Regulation of the expression of human ornithine decarboxylase gene and ornithine decarboxylase promoter-driven reporter gene in transgenic mice

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We have studied the regulation of the expression of ornithine decarboxylase with the aid of transgenic mice harbouring either functional human ornithine decarboxylase genes or the mouse ornithine decarboxylase promoter-driven chloramphenicol acetyltransferase fusion gene in their genome. We used three different stimuli which are well known to enhance ornithine decarboxylase activity in their appropriate target tissues: (i) testosterone in female kidney, (ii) a phorbol ester in epidermis and (iii) partial hepatectomy in liver. Endogenous mouse ornithine decarboxylase activity was strikingly stimulated in response to these treatments. Even though containing the 5′ flanking region of the mouse ornithine decarboxylase gene, known to possess full promoter activity, the chloramphenicol acetyltransferase reporter gene was entirely insensitive to any of these stimuli. The human transgene-derived ornithine decarboxylase activity in kidney was unaffected by testosterone treatment, but responded in skin to application of the phorbol ester and likewise was clearly enhanced in regenerating liver. Although mouse endogenous ornithine decarboxylase mRNA levels were distinctly elevated after testosterone, this treatment did not influence the accumulation of the human transgene-derived mRNA. The phorbol ester enhanced the accumulation of mouse endogenous ornithine decarboxylase mRNA and also that derived from the human transgene; however, the enzyme activity was stimulated in regenerating liver without appreciable changes in the levels of endogenous or transgene-derived message. Our present results strongly emphasize the central role of the coding sequence of ornithine decarboxylase gene in the induction of the enzyme activity.

INTRODUCTION

Although ornithine decarboxylase (EC 4.1.1.17) is one of the most inducible mammalian enzymes, the mechanisms involved in the induction in response to anabolic stimuli or the repression of the enzyme activity in response to catabolic stimuli are poorly understood. The promoter region of the rodent [1–3] as well as the human [4,5] ornithine decarboxylase gene appears to contain all the genetic elements required for effective transcriptional regulation, yet the experimental evidence supporting a transcription-based regulation, although existing [6], is rather scanty. A further puzzling feature linked to the regulation of the expression of this enzyme is the fact that ornithine decarboxylase is an extremely low-abundance protein in mammalian tissues, even though numerous transfection experiments have shown that the strength of its promoter is fully comparable with strong viral promoters [1,7]. In addition to revealing the strength of its promoter, these transfection experiments with various fusion reporter genes have indicated that the coding region of the gene is indispensable for the proper regulation of its expression in response to growth-promoting stimuli [8].

We have recently generated several transgenic mouse lines harbouring a functional human ornithine decarboxylase gene [9,10] and also mouse lines carrying a fusion gene consisting of the promoter of the mouse ornithine decarboxylase gene operationally linked to the bacterial chloramphenicol acetyltransferase reporter gene [10]. The human ornithine decarboxylase gene is peculiarly expressed in a position-independent, gene-copy-number-dependent, fashion in most of the tissues of transgenic mice [10]. The tissue-specific expression of the human transgene greatly differs from the expression pattern of the endogenous mouse ornithine decarboxylase gene, but is almost identical with that of mouse ornithine decarboxylase promoter-driven chloramphenicol acetyltransferase gene [10].

Transfection experiments are easy to carry out and well suited for most studies aimed to elucidate the strength and regulation of a given promoter, yet for ornithine decarboxylase they have an inherent limitation, as only a few means are available to modulate the expression of ornithine decarboxylase in cultured mammalian cells. In this respect, transgenic animals offer vastly better opportunities to study the regulation of the expression in different tissues in a living creature. With the aid of three different transgenic mouse lines, two harbouring an active human ornithine decarboxylase gene with different lengths of the promoter region and one mouse ornithine decarboxylase promoter chloramphenicol acetyltransferase fusion gene, we have compared the regulation of these transgenes with the control of the mouse endogenous ornithine decarboxylase gene. We selected three different means to induce the enzyme activity: testosterone-induced stimulation of ornithine decarboxylase activity in female kidney, a process that apparently does not involve a general tissue proliferation, phorbol 12-myristate 13-acetate induction of the enzyme activity in epidermis, and that elicited by partial hepatectomy in the liver. The last two processes obviously induce a mitogenic response in the target tissues. Although mouse endogenous ornithine decarboxylase activity was strikingly enhanced in response to all of these treatments, the human transgene-derived ornithine decarboxylase responded only to the mitogenic stimuli and the reporter gene responded to none of the treatments.

EXPERIMENTAL

Production of transgenic mice

The transgenic mice were generated by using the pronuclear microinjection technique [11]. A human ornithine decarboxylase gene containing, in addition to all the 12 exons and 11 intervening sequences of the transcription unit, some 800 and 1000

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nucleotides of the 5′- and 3′-flanking regions respectively [9], a human ornithine decarboxylase gene containing the whole transcription unit plus some 8000 nucleotides upstream from the transcription start site together with 1000 nucleotides of the 3′-flanking region, and a fusion gene consisting of mouse ornithine decarboxylase gene 5′-flanking DNA (−1658 to 13 relative to the transcription start site) operationally linked to the bacterial chloramphenicol acetyltransferase gene [7], were used as the transgenes.

Animals and treatments

Female mice (transgenic and their non-transgenic littermates) were used in all experiments. Testosterone propionate (in olive oil) was given intraperitoneally at 1 mg/day for 5 days and the analyses were carried out 24 h after the last injection. Phorbol 12-myristate 13-acetate (in acetone) (10 nmol) was applied on to shaved dorsal skin. Acetone was applied on to contralateral skin, serving as vehicle control. Partial hepatectomy was carried out under general anaesthesia. The left lateral, left median and right median lobes (about 70% of the liver) were removed during the operation. Sham-operated animals served as controls.

Determination of mouse- and human-specific ornithine decarboxylase mRNA levels

The detection and semi-quantification of mouse and human ornithine decarboxylase mRNA was accomplished with the aid of reverse transcription/PCR [12], using species-specific primers essentially as described in [9]. The quantification of the u.v.-illuminated PCR product was performed by scanning the photographs with a Hewlett–Packard ScanJet Plus scanner. Total RNA was isolated by the guanidinium thiocyanate method [13].

Enzyme assays

The renal and hepatic tissue samples were homogenized with 2–3 vol. of buffer containing 25 mM Tris/HCl (pH 7.4), 0.1 mM EDTA and 1 mM dithiothreitol. The homogenates were centrifuged at 15000 *g* for 30 min at 4 °C and the resulting supernatants were used for assay of ornithine decarboxylase activity. Epidermal tissue samples were obtained and processed as described in [14]. Before assay of chloramphenicol acetyltransferase activity, the supernatants were heated at 65 °C for 10 min to inactivate the endogenous acetyltransferase activities, followed by centrifugation for 5 min at 15000 *g*. A portion of the supernatant corresponding to 12.5 mg of the renal or hepatic tissue was used for assay of the acetyltransferase activity. Corresponding tissues, treated as described above, from non-transgenic animals served as blanks for the chloramphenicol acetyltransferase assay. The activity of ornithine decarboxylase was assayed as described in [15], and that of chloramphenicol acetyltransferase by the method of Gorman et al. [16]. The acetyltransferase activity was expressed per mg of tissue weight, except the epidermal activity, which was expressed per mg of protein. For statistical analyses, Student's two-tailed *t* test was used.

RESULTS

As shown in Table 1, the basal renal ornithine decarboxylase activity was 10 times higher in transgenic mice in comparison with their wild-type littermates, yet the maximum activities after 5 days' testosterone treatment were practically identical, thus indicating that the transgene did not contribute anything to the stimulated activity.

Figure 1 depicts the results of a reverse transcriptase/PCR analysis of the accumulation of mouse- and human-specific ornithine decarboxylase mRNA in response to the testosterone treatment. A cDNA dilution analysis (Figure 1) revealed that, although the level of the mouse-specific mRNA was increased

Table 1 Effect of testosterone on renal ornithine decarboxylase activity in non-transgenic mice and transgenic mice harbouring the human ornithine decarboxylase gene

The animals received testosterone (or oil) for 5 days before assay of the enzyme activity. Values are means ± S.D.; $n = 4$ mice in each group: [a] $P < 0.001$.

Mouse line	Treatment	Ornithine decarboxylase activity (pmol of CO_2/60 min per mg of tissue)
Non-transgenic	Vehicle	9 ± 10
Transgenic	Vehicle	89 ± 48
Non-transgenic	Testosterone	22800 ± 4000[a]
Transgenic	Testosterone	20000 ± 5200[a]

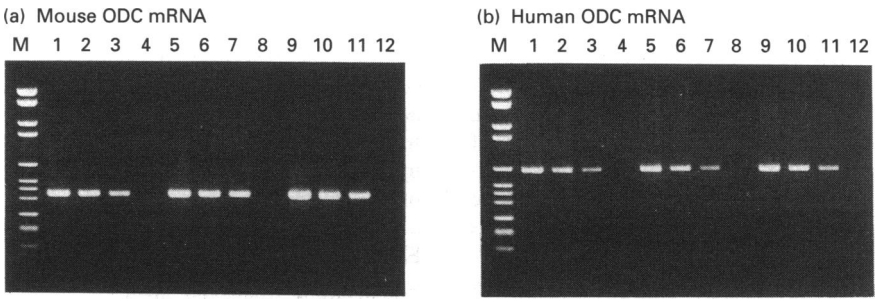

Figure 1 Levels of endogenous mouse ornithine decarboxylase (ODC) mRNA and the human transgene-derived mRNA in kidney after testosterone treatment

The transgenic animals carrying the 800-nucleotide promoter fragment were treated for 5 consecutive days with olive oil (lanes 1–4) or with 1 mg of testosterone propionate daily (lanes 5–12). DNAase-treated total RNA was reverse-transcribed [AMV reverse transcriptase and oligo(dT) primer]. cDNA dilutions corresponding to 200 ng (lanes 1, 5 and 9), 40 ng (lanes 2, 6 and 10) or 8 ng (lanes 3, 7 and 11) of total RNA were amplified together with blank samples (lanes 4, 8, 12) (200 ng of total RNA without cDNA synthesis) for 26 cycles with mouse or human ornithine decarboxylase mRNA-specific primers by using the hot start technique. PCR products were electrophoresed in 1.5% agarose gels, photographed and scanned. Samples 1–4 represent control mouse and samples 5–8 and 9–12 two different testosterone-treated mice. Altogether two control mice and two testosterone-treated mice were analysed with essentially the same results. *Bg*/I-and *Hin*fI-digested pBR328 was used as a molecular-mass marker (M).

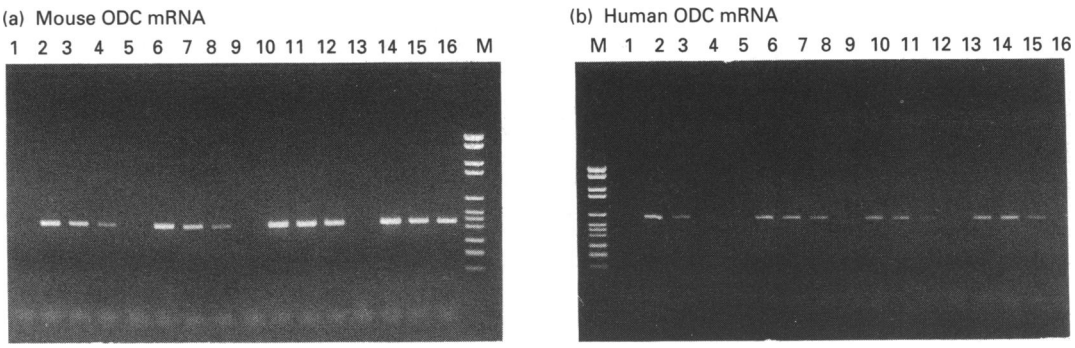

Figure 2 Levels of endogenous mouse ornithine decarboxylase (ODC) mRNA and the human transgene-derived mRNA in kidney after testosterone treatment

The transgenic animals carrying the 8000-nucleotide promoter fragment were treated with testosterone and the samples were reverse-transcribed as described in the legend to Figure 1. cDNA dilutions corresponding to 200 ng (lanes 2, 6, 10 and 14), 50 ng (lanes 3, 7, 11 and 15) and 12.5 ng (lanes 4, 8, 12 and 16) of total RNA were amplified together with blank samples (200 ng of total RNA without cDNA synthesis) (lanes 1, 5, 9 and 13) as described in the legend to Figure 1. Samples 1–8 represent two control animals and samples 9–16 two testosterone-treated animals. Altogether three control mice and three testosterone-treated mice were analysed, with essentially the same results. *Bgl*I- and *Hin*fI-digested pBR328 was used as molecular-mass marker (M).

Table 2 Effect of testosterone on endogenous renal ornithine decarboxylase activity and chloramphenicol acetyltransferase activity in transgenic mice harbouring the ornithine decarboxylase promoter-driven chloramphenicol acetyltransferase gene

The animals received testosterone (or oil) for 5 days before the enzyme assays. Values are means ± S.D.; $n = 4$ mice in each group: [a] $P < 0.001$.

Treatment	Ornithine decarboxylase activity (pmol of CO_2/ 60 min per mg of tissue)	Chloramphenicol acetyltransferase activity (c.p.m./ 60 min per mg of tissue)
Vehicle	16 ± 7.2	42 ± 19
Testosterone	20800 ± 6600[a]	19 ± 9.5

Table 3 Effect of phorbol 12-myristate 13-acetate (PMA) on epidermal ornithine decarboxylase activity in non-transgenic mice and in transgenic mice harbouring the human ornithine decarboxylase gene

The animals received a single topical application of PMA (10 nmol) (or acetone) on to shaved dorsal skin at the time points indicated. Values are means ± S.D.; $n = 4$ mice in each group: [a] $P < 0.001$.

Mouse line	Treatment	Time after treatment (h)	Ornithine decarboxylase activity (pmol of CO_2/ 60 min per mg of protein)
Non-transgenic	Vehicle	4.5	2.7 ± 2.8
Transgenic	Vehicle	4.5	43.6 ± 23
Non-transgenic	PMA	4.5	857 ± 313
Transgenic	PMA	4.5	966 ± 393
Non-transgenic	PMA	8	382 ± 121
Transgenic	PMA	8	869 ± 108[a]

roughly by a factor of 5 (the enzyme activity was stimulated by a factor of more than 2000), the amount of human-specific mRNA remained unaltered after testosterone. However, as the hormone-responsive element 5′-AGTCCnnnTGTTCT-3′ (n = any nucleotide) of the mouse ornithine decarboxylase gene is located at about −900 nucleotides relative to the transcription start site [7], the possibility remained that the human promoter (800 nucleotides) used was too short to respond to testosterone. We therefore tested another transgenic mouse line harbouring a human ornithine decarboxylase gene with about 8000 nucleotides of the 5′ flanking region, for its response to testosterone. We have so far sequenced about 6000 nucleotides of the 5′-flanking region of this transgene (GenBank accession no. L12582) and found an incomplete 3′ hormone-responsive element (5′-CGTTCT-3′) at about −1700 nucleotides and another element (5′-AGTTCT-3′) at about −2300 nucleotides from the transcription start site. The sequencing also revealed that all the transcriptionally important consensus sequences [5] were located within 800 nucleotides from the transcription start site. The reverse-transcription/PCR analysis shown in Figure 2 indicated that testosterone elicited about a 16-fold increase in the accumulation of the endogenous ornithine decarboxylase mRNA in the transgenic mice carrying the human ornithine decarboxylase gene with 8000 nucleotides of the 5′-flanking region, whereas the level of the transgene-derived message did not show any changes whatsoever.

Table 2 shows the effect of testosterone treatment on mouse endogenous ornithine decarboxylase activity and mouse ornithine decarboxylase promoter-driven chloramphenicol acetyltransferase activity in kidney of transgenic mice harbouring the latter fusion gene in their genome. In contrast with the dramatic stimulation (more than 1200-fold) of the endogenous renal ornithine decarboxylase activity, the reporter gene showed an insignificant decline in its activity in response to the androgen.

Table 3 summarizes the results of an experiment in which phorbol ester was used to stimulate epidermal ornithine decarboxylase activity in transgenic mice carrying the human ornithine decarboxylase gene (with the 800-nucleotide promoter) and their non-transgenic littermates; as indicated, the basal epidermal ornithine decarboxylase activity was nearly 20 times higher in the transgenic mice than in their syngenic littermates. At 4.5 h after the phorbol ester application, the contribution of the transgene-derived activity was not obvious. However, at 8 h after application the enzyme activity was significantly higher in the transgenic animals, indicating a contribution of the transgene. This difference was even more distinct at 24 h after the treatment (results not shown). It thus appears that the transgene was responding to the drug, yet with somewhat slower kinetics.

Figure 3 depicts the effect of the phorbol ester on accumulation of the mouse- and human-specific ornithine decarboxylase

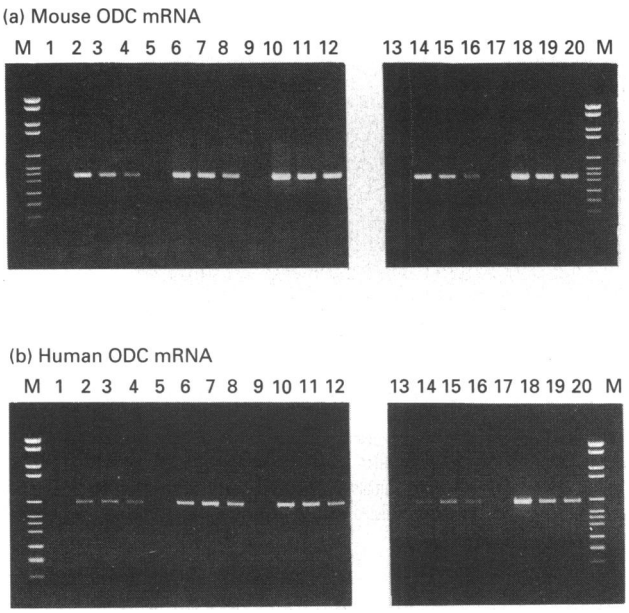

Figure 3 Levels of endogenous mouse ornithine decarboxylase (ODC) mRNA and the human transgene-derived mRNA in epidermis after a topical application of phorbol 12-myristate 13-acetate (PMA)

Lanes 1–4 represent the mRNA levels without any treatment, lanes 5–8 4.5 h after acetone application, lanes 13–16 8 h after acetone application, lanes 9–12 4.5 h after application of PMA and lanes 17–20 8 h after application of PMA. Samples 1, 5, 9, 13 and 17 are blanks (no cDNA synthesis, 200 ng of total RNA). Samples 2, 6, 10, 14 and 18 corresponded to 200 ng, 3, 7, 11, 15 and 19 to 40 ng, and 4, 8, 12, 16 and 20 to 8 ng of total RNA. Each sample was pooled from tissue pieces obtained from three mice. Reverse transcriptase/PCR was carried out as described for Figure 1, except that 32 (**a**) or 34 (**b**) cycles were run. Lanes M are as in Figures 1 and 2.

Table 4 Effect of phorbol 12-myristate 13-acetate (PMA) on endogenous epidermal ornithine decarboxylase activity and chloramphenicol acetyltransferase activity in transgenic mice harbouring the ornithine decarboxylase promoter-driven chloramphenicol acetyltransferase gene

The animals received a single topical application of PMA (10 nmol) (or acetone) on to shaved skin 4.5 h before the enzyme assays. Values are means ± S.D.; $n = 4$ mice in each group: $^{a}P < 0.001$.

Treatment	Ornithine decarboxylase activity (pmol of CO_2/ 60 min per mg of protein)	Chloramphenicol acetyltransferase activity (c.p.m./ 60 min per mg of protein)
Vehicle	5.6 ± 3.2	10760 ± 1503
PMA	2670 ± 425[a]	739 ± 389[a]

Table 5 Effect of partial hepatectomy on liver ornithine decarboxylase activity in non-transgenic mice and transgenic mice harbouring the human ornithine decarboxylase gene

The animals were partially hepatectomized (or sham operated) 24 h before the enzyme assay. Values are means ± S.D.; $n = 5$ mice in each group: $^{a}P < 0.05$; $^{b}P < 0.001$.

Mouse line	Operation	Ornithine decarboxylase activity (pmol of CO_2/ 60 min per mg of tissue)
Non-transgenic	Sham	2.5 ± 1.7
Transgenic	Sham	7.6 ± 2.7
Non-transgenic	Partial hepatectomy	12.1 ± 4.0[a]
Transgenic	Partial hepatectomy	52.9 ± 8.8[b]

Table 6 Effect of partial hepatectomy on endogenous liver ornithine decarboxylase activity and chloramphenicol acetyltransferase activity in transgenic mice harbouring the ornithine decarboxylase promoter-driven chloramphenicol acetyltransferase gene

The animals were partially hepatectomized (or sham operated) 24 h before the enzyme assay. Values are means ± S.D.; $n = 4$ sham operated and 5 partially hepatectomized mice: $^{a}P < 0.001$.

Operation	Ornithine decarboxylase activity (pmol of CO_2/ 60 min per mg of tissue)	Chloramphenicol acetyltransferase activity (c.p.m./ 60 min per mg of tissue)
Sham	0.15 ± 0.01	29 ± 33
Partial hepatectomy	3.02 ± 0.99[a]	60 ± 33

mRNA in epidermis. Interestingly, although the enzyme activity was never stimulated by acetone (used as the vehicle for the phorbol ester), the vehicle nevertheless brought about a 4–5-fold increase in the amount of both mouse- and human-specific ornithine decarboxylase mRNA at 4.5 h after the application. The accumulation of the mouse-specific mRNA was further increased by a factor of about 4 at 4.5 h after application of the phorbol ester, whereas that of human transgene-derived mRNA remained at the level already achieved with the vehicle alone (Figure 3). At 8 h after the phorbol ester, the human transgene-derived mRNA level was also distinctly higher than that resulting from application of the vehicle (Figure 3).

Table 4 shows the effect of the phorbol ester on endogenous epidermal ornithine decarboxylase activity and epidermal chloramphenicol acetyltransferase activity in transgenic mice harbouring the fusion gene. In contrast with the nearly 500-fold increase in the endogenous ornithine decarboxylase activity, the treatment profoundly decreased the transgene-derived reporter gene activity (Table 4). The chloramphenicol acetyltransferase activity remained much below the control level, at least up to 24 h after application of the drug (results not shown).

Table 5 indicates that the basal ornithine decarboxylase activity in liver of transgenic mice carrying the human transgene (with the 800-nucleotide promoter) was 3 times higher in comparison with that of the wild-type littermates. In both mouse lines partial hepatectomy resulted in a distinct stimulation of the enzyme activity in the regenerating liver remnant (Table 5). In fact, the magnitude of the response in transgenic animals was greater than that in non-transgenic animals.

In spite of the enhancement of the enzyme activity in regenerating liver, the levels of mRNA remained unchanged at 24 h postoperatively in both syngenic and transgenic animals (results not shown).

Table 6 summarizes the results of an experiment where transgenic mice carrying the ornithine decarboxylase promoter-driven chloramphenicol acetyltransferase reporter gene were subjected to partial hepatectomy. Analysis of the endogenous ornithine decarboxylase activity and the reporter gene activity revealed, that whereas the endogenous activity was stimulated by

a factor of 20, there was only an insignificant increase in the reporter gene activity.

DISCUSSION

Mammalian ornithine decarboxylase apparently belongs to the group of 'housekeeping' enzymes that are expressed in practically all tissues. Its expression rate, however, is generally very low, with wide variations between different tissues. Ornithine decarboxylase is also considered as one of the most inducible enzymes in mammals. With the advent of cloning of its cDNA and the availability of molecular probes for detection of its mRNA, it has become increasingly evident that transcriptional regulation, although existing [6], may not be the major mode to control the expression of ornithine decarboxylase.

A number of studies [17–21] has suggested that the rapid fluctuations of the enzyme activity are based on a direct translational regulation mainly exerted by the polyamines, but even this has been disputed with good reasons [22,23]. Thus the view that the major regulation of the gene expression of ornithine decarboxylase occurs post-translationally has lately gained more and more strength.

Mammalian ornithine decarboxylase has a very short molecular half-life [24], attributable to the so-called PEST amino acid sequences typical of rapidly degraded mammalian proteins [25]. Transfection experiments carried out with various heterologous gene constructs have emphasized the importance of the coding region, and peculiarly not the promoter region, in regulation of the expression of mammalian ornithine decarboxylase [8]. In fact, from the transfection experiments [8] little or no experimental evidence was found that sequences flanking the coding region would participate in the regulation of ornithine decarboxylase activity. A further piece of evidence emphasizing the role of the rapid turnover rate of mammalian ornithine decarboxylase in its regulation came from experiments where the sequence encoding the C-terminus of mouse ornithine decarboxylase was cleaved off before transfection. This truncation not only rendered the enzyme stable, but also greatly attenuated, and almost abolished, its response to mitogenic stimuli [8]. Our present results with the transgenic mouse lines likewise strongly support the importance of the post-translation control mode in the regulation of ornithine decarboxylase. We employed three different means to stimulate the enzyme activity in the transgenic animals. Testosterone is known to elicit its effect on renal ornithine decarboxylase through an array of different mechanisms. It induces an enhanced accumulation of ornithine decarboxylase mRNA, possibly through a transcriptional activation, but certainly by stabilization of the mRNA and also of the enzyme protein itself [26,27]. This process apparently does not involve any general mitogenic response in the renal tissue. Phorbol esters and a partial resection of rodent liver obviously elicit a general mitogenic response in their target tissues. It is noteworthy that the reporter gene chloramphenicol acetyltransferase driven by the 5′-flanking region of the mouse ornithine decarboxylase gene, possessing the full promoter activity [7], was completely insensitive to these three stimuli that strikingly enhanced the endogenous ornithine decarboxylase activity in the same animals. These results support the notion that the promoter region of the mammalian ornithine decarboxylase gene is not necessarily governing the modulation of the enzyme activity in response to these stimuli. A comparison of the responses of mouse endogenous ornithine decarboxylase and the human transgene-derived activities revealed some interesting differences. The human transgene-derived renal ornithine decarboxylase expression was insensitive to testosterone treatment, as regards both the accumulation of mRNA and the enzyme activity. However, the possibility remains that, unlike the mouse ornithine decarboxylase gene, the human gene may not be androgen-regulated at all. The two mitogenic stimuli, the phorbol ester and partial hepatectomy, elicited partial (epidermis) or complete (regenerating liver) responses in the transgene-derived ornithine decarboxylase activity. Interestingly, whereas the phorbol ester distinctly elevated the level of mouse endogenous ornithine decarboxylase mRNA and later also that of human transgene-derived mRNA, the enhanced enzyme activity in the regenerating liver was not associated with any changes in mRNA levels in either syngenic or transgenic mice.

The results of the experiments with the transgenic mice harbouring the human ornithine decarboxylase genes in their genome are in full agreement with the view that the short half-life, as determined by its primary structure, of ornithine decarboxylase is the major factor responsible for its stimulation upon application of growth-promoting stimuli, such as the phorbol ester and partial hepatectomy. A mitogenic stimulus associated with an increase in general protein synthesis will inevitably lead to a prompt induction of the synthesis of proteins with rapid degradation rate, as defined by the mathematical model already presented in 1970 [28] and specifically applied to ornithine decarboxylase in 1976 by Tabor and Tabor [29]. The overall contribution of the promoter activation to the stimulation of the enzyme activity is difficult to judge, but it may be of minor significance. This is exemplified by the fact that, whereas the endogenous enzyme activity was stimulated by a factor of more than 2000-fold in kidney after testosterone treatment (Table 1), the steady-state level of mRNA was only increased by a factor of about 5–16 (Figures 1 and 2). Similarly, the more than 300-fold stimulation of the endogenous enzyme activity elicited by the phorbol ester in the skin (Table 3) was accompanied by only a 4-fold increase in the accumulation of mRNA (Figure 2). It is likewise evident that ornithine decarboxylase activity was distinctly stimulated in regenerating liver without any changes in the mRNA levels. Except for the application of acetone and phorbol ester on to the skin, human transgene-derived mRNA did not display changes in response to any of the treatments. This may be related to the fact that the human transgene-derived ornithine decarboxylase was already over-expressed, in comparison with the endogenous enzyme activity, in all of the tissues included in this study. The fact, however, remains that the promoter region is essential for the expression of mammalian ornithine decarboxylase, as we recently generated transgenic mice carrying a promoterless human ornithine decarboxylase gene in their genome and found no signs whatsoever of the presence of human-specific mRNA in their tissues [9].

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