it was undetectable at low concentrations of nuclear extracts that were nevertheless sufficient for the formation of complexes B and C (compare Figure 2a, lanes 1 and 2). Addition of a 100-fold molar excess of the non-radioactive probe abolished complexes A, B and C as well as two of the faster-migrating ones denoted by D and E (Figure 2b, lane 2). Competition experiments with oligonucleotides $-62/-33$ and $-52/-3$ provided additional evidence that complexes A, B and C were formed through interaction with the CREL element (Figure 2b, lanes 5 and 6). Surprisingly, an excess of oligomer $-138/-89$ was not only able to eliminate complexes D and E but also abolished complex A (Figure 2b, lane 3). This oligomer did not, however, influence the formation of complexes B and C. A possible explanation is that the factors occupying the region $-59/-39$ are able to form protein–protein interactions with those associated with the region $-136/-89$. This GC-rich region contains a consensus Sp1 site at -112 and, indeed, not only complex E but also complex A was competed for by an Sp1

Table 1 Effect of mutations in CREL on basal and cAMP-induced expression of pODCcat(−1658/+16) constructs

CREL was modified by mutating the three G residues (−55, −47, −44) to T (denoted by pM) or by deleting the sequence −59/−39 (denoted by pD) from pODCcat(−1658/+16) (denoted by pWT). CV-1, CHO, NIH/3T3 or HeLa cells were transfected with 7 µg of the indicated pODCcat constructs as described in the Materials and methods section. The cells received, 18 h after transfection, fresh medium supplemented with or without 1 mM 8Br-cAMP for the ensuing 30 h. All chloramphenicol acetyltransferase values were normalized with either β-galactosidase activity or protein concentration in the same extract, and the values for the pWT construct pODCcat(−1658/+16) in the absence of cAMP were set as 100 for each cell line separately. pPL corresponds to a promoterless reporter and p-97 refers to pODCcat(−97/+16) construct. Means ± S.E.M. values for three to six experiments are given, except for HeLa cells when mean values from duplicate experiments are shown.

Cell line		Reporter construct (relative chloramphenicol acetyltransferase activity)				
		pWT	pM	pD	p-97	pPL
CV-1	−cAMP	100 ± 5	53 ± 5	23 ± 3	70 ± 2	0.2 ± 0.1
	+cAMP	228 ± 24	132 ± 16	44 ± 6	222 ± 22	0.2 ± 0.1
CHO	−cAMP	100 ± 10	434 ± 8	382 ± 17	50 ± 4	6 ± 1
	+cAMP	473 ± 32	1733 ± 172	1001 ± 150	248 ± 6	16 ± 3
HeLa	−cAMP	100	145	63	38	3.7
	+cAMP	201	197	66	55	2.2
NIH/3T3	−cAMP	100 ± 17	273 ± 31	223 ± 26	65 ± 24	6 ± 1
	+cAMP	185 ± 33	557 ± 112	305 ± 87	218 ± 62	14 ± 6

consensus oligomer (Figure 2b, lane 9). In addition, purified Sp1 protein bound to the above promoter region (Figure 2c), indicating that complex E contains an Sp1-like protein. In view of this, it is likely that Sp1 and/or related proteins interact with the proximal ODC promoter and form protein–protein complexes with factors binding to CREL. Sp1 did not, however, bind under our experimental conditions directly to oligomer −64/−37 containing CREL (results not shown).

As CREL-associated proteins seemed to be distinct from CREB [24], we examined the possibility that other members of the CREB/ATF family recognizing the sequence 5'-TGAGCTCA-3' [30] could assemble into complexes A, B and/or C. As shown in Figure 3, none of the antibodies specific for activating transcription factors (anti-ATF-1, -2 or -3) or CRE-binding proteins (anti-CREB-1 or -2) altered the mobility or intensity of complexes B and C, when an oligonucleotide covering the region −64/−37 was used as a probe.

Effect of mutations in the CREL motif on ODC promoter activity

We assessed next how CREL mutations and/or deletion in the context of the 1.6 kb ODC promoter affected basal activity and cAMP regulation of the promoter under transient transfection conditions. Point mutations within CREL that abolished protein binding to this motif, as judged by EMSA experiments, decreased the promoter activity by approx. 50% in CV-1 cells, whereas in CHO, HeLa and NIH/3T3 cells the effect was opposite, in that mutated promoters exhibited approx. 50–300% increased activities (Table 1). In CV-1 cells, deletion of CREL had an even more drastic effect than point mutations as it decreased promoter activity to one-quarter of the wild-type activity. This latter construct exhibited reduced activity in HeLa cells too. In CHO and NIH/3T3 cells, however, the behaviour of the deletion mutant did not differ significantly from that of the point-mutated construct. These results imply that CREL motif, which is recognized by specific nuclear proteins, contributes significantly to cell-specific regulation of the ODC promoter.

Table 2 Effect of mutations in CREL on basal and cAMP-induced expression of pODCluc(−133/+16) constructs

CV-1, CHO, NIH/3T3 or HeLa cells were transfected with 7 µg of the indicated pODCluc constructs as described in the Materials and methods section and in Table 1. All luciferase values were normalized with either β-galactosidase activity or protein concentration in the same extract, and the values for pODCluc(−133/+16) (denoted by pWTS) in the absence of 8Br-cAMP were set as 100 for each cell line separately. pGL3 is a promoterless reporter, pMS is pODCluc(−133/+16) construct in which the three G residues (−55, −47, −44) are replaced by T, and pDS is pODCluc(−133/+16) without the sequence −59/−39. Means ± S.E.M. values for three separate experiments are shown, except for pGL3 where mean values from duplicate experiments are given. The relative activity of the pODCluc(−133/+16) construct in CV-1 cells was 32 ± 8% of that of the pGL3control vector driven by SV40 promoter and SV40 enhancer.

Cell line		Reporter construct (relative luciferase activity)			
		pWTS	pMS	pDS	pGL3
CV-1	−cAMP	100 ± 6	40 ± 1	17 ± 1	0.3
	+cAMP	59 ± 4	36 ± 2	14 ± 1	0.2
CHO	−cAMP	100 ± 3	47 ± 2	47 ± 2	4.4
	+cAMP	200 ± 15	104 ± 1	78 ± 2	7.7
HeLa	−cAMP	100 ± 2	25 ± 1	10 ± 1	0.2
	+cAMP	83 ± 3	18 ± 1	10 ± 1	0.2
NIH/3T3	−cAMP	100 ± 14	40 ± 13	25 ± 10	0.9
	+cAMP	128 ± 24	30 ± 7	31 ± 4	1.5

The above mutations were expected to affect cAMP regulation of the promoter; however, activities of mutated constructs were still induced by cAMP and, in particular, replacement of the three G residues by T had only a marginal effect on this induction (Table 1). The construct pODCcat(−1658/+16)M was stimulated by cAMP as efficiently as the wild-type promoter in CV-1, CHO and NIH/3T3 cells. Deletion of CREL impaired only slightly the stimulation by cAMP in CV-1 and CHO, but blunted it in NIH/3T3 and HeLa cells (Table 1). A plausible explanation for these results is that factors binding to distal elements within the promoter are capable of compensating for the loss of CREL.

Because a short ODC promoter construct covering the region −97/+16 was induced by cAMP at least as well as the 1.6 kb construct in most cases (Table 1) [24], it was assumed that an element mediating the response to cAMP was located in the most proximal promoter. To address this question, we used luciferase reporters driven by the region −133/+16 of wild-type and mutated promoters. The two CREL-mutated forms of pODCluc(−133/+16) showed reduced basal activity in all cell lines examined (Table 2). The activities of CREL-deleted construct (pDS) were 10–50% of those of the wild-type, and the corresponding values were 25–50% of wild-type activity for the construct with three mutated G residues (pMS). This decreased promoter strength brought about by alterations in CREL sequence was in agreement with the result that formation of protein complexes A, B and C was abolished by the above mutations (Figure 2a). Thus, in the absence of upstream promoter sequences (from −1658 to −134), the CREL motif behaves as an activating element.

Neither the wild-type pODCluc(−133/+16) nor the corresponding mutated constructs was significantly induced by cAMP in CV-1 or HeLa cells (Table 2). The activity of the wild-type construct (pWTS) in CV-1 cells was actually decreased in the presence of cAMP. All luciferase reporter constructs, including the promoterless pGL3 vector, were stimulated by cAMP approx. 2-fold in CHO cells. In agreement with our previous results on pODCcat(−97/+16) reporter [24], the wild-type

pODCluc(−133/+16) construct was activated somewhat by cAMP in NIH/3T3 cells. In this latter case, CREL mutations did abolish the response to cAMP (Table 2).

DISCUSSION

The murine ODC promoter containing 1.6 kb of 5'-flanking DNA to the gene is highly active in cultured mouse cells [24]. The present results show that a region in the proximal promoter containing a CREL, which is protected by ubiquitous nuclear proteins in DNase footprinting assays [24], plays an important role in promoter activation *in vivo*. In CV-1 cells, mutation of three G residues to T within and around CREL diminished ODC promoter activity to one-half, and deletion of the sequence −59/−39 to one-quarter, of wild-type activity. Although this region is of such a crucial importance for basal promoter activity in CV-1 cells, neither of the two mutations significantly influenced cAMP activation of the promoter. In CHO, NIH/3T3 and HeLa cells, however, these mutations in CREL enhanced the promoter strength 1.5–3-fold. Within the extent of their resolution, protein–DNA interaction experiments did not support the notion that cell-type-specific effects, exerted by the mutations, were due to differences in protein(s) binding to CREL, or to the absence of specific nuclear proteins from distinct cell types. Our previous work has suggested that the distal part of the murine ODC promoter contains DNA element(s) involved in silencing its activity in NIH/3T3 cells [24]. It is tempting to speculate that an element of this nature would inhibit ODC promoter activity in co-operation with the proximal motif characterized in the present study.

Factors that interact with the CREL motif of the promoter are clearly distinct from CREB or ATF proteins ([24], and this study). Proteins binding to CREL were also able to interact with factors associated with GC-rich sequences upstream from CREL. There is a perfect Sp1 motif at nt −112 from the transcription start site, and inhibition of Sp1 binding (or related activity) affected the composition of protein complexes assembled on CREL. Li et al. [31] have recently postulated that the ODC promoter contains a novel protein-binding motif, 5'-GCCCTCCCC-3', which overlaps in part with the Sp1 site. As with CREL, mutations of this motif elicited cell-specific consequences that did not correlate with the DNA-binding activities of corresponding nuclear extracts [31]. Sp1 has been implicated in the regulation of the rat ODC promoter, but the sequences mediating those responses in co-transfection assays were located further upstream, between nt −345 and −168 [32].

The consequences of CREL mutations in the context of the proximal −133 nt did not show cell specificity. A significantly decreased basal promoter activity was observed in all cell types, suggesting that the cell-type specificity of the 1.6 kb promoter derives from interactions between cell-specific factors binding to distal elements and ubiquitous proteins associated with CREL in the proximal promoter. This notion is supported by Moshier et al. [33], who have reported co-operative interactions between different functional regions of the human ODC promoter. Antibodies specific for various CREB and ATF proteins did not recognize CREL–protein complexes, thus confirming our previous findings that a consensus CRE sequence is incapable of interacting with protein complexes bound to CREL. In contrast, Abrahamsen et al. [34] have presented results implying that CREB is among the proteins that regulate ODC promoter activity through interaction with the CREL element. These authors were able to detect a retarded complex with a CREL-containing probe by using high concentrations of purified recombinant CREB protein. However, in the same study, consensus

CRE competed poorly with CREL for protein complexes interacting with the latter element. Our attempts to purify CREL-binding proteins have so far been unsuccessful, possibly owing to their labile and heteromeric nature.

Other transcription factors known to interact with GC-rich sequences include Egr-1, WT-1 and the retinoblastoma gene product (Rb) [35–38]. The region −106/−98 of the murine ODC promoter contains, adjacent to the Sp1 motif, an 8 of 9 nt match to the consensus element (5'-GCGGGGCG-3') for Egr-1, also known as Krox or Zif268 [36]. Egr-1 function may also exhibit cell type selectivity, in that this factor regulated the platelet-derived growth factor A chain promoter oppositely in 293 and in NIH/3T3 cells [39]. WT-1 protein, in turn, has been postulated to function as a transcriptional repressor when bound to an Egr-1 site [37]. Overexpression of WT-1 or Rb proteins in CV-1 cells did not, however, markedly influence the activity of ODC promoter constructs examined in the present study (J. J. Palvimo and O. A. Jänne, unpublished work).

In conclusion, the results of this and other studies [31,33,34] stress the importance of the proximal promoter in ODC gene transcription. A remarkable feature of elements in this region is their ability to confer cell-type specificity on ODC gene expression. Even though ODC protein production may be mainly regulated at multiple levels after transcription [1–11], basal expression of this housekeeping gene is also under complex transcriptional regulation.

We thank Ms. Saija Roine and Ms. Leena Pietilä for technical assistance. This work was supported by grants from the Medical Research Council of the Academy of Finland, the Finnish Cancer Foundation, the Sigrid Jusélius Foundation and the University of Helsinki.

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