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ANDROGEN-REGULATION OF ORNITHINE DECARBOXYLASE AND *S*-ADENOSYLMETHIONINE DECARBOXYLASE GENES

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Summary—Ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (AdoMetDC) are two key enzymes in polyamine biosynthesis. Both the ODC and the AdoMetDC gene is regulated by androgens in accessory sex organs of mice and rats, whereas only the ODC gene is androgen-responsive in rodent kidney. Androgenic responses in murine and rat kidneys are, however, dissimilar in that the induction of ODC activity and ODC mRNA accumulation is transient in the rat but sustained in the murine renal cells. In addition, *in situ* hybridization experiments with single-stranded cRNA probes revealed that ODC gene expression occurs in different subpopulations of epithelial cells of the proximal tubules in mice and rats. ODC and AdoMetDC genes are androgen-regulated in the same cell types of the accessory sex organs, as judged by hybridization histochemistry. Sequencing of the promotor region of the murine ODC gene has indicated the presence of several DNA elements for binding of transcription factors/regulatory proteins, including a putative androgen-response element at about 900 nucleotides upstream of the transcription start site.

INTRODUCTION

Androgens are essential steroid hormones which control the differentiation, development and maintenance of male reproductive functions. Sexually dimorphic responses in non-genital tissues are also elicited by male sex steroids via mechanisms identical with those in the reproductive tract organs. Androgens, such as testosterone and 5α-dihydrotestosterone, mediate their actions through binding to intracellular receptor proteins, which belong to a family of ligand-responsive transcription factors, including multiple receptors for different classes of steroid hormones, thyroid hormones, retinoic acids and vitamin D [1–6]. Receptor–androgen complexes interact with regulatory regions of specific genes and modulate their activity, which eventually results in the expression of the androgen-induced phenotype. This phenotype is acquired by cell- and tissue-specific mechanisms: in some tissues, including the accessory sex organs, androgens promote both hyperplastic and hypertrophic growth responses, whereas most non-reproductive organs undergo only a hypertrophic response to androgen administration [1, 7, 8].

Ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (AdoMetDC) are two key enzymes in polyamine biosynthesis [9–11]. ODC and AdoMetDC are ubiquitous "housekeeping" enzymes that are highly regulated and respond to a large number of stimuli affecting growth and differentiation. In addition, activities of these two enzymes respond to alterations in the cellular content of putrescine and polyamines [9–11]. Androgen-regulation of ODC occurs in target tissues exhibiting both hyperplastic and hypertrophic responses to male sex steroids, such as rat prostate, seminal vesicles, epididymis and mouse kidney. By contrast, stimulation of AdoMetDC activity by androgens appears to be limited to accessory sex organs [12–15]; and only minor or no changes in its activity take place in murine kidney after androgen administration [16, 17].

In the present chapter, we will summarize data on androgen-regulation of ODC and AdoMetDC genes in kidneys and accessory sex organs of mice and rats. In addition, we will describe our initial studies on the structure and function of the murine ODC gene promoter.

ANDROGEN-REGULATION OF ODC mRNA ACCUMULATION

Mouse kidney

There are two ODC mRNA species present in rodent tissues, with sizes of about 2.2 and 2.7

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kilobases (kb), which originate from alternative utilization of two different polyadenylation signals in the ODC gene [18, 19]. In contrast to the rodent tissues, only a single ODC mRNA is present in human tissues and cell lines [20–23]. Administration of testosterone at pharmacological doses increases the accumulation of the two ODC mRNA species on co-ordinate fashion in all tissues studied so far. In the murine kidney, the induction of ODC mRNA parallels closely that of the immunoreactive enzyme protein, with the initial increase in the mRNA content being detected between 2 and 6 h after steroid administration [16, 24, 25]. When cycloheximide was administered concomitantly with the androgen, no induction of the enzymatic activity occurred, indicating that androgen-regulation of ODC activity involves new protein synthesis rather than activation of preexisting enzyme molecules [25]. In similar experiments, administration of cycloheximide with testosterone decreased, but did not abolish, the androgen-elicited increase in ODC mRNA content in murine kidney [25]. Long-term androgen treatment of mice with testosterone-releasing implants brought about co-ordinate changes in ODC activity, immunoreactive enzyme protein concentration and ODC mRNA species in a manner similar to those after the exposure to a single steroid dose, implying that posttranslational modification of ODC activity is not a component of its androgenic regulation [24].

Administration of androgen to mice at pharmacological doses for several days increased ODC mRNA accumulation 10- to 50-fold (depending on the mouse strain, see [26]) over the values in kidneys of castrated male or intact females, whereas the corresponding increases in the enzyme activity of immunoreactive enzyme protein concentration were 200- to 500-fold [24]. This disparity is understood by the finding that prolonged administration of androgens decreases the turn-over rate of the ODC protein in murine kidney by a factor of 5-10, with the androgen-induced half-life of the enzyme being 100–150 min as opposed to about 15 min prior to steroid administration [24, 27]. The above-mentioned experiments have only been carried out on mouse kidney, and it is currently not known whether the same mechanisms apply to other murine tissues or to other species. It is worth pointing out, however, that in most of the cases that we have studied, the relative changes in ODC activity have exceeded those in ODC mRNA accumulation.

Even though the response in ODC to androgen administration in mouse kidney (or accessory sex organs; our unpublished observations) is relatively fast, with the initial changes being observed by the first 6 h after hormone injection [16, 24], the induction kinetics are still sluggish in comparison to some other steroid-regulated genes. At least two mechanisms could be envisioned to explain this phenomenon. First, only a few renal cells may initially respond to androgen, after which additional cells are recruited for the full response that peaks around 24 h after hormone exposure; or second, the androgen-regulated expression of the ODC gene may be indirect in that receptor–androgen complexes do not interact with the ODC promoter but increase the synthesis and/or activity of a transcription factor that subsequently activates the ODC gene. There are precedents amongst the hormone-regulated genes for both of these possibilities. By using *in situ* hybridization histochemistry to identify those cells in mouse kidney that express ODC mRNA, we evaluated the possibility of cell recruitment being a component of the androgenic response [17]. The data indicated clearly that no major recruitment takes place during the acquisition of androgen induction of ODC mRNA in that there was only a progressive increase in the mRNA content per cell during the response, which occurred in all cells that exhibited the initial response. The cells in murine kidney that responded to testosterone administration by increasing their steady-state ODC mRNA levels were identified to be epithelial cells of the proximal tubules, mainly those cells in the more cortical regions (so-called S1 and S2 cells) of the tissue [Fig. 1(A) and (B)].

The question of whether androgens regulate the steady-state level of ODC mRNA at the transcriptional level has not so far been unequivocally answered. Independent of the site of this regulation, it is clear that the androgen receptor is involved, since no response in ODC activity, immunoreactive protein concentration or ODC mRNA accumulation has been observed in androgen receptor deficient (Tfm/Y) mice [16, 25]. Some recent studies have suggested that androgens increase the rate of ODC gene transcription [28, 29]; however, there are reports in the literature to indicate that no or minimal changes are seen in the rate of transcription of the ODC gene after testosterone treatment, as measured by nuclear run-on assays [30, 31]. In view of the fact that mouse genome contains multiple ODC pseudogenes (at

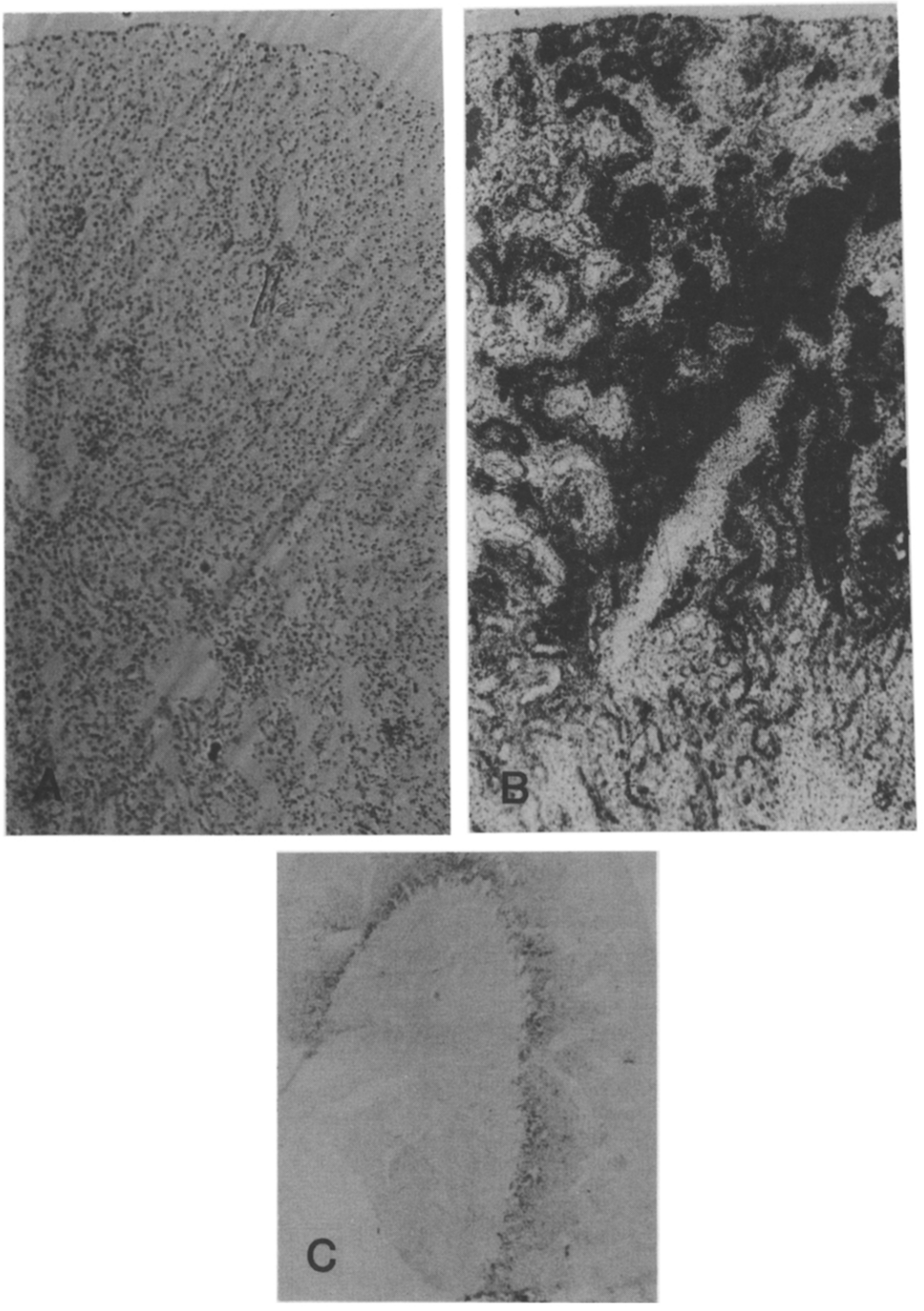

Fig. 1. Hybridization histochemical analysis of ODC mRNA expression in mouse and rat kidneys. In each case, the tissue sections were hybridized with an ^{35}S-labeled ODC cRNA probe, washed, treated with RNase and exposed to Kodak NTB-2 photographic emulsion as described previously [17, 46]. The slides were counterstained with hematoxylin and eosin. (A) Male mouse after 4 days of castration, (B) castrated mouse treated with testosterone-releasing implant for 48 h and (C) castrated rat treated with testosterone implant for 48 h. Magnification; ×40 (A and B) and ×10 (C).

least 10 different loci; our unpublished observations), it is possible that their presence has interfered with the accuracy of nuclear run-on measurements and possibly masked androgen-induced changes, *e.g.* due to transcription of an active but unrelated locus containing an ODC pseudogene. This notion is supported by the fact that we, and other investigators, using strand-specific probes, have measured significant transcription rates for the opposite strand of the ODC gene [29, 31].

Androgen-induced increase in mature ODC mRNA species occurs not only in the cytoplasmic but also nuclear RNA [31]. Moreover, there is not preferential accumulation of ODC mRNA precursors in nuclei of intact or androgen-treated animals, suggesting that the processing of primary ODC gene transcripts is neither rate-limiting nor an androgen-regulated event. When the relative concentrations of cytoplasmic and nuclear ODC mRNAs were calculated prior to and after androgen treatment, it was found that the cytoplasmic mRNA concentration was approx. 50 times higher than the nuclear one independent of the hormonal status of the animals [31]. This result implies that if stabilization of ODC mRNA is indeed an androgen receptor-dependent event, it has to occur already in the nuclei and involve only fully processed, mature mRNA species. An alternative explanation is that the dramatic increase in the mature ODC mRNA content in nuclei after androgen administration results from an elevated rate of ODC gene transcription, which has been, however, difficult to verify by transcription rate measurements due to technical problems alluded to above. In order to evaluate these mechanisms in greater detail, and to elucidate the nature of basal transcription factors governing the activity of the murine ODC gene, we have isolated and sequenced about 1650 nucleotides (nt) of the 5′-flanking region of the murine ODC gene (see below).

Rat kidney

Implantation of castrated male rats with rods releasing pharmacological doses of testosterone (400 μg/day) resulted in an increase in both ODC activity and steady-state ODC mRNA content in renal tissue. The response in rat kidney was, however, different from those in the murine kidney at least in three aspects [17]. First, the induction kinetics were faster than those in the mouse kidney, with the maximal increases in the enzyme activity and mRNA accumulation being achieved by 12 h of testosterone implantation. Second, the response in rat kidney was less extensive and maximally resulted in 3- and 30-fold higher ODC mRNA and enzyme levels, respectively. Third, the response in rat kidney was short-lived and pretreatment levels in both enzyme activity and mRNA content were regained by about 72 h after steroid exposure, despite the fact that the animals received androgen continuously via testosterone-releasing implants. The biological basis for these differences between the mouse and the rat is not currently known. It is of interest to point out, however, that although the cells expressing ODC mRNA in rat kidney are also located in the proximal tubular epithelium as is the case with murine kidney they are confined to the juxtamedullary region and correspond to the so-called S3 cells ([17, 32] and Fig. 1(C)).

Rodent prostate and seminal vesicles

Previous studies by several groups have shown that androgen treatment of castrated rats results in a dramatic increase in ODC activity of rat prostate and seminal vesicles [12–15, 33]. Although murine accessory sex organs are also androgen-responsive, the extent of enzyme induction is smaller than that in the rat. Studies on ODC mRNA in rodent accessory sex organs yielded results that were in agreement with those in enzyme activities; there was a clear induction of ODC mRNA accumulation in both rat and murine tissues and the relative changes were most pronounced in the rat prostate [17]. In accordance with the report by Blackshear *et al.* [32], our hybridization histochemical analyses localized androgen-induced accumulation of ODC mRNA mainly in the epithelial cells of the accessory sex organs. This distribution of male sex steroid-responsive cells in accessory sex organs of adult rats and mice is in agreement with the findings that the androgen receptor protein [34–36] and its mRNA (our unpublished data) are also concentrated in the epithelial cells.

ANDROGEN-REGULATION OF AdoMetDC mRNA ACCUMULATION

Although both murine and rat renal tissue exhibits a relatively high AdoMetDC activity and mRNA levels that can be easily detected in RNA blots, neither tissue responded to androgen treatment to a significant degree [16, 17]. It was, therefore, interesting to observe that an-

drogen exposure led to a marked stimulation in both AdoMetDC activity and mRNA content in rodent accessory sex organs. In the case of prostate and seminal vesicles, AdoMetDC mRNA accumulation was induced with a time course indentical to that of ODC mRNA, and even the extent of induction was quite similar. For example, in the rat prostate, the mRNAs encoding ODC and AdoMetDC were induced approx. 10-fold by 24 h of testosterone implantation and stayed at this new steady-state at least for the ensuing 72 h [17, 31]. There are two AdoMetDC mRNA species in all tissues (and species) so far studied, which originate from alternative utilization of two polyadenylation signals in the AdoMetDC gene ([37], and our unpublished data). As is the case with the ODC mRNA species, the two AdoMetDC mRNAs (2.0 and 3.5 kb in size) are co-ordinately induced by androgens in mouse and rat tissues.

In order to identify the cells exhibiting androgen-regulation of AdoMetDC mRNA, hybridization histochemistry with single-stranded AdoMetDC cRNA probes was used [17]. In both rat and mouse prostate and seminal vesicles, a dramatic increase in AdoMetDC cRNA hybridization was detected after androgen treatment. This response took place in the glandular

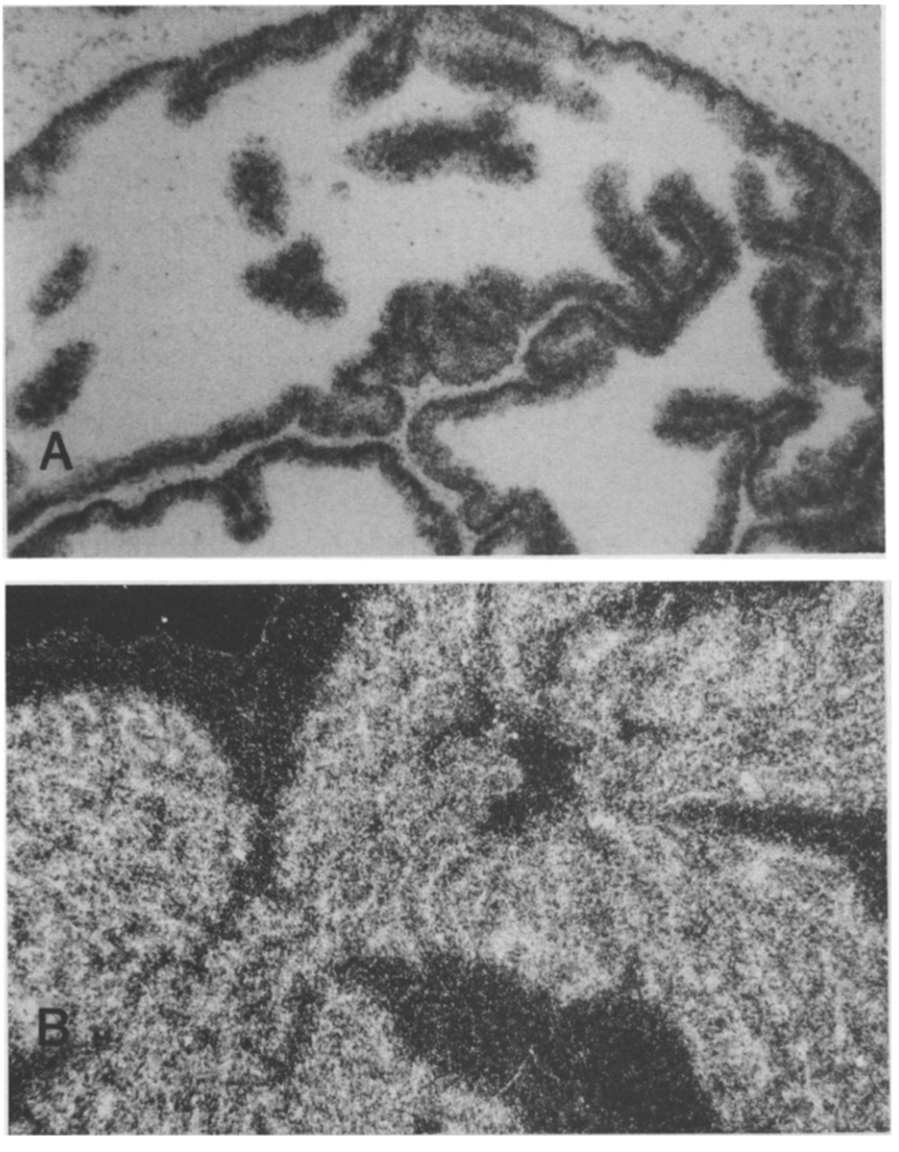

Fig. 2. Identification of the cells in rat ventral prostate (A) and mouse seminal vesicle (B) expressing the AdoMetDC gene. Hybridization histochemistry was carried out using an ^{35}S-labeled AdoMetDC cRNA probe as described in [17, 46] and in the legend to Fig. 1. The slides were exposed to Kodak NTB-2 photographic emulsion and counterstained with hematoxylin and eosin. Bright field (A) and dark field (B) images are shown. Magnification: ×20 (A) and ×40 (B).

epithelial cells, and the stromal cells showed very little or no androgen response [17]. Examples of these data are illustrated in Fig. 2(A) and (B), which show intensive hybridization signals in the rat ventral prostate [Fig. 2(A)] and mouse seminal vesicle [Fig. 2(B)] 48 h after testosterone implantation. In castrated animals, only a weak hybridization signal was seen, and the androgen-elicited changes in the mRNA levels revealed by *in situ* hybridization appeared to be more marked than those detected by Northern blots. However, no quantitative image analysis has yet been carried out to validate this visual impression. In agreement with data from RNA blotting studies, hybridization histochemistry did not reveal any androgen-induced changes in AdoMetDC mRNA in rodent kidney. The reasons for the phenomenon that the AdoMetDC gene is androgen-regulated in accessory sex tissues but not in the kidney are not known at present. One possible explanation is that the former response involves cellular hyperplasia and increased rate of DNA replication, which are usually associated with an increased demand of cellular polyamines [9–11]. In any event, it will be of interest to elucidate in the future the nature of the tissue-specific factors that mediate the androgen induction of the AdoMetDC gene in rodent accessory sex organs, or prevent this from occurring in the rodent kidney.

A salient feature in both ODC- and AdoMetDC-regulation is that, after an initial and extensive response, a new steady-state is reached by 24–48 h of hormone treatment. This level was maintained despite the fact that the animals continuously received pharmacological doses of testosterone. An explanation for this finding may be that expression of the androgen receptor gene is under a homologous down-regulation, with both the receptor mRNA and immunoreactive protein levels decreasing after androgen exposure [34, 38, 39]. As a consequence, the concentration of biologically active receptor–androgen complexes should be decreased, even though the circulating hormone levels may still be above those under physiological conditions.

MOUSE ODC GENE PROMOTER

To elucidate the nature of *trans*-acting nuclear protein factors and *cis*-acting DNA elements involved in the function and regulation of the murine ODC gene, we have isolated and sequenced approx. 1650 nt of the 5′-flanking region of the expressed mouse gene [40]. The mouse and rat ODC promoters are very similar,

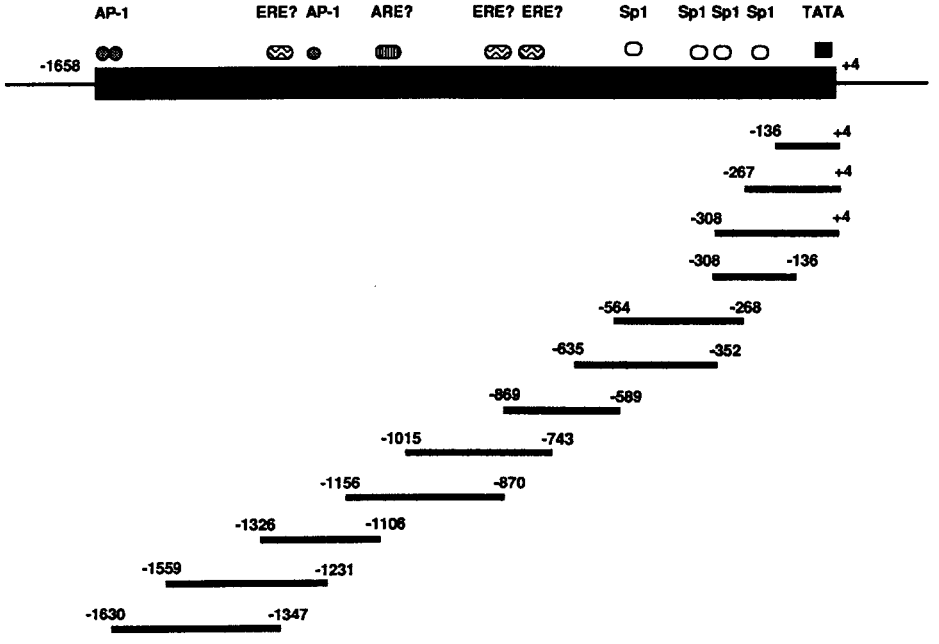

Fig. 3. Localization of some transcription factor-binding sites in the murine ODC promoter. The consensus sequences used in the computer search for the DNA elements were those defined by Mitchell and Tjian [45]. The lines under the promoter sequence [40] corresponds to fragments used for gel-retardation studies, and their 5′ and 3′ ends with respect to the transcription start site are identified by numbers above the lines.

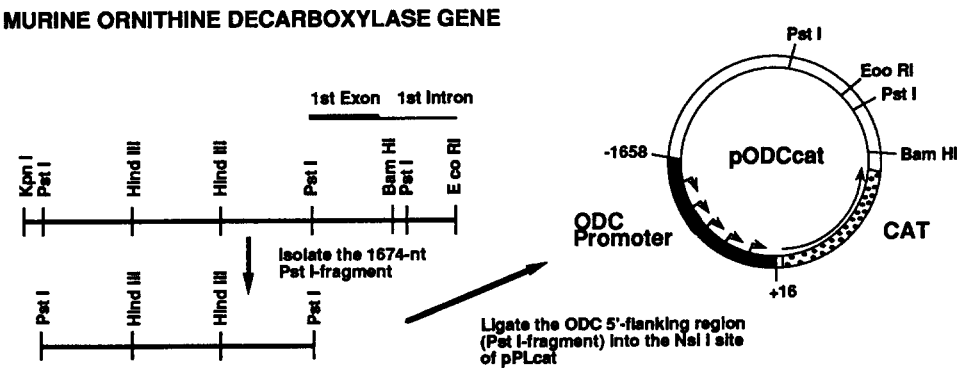

Fig. 4. Construction of the pODCcat reporter vector containing 1658 nt of the 5′-flanking region of the murine ODC gene. The ODC promoter region is indicated by the dark thick line, while the CAT gene is identified by the stippled area. A 50-nt long multiple cloning site was first inserted in front of the CAT gene, after which the ODC promoter was cloned into the *Nsi* I site. Arrowheads depict the approximate locations of the several 5′-deletion mutants used for transient expression studies.

with about 92% sequence conservation in the first 380 nt [40, 41]. Moreover, the proximal promoter of the human ODC gene exhibits over 70% sequence identity with the murine one [19, 21–23]. The mouse promoter contains a great number of DNA motifs for binding of various transcription factors [40, 41]; some of these are depicted in Fig. 3, which also illustrates some of the fragments that we have isolated and used for gel-retardation experiments. From the androgen-regulation point of view, it was of interest to find that the promoter contains a putative androgen-response element (ARE) at about −910 from the cap site ([40] and Fig. 3). This sequence (5′-AGTCCCACT-TGTTCT-3′) is not completely identical with the consensus sequence of the 15-mer glucocorticoid-response element that also functions as an ARE [3, 42, 43]. In the case of another androgen-regulated gene, the sequence identical with the hexamer 5′-TGTTCT-3′ has been sufficient to confer its androgen responsiveness [44]. Our initial band-shift studies with a 50-nt long oligomer containing the above-mentioned putative ARE using nuclear extracts from mouse kidney have shown that this DNA element binds nuclear proteins (Palvimo and Jänne, to be published). It is not currently known whether the interacting proteins are related to steroid receptors; however the same oligomer forms weak complexes with the androgen receptor protein that has been expressed in the Baculovirus system (our unpublished observations).

The 1658-nt long mouse ODC promoter sequence plus the first 13 nt of exon I were isolated as a single *Pst* I fragment and inserted into the *Nsi* I site of a promoterless chloramphenicol acetyltransferase reporter vector (pPLcat), which also contained a multiple cloning site in front of the CAT gene (Fig. 4). In transient expression studies with a variety of cells, the mouse promoter (pODCcat, Fig. 4) exhibited very strong promoter activity and yielded Cat values that were up to 50% of those obtained using viral promoters such as RSV, CMV and SV-40 (Palvimo, Eisenberg and Jänne, to be published). It was intriguing to observe that 5′-deletions of pODCcat increased its promoter activity over that containing the entire 1.6 kb 5′-flanking region, with the highest activity being observed with a reporter gene construct containing about 700 nt of the ODC promoter. These findings suggest that the distal part of the promoter contains DNA elements involved in silencing of the promoter activity. Interestingly, the putative ARE is localized in this part of the distal ODC promoter.

Detailed studies on the nuclear proteins interacting with the promoter elements are currently under way, and their results should eventually uncover the DNA motifs of the ODC promoter required for its basal activity and the sequences/nuclear factors that are necessary for the hormonal regulation of the ODC gene.

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