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REGULAR ARTICLE

Expression of the Oct-2 transcription factor in mouse mammary gland and cloning and characterization of a novel Oct-2 isoform

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Abstract Oct-2 is a member of the POU family of transcription factors, which specifically bind to the octamer DNA motif ATGCAAAT and its closely related sequences. Unlike its ubiquitous counterpart Oct-1, Oct-2 is thought to be expressed only in B lymphocytes and neuronal cells and is mainly involved in immunoglobulin gene expression. We show here that Oct-2 is also expressed in the epithelial cells of mouse mammary gland, and that this expression is developmentally regulated. Rapid amplification of cDNA ends and subsequent cDNA cloning indicate that the mammary gland expresses multiple Oct-2 isoforms, including a novel isoform, named Oct-2.7. Compared with Oct-2 (isoform 2.1), the deduced Oct-2.7 sequence has an additional 22 amino acids close to the N-terminus and a novel 76-amino-acid C-terminus resulting from alternative splicing, with retention of the last intron that is spliced out in all other isoforms. Although Oct-2.7 has intact POU-specific and POU-homeo domains, it is unable to bind to the octamer motif, unlike all other known isoforms. Like Oct-1, both Oct-2.1 and Oct-2.7 can activate basal β-casein gene promoter activity. However, activation by Oct-2.7, which is independent of DNA binding, is significantly lower than that by Oct-2.1. Moreover, deletion of the first 114 amino acids at the N-terminus of Oct-2.1 has no effect on activation; this does not support previous reports of the presence of an inhibitory domain in this region.

Keywords Differential splicing · Gene expression · Genomic organization · Oct-2 isoforms Transcriptional regulation · Mouse

Introduction

The octamer-binding proteins (Oct) are a family of highly conserved transcription factors that are able to bind to a specific octamer DNA motif, ATGCAAAT, and closely related sequences. These sequences are *cis*-acting elements that are widely distributed in the promoter and enhancer regions of both cell type-specific and ubiquitously expressed genes (Scholer 1991). Oct factors share extensive homology in the DNA-binding POU domain (Verrijzer and van der Vliet 1993). This domain has a characteristic bipartite structure: a 75- to 80-amino-acid amino-terminal (N-terminal)-specific subdomain (POU_S) separated by a 14–26 variable amino-acid linker from a 60-amino-acid carboxyl-terminal (C-terminal) homeo subdomain (POU_H) that is closely related to homeodomain proteins. Both subdomains are required for high-affinity site-specific DNA binding (Verrijzer et al. 1992a; Schonemann et al. 1998), and the mechanism of binding is distinct from that of the classic homeodomain proteins. The POU domain also mediates specific protein-protein interactions with other transcription factors or cofactors (Verrijzer et al. 1992b; Inamoto et al. 1997; Prefontaine et al. 1999). The other regions of the POU factors are generally much less homologous with one another and bear the transactivation and inhibitory domains of the protein.

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The nucleotide sequence of the novel Oct-2 isoform 2.7 reported in this paper has been submitted to GenBank with assigned accession number AY746974.

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Up to 11 Oct factors (Oct-1 to Oct-11) have been assigned by distinct band shifts in various mouse tissues at different stages of development (Scholer et al. 1989; Scholer 1991; Goldsborough et al. 1993). To date, only five genes that encode these Oct-binding proteins, viz., Oct-1 (Sturm et al. 1988), Oct-2 (Clerc et al. 1988), Oct-3/4 (Rosner et al. 1990), Oct-6 (Meijer et al. 1990), and Oct-11 (Goldsborough et al. 1993), have been cloned and characterized. At least some of the remaining band-shift activities may represent degraded products or the splicing variants of these genes. Unlike the apparent ubiquitous expression pattern of Oct-1, all other members of Oct factors exhibit a developmental and tissue-specific expression pattern (Veenstra et al. 1997; Latchman 1999).

Oct-2 is one of the best characterized members of the POU factors and is considered to be expressed only in B lymphocytes and neuronal cells (Latchman 1996). It is encoded by a single gene on mouse chromosome 7, but multiple cell-type-specific, alternatively spliced isoforms have been identified (Hatzopoulos et al. 1990; Wirth et al. 1991; Liu et al. 1995). B lymphocytes predominantly express Oct-2.1, which includes a C-terminal activation domain that overcomes the effect of an N-terminal inhibitory domain and stimulates transcription of its target genes (Lillycrop and Latchman 1992; Lillycrop et al. 1994). In contrast, neuronal cells predominantly express Oct-2.4 and Oct-2.5, which lack the C-terminal activation domain but contain an intact N-terminal inhibitory domain, resulting in a generally repressive effect on transcription (Lillycrop and Latchman 1992). Because of its cell-specific expression, Oct-2 was originally believed to play a critical role in determining the B-cell-specific expression of immunoglobulin genes. However, Oct-2 has been shown not to be essential in immunoglobulin gene expression by the observation that the Oct-2 knock-out mice retain the expression of immunoglobulin, although they do have an impacted B cell lineage and reduced serum Ig levels (Corcoran et al. 1993; Schubart et al. 2001).

In this paper, we report the expression of Oct-2 in mouse mammary gland, the cloning of a novel Oct-2 isoform, and the genomic organization of the Oct-2 gene. We also describe the properties of the various Oct-2 isoforms with respect to binding to the octamer consensus oligonucleotide and their transcriptional activation of basal mouse β-casein gene promoter activity.

Materials and methods

Immunofluorescence microscopy

Mammary gland tissues from a mid-lactation mouse were fixed in 4% (w/v) paraformaldehyde at 4°C for 4 h followed by immersion in 0.5 M sucrose in phosphate-buffered saline (PBS) overnight. Following cryoprotection, fixed tissues were embedded in OCT compound (Sakura Finetek), frozen in liquid-nitrogen-chilled isopentane, and stored at −80°C. For immunohistochemical staining, tissue sections (10 μm) were cut, thaw-mounted onto the surface of gelatine-coated slides, and incubated in 10% normal goat serum for 30 min at room temperature to block non-specific antibody binding. The sections were then incubated with either diluted (2 μg/ml in PBS with 1% bovine serum albumin [BSA]) anti-Oct-2 antibody raised against an epitope mapping at the C-terminus of Oct-2 (no. sc-233X, Santa Cruz Biotechnology) or normal rabbit IgG for 1 h at 37°C. For an additional control, some sections were incubated with the anti-Oct-2 antibody that had been pre-incubated with a 5-fold excess of the control polypeptide (Santa Cruz Biotechnology) at 4°C overnight. After being washed twice in PBS with 1% BSA for 5 min, the sections were incubated for 1 h at room temperature in the dark with secondary antibody conjugated with Alexa Fluor 647 (Molecular Probes) diluted 1:400. Following three washes in PBS with 1% BSA for 5 min, some sections were counterstained with Sytox (Molecular Probes) diluted 1:10,000 in PBS for 5 min at room temperature. Finally, the sections were washed twice in PBS with 1% BSA for 15 min, once in PBS, and once in distilled water before being mounted on glass microscope slides and examined under a confocal microscope (Bio-Rad).

HC11 mouse mammary epithelial cells were grown in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal calf serum (Gibco), 10 ng/ml murine epidermal growth factor (Sigma), 5 μg/ml bovine insulin (Sigma), and 50 μg/ml gentamicin (Gibco). Cells were grown on glass coverslips to confluency, fixed with 4% paraformaldehyde in PBS at room temperature for 20 min, washed twice in PBS, and permeabilized in 0.1% Triton X (in PBS with 0.5% BSA) for 15 min at room temperature, followed by two washes in PBS with 1% BSA for 10 min. Immunofluoresence staining of the cells was carried out as described above, except with secondary antibody conjugated to Alexa Fluor 568 (1:1,000 dilution in PBS with 1% BSA).

Rapid amplification of cDNA ends and cloning of mouse Oct-2 isoforms

The sequences of all primer oligonucleotides used in this study are listed in Table 1.

The 5′ and 3′ sequences of mouse Oct-2 were obtained by rapid amplification of cDNA ends (RACE) with the SMART RACE cDNA Amplification Kit (Clontech). The 5′ and 3′ RACE-ready first-strand cDNAs were synthesized by using 1 μg poly(A)$^+$ RNA isolated from the mammary glands of late pregnant and early lactation mice. The 5′

Table 1 Sequences of oligonucleotide primers used for rapid amplification of cDNA ends and for the polymerase chain reaction

Primer name	Type	Sequence
mOct2B-211F	Forward	5′-GGCAGCATGGTTCATTCCAG-3′
mOct2B-229F	Forward	5′-AGCATGGGGGCTCCAGAAAT-3′
mOct2B-399F	Forward	5′-CAAGATCAAGGCTGAAGACC-3′
mOct2B-575F	Forward	5′-TCCAGCCACCTGCTCAGTTC-3′
mOct2B-1056F	Forward	5′-GAGACGCAAGAAGAGGACCA-3′
mOct2B-1528F	Forward	5′-CACTCGGCTATTGGCTTGTC-3′
mOct2.7-1503F	Forward	5′-CCATTGCCACCAGTCCTGTC-3′
mβActin-F	Forward	5′-TAGACTTCGAGCAGGAGATG-3′
mOct2B-540R	Reverse	5′-CTGGAGGAGTTGCTGTATGT-3′
mOct2B-973R	Reverse	5′-CTGCGTCGTTGAGCCACTTC-3′
mOct2B-1074R	Reverse	5′-GGTCCTCTTCTTGCGTCTCC-3′
mOct2B-2019R	Reverse	5′-GGACCAAGGTCGGGAGATTC-3′
mOct2B-2048R	Reverse	5′-ACCAGCCTCCTTACCCTCTC-3′
mOct2.7-1606R	Reverse	5′-GAGGCGAGCAATGCAGTGAG-3′
mβActin-R	Reverse	5′-CCACCAGACAGCACTGTGTT-3′

sequences of mouse Oct-2 were amplified with the provided universal primer (UPM, Clontech) and a reverse primer, mOct2B-1074R, designed from mouse Oct-2B cDNA (GenBank accession no. X53654). The reaction was reamplified with the provided nested universal primer (NUP, Clontech) and the nested mouse Oct-2-specific primer, mOct2B-540R. The 3′ sequence of mouse Oct-2 was first amplified with the UPM and the mouse Oct-2-specific forward primer mOct2B-1056F and then reamplified with the NUP and the nested primer, mOct2B-1528F. The resulting polymerase chain reaction (PCR) products were gel-purified and cloned into pCR2.1 vector (Invitrogen) and sequenced by using an ABI 377 automated sequencer (Applied Biosystems). Each individual sequence of the RACE products was verified in at least three independent clones.

Based on our RACE results, mouse full-length Oct-2 cDNAs were amplified from the mouse mammary gland 5′ RACE-ready first-strand cDNA library above by PCR with Herculase-enhanced DNA polymerase (Stratagene). The forward primers for amplifying Oct-2, Oct-2ΔN5, Oct-2ΔN114, and Oct-2ΔN223 were mOct2B-211F, mOct2B-229F, mOct2B-399F, and NUP, respectively. The reverse primer was mOct2B-2019R for Oct-2, Oct-2ΔN5, and Oct-2ΔN223, with mOct2B-2048R being used for Oct-2ΔN114. The Oct-2.7 was first amplified with primers UPM and mOct2B-2048R and then reamplified with primers mOct2B-229F and mOct2B-2019R. The PCR products were gel-purified and cloned into pCR2.1 vector (Invitrogen). The Oct-2 cDNAs were sequenced and verified in at least three independent clones.

The novel Oct-2.7 sequence has been submitted to GenBank with the assigned accession number AY746974.

DNA sequence analysis

The analysis of the cDNA sequences and the genomic organization of Oct-2 gene was conducted by using the computer programs of DNAstar (DNASTAR) and the National Center for Biotechnology Information (NCBI) BLAST web tools (http://www.ncbi.nlm.nih.gov/BLAST/).

Reverse transcription/PCR

Isolation of total RNA from lactating and virgin mouse tissues, reverse transcription of RNAs, and amplification of mouse β-actin cDNA were carried out as described previously (Zhao et al. 2004). PCR for amplification of Oct-2 cDNA fragments was carried out with the Herculase-enhanced DNA polymerase for high GC conditions (with 2%–4% dimethyl sulfoxide [DMSO]). Oct-2.7 cDNA was amplified with mouse Oct-2.7-specific primers mOct2.7-1503F and mOct2.7-1606R. The cDNA of all expressed Oct-2 isoforms was amplified with primers mOct2B-575F and mOct2B-973R, which are present in all Oct-2 isoforms. Primers for mouse β-actin were mβActin-F and mβActin-R to produce a 244-bp product.

In vitro transcription and translation of mouse Oct-2 cDNAs

The cDNAs of mouse Oct-2 were excised from the plasmids of pCR2.1 and subcloned into the mammalian expression vector plasmid pcDNA3.1(−) (Invitrogen). These plasmids were transcribed and translated in the presence of L-[^{35}S] methionine (Amersham) following the technical manual of the TNT coupled Reticulocyte Lysate System (Promega). The synthesized proteins were resolved by 10% SDS-polyacrylamide gel electrophoresis and imaged by means of a phosphor-capture screen and Quantity One software on a Molecular Imager FX (Bio-Rad).

Cell culture, transient transfections, and reporter gene assays

The culture of COS-7 cells (ATCC), the transient transfection with mouse Oct-1 or Oct-2 expression vector and mouse β-casein promoter (−258 to +7) luciferase construct (mβCnP_WT/pGL3), and the luciferase reporter assays were carried out as described previously (Zhao et al. 2004).

Preparation of tissue and cell nuclear extracts and gel electrophoresis mobility shift assay

Nuclear extracts from transfected cells were prepared as described by Schreiber et al. (1989). The oligonucleotides used in the gel electrophoresis mobility shift assay (EMSA)

were as described previously (Zhao et al. 2004). For the binding reactions with antibody, antibody was added to the binding reaction and incubated on ice for 30–60 min before addition of labeled probe. Incubation was subsequently continued for an additional 20 min at room temperature.

Western blot analysis

Nuclear extracts from mouse tissues were prepared as previously described (Zhao et al. 2002). Nuclear extracts from the transfected cells (10 μg) or tissues (50 μg) were resuspended in Laemmli sample buffer (Laemmli 1970) and resolved on a 10% (w/v) SDS-polyacrylamide gel by means of the Bio-Rad Mini-PROTEAN III Cell System. The proteins were electrophoretically transferred to a polyvinylidene fluoride (PVDF) membrane (Amersham). The blot was blocked overnight at 4°C in TRIS-buffered saline (TBS) containing 5% (w/v) non-fat dried milk and incubated for 1 h at room temperature in TBS, 0.5% non-fat dry milk, containing 0.2 μg/ml anti-Oct-2 antibody. The membrane was then washed twice at room temperature for 15 min in TBS and incubated for 1 h at room temperature in TBS, 0.5% non-fat dried milk, with a 1:2,000 dilution of goat anti-rabbit secondary antibody conjugated with horseradish peroxidase (Amersham). The immune complex was detected by using the SuperSignal West Pico Chemiluminescent substrate (Pierce).

Statistical analysis

Statistical analysis was carried out by using the one-way ANOVA function of Minitab software. Significance was declared at $P<0.05$.

Results and discussion

Expression and localization of Oct-2 in the mouse mammary gland

Oct-2 is thought to be expressed in B lymphocytes and neuronal cells, but not in other cell types (Latchman 1996). However, in our previous study Oct-2 was amplified from mouse mammary gland, in addition to Oct-1, with degenerate oligonucleotide primers designed for the POU_S and POU_H subdomains, which are highly conserved but flank highly specific link among octamer factors (Zhao et al. 2002). To confirm the expression of Oct-2 in the mammary gland, Western blot analysis in mouse mammary tissue was performed with a specific Oct-2 antibody raised against an epitope mapping at the C-terminus of Oct-2 (Fig. 1a). Western blot analysis indeed detected specific Oct-2 bands in the mammary gland, including a 76-kDa band and two 62-kDa and 60-kDa bands as reported in B cells by using two different anti-Oct-2 antibodies (Wirth et al. 1991). However, the 72-kDa band was the dominant form in the mammary gland, whereas B cells (Wirth et al. 1991) and Chinese hamster ovary cells (Fig. 1a) mainly expressed the 62-kDA and 60-kDa bands. None of these bands could be detected in liver nuclear extract (Fig. 1a), and all the bands could be completely blocked by the control peptide (data not shown). In addition, the expression of Oct-2 in the mammary gland was developmentally regulated. The Oct-2 protein levels in the mammary nuclear extracts were barely detectable in virgin mice, increased during pregnancy, reached a maximal level during late pregnancy, then decreased during lactation, and became extremely low in the post-lactation stage (Fig. 1a). This expression pattern coincides with that of the milk protein β-casein (Rijnkels et al. 1997). Furthermore, the band pattern of Oct-2 in the mammary gland seemed to change with the different developmental stages. The 62-kDa and 60-kDa bands were strongest in early lactation, unlike the 72-kDa band (Fig. 1a).

To localize Oct-2 expression in the mammary gland further, immunofluoresence staining was used to examine Oct-2 expression and localization in mammary epithelial cells in both tissue sections and in a mouse mammary epithelial cell line, HC11. As shown in Fig. 1b, Oct-2 is expressed in the alveolus epithelial cells of the mammary gland with subcellular localization to the nucleus and cytoplasm (Fig. 1b-1) and in HC11 cells with subcellular localization to the nucleus and peri-nuclear regions (Fig. 1b-3). Control staining with normal rabbit IgG (data not shown) or anti-Oct-2 pre-incubated with the control peptide reduced the signal to autofluorescence levels (Fig. 1b-2 and b-4; autofluorescence data not shown).

Expression and cloning of Oct-2 isoforms in mouse mammary gland

Several Oct-2 isoforms have been reported and found to be tissue-specific (Lillycrop and Latchman 1992; Lillycrop et al. 1994; Liu et al. 1995). As shown in Fig. 1a, multiple Oct-2 bands were detected in the mammary gland; these might have resulted from different isoforms being expressed. To examine and isolate the Oct-2 isoforms expressed in the mammary gland, we performed RACE experiments with RNA isolated from both pregnant and lactating mouse mammary gland and specific Oct-2 primers designed from the published mouse Oct-2B sequence (GenBank no. X53654). The 5′ sequences of mouse Oct-2 cDNAs were amplified with the UPM adapter primer and an Oct-2 reverse primer located in the conserved POU_H subdomain of exon 11 and re-amplified with the nested UPM primer NUP and a second Oct-2 reverse primer within exon 6 (Fig. 2). 5′ RACE resulted in five different 5′

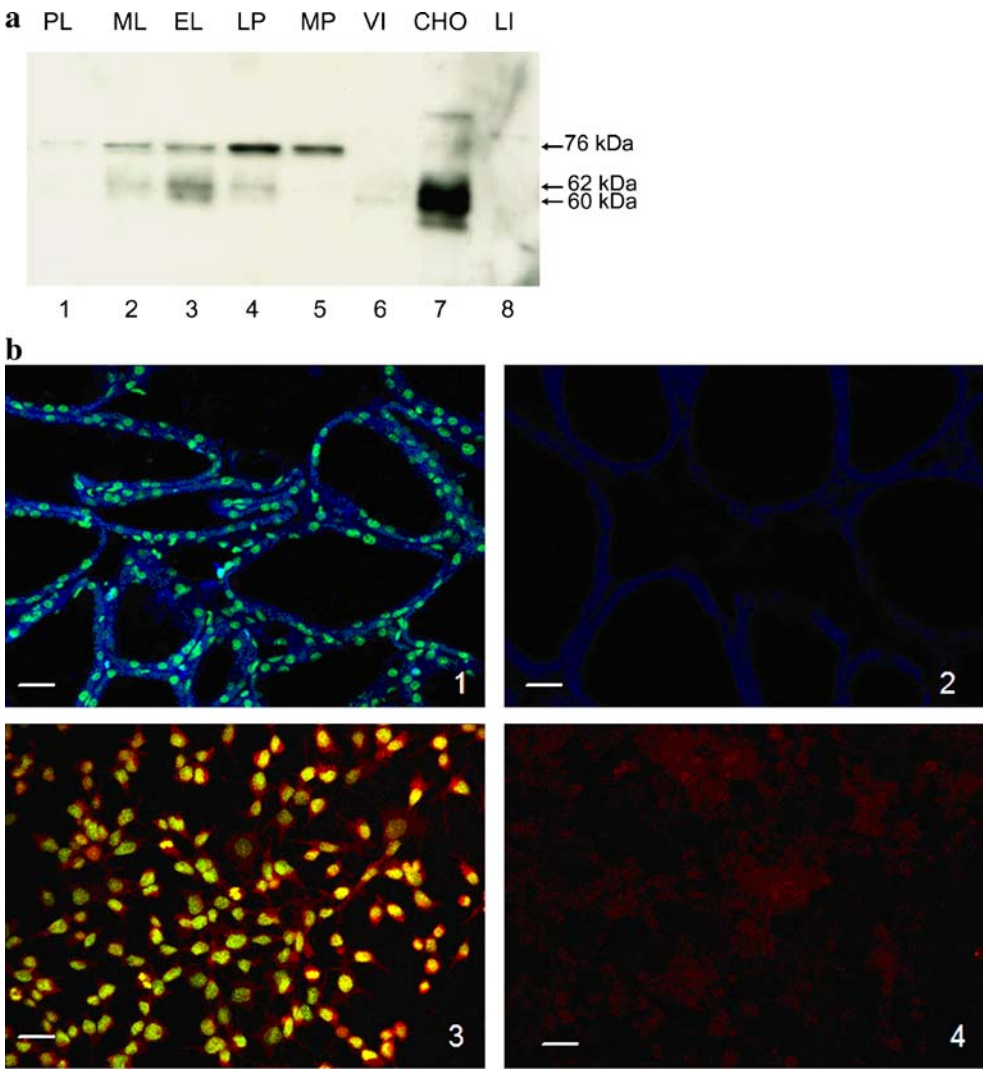

Fig. 1 Expression and localization of Oct-2 in the mouse mammary gland. **a** Western blot analysis of nuclear extracts from mouse mammary tissue; 50 μg nuclear extract from mice mammary glands at the virgin stage (*VI*, *lane 6*), mid-pregnancy (*MP*, day 13–15, *lane 5*), late pregnancy (*LP*, day 17–19, *lane 4*), early lactation (*EL*, day 2–4, *lane 3*), mid-lactation (*ML*, day 7–10, *lane 2*), and post lactation (*PL*, *lane 1*), together with nuclear extracts from the liver of late pregnant mouse (*LI*, *lane 8*) and Chinese hamster ovary cells (*CHO*, *lane 7*), were fractionated by SDS-polyacrylamide gel electrophoresis and transferred to PVDF filters. The filters were incubated with a polyclonal rabbit anti-Oct-2 antibody. The sizes of the specific Oct-2 bands are shown in kilodaltons (*kDa*, *right*). **b** Expression and localization of Oct-2 in the mouse mammary gland and HC11 cells by immunocytochemistry. Sections of mammary tissue from a mid-lactation mouse (*1*, *2*) and HC11 cells (*3*, *4*) were incubated with either anti-Oct-2 antibody (*1*, *3*) or anti-Oct-2 pre-incubated with the corresponding control peptide (*2*, *4*). Antibody complexes were detected with fluorochrome-coupled secondary antibodies (*blue* in *1*, *2*; *red* in *3*, *4*) and analyzed with a confocal microscope. In *1*, *3*, the tissue section or cells were counter-stained with Sytox nuclear stain (*green*). *Bars* 20 μM

sequences: the first three started at positions 1a, 1b, and 1c (Fig. 3), respectively, and might have been the products of an imprecise Oct-2 promoter; the last two started at positions 2 and 3 (Fig. 3) and might have been the result of the degradation of the full-length Oct-2 mRNA. However, the possibility that these different sequences were transcribed from different promoters could not be ruled out. The 3′ sequences of Oct-2 cDNAs were amplified with the UPM primer and a mouse Oct-2 forward primer in exon 11 and then re-amplified with the NUP primer and the second Oct-2 forward primer at the end of exon 13. 3′ RACE resulted in only one 3′ sequence identical to the major Oct-2.1 isoform. The longer and high GC 3′ sequence in isoform 2.7 reported in this paper could not be amplified under our regular RACE PCR conditions (without DMSO in the reaction).

Based on our RACE data, the full-length mouse Oct-2 cDNAs with the different 5′ ends were amplified from our RACE libraries by using the primers designed from the far ends (untranslated regions) of our 5′ and 3′ RACE sequences. Five cDNAs were obtained, including the full-length Oct-2.1 (BC050258 or X57937) and three truncated

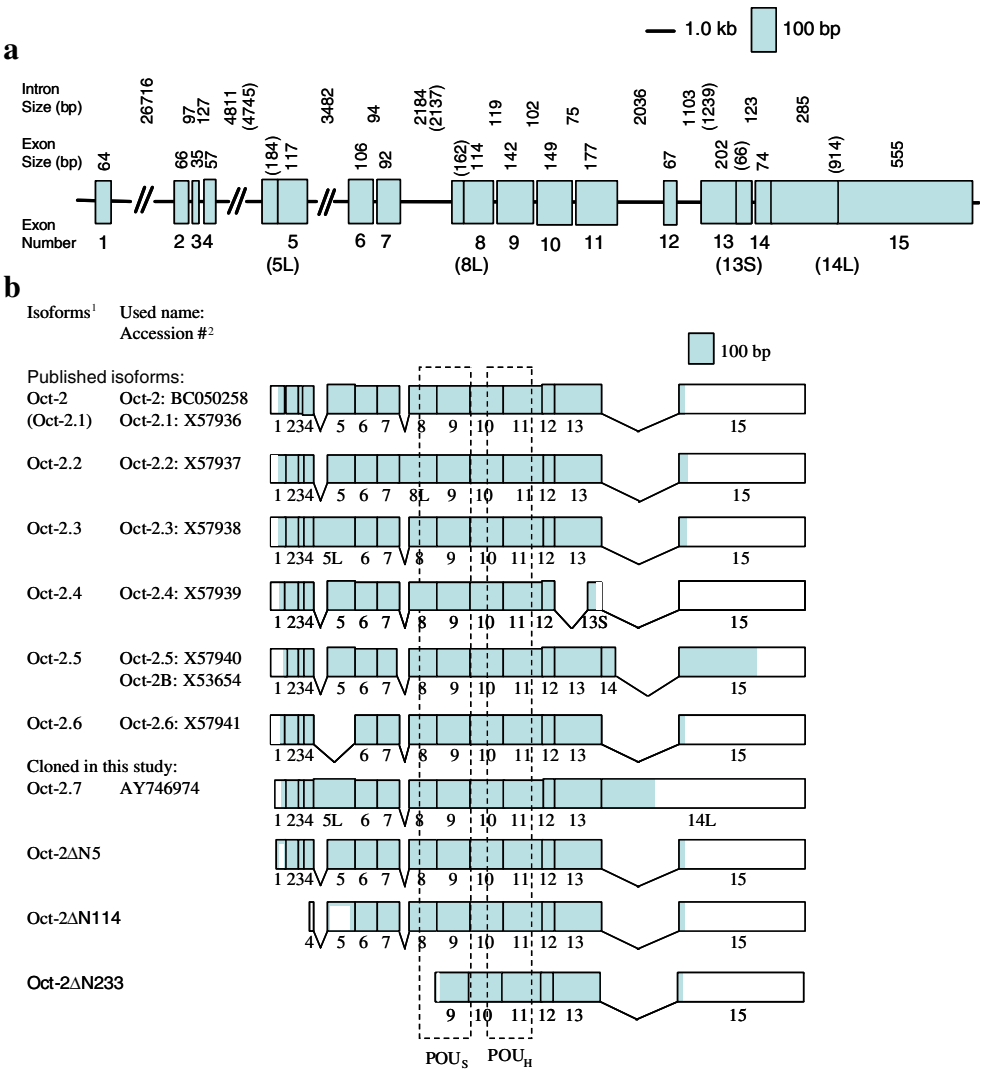

Fig. 2 Genomic structure of the mouse Oct-2 gene and its alternatively spliced isoforms. **a** Representation of the genomic structure of mouse Oct-2 as deduced from the published data of the mouse genomic sequence in GenBank (accession no. NT_039413.2). Exons are depicted as *boxes*. The number and size of each exon and the size of each intron are indicated. Alternatively spliced products for the exon are indicated in *parentheses*. **b** Representation of the alternatively spliced isoforms of mouse Oct-2. Isoform 2 (or 2.1), 2.2, 2.3, 2.4, 2.5 (or 2b), and 2.6([1]) represent the GenBank sequences as indicated ([2]). The *number below* each *box* indicates the exon number (*filled boxes* protein-coding sequences, *open boxes* untranslated regions, *white portion of exons* untranslated regions of exons, *dashed rectangles* POU-specific [POU_S] and POU-homeo [POU_H] domains)

forms, which potentially lacked the 5, 114, or 233 amino acids at the N-terminus based on the predicted open reading frames (ORF) of these cDNAs; these were designated as mOct-2ΔN5, mOct-2ΔN114, and mOct-2ΔN233, respectively (Fig. 2b). The truncated Oct-2 transcripts were cloned to study the functional properties of the different N-terminal structural domains of Oct-2. The possibility that these truncated transcripts might have biological function in tissue cannot be ruled out. In addition, a novel Oct-2 isoform was obtained, designated as mOct-2.7 (Fig. 3). The deduced amino-acid sequence of mOct-2.7 is 544 amino acids long with a calculated molecular weight of 57.5 kDa, as compared with the 463-amino-acid (49.4 kDa) product of the Oct-2.1 transcript. Of note, the Oct-2.7 sequence was unable to be amplified with the 5′ mOct2B-211F primer, which did amplify Oct-2.1 and covered the first ATG initiation codon in the Oct-2.1 transcript, but rather was amplified by using the mOct2B-229F primer covering the second ATG site in Oct-2.1. If Oct-2.7 is transcribed from the start site of 1c (Fig. 3), then this isoform may lack the first 5 amino acids of Oct-2.1, as in the case of Oct-2ΔN5.

Genomic structure of the mouse Oct-2 gene

A BLAST search of the NCBI mouse genome database reveals that the murine Oct-2 is a single copy gene located on chromosome 7 (between 7A2-7A3 in the Ideogram). However, multiple alternatively spliced isoforms of Oct-2 have been reported in mouse tissues (Hatzopoulos et al. 1990; Wirth et al. 1991). By aligning our mOct-2.7 cDNA and the cDNA sequences of other published mouse Oct-2 isoforms with the mouse Oct-2 genomic sequence in GenBank (NT_039413.2), we have obtained the exact sizes of exons and introns of the mouse Oct-2 gene and their locations.

As depicted in Fig. 2a, the mOct-2 gene spans approximately 43 kb and is composed of at least 15 exons. The diagram in Fig. 2b represents the alternatively spliced

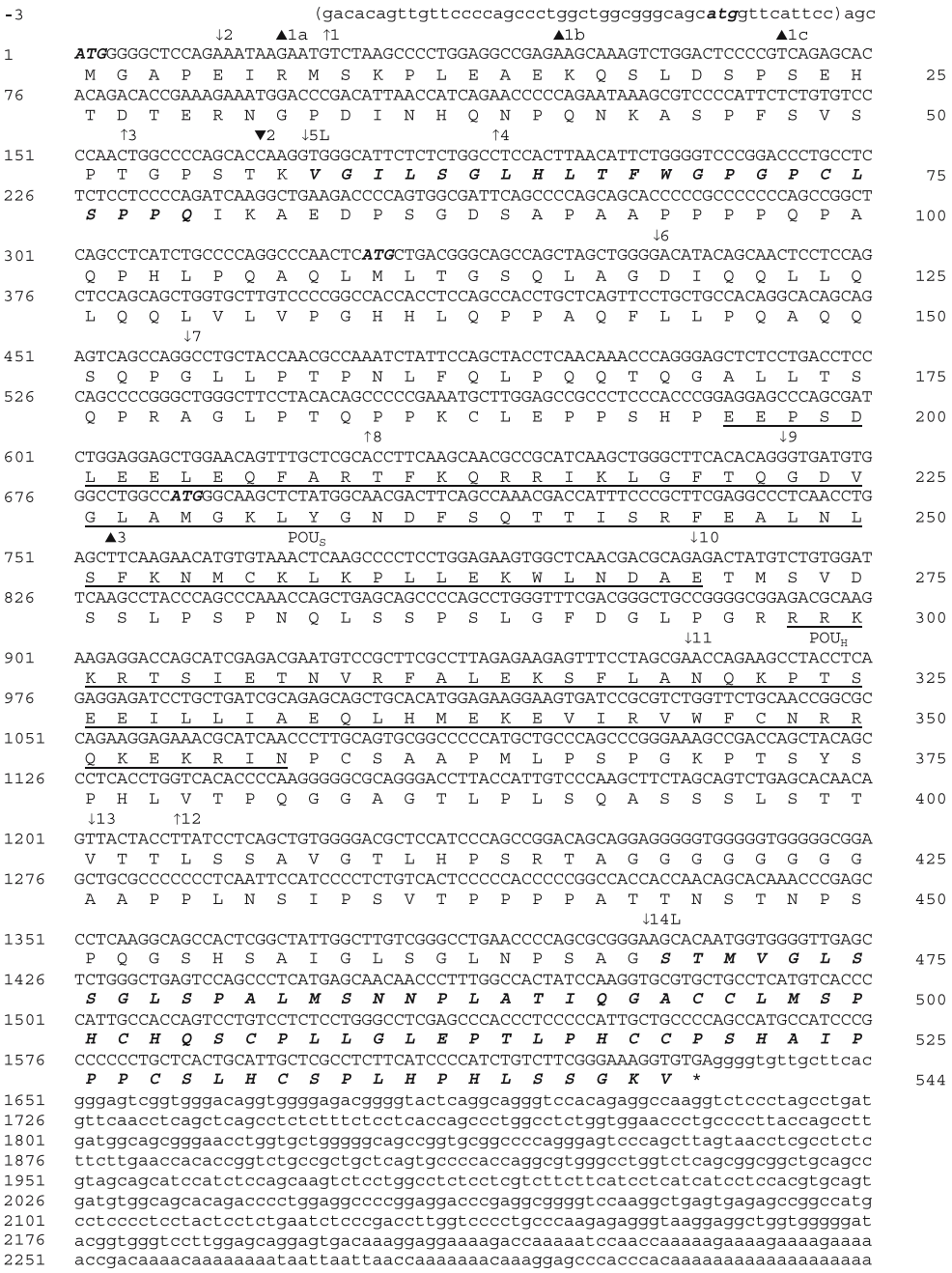

Fig. 3 Full-length cDNA and deduced amino-acid sequences of mouse Oct-2.7 (*lower case letters* 5′ and 3′ untranslated regions, *upper case letters* coding sequence and predicted amino-acid sequence, *letters in bold* the novel C-terminus sequence of mOct-2.7 and the extra sequence from exon 5L compared with mOct-2, *underlined letters* POU-specific [N-terminus side] and POU-homeo [C-terminus side] domains). The sequence in *parentheses* is the potential sequence but this could not be amplified (*arrowheads* five different 5′ start sites of mRNAs detected by our RACE, *arrows with numbers* start sites of the individual exons). The potential translation start sites are given in *bold italics*. The nucleotide sequence of mOct-2.7 mRNA has been submitted to the GenBank nucleotide database with the accession number AY746974

isoforms of the mOct-2 transcript showing the locations of the POU-specific and the POU-homeo domains. All isoforms contain intact POU-specific and the POU-homeo domains, except for the truncated form mOct-2ΔN233. The differences are found in exons 5, 8, 13, and 14. Compared with the major isoform Oct-2.1, isoform 2.2 has a larger exon 8 (8L); 2.3 has a larger exon 5 (5L); 2.4 and 2.5 have either a shorter exon 13 (13S) or an extra exon 14, which result in proteins with completely different C-terminal tails; the 2.6 lacks exon 5; and 2.7 has the same exon 5L as 2.3 and a novel exon 14L, which results from the failure to splice out intron 14. Thus, relative to Oct-2.1, Oct-2.7 has an extra 22 amino acids translated from exon 5L and a novel 76-amino-acid C-terminal tail translated from exon 14L, in addition to lacking the first 5 amino acids in the N-terminus.

Both the 5′ and 3′ splice junctions and the sizes of each exon and intron are shown in Table 2. In each case, the sequences at the boundaries comply with the canonical AG..GT acceptor/donor splice sites. The sizes of exons range from 35 bp for exon 3 to 914 bp for exon 14L. The intron sizes range from 75 bp to 26.7 kb.

Ubiquitous expression of Oct-2 mRNA in mouse tissues

B cells and neuronal cells have been shown to express different Oct-2 isoforms (Lillycrop and Latchman 1992;

Table 2 Nucleotide sequence of the intron-exon junction of the mouse Oct-2 gene[a]

Exon number[b]	3′ Intron	Exon sequence	5′ Intron	Size (bp)	
				Exon (location)	Intron[c]
1	N/A	GAC ACA GTT ..... GGG CTC CAG	gtaagaggct	20–64[d] (1060603–1060666)	26715
2	ctgttcacag	AAA TAA GAA ..... AGC ACA CAG	gtgagcgtgg	66 (1087382–1087447)	97
3	ctctttacag	ACA CCG AAA ..... AAC CAT CAG	gtcaggctct	35 (1087545–1087579)	127
4	ctcttcacag	AAC CCC CAG ..... AGC ACC AAG	gtaagcaccc	57 (1087707–1087763)	4811 (4745)
5L	tggttcacag	GTG GGC ATT ..... CTA GCT GGG	gtaagtatct	184 (1092509–1092692)	3481
5	tcctccccag	ATC AAG GCT ..... CTA GCT GGG	gtaagtatct	117 (1092575–1092692)	3481
6	tctctgccag	GAC ATA CAG ..... GTC AGC CAG	gtgagagctc	106 (1096174–1096279)	93
7	gcctctccag	GCC TGC TAC ..... CCT ACA CAG	gtgagactgt	92 (1096373–1096464)	2185 (2137)
8L	tccccaccag	GCT ATG ACT ..... TTC ACA CAG	gtctgggacc	162 (1098602–1098763)	119
8	acacccgcag	CCC CCG AAA ..... TTC ACA CAG	gtctgggacc	114 (1098650–1098763)	119
9	tctggaccag	GGT GAT GTG ..... ACG ACG CAG	gtgagcctgg	142 (1098883–1099024)	102
10	gttttcacag	AGA CTA TGT ..... TTC CTA GCG	gtgagtttct	149 (1099127–1099275)	75
11	ctgcctgtag	AAC CAG AAG ..... CCT CAC CTG	gtacccagaa	177 (1099351–1099527)	2036
12	ctcttcccag	GTC ACA CCC ..... GCA CAA CAG	gttagaggca	67 (1101564–1101630)	1103 (1239)
13	tctccggcag	TTA CTA CCT ..... CAG CGC GGG	gtaagtgtgc	202 (1102734–1102935)	123
13S	ccaccaacag	CAC AAA CCC ..... CAG CGC GGG	gtaagtgtgc	66 (1102870–1102935)	123
14L	gctcttgcag	AAG CAC AAT ..... AAA AAA AAA	N/A	914 (1103059–1103972)	N/A
14	gctcttgcag	AAG CAC AAT ..... CTA TCC AAG	gtgcgtgctg	74 (1103059–1103132)	285
15	tcctcaccag	CCC TGG CC ..... AAA AAA AAA	N/A	555 (1103418–1103972)	N/A

[a] Exact sizes of exons and introns and their locations were obtained from the mouse genomic sequence in GenBank (Accession no. NT_039413.2)
[b] *L* long, *S* short
[c] Each *number* in *parentheses* is the size of the alternative intron
[d] Exact sizes of exon 1 based on our 5′ RACE data

Lillycrop et al. 1994). To examine whether Oct-2.7 expression is tissue-specific, we have performed reverse transcription/PCR (RT-PCR) in the mammary gland, liver, lung, kidney, spleen, and intestine of a lactating mouse and in the mammary gland, liver, lung, kidney, intestine, uterus, and ovary of a virgin mouse. Specific primers located in the unique exon 14L region of Oct-2.7 amplified a PCR product in all tissues examined, with the highest expression in spleen and lung (Fig. 4, top). The PCR product was verified as being Oct-2.7 by sequencing. Similarly, by using primers located in exons 6 and 9, which are present in all known isoforms (Fig. 2b), a PCR product was strongly detected in all tissues (Fig. 4, middle). The PCR products for Oct-2.7 and for all Oct-2 isoforms were also amplified by using two different primer sets for each product (data not shown).

Our data thus indicate that Oct-2 is another ubiquitously expressed POU factor, like Oct-1, at least at the mRNA level, and challenge the current view of the tissue-specific expression of Oct-2. The Oct-2 products are unlikely to have been amplified from the B cells and neuronal cells in the different tissues because of the high levels of expression detected in all tissues, and at least in the mammary gland, expression of Oct-2 is mainly seen in the mammary

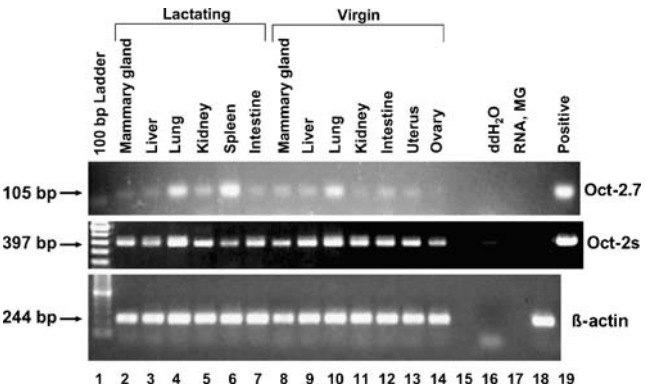

Fig. 4 Tissue distribution of Oct-2 mRNA in lactating and virgin mice as revealed by RT-PCR. The first-strand cDNAs were synthesized from 5 μg total RNA isolated from the mammary gland (*lane 2*), liver (*lane 3*), lung (*lane 4*), kidney (*lane 5*), spleen (*lane 6*), and small intestine mucosa (*lane 7*) of one lactating mouse and from the mammary gland (*lane 8*), liver (*lane 9*), lung (*lane 10*), kidney (*lane 11*), small intestine mucosa (*lane 12*), uterus (*lane 13*), and ovary (*lane 14*) of one virgin mouse. The cDNAs, together with double-distilled (*dd*) H_2O (*lane 16*) and RNA from the mammary gland (*MG*) of the lactating mouse (*lanes 17*, *top* and *middle* gels; negative controls) and 1 ng mouse Oct-2.7 plasmid DNA (*lanes 19*, *top* and *middle* gels; positive controls) or mouse β-actin control template (*lane 18*, *bottom* gel; positive control), were amplified by using specific primers for mouse Oct-2.7 (*top*), all Oct-2 isoforms (*middle*), or β-actin (*bottom*). *Lane 15* of all gels, *lanes 17* of the *bottom* gel, and *lane 18* of the *top* and *middle* gels are empty lanes. Sizes of RT-PCR products are indicated *left* (*arrows*)

epithelial cells, as shown in Fig. 1b. Consistent with our findings, Oct-2 transcripts have been reported in testis, kidney, and intestine, in addition to lymphocytes and neuronal cells (Hatzopoulos et al. 1990).

Binding of Oct-2 isoforms to the Oct motif oligonucleotide

Coupled in vitro transcription and translation were used to characterize the mouse Oct-2 cDNAs (Fig. 5a) and their products. All mouse Oct-2 cDNAs cloned in this study showed transcription and translation products with apparent molecular weights close to the predicted peptide sizes from their cDNA sequences, except for Oct-2.7, which had an apparent weight of 70 kDa rather than the predicted size of 57.5 kDa. This difference may have been the result of post-translational modifications. Gel EMSAs were performed to test whether these different Oct-2 products could effectively bind to the octamer consensus oligonucleotide (Fig. 5b). The in vitro translated mOct-2 (Fig. 5b, lane 1), mOct-2ΔN5 (lane 4), and mOct-2ΔN114 (lane 3) were able to bind to an octamer consensus oligonucleotide to form a single binding complex with a faster mobility than the mouse Oct-1B (mOct-1B) complex (lane 7). However, no specific binding complex was formed by mOct-2ΔN233 (Fig. 5b, lane 2) and mOct-2.7 (lane 8). The mOct-2 binding was specific as confirmed by a supershift assay with the anti-Oct-2 antibody (Fig. 5b, lane 6). The empty vector (pcDNA) transcription and translation reaction was used as a negative control (Fig. 5b, lane 5).

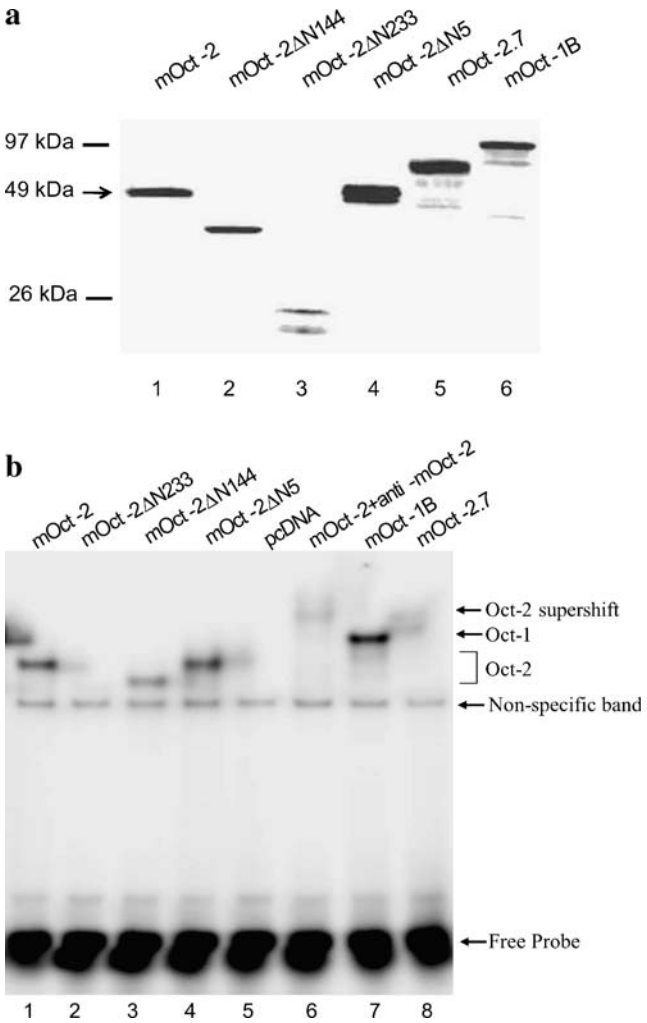

Fig. 5 In vitro transcription and translation of mOct-2 cDNAs and binding activity of in vitro translated products to [^{32}P]-labeled double-stranded octamer consensus oligonucleotide probe. **a** In vitro transcribed and translated products of mOct-2 (*lane 1*), mOct-2ΔN144 (*lane 2*), mOct-2ΔN233 (*lane 3*), mOct-2ΔN5 (*lane 4*), and mOct-2.7 (*lane 5*) cDNAs. mOct-1B cDNA (*lane 6*) was used as a positive control. **b** Binding activity of in vitro translated mOct-2 proteins to [^{32}P]-labeled double-stranded octamer consensus oligonucleotide probe by EMSA. In vitro translation reaction of empty vector (*pcDNA*, *lane 5*) and mOct-1B (*lane 7*) were used as negative and positive controls. In *lane 6*, the anti-Oct-2 antibody was added to the reaction of mOct-2 to verify the specific binding. Binding complexes are indicated *right* (*arrows*)

The mouse Oct-2 cDNA products were further tested in vivo. Plasmids expressing mOct-2, mOct-2ΔN5, mOct-2ΔN114, mOct-2ΔN233, or mOct-2.7, together with the control pcDNA vector plasmid, were transfected into COS-7 cells. Nuclear extracts from these cells were subjected to Western blot analysis with the anti-Oct-2 antibody raised against an epitope mapping at the C-terminus of Oct-2 (Fig. 6a). All the recombinant Oct-2 proteins were strongly detected except for mOct-2.7, which, because of its different C-terminal tail, could not be recognized by the antibody used (Fig. 6a, lane 7). Multiple bands were detected in positive extracts. The smaller bands might have represented the proteolytic products of the largest band but might also have resulted from the use of different translation initiation sites within the same transcript, because the patterns of these bands matched well between nuclear extracts. For example, the smallest Oct-2 band was detected in mOct-2, mOct-2ΔN5, and mOct-2ΔN114 cells, as in the cells transfected with the smallest truncated form, mOct-2ΔN233. Of note, the top bands in mOct-2, mOct-2ΔN5, and mOct-2ΔN114 cells were all larger than their corresponding in vitro translated products (the top Oct-2 band had a similar size to the weak 60-kDa to 62-kDa Oct-2 bands detected in mouse mammary tissue in Fig. 1a), indicating the occurrence of post-translational modifications of these isoforms in vivo and that these modifications were different from the putative modification of mOct-2.7 during in vitro translation. In contrast, the mOct-2ΔN233 band had a similar size to the in vitro translated product, suggesting that any post-translational modification of mOct-2, mOct-2ΔN5, and mOct-2ΔN114 occurred between amino-acid residues 114 and 233. Consistent with this idea, Ahmad et al. (2006) have recently reported the phosphorylation and glycosylation at the Ser191 of human Oct-2 (Ser175 of mouse Oct-2). In addition, the major 76-

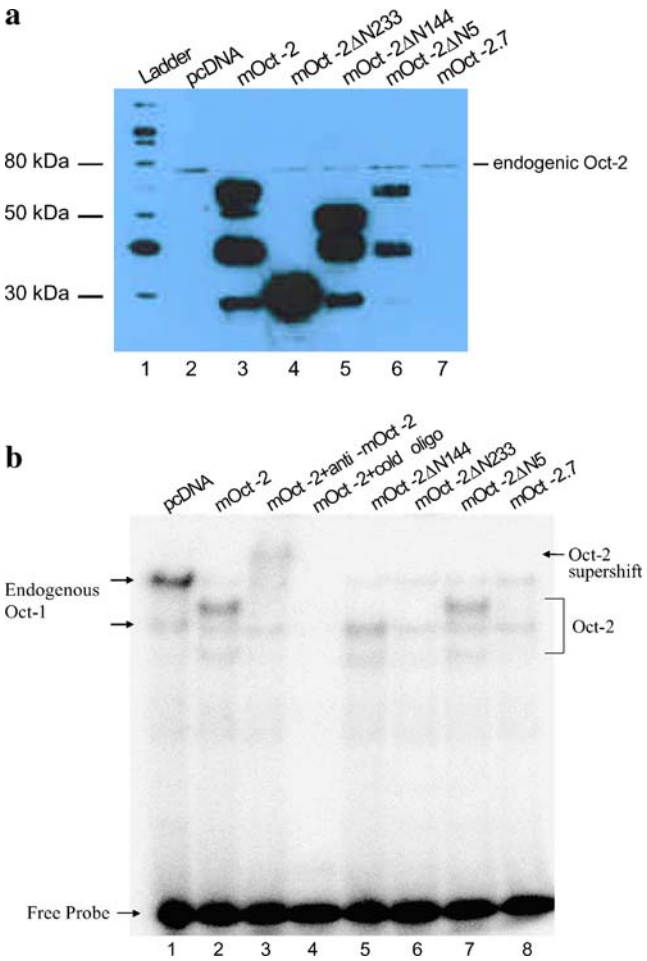

Fig. 6 In vivo products of mOct-2 cDNAs and their binding activity to [^{32}P]-labeled double-stranded octamer consensus oligonucleotide probe. **a** COS-7 cells were transfected with either pcDNA vector plasmid (*lane 2*) or the expression plasmid of mOct-2 (*lane 3*), mOct-2ΔN233 (*lane 4*), mOct-2ΔN144 (*lane 5*), mOct-2ΔN5 (*lane 6*), or mOct-2.7 (*lane 7*). The nuclear extracts of these cells (10 μg in each lane) were subjected to Western blot analysis with an anti-Oct-2 antibody raised against an epitope mapping at the C-terminus of mOct-2 (*lane 1* protein size markers). The positions of three size markers are indicated *left*. **b** Binding activity of the transfected COS-7 cell nuclear extracts to [^{32}P]-labeled double-stranded octamer consensus oligonucleotide probe by EMSA. In *lanes 3*, *4*, anti-Oct-2 or unlabeled competitors for the octamer consensus oligonucleotide was added to verify the specificity of binding. The binding complexes of Oct-2 proteins and endogenous Oct-1 are indicated (*arrows*)

kDa Oct-2 band detected in the mammary gland in the pregnant state (see Fig. 1a) was detected in COS-7 cells endogenously but not from any transfected Oct-2 cDNA, suggesting that the mammary gland at the pregnant stage mainly expressed another isoform, potentially Oct-2.5 (Oct-2B), as a similar sized band was detected in B cells by using an antibody raised against the Oct-2.5 isoform (Wirth et al. 1991). Although, in the mammary tissue, the full-length Oct-2.5 sequence was not amplified in our high GC PCR conditions, and although the 3′ RACE products containing exon 14 (unique to Oct-2.5; Fig. 2b) was not amplified under regular RACE PCR conditions, the exon 14 sequence was amplified in all mouse tissues mentioned in Fig. 4 by RT-PCR under high GC conditions (data not shown).

The nuclear extracts of the transfected cells were also analyzed for binding activity to the octamer consensus oligonucleotide (Fig. 6b). The results matched well with the binding of in vitro translated products, except that smaller binding complexes were also seen in mOct-2 (Fig. 6b, lane 2), mOct-2ΔN5 (lane 7), and mOct-2ΔN114 (lane 5) cells, consistent with the multiple bands seen in our Western blot analysis. The cells transfected with mOct-2ΔN233 (Fig. 6b, lane 6) or mOct-2.7 (lane 8) showed no specific Oct-2 binding activity.

mOct-2ΔN233 lacks an intact POU_S domain (Fig. 2b). Its loss of binding activity to the octamer motif demonstrates the importance of POU_S in DNA binding. However, mOct-2.7 has intact POU_S and POU_H domains. The failure to detect binding of Oct-2.7 to the octamer motif is surprising and interesting. All of the other known isoforms of Oct-2 are able to bind to the octamer motif (Wirth et al. 1991). As mentioned above, compared with Oct-2ΔN5, which lacks the first 5 amino acids in the N-terminus and can effectively bind to the octamer motif, Oct-2.7 has an extra 22 amino acids from exon 5L in the N-terminal sequence and a different 76-amino-acid C-terminal tail. Oct-2.3, which has the same 22 amino acids from exon 5L, can also effectively bind to the octamer motif (Wirth et al. 1991), suggesting that the unique C-terminal tail of Oct-2.7 inhibits this protein binding to DNA. Furthermore, the inhibition of binding could be a unique property of the C-terminal tail in Oct-2.7 or through its interaction with the 22 amino acids at the N-terminal side, since Oct-2.4 and 2.5,

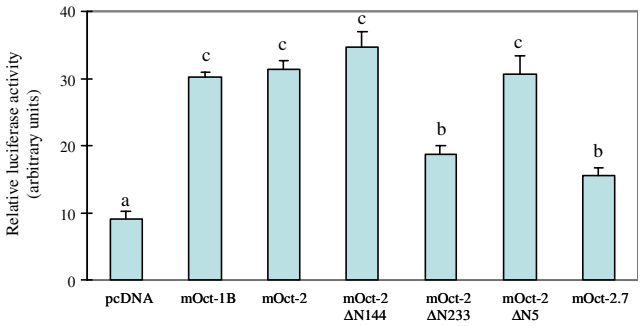

Fig. 7 Transcriptional activation of basal mouse β-casein gene promoter activity by Oct-2 in COS-7 cells. COS-7 cells were transfected with a mouse β-casein gene promoter luciferase construct and either pcDNA vector plasmid or the expression plasmid of mOct-1B, mOct-2, mOct-2ΔN144, mOct-2ΔN233, mOct-2ΔN5, or mOct-2.7. Luciferase activity was measured and normalized to control *renilla* luciferase activity. Results represent the mean±SEM of one experiment in triplicate. Three independent experiments were carried out. Data from one representative experiment are shown. *Bars* not sharing a common *letter* are significantly different ($P<0.05$)

which also have different C-terminal tails, show effective binding to the octamer motif (Wirth et al. 1991). In addition, Oct-1Z, which has a shorter and hence different C-terminal sequence compared with Oct-1, can also effectively bind to the octamer motif (Zhao et al. 2004).

Transactivation of the basal mouse β-casein gene promoter activity by Oct-2 in COS-7 cells

Our previous study showed that Oct-1 activated the basal activity of the mouse β-casein gene promoter by binding to an octamer sequence in a region defined as block C of the promoter (Zhao et al. 2004). To examine whether the Oct-2 isoforms cloned from the mammary gland in this study had the same effect, the Oct-2 expression plasmids and pcDNA control plasmid were each transiently cotransfected into COS-7 cells with a β-casein promoter/luciferase construct (Zhao et al. 2004). As shown in Fig. 7, all Oct-2 isoforms examined induced luciferase activity, including both mOct-2ΔN233 and mOct-2.7, which had no DNA binding activity, although their inductions were significantly lower than other isoforms. mOct-2, mOct-2ΔN5, and mOct-2ΔN114 had similar activities as Oct-1B in the transactivation of basal mouse β-casein gene promoter activity.

These results indicate the following. (1) Like Oct-1, all forms of Oct-2 activate basal β-casein gene promoter activity. This is consistent with the previous observation that all Oct-2 splicing variants (2.1–2.6) retain the ability to activate an octamer-containing promoter (Wirth et al. 1991). (2) Our N-terminal truncated variants demonstrate that at least the first 114 amino acids of Oct-2 at the N-terminus are not required for the activation of basal β-casein gene promoter activity. (3) Activation by Oct-2 is partially independent of their binding to the promoter, as both mOct-2ΔN233 and mOct-2.7 retain partial activation of β-casein gene promoter activity, even though they lack the ability to bind to DNA. This independence has also been observed in Oct-1 (Dong and Zhao 2007).

The C-terminal sequence of Oct-2 is serine-, threonine-, and proline-rich and has been shown to contain a transcriptional activation domain because of its ability to activate transcription from a distal position (Muller-Immergluck et al. 1991; Annweiler et al. 1994; Sharif et al. 2001). The C-terminal domain of Oct-2 has been shown to be essential for all known in vivo functions (Corcoran et al. 2004). Mice with a mutated allele of Oct-2 that encodes Oct-2 lacking all sequences from the C-terminal to the POU domains are unable to rescue any of the defects exhibited by Oct-2 null mice, including death shortly after birth and reduced B cell maturation and serum Ig levels. Thus, Oct-2.7, which has a markedly different C-terminus from the major isoform Oct-2.1, and which lacks the ability to bind the octamer motif, may have special physiological significance compared with the other isoforms (Chapman and Latchman 1998). It may functionally compete with other more potent isoforms through some non-DNA-binding mechanism, such as a passive repressor.

Three distinct regions in the N-terminus of Oct-2 have been identified that inhibit the activity of the C-terminal activation domain. The first inhibitory domain is located between amino acids 42 and 64 of Oct-2.1 (Friedl and Matthias 1995). The second is between amino acids 142 and 181 (Lillycrop et al. 1994), and the third is the additional 22 amino acids from exon 5L in Oct-2.3 and 2.7 (Wirth et al. 1991; Annweiler et al. 1994). These three domains have distinct functional differences (Gay et al. 1997). However, our data do not support the presence of any inhibitory domain in the first 114 amino acids of Oct-2, since mOct-2ΔN114, which lacks the first 114 amino acids, activates basal β-casein gene promoter activity no less effectively than Oct-2.1. An alternative explanation is that the effect of each inhibitory domain may be cell- or promoter-specific (Lillycrop and Latchman 1995).

In conclusion, the mammary gland expresses multiple isoforms of the Oct-2 transcription factor, including the novel isoform Oct-2.7. The expression levels and pattern of these isoforms are developmentally regulated. Isoform 2.7 has a unique C-terminal sequence that inhibits its binding to the octamer motif. In the mammary gland, Oct-2 may potentially activate milk protein β-casein gene expression with Oct-1.

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References

Ahmad I, Hoessli DC, Walker-Nasir E, Rafik SM, Shakoori AR, Nasir-ud-Din (2006) Oct-2 DNA binding transcription factor: functional consequences of phosphorylation and glycosylation. Nucleic Acids Res 34:175–184

Annweiler A, Zwilling S, Wirth T (1994) Functional differences between the Oct2 transactivation domains determine the transactivation potential of individual Oct2 isoforms. Nucleic Acids Res 22:4250–4258

Chapman CM, Latchman DS (1998) The different alternatively spliced isoforms of the Oct-2 transcription factor repress the involucrin promoter in a cell type-specific manner. Mol Biol Rep 25:253–257

Clerc RG, Corcoran LM, LeBowitz JH, Baltimore D, Sharp PA (1988) The B-cell-specific Oct-2 protein contains POU box- and homeo box-type domains. Genes Dev 2:1570–1581

Corcoran LM, Karvelas M, Nossal GJ, Ye ZS, Jacks T, Baltimore D (1993) Oct-2, although not required for early B-cell development, is critical for later B-cell maturation and for postnatal survival. Genes Dev 7:570–582

Corcoran LM, Koentgen F, Dietrich W, Veale M, Humbert PO (2004) All known in vivo functions of the Oct-2 transcription factor require the C-terminal protein domain. J Immunol 172:2962–2969

Dong B, Zhao F-Q (2007) Involvement of the ubiquitous Oct-1 transcription factor in hormonal induction of beta-casein gene expression. Biochem J 401:57–64

Friedl EM, Matthias P (1995) Transcriptional activation and repression, two properties of the lymphoid-specific transcription factor Oct-2a. Eur J Biochem 234:308–316

Gay RD, Dawson SJ, Latchman DS (1997) The different inhibitory domains of the Oct-2 transcription factor have distinct functional activities. FEBS Lett 416:135–138

Goldsborough AS, Healy LE, Copeland NG, Gilbert DJ, Jenkins NA, Willison KR, Ashworth A (1993) Cloning, chromosomal localization and expression pattern of the POU domain gene Oct-11. Nucleic Acids Res 21:127–134

Hatzopoulos AK, Stoykova AS, Erselius JR, Goulding M, Neuman T, Gruss P (1990) Structure and expression of the mouse Oct2a and Oct2b, two differentially spliced products of the same gene. Development 109:349–362

Inamoto S, Segil N, Pan ZQ, Kimura M, Roeder RG (1997) The cyclin-dependent kinase-activating kinase (CAK) assembly factor, MAT1, targets and enhances CAK activity on the POU domains of octamer transcription factors. J Biol Chem 272:29852–29858

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 277:680–685

Latchman DS (1996) The Oct-2 transcription factor. Int J Biochem Cell Biol 28:1081–1083

Latchman DS (1999) POU family transcription factors in the nervous system. J Cell Physiol 179:126–133

Lillycrop KA, Latchman DS (1992) Alternative splicing of the Oct-2 transcription factor RNA is differentially regulated in neuronal cells and B cells and results in protein isoforms with opposite effects on the activity of octamer/TAATGARAT-containing promoters. J Biol Chem 267:24960–24965

Lillycrop KA, Latchman DS (1995) The inhibitory domain in the Oct-2 transcription factor represses gene activity in a cell type-specific and promoter-independent manner. Mol Biol Rep 21:87–94

Lillycrop KA, Dawson SJ, Estridge JK, Gerster T, Matthias P, Latchman DS (1994) Repression of a *Herpes simplex* virus immediate-early promoter by the Oct-2 transcription factor is dependent on an inhibitory region at the N terminus of the protein. Mol Cell Biol 14:7633–7642

Liu YZ, Lillycrop KA, Latchman DS (1995) Regulated splicing of the Oct-2 transcription factor RNA in neuronal cells. Neurosci Lett 183:8–12

Meijer D, Graus A, Kraay R, Langeveld A, Mulder MP, Grosveld G (1990) The octamer binding factor Oct6: cDNA cloning and expression in early embryonic cells. Nucleic Acids Res 18:7357–7365

Muller-Immergluck MM, Schaffner W, Matthias P (1991) Transcription factor Oct-2A contains functionally redundant activating domains and works selectively from a promoter but not from a remote enhancer position in non-lymphoid (HeLa) cells. EMBO J 9:1625–1634

Prefontaine GG, Walther R, Giffin W, Lemieux ME, Pope L, Hache RJ (1999) Selective binding of steroid hormone receptors to octamer transcription factors determines transcriptional synergism at the mouse mammary tumor virus promoter. J Biol Chem 274:26713–26719

Rijnkels M, Wheeler DA, de Boer HA, Pieper FR (1997) Structure and expression of the mouse casein gene locus. Mamm Genome 8:9–15

Rosner MH, Vigano MA, Ozato K, Timmons PM, Poirier F, Rigby PW, Staudt LM (1990) A POU-domain transcription factor in early stem cells and germ cells of the mammalian embryo. Nature 345:686–692

Scholer HR (1991) Octamania: the POU factors in murine development. Trends Genet 7:323–329

Scholer HR, Hatzopoulos AK, Balling R, Suzuki N, Gruss P (1989) A family of octamer-specific proteins present during mouse embryogenesis: evidence for germline-specific expression of an Oct factor. EMBO J 8:2543–2550

Schonemann MD, Ryan AK, Erkman L, McEvilly RJ, Bermingham J, Rosenfeld MG (1998) POU domain factors in neural development. Adv Exp Med Biol 449:39–53

Schreiber E, Matthias P, Muller MM, Schaffner W (1989) Rapid detection of octamer binding proteins with "mini-extracts", prepared from a small number of cells. Nucleic Acids Res 17:6419

Schubart K, Massa S, Schubart D, Corcoran LM, Rolink AG, Matthias P (2001) B cell development and immunoglobulin gene transcription in the absence of Oct-2 and OBF-1. Nat Immunol 2:69–74

Sharif MN, Radomska HS, Miller DM, Eckhardt LA (2001) Unique function for carboxyl-terminal domain of Oct-2 in Ig-secreting cells. J Immunol 167:4421–4429

Sturm RA, Das G, Herr W (1988) The ubiquitous octamer-binding protein Oct-1 contains a POU domain with a homeo box subdomain. Genes Dev 2:1582–1599

Veenstra GJ, van der Vliet PC, Destree OH (1997) POU domain transcription factors in embryonic development. Mol Biol Rep 24:139–155

Verrijzer CP, van der Vliet PC (1993) POU domain transcription factors. Biochim Biophys Acta 1173:1–21

Verrijzer CP, Alkema MJ, van Weperen WW, van Leeuwen HC, Strating MJ, van der Vliet PC (1992a) The DNA binding specificity of the bipartite POU domain and its subdomains. EMBO J 11:4993–5003

Verrijzer CP, Oosterhout JA van, Vliet PC van der (1992b) The Oct-1 POU domain mediates interactions between Oct-1 and other POU proteins. Mol Cell Biol 12:542–551

Wirth T, Priess A, Annweiler A, Zwilling S, Oeler B (1991) Multiple Oct2 isoforms are generated by alternative splicing. Nucleic Acids Res 19:43–51

Zhao F-Q, Adachi K, Oka T (2002) Involvement of Oct-1 in transcriptional regulation of beta-casein gene expression in mouse mammary gland. Biochim Biophys Acta 1577:27–37

Zhao F-Q, Zheng Y, Dong B, Oka T (2004) Cloning, genomic organization, expression, and effect on beta-casein promoter activity of a novel isoform of the mouse Oct-1 transcription factor. Gene 326:175–187