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# POSITION-INDEPENDENT, ABERRANT EXPRESSION OF THE HUMAN ORNITHINE DECARBOXYLASE GENE IN TRANSGENIC MICE

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SUMMARY: We have generated transgenic mouse lines carrying the human ornithine decarboxylase (ODC) gene in their genome. Six of 7 transgenic lines overexpressed ODC in most of their tissues, which was most strikingly manifested as a highly ectopic enzyme activity in the testis and brain of transgenic mice. A close correlation existed between enzyme activity (or ODC mRNA level) and gene copy number in testis and brain, indicating that the expression occurred independently of the transgene's chromosomal integration site. Transgenic mice carrying the mouse ODC promoter fused to the bacterial chloramphenicol acetyltransferase gene expressed the reporter gene in a similarly aberrant fashion. Even though the human ODC gene construct contained 5'-flanking sequences (800 nt), sufficient to confer maximal promoter activity in transfected cells, and about 1000 nt of 3'-flanking DNA, it is improbable that the observed gene copy number-dependent expression was due to the presence of so-called DNA attachment elements. In contrast, our data suggest that expression of the mammalian ODC gene is governed by distal silencer elements that were missing in the transgene constructs, which permitted an apparently position-independent expression of the transgene. 

Mammalian ornithine decarboxylase (ODC, EC 4.1.1.17) is a unique enzyme that is regulated with a great complexity. The ODC gene appears to be poorly expressed in mammalian cells in normal circumstances, although the activity of its promoter in transfection experiments has proven to be almost as strong as that of many viral promoters (1, 2). Poor expression of this gene *in vivo* is illustrated by the fact that ODC is a very low abundance protein and amounts only to a minute fraction of total soluble protein even in maximally stimulated animal cells (3). Although not accurately quantified in any studies, ODC mRNA also appears to represent a low abundance mRNA under maximally induced conditions. The activity of the enzyme is increased by a vast number of growth-promoting stimuli; however, even under these conditions, there is no direct proof for a predominantly transcriptional regulation of the gene encoding this protein (3). Some recent studies have suggested that modulation of the strong promoter of this gene is not the primary mechanisms by which induction of ODC activity is governed, but that sequences within the protein-coding region of the mRNA along with the short half-life of the protein are mainly responsible for the rapid induction kinetics of the enzyme (4).

We have recently isolated and sequenced the human chromosome 2-derived ODC gene (5), which represents an actively expressed gene as proven by transfection studies (6, 7). The isolated gene with 12 exons and 11 introns together with 800 and 1000 nt of the 5'- and 3'-flanking

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sequences, respectively, has been used to produce transgenic mice overexpressing the human transgene (8). After the generation of the initial transgenic mouse lines aberrantly expressing the human ODC gene and showing testicular abnormalities, we have produced 5 additional transgenic lines carrying this gene. With the exception of one line, the progeny of the other lines overexpressed ODC in most of their tissues, especially in the testis and the brain. Transgene dosage and expression of enzyme activity exhibited a highly significant correlation in these two tissues, implying that the human gene was expressed in a position-independent fashion. Additional transgenic mouse lines were produced with a chimeric construct comprising the mouse ODC promoter-driven chloramphenicol acetyltransferase (CAT) gene, and this construct was expressed in a fashion almost identical with that of the human ODC transgene.

## MATERIALS AND METHODS

The transgenic animals were produced by the pronuclear microinjection technique (9). Fertilized oocytes were obtained from superovulated BALB/c x DBA/2 mice mated with males of the same strain. The gene constructs used for microinjections were the human ODC gene containing, in addition to 12 exons and 11 introns of the transcription unit, 800 and 1000 nt of the 5'- and 3'-flanking DNA, respectively (5), and the mouse ODC promoter (corresponding to nt -1658 to +13) cloned in front of the bacterial CAT gene (2). Previous transfection studies with chimeric gene constructs using both human and murine promoter sequences with different reporter genes have indicated that 800 nt of the 5'-flanking DNA of both genes are sufficient to confer maximal promoter activity upon the reporter genes in a number of cell lines (2, and our unpublished observations).

Genomic DNA for gene copy number determinations was isolated from kidney of the transgenic animals with the aid of a 341 Nucleic Acid Purification System (Genepure™, Applied Biosystems) using the organic cycle. DNA was digested with *Msp* I to yield human-specific (10) restriction fragments of 2.3-2.4 and 0.8 kbp in size and subjected to agarose gel electrophoresis followed by hybridization to mouse and human ODC cDNAs as previously described (8, 10). The signal intensity of the human- and mouse-specific fragments was determined with a Shimadzu Dual Wavelength Chromato Scanner. Mouse-specific fragments were used as internal standards for the normalization of the amount of human DNA.

Total RNA was isolated by the guanidinium-thiocyanate method (11). Human-specific ODC mRNA was measured by employing amplification with the combined use of reverse transcriptase and polymerase chain reaction essentially as described by Hyttinen *et al.* (12). The 5' primer for the polymerase chain reaction (5'-CCTTCGTGCAGGCAATCTCT-3') recognizes a sequence in exon 7, whereas the 3' primer (5'-GCTGCATGAGTTCCCACGCA-3') corresponds to a sequence that spans over the junction between exons 10 and 11 of the human ODC gene, and thus prevented genomic DNA sequences from being included in the amplified bands. The oligonucleotides were synthesized with a 381A DNA synthesizer (Applied Biosystems).

ODC activity was measured by the method of Jänne and Williams-Ashman (13) and that of CAT as described by Gorman *et al.* (14).

## RESULTS

Out of 7 transgenic mouse lines carrying the human ODC gene, 6 overexpressed (10 to 100 times higher activity than that of the endogenous murine gene in normal littermates of the same sex) the transgene in all the other tissues examined (testis, brain, spleen, liver, muscle and heart) except in the kidney. The aberrant expression was most strikingly manifested in the testis and brain (Fig. 1). Hybridization histochemistry studies showed that, at least in the testis, accumulation of ODC mRNA (murine or human) occurred almost exclusively in the Sertoli cells, *i.e.*, the high testicular activity in transgenic animals was not due to expression of the transgene in cell types different from those expressing the endogeneous gene (our unpublished results).

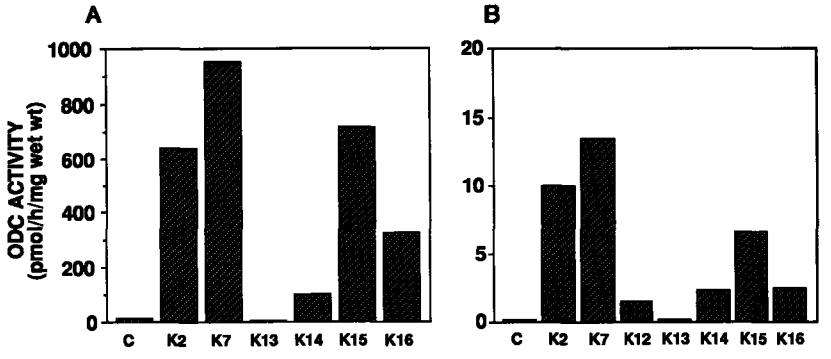


Fig. 1. **ODC activity in testis (A) and brain (B) of the members of different transgenic mouse lines carrying the human ODC gene.** C, non-transgenic; K2 through K16, transgenic mice carrying the human ODC gene (K12 did not have male descendants).

As depicted in Fig. 2, a highly significant correlation existed between the transgene copy number and ODC activity in the testis ($r = 0.97$; $p < 0.001$) and the brain ($r = 0.95$; $p < 0.001$), but not in the kidney ($r = 0.53$; not significant) among the male members of different transgenic mouse

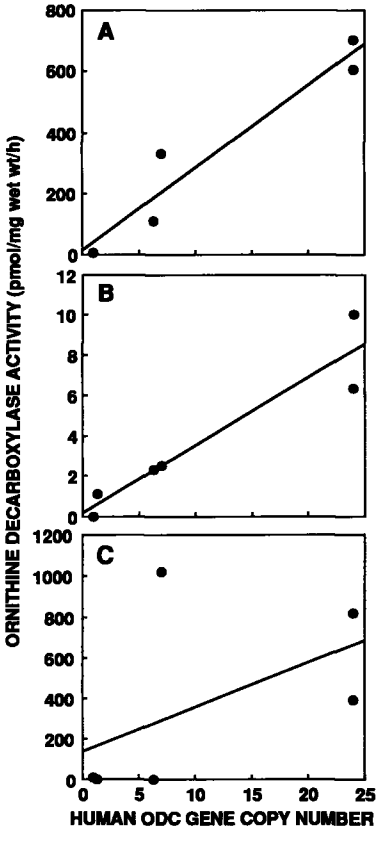


Fig. 2. **Correlation between human ODC gene copy number and enzyme activity in testis (A), brain (B) and kidney (C) of different transgenic mouse lines.** ODC activity in transgenic animals represents the total tissue activity minus the endogenous mouse activity (the mean enzyme activity in normal littermates of the same sex). The correlation coefficients were as follows: (A) testis, $r = 0.95$ ($p < 0.001$); (B) brain, $r = 0.97$ ($p < 0.001$) and (C) kidney, $r = 0.54$ (not significant).

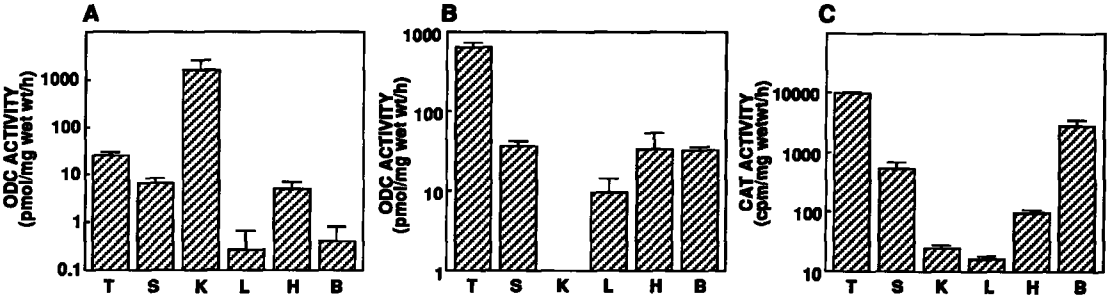


Fig. 3. **Tissue distribution of mouse endogenous ODC activity (A), the human transgene-derived ODC activity (B), and the mouse ODC gene promoter-driven chloramphenicol acetyltransferase (CAT) activity (C).** Three to four animals were included in each group. The vertical bars represent standard deviations. Abbreviations: T, testis; S, spleen; K, kidney; L, liver; H, heart; and B, brain.

lines. The experiment was subsequently repeated by measuring brain enzyme activity in relation to the transgene copy number in female members of the transgenic lines. Again, a highly significant correlation was found, ruling out the possibility that, at least in the brain, sex-specific factors were responsible for the observed correlations.

Expression of human ODC mRNA was also studied in the brain, using amplification with primers specific to the human mRNA, and it was found to be expressed in all of the transgenic lines (results not shown). Comparison of the amount of the human ODC mRNA to the gene copy number revealed again that a positive correlation existed between these parameters.

We next generated two transgenic mouse lines (K3 and K21) carrying the murine ODC promoter-driven CAT gene in their genome. Comparison of the expression of the reporter gene (Fig. 3C; line K3) with that of the ODC transgene (Fig. 3B; line K2) in different tissues showed a striking similarity in their expression, which was markedly different from the normal mouse expression pattern (Fig. 3A). The two transgenes were most actively expressed in the testis (note that the scales in Fig. 3 are logarithmic) while in the kidney, which tissue displays endogenous ODC activity that is normally 100 and 1000 times higher than testicular and brain activities, respectively (Fig. 3A), there was practically no transgene expression (Fig. 2B and C). Both transgenes also conferred high enzyme activities upon the brain (Fig. 2B and C), which tissue normally contains barely detectable ODC activity (Fig. 3A).

## DISCUSSION

A number of studies with transgenic mice generated through microinjection of foreign genes into fertilized oocytes have clearly indicated that there is generally no direct relationship between the copy number of the integrated transgene and the level of expression (15-17). This lack of correlation is usually attributed to a chromosomal position effect that originates from a random integration of the transgene. Position-independent expression of a transgene has been reported in a few cases, such as the human β-globin gene (18) and, more recently, the chicken lysozyme gene (19). The position-indenpendent expression has been attributed to so-called matrix attachment elements flanking the 5'- and 3'-regions of the transgene and topologically sequestering a functional unit (20, 21). A gene construct containing all the functional enhancers and attachment elements is believed to form a chromosomal loop that is capable of high-level, tissue-specific and position-independent expression (19). As a consequence, there is usually a fairly good correlation

between the copy number of the transgene and the level of its expression. A similar gene copy number-dependent, efficient expression has also been reported for the human CD2 gene due to the presence of a DNase I hypersensitive 3'-flanking region (22).

It is difficult to imagine that, in the case of the human ODC gene construct, the high-level and gene copy number-dependent expression would result from the presence of these attachment elements. In all the cases described so far, the attachment elements have been located several kbp apart from the transcribed region (18-22), yet the human gene construct that we used in this work contained less than 1000 nt of both 3'- and 5'-flanking DNA. The constructs including the attachment elements usually display a tissue-specific expression that is typical of the gene in question. This general rule is in striking contrast to the expression of the ODC transgene, which was greatly distorted from that of the endogenous gene. Moreover, a chimeric gene construct containing the mouse ODC promoter-driven reporter gene displayed an expression pattern that was almost identical with that of the human transgene (Fig. 3). As there is no nucleotide sequence information available for the attachment elements, our data do not formally prove that the human and mouse ODC gene constructs were devoid of such elements. However, if these elements were present and were responsible for the observed effects, an attachment element in the 5'-flanking region would suffice, as the mouse gene construct contained only this region and the first 13 nt of exon 1.

Our present results are more easily interpreted by assuming that mammalian ODC gene expression is governed by strong silencer elements that were not included in the gene constructs used in this study to generate transgenic animals. Some indication of such silencing activity in the distal promoter of the mouse gene was seen in transfection experiments, which showed doubling of the promoter activity in NIH/3T3 murine cells when the sequence between -750 and -1,658 was deleted (2). It is entirely possible that these silencer elements are so strong in some tissues, such as the brain, that the release from their influence results in an apparent gene copy number-dependent expression in transgenic animals. In fact, Rauth *et al.* (23) recently reported that the adenosine deaminase gene promoter fused with a reporter gene resulted in its efficient expression in the brain of transgenic mice. This is in contrast to endogenous adenosine deaminase activity that is hardly detectable in mouse brain (23). Interestingly, the proximal promoter of the adenosine deaminase gene is highly GC-rich, which is a feature also characteristic of both the human (5) and the murine (1,2) ODC promoters. Experimental evidence has also been presented indicating that the human apolipoprotein E gene contains regulatory elements in the 3'-flanking region, the removal of which leads to an aberrant expression and loss of tissue specificity in transgenic mice (24). It is also of interest to note that, although the putative attachment elements are supposed to govern the tissue-specific expression of a transgene, the complete gene domain for chicken lysozyme was ectopically expressed in the brain of transgenic mice (19).

An additional puzzling feature is involved in the expression of the human ODC gene construct that we have used. We have previously studied the same gene construct in transfections of cultured cells, using Chinese hamster ovary cells devoid of endogenous ODC activity. In the course of these studies, we isolated a number of cell clones expressing the human enzyme to varying extent, but could not show any correlation between the level of enzyme activity or mRNA accumulation and the number of integrated copies of the human ODC gene (6, 25). These observations may imply that the gene copy number-dependent expression of the transgene has been achieved during the embryonic development and differentiation.

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