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Isoforms of Transcription Factor Oct-1 Synthesized in Lymphocytes

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Abstract—Transcription factor Oct-1 is ubiquitous, participating in expression of the cell housekeeping genes as well as in differentiation of lymphocytes and activation of transcription of immunoglobulin genes in B cells. A new tissue-specific form of Oct-1 (Oct-1L) was found in lymphoid cells of bone marrow, lymph nodes, spleen, thymus, as well as in the cell lines of B and T lymphocytes at different stages of differentiation. This isoform was not found in embryonal and nonlymphoid tissues and cell lines. The complete structure of the *oct-1L* mRNA was determined, which generally corresponds to the structure of mouse Oct-1b. The difference between *oct-1L* and isoforms *oct-1a*, *b*, and *c* functioning in all cells is replacement of the long first 5'-terminal exon (the 1U exon) encoding 21 amino acid residues with a short one encoding 10 amino acids in the isoforms Oct-1L and Oct-1R. Interestingly, attempts to find mature *oct-1* mRNA simultaneously containing the 1L and 1U exons were unsuccessful. The parallel synthesis of "ubiquitous" isoforms Oct-1a, b, and c as well as tissue-specific Oct-1L and Oct-1R in lymphocytes and their precursors may be due either to a high demand of these cells for Oct-1 or to selective participation of different Oct-1 isoforms in regulation of the housekeeping genes and genes involved in the B and T cell differentiation and synthesis of imminoglobulins.

Key words: transcription factor Oct-1, the Oct-1L isoform, tissue-specific expression, lymphocytes

INTRODUCTION

The Oct proteins are members of a family of proteins containing the POU domain first detected in transcription factors Pit-1, Oct-1, and Unc [1, 2]. Oct-1 has numerous functions in the cell, it participates in positive and negative regulation of transcription, DNA replication [1, 2], attachment of chromatin loops to the nuclear matrix [3, 4], as well as in apoptosis [5]. The *oct-1* gene is one of the first genes expressed in the embryo [6]. The variety of Oct-1 functions is due to the fact that it controls many housekeeping genes, such as those for histones H2B [7], H3B [8], snRNA [2, 9–11], as well as tissue-specific genes. In particular, Oct-1 interacts with promoters and enhancers of genes of the light and heavy inmmunoglobulin chains [12, 13], interleukins 2 (IL-2) [14, 15] and 8 (IL-8) [16], and granulocyte-stimulating factor [17]. In the endocrine system, Oct-1 controls the expression of genes of some hormones and of proteins regulating their expression [18–22].

The "ubiquity" of Oct-1 and simultaneously its participation in the tissue-specific transcription may be realized in different ways. Oct-1 is able to establish complexes with different nuclear proteins. Its interaction with transcription factors and tissue-specific coregulators provides for fine regulation of gene expression. Oct-1 was shown to form complexes with several transcription factors such as Sp1 [23, 24], Ap-1 [14, 15], NF-1 [25], receptor of steroid hormones [26], Pit-1 [21], and homeoprotein Pbx [27]. In lymphocytes, Oct-1 interacts with a tissue-specific coactivator OCA-B (synonyms OBF or Bob.1) [28–30]. The POU domain and other regions of Oct-1 may be involved in these contacts [31–33].

The multiplicity of Oct-1 functions and fine regulation of expression may be ensured by its interaction with different DNA sites, such as canonical ATGCAAAT, noncanonical *oct* sites with single mutations, and homeospecific sites [34–38]. The Oct-1 affinity to all these sites is strongly dependent on the context of a sequence to be recognized. In its turn, the interaction with cofactors is strictly dependent on the site to which Oct-1 is bound. Thus, the VP16 protein interacts with Oct-1 only if the latter is associated with TAATGARAT [39], whereas OCA-B (OBF, Bob.1) interacts only with Oct-1 bound to the canonical *oct* site ATGCAAAT [40]. The glucocorticoid receptor specifically interacts with Oct-1 bound to an A/T-rich site, but does not contact Oct-1 bound to the canonical *oct* site in DNA [41].

Previously [42], in myeloma cells we revealed alternative tissue-specific splicing of exons localized

in the 5' region of *oct-1* pre-mRNA. Here we show that in lymphocytes, already at the early stages of their differentiation, a heretofore unknown isoform Oct-1L is synthesized (L for lymphocyte). Screening of a broad spectrum of cell lines and tissues shows that Oct-1L is synthesized as the main isoform in B cells and as a minor one in T cells. It is also shown that the earlier identified isoform Oct-1R [43] is present only in B cells. The Oct-1L structure has been determined and compared with those of different mouse and human isoforms. The possible role of lymphocyte-specific Oct-1 isoforms in lymphocyte maturation and formation of immune response is discussed.

EXPERIMENTAL

Cell lines. The following mouse cell lines were used in this work: myeloma NS/0, neuroblastoma NB41A3, embryonal carcinoma F9, fibroblasts 10(1), bone marrow stem cells FDCP-2, thymoma EL-4. Cells were grown in DMEM supplemented with 10% fetal calf serum and 40 μg/ml Gentamycin.

RNA isolation. RNA was isolated from cell lines and lymph nodes, spleen, thymus, and bone marrow of C57BL/6 mice using the guanidine isothiocyanate technique [44].

Oligonucleotides. The following oligonucleotides were synthesized:

αIL: 5'-GTCACTGCAGTCCAGCATGGC-3' to exon 1L,

ex2: 5'-GGTTTCTGATGGATTATTCATTC-3' to exon 2,

1L: 5'-GCCATGCTGGACTGCAGTGAC-3' to exon 1L,

1U: 5'-GAGCAGCGAGTCAAGATGAG-3' to exon 1U,

(5'-1L): 5'-ACTAAGCTTCTCTTCCCACCCTTGTT-3' to the 5'-UTR of exon 1L,

1A: 5'-TAGCCAGCCTATCACCCTGTAGT-3' to the 3' end of *oct-1a*,

1R: 5'-ATAGGATCCTTAAGTGCAAACCCATCT-3' to the 3' end of *oct-1R*,

T13: 5'-GGGAGGCCCTTTTTTTTTTTTT-3' to the poly(A) end.

Reverse transcription was carried out as described elsewhere [42]. Reaction products were analyzed in 6% PAG in the presence of 8 M urea. The sequencing reaction products of known nucleotide sequence (5'-untranslated region of *oct-1* exon 1L) were used as markers. To identify products of alternative splicing, oligo ex2 to the second exon was used. The full-sized *oct-1* cDNA were obtained using oligo T13.

Polymerase chain reaction. The single-stranded cDNA obtained by reverse transcription (1/5–1/10 part) was used as template in PCR. Oligo ex2 was labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase (Fermentas, Lithuania) according to the supplier's protocol. PCR was carried out using Hi-Fi polymerase (Silex-M) as described by the producer: 95°C, 2 min; (95°C, 40 s; 47°C, 1 min; 72°C, 2 min), 5 cycles; (95°C, 40 s; 57°C, 1 min; 72°C, 2 min), 30 cycles. The PCR products were analyzed in a denaturing γ-PAGE. Products of sequencing reactions with known base sequence were used as markers.

The full-sized *oct-1* cDNA were obtained in two steps. The first was PCR with the oligo 1L–T13 pair, 95°C, 2 min; (95°C, 1 min; 47°C, 1 min; 72°C, 4–6 min), 5 cycles; (95°C, 40 s; 57°C, 1 min; 72°C, 4–6 min), 30 cycles. At the second stage, 1 μl of mixture from the first PCR was taken for reaction in 50 μl with two different pairs of oligos (1L–1A and 1L–1R), 95°C, 2 min; (95°C, 1 min; 57°C, 1 min; 72°C, 4–6 min), 35 cycles. PCR products were analyzed by Southern blotting with a ^{32}P DNA probe (175 bp of 5'-untranslated region of exon 1L).

Sequencing. Nucleotide sequences were determined by PCR using a sequencing kit (Fermentas, Lithuania) as described by the producer.

Northern blotting. Total RNA (20 μg) was denatured at 68°C, resolved by electrophoresis in 1% agarose–formaldehyde gel, and transferred onto Hybond membrane (Amersham) in 10× SSC. Prehybridization conditions were as follows: 50% formaldehyde, 6× SSC, fivefold Denhardt's solution, 0.1% sodium dodecylsulfate (SDS), and 100 μg/ml denatured salmon sperm DNA, 55°C, 4 h. Hybridization was carried out for 12 h under the same conditions in the presence of [^{32}P]-RNA probe. The filter was washed under standard conditions and exposed for six days. An antisense probe for Northern analysis was obtained using a *Pst*I–*Eco*RI fragment of *oct-1R* cDNA cloned in pGEM4 vector. RNA was labeled using the Riboprobe system (Promega) according to the producer's protocol.

RESULTS

Cloning of cDNA and analysis of a new Oct-1 isoform. The cDNA encoding a new Oct-1 isoform was cloned from the NS/0 myeloma cells by reverse transcription and PCR. The 5'-terminal region of this cDNA is identical to *oct-1R* cDNA cloned previously from myeloma cells [43], whereas the rest of the sequence is completely homologous to mouse *oct-1b* cDNA [45] (Fig. 1). The 5' regions of *oct-1R* and *oct-1L* cDNA differ from those of all known mouse and human full-sized *oct-1* cDNA (*oct-1a, b, c*, *oct-1A, B*) [24]. The 5' exon of *oct-1R* and *oct-1L* cDNA encodes ten N-terminal amino acid residues, whereas that of

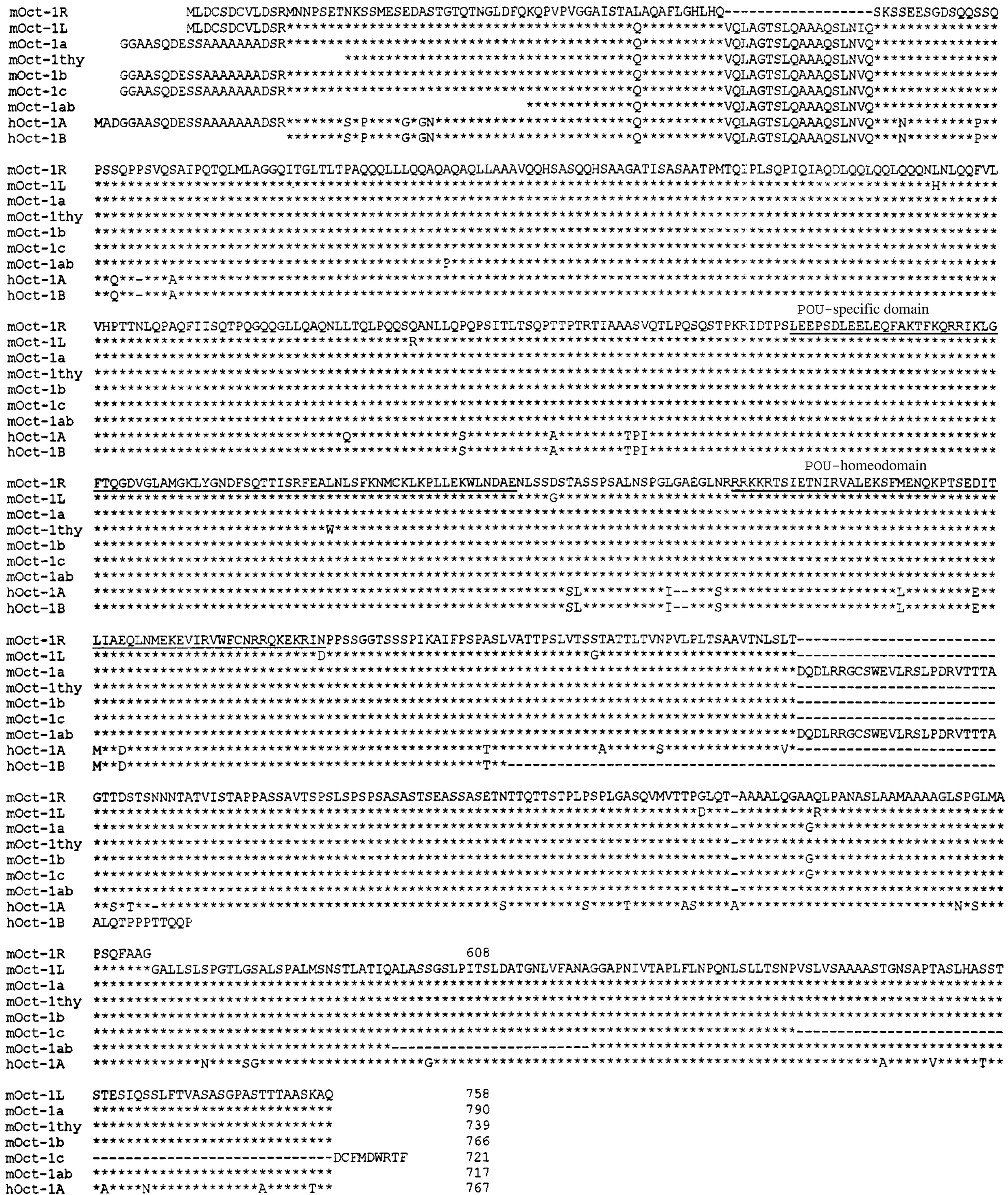

Fig. 1. Comparison of amino acid sequences of isoforms of (m) mouse and (h) human Oct-1 isoforms. The POU-homeodomain and POU-specific domain are underlined, and the lengths of amino acid sequences are indicated.

subforms *oct-1a, b, c* encodes 21 residues. We have called these exons 1L and 1U, respectively. The 1L exon was also found [46] in the mouse locus *otf-1* responsible for synthesis of Oct-1.

Tissue-specific synthesis of *oct-1L* and *oct-1R* RNA. The subforms of *oct-1* mRNA containing the 1L exon were revealed by reverse transcription and PCR using two pairs of oligonucleotides as described

elsewhere [42]. It has been shown that the products corresponding in size to the distance between primers in mature *oct-1* mRNA (60 and 196 bp) are synthesized in the cells of myeloma NS/0, spleen, lymph nodes, bone marrow, thymus, and T-cell lymphoma EL-4, while in T cells (thymus and EL-4) this mRNA is present in a negligible amount, and a signal is detected only after a long exposure. These PCR products were not found in other cell lines (Fig. 2).

A different pair of oligos (1U and [^{32}P]ex2) were used to determine the mRNA containing the 1U exon. The 77-bp PCR product corresponding to the 5' end of mature *oct-1a, b, c* mRNA was found in all cell lines and tissues studied, both lymphoid and nonlymphoid (Fig. 2): in myeloma NS/0, thymoma EL-4, as well as in mouse stem hemopoietic cells FDCP-2, bone marrow cells, spleen, lymph nodes, and thymus.

Thus, *oct-1* mRNA containing the 1L exon, unlike *oct-1a, b, c* mRNA, is expressed in lymphoid cells only: strong expression of subforms containing the 1L exon is observed in cells of the B series, such as those of myeloma NS/0, spleen, bone marrow (in our experiment they are enriched in B cells), and lymph nodes. Weak expression is observed in cells of the T series, thymocytes (immature T lymphocytes) and the T-cell lymphoma EL-4 (corresponds to mature T cells). In all other cell lines tested, no such *oct-1* mRNA was found.

Expression of *oct-1* mRNA in T and B lymphocytes. To reveal in T and B lymphocytes the mature forms of *oct-1* mRNA containing the 5'-terminal 1L exon, full-sized *oct-1* cDNA were synthesized by RT-PCR.

The use of oligo T13 allowed us to obtain a single-stranded cDNA and perform two successive rounds of PCR, the first with oligos 1L–T13 and the second one with two different pairs of oligos, 1L–1A and 1L–1R. The products of these reactions were analyzed by Southern blotting with a radioactive probe specific toward the exon 1L 5'-UTR (176 bp) (Fig. 3).

Two *oct-1* mRNA with identical 5'-terminal exon 1L but different 3'-terminal sequences were found. They were identified as *oct-1L* and *oct-1R*. The *oct-1L* mRNA is expressed both in B and T lymphocytes, but B lymphocytes contain many times more this RNA. Probably, very quick specific splicing of *oct-1L* mRNA takes place in T lymphocytes (Southern blotting).

The other lymphoid-specific form *oct-1R* mRNA is expressed only in the B-cell line of myeloma NS/0, and in this case its amount is many times lower than that of *oct-1L*. This form is absent from T cells (Fig. 3).

It is important that Southern blotting shows an approximately equal content of *oct-1L* and *oct-1R* pre-mRNA in T and B cells. Differences in the content of mature *oct-1L* and *oct-1R* mRNA in T and B cells are probably due either to the efficiency of alternative splicing or to the stability of these mRNA.

To identify the subforms of *oct-1* RNA (including *oct-1* pre-mRNA) and determine their size, Northern blotting of RNA isolated from myeloma NS/0 and HeLa cells was applied. A fragment of *oct-1* cDNA corresponding to the POU domain region was used as a probe. The 14.6–16, 2.4, and 0.7 kbp transcripts were found in RNA from NS/0 cells (Fig. 4). The PCR products of approximately the same size are also revealed by Southern blotting. Small size differences between PCR products and RNA transcripts are due to the presence of an additional 3'-terminal sequence in mRNA and to the use in Northern blotting of DNA markers whose mobility in agarose gel in the presence of formaldehyde differs somewhat from that of RNA molecules, which hinders precise determination of the size of RNA transcripts.

The results of Southern blotting of PCR products and Northern blotting of RNA from NS/0 cells agree nicely. The differences in the intensities of bands corresponding to the high- and low-molecular-weight nucleotide sequences detected by Southern and Northern blotting are probably due to the fact that the PCR conditions were optimized for the 2-3-kbp DNA: under these conditions the yield of high-molecular-weight products is much lower than that of low-molecular ones. It is seen in Fig. 4 (Northern blotting) that the composition of *oct-1* mRNA isoforms in NS/0 cells differs from that in HeLa cells. Transcripts of 14.5–16 and 2.3 kbp were found in HeLa cells. These differences may be due both to the tissue and species specificities of the cells. It also follows from the results of Northern blotting that splicing of mRNA for the ubiquitous Oct-1 may proceed differently in different cell lines.

DISCUSSION

Transcription factors, like other proteins, may be represented by a set of isoforms resulting from alternative splicing and differing in both structure and function. The interaction of these related proteins with DNA is defined by the context of a sequence to be recognized as well as by other nuclear proteins, coactivators and transcription factors.

At different stages of development and in different tissues, protein isoforms may have both similar and unique functions inherent only to the given isoform [47, 48]. Thus, Pit-1 protein plays an important role in the development of adenohypophysis. Alternative splicing of *pit-1* mRNA gives rise to two isoforms Pit-1 and Pit-1a exhibiting different transactivation abilities. Unlike Pit-1, Pit-1a is not able to activate promoters of the prolactin gene and of its own gene [49]. Expression of *oct-2* gene in B cells results in forma-

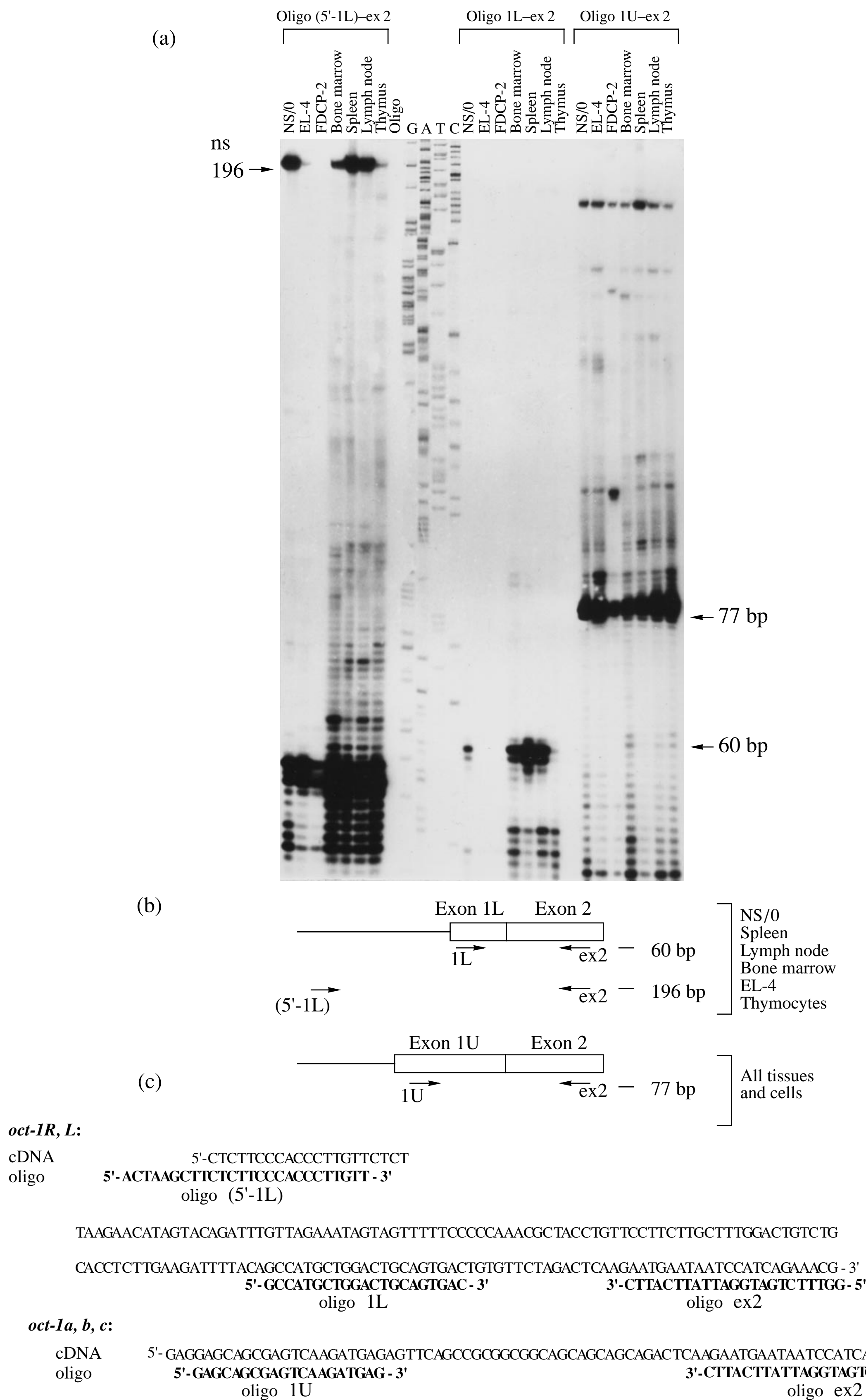

Fig. 2. Alternative splicing of 5' exons of *oct-1* RNA. (a) Products of RT-PCR. In the upper part, pairs of primers used in PCR. (b) Scheme of experiment. (c) Primers used to identify mRNA *oct-1a, b, c* and *oct-1R*, *L* and corresponding sites of *oct-1L* cDNA.

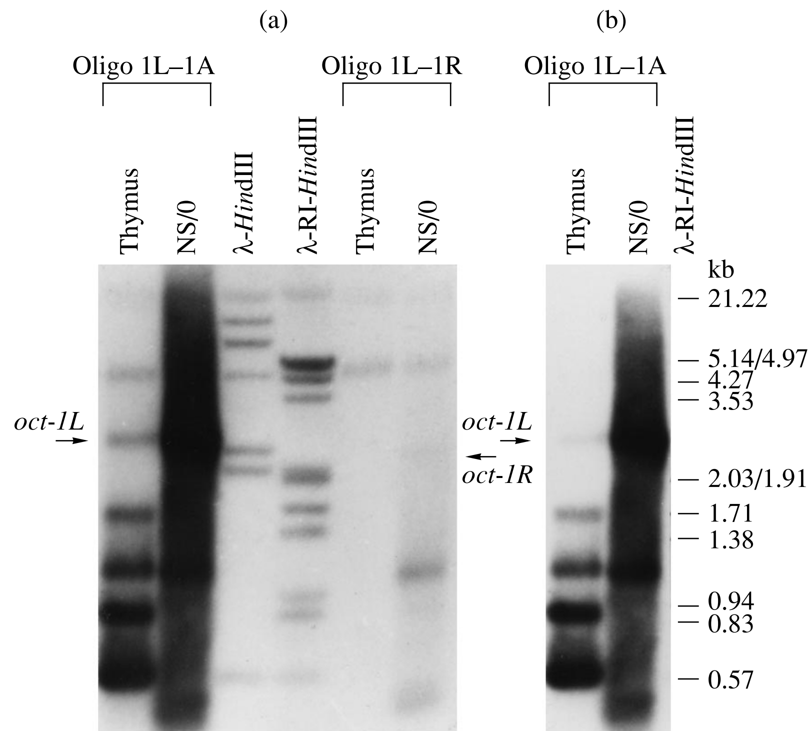

Fig. 3. Southern blotting of *oct-1L* and *oct-1R* isoforms obtained by RT-PCR. PCR products were revealed in the myeloma NS/0 cells and thymocytes by hybridization with the *oct-1* cDNA labeled exon 1L UTR (196 bp). Exposure time 3 days (a) and 12 h (b).

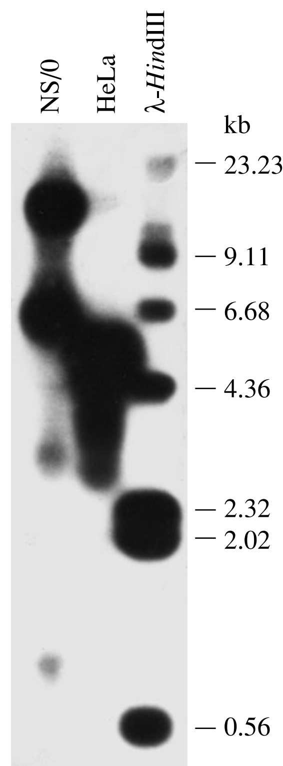

Fig. 4. Northern blotting of *oct-1* mRNA from NS/0 and HeLa cells with labeled *Pst*I–*Eco*RI fragment of *oct-1* cDNA. Labeled fragments of phage λ DNA obtained by cleavage with *Hin*dIII or *Hin*dIII/*Eco*RI were used as markers.

tion of a set of mRNA encoding functionally different isoforms of Oct-2 [50–52].

Oct-1 is known to consist of a set of isoforms synthesized in all tissues and cell lines [45, 53]. Here, we have characterized for the first time Oct-1L, a new isoform of Oct-1, and have shown that synthesis of Oct-1L and Oct-1R is tissue-specific and up to now these isoforms are found only in lymphocytes. The *oct-1L* and *oct-1R* mRNA containing exon 1L are generated at the early stages of differentiation, namely, in a population of bone marrow cells containing immature B lymphocytes. A high level of *oct-1L* mRNA is preserved in spleen and lymph node cells. The myeloma NS/0 cells corresponding to the terminal stage of B lymphocyte differentiation are characterized by a high content of *oct-1L* mRNA. Small amounts of this mRNA are synthesized in thymus cells and T-cell lymphoma EL-4.

Proteins Oct-1L and the earlier discovered Oct-1R [42, 43] are identical in the region encoded by the 5'-terminal exon (the 1L exon) but differ much in the rest of the sequence (Fig. 1). The 1L exon encoding ten amino acid residues was not found in mRNA of other known isoforms of Oct-1, which contain at their 5' end a longer exon 1U not homologous to the 1L exon. No mature mRNA were found to simultaneously contain both exons [42]. Proteins Oct-1 and Oct-2 recognize with the highest affinity (as compared with other POU

proteins) the ATGCAAAT motif playing the leading role in expression of immunoglobulin genes and others expressed in lymphocytes [13]. Probably, both proteins participate in lymphocyte differentiation in the immune response [13]. In the POU protein family, transcription factors Oct-1 and Oct-2 have both functional and highest structural similarity. The previous comparison [54] of base sequences of exons of human *oct-1* and mouse *oct-2* revealed significant structural similarity of all exons except 1 and 6. In that work, the long (64 bp) human exon 1U was compared with the short (31 bp) first exon of mouse *oct-2*. We have compared the first exon of *oct-2* with the 1L exon of mouse *oct-1*. It turned out that the 1L exon and the first exon of *oct-2* not only coincide in length, but have appreciable sequence homology (57%):

ATGGTTCACTCCAGCATGGGGGCTCCAG in *oct-2*

ATGCTGGACTGCAGTGACTGTGTTCTAG in *oct-1L*.

In the immune system cells, Oct-1 takes part in regulation of immunoglobulin genes as well as of other tissue-specific genes, such as genes of interleukins 2 [14, 15], 3, 5 [17], and 8 [16] and receptor proteins of T cells. This is indicative of the necessity of Oct-1 for the B-cell differentiation and formation of both antigen-dependent and antigen-independent immune response [13]. A special role in these processes may belong to isoforms Oct-1L and Oct-1R synthesized beginning from the early stages of B lymphocyte differentiation which includes formation of their specific functions, such as synthesis of immunoglobulin-like receptors and "maturation" of immune response, switching of antibody isotypes, and antigen-dependent synthesis of antibodies. The Oct-1 isoforms "containing" the 1L exon are synthesized in lymphocytes simultaneously with nonspecific isoforms "containing" the 1U exon. Additional synthesis of a multifunctional transcription factor Oct-1 may be necessary in intensely functioning lymphocytes to ensure contacts with regulatory elements. This may be combined with functions characteristic only of Oct-1 isoforms "containing" the 1L exon. Assessment of this alternative will be the subject of our further investigations.

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