Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochimie 85 (2003) 715–718
BIOCHIMIE
www.elsevier.com/locate/biochi

Short communication

Regulation of oct-1 gene transcription is different in lymphoid and non-lymphoid cells

Svetlana Zhenilo, Igor Deyev, Sergey Serov, Oleg Polanovsky *

Engelhardt Institute of Molecular Biology, ul. Vavilova 32, Russian Academy of Sciences, 117984 Moscow, Russia

Received 30 April 2003; accepted 23 May 2003

Abstract

Transcription factor Oct-1 is expressed in all eukaryotic cells acting as a positive or negative regulator of gene transcription and DNA replication. Being a ubiquitous nuclear protein, Oct-1 also takes part in the regulation of tissue-specific gene expression. In this paper, we have found that human oct-1 gene is regulated by two promoters, located in OTF-1 locus upstream of 1U and 1L exons, respectively. The DNA region preceding U exon has a pattern typical of the constitutive gene promoters. The 5'-region upstream of 1L–exon is AT-rich, contains no TATA box, but has two octamer sequences targeted by Oct-1 and Oct-2 proteins. Analysis of promoter activity is carried out by transfection of recombinant plasmids in non-lymphoid HEK293 and lymphoid Raji cells. In non-lymphoid cells, efficiency of transcription from the 1U promoter several times exceeded that from the 1L promoter. The 1U promoter activity is little increased in the presence of an external enhancer. A different expression pattern was observed if the same constructs were transfected into lymphoid Raji cells. In this case, the level of transcription from the 1L promoter (the L–2 fragment, containing a proximal octamer site) in the presence of the enhancer was significantly higher than that of any fragments containing 1U promoter. It was shown that distal regions of both 1U and 1L were capable of silencing activity. In Raji cells, the enhancer completely overcomes the activity of U silencer, but only partly overcomes that of L silencer. Our data on tissue-specific features of 1L promoter and interaction of both oct-1 promoters with enhancer and silencers in different cell types point to a fine tissue-specific regulation of the oct-1 gene expression, especially in lymphoid cells.

Keywords: *Oct*–1 gene; Tissue-specific transcription; Promoters; Silencers

1. Introduction

Transcription factors Oct-1 and Oct-2 were originally identified in the late 1980s [1–3]. These factors belong to the large family of POU domain proteins, which has first been revealed in transcription factors Pit-1, Oct-1, and Unc-86 [4]. In double-stranded DNA, the optimal target sequence of these proteins is the ATGCAAAT octamer, first revealed in immunoglobulin (Ig) gene promoters [5]. Later, this sequence was found in promoters and enhancers of many other genes [6–8]. In particular, Oct-1 participates in the expression control of many housekeeping genes such as those for snRNA and histone H2B [9,10]. This implicates Oct-1 in cell proliferation and differentiation. The mechanism of transcription activation by Oct-1 involves co-activators and other transcription factors [6–8]. Recently, it has been shown that POU domain of the Oct-1 protein could form direct contacts with TATA-binding protein (TBP), which was found to be essential for the interaction with a distant enhancer [9]. Multiple functions of Oct-1 may be explained by the context of recognized DNA sequence, by associations with other proteins, and also by specific features of its own structure either in POU domain or in other functionally important regions [10,11]. We have previously revealed oct-1 mRNA isoforms specific for lymphoid cells and tissues [12,13] differing from the set of the so-called ubiquitous Oct-1 isoforms by the structure of 5'-terminal exon. It was shown that tissue-specific isoforms contain a short 5'-terminal exon 1L, whereas the ubiquitously expressed Oct-1 isoforms contain at 5'-end exon 1U. Location of both exons was established in human and mouse OTF-1 loci [14]. This work is focused on the study and on the comparison of the structure and specific features of transcription of the 5'-terminal regions located

Abbreviations: Ig; immunoglobulin; PCR; polymerase cycle reaction; snRNA; small nuclear RNA; TBP; TATA-binding protein.

* Corresponding author. Tel.: +7-95-135-9903; fax: +7-95-135-1405.
E-mail address: pol@genome.relarn.ru (O. Polanovsky).

doi:10.1016/S0300-9084(03)00121-4

upstream of exons 1U and 1L in human lymphoid and non-lymphoid cells.

2. Materials and methods

2.1. Cell lines

The HEK293 cells (embryonal kidney carcinoma) were maintained in DMEM medium containing 10% fetal calf serum and 40 μg/ml kanamycin. Raji (B–lymphocytes) cells were maintained in RPML-1640 medium containing 10% fetal calf serum and 40 μg/ml kanamycin.

2.2. Plasmid constructs

Fragments of 5'-terminal regions of exons 1L and 1U were generated by PCR using Hi-Fi polymerase (Sileks M, Russia) and human genomic DNA (Promega) as template. The L–1 construct was generated with primers 3L(5'-aaaggtaccgttaggtccaagaagccagg-3')–2L(5'-gagcagagagtccaaacaag-3'); construct L–2, with primers 4L(5'-aaaggtacctatttatgcaacgatttaaatt-3')–2L; construct L–3, with primers 5L(5'-aaaggtaccaagcttcattatggaaactgcc-3')–2L; construct U–1, with primers 1U(5'-aaaggtacctttccccaataccat-3')–2U (5'-gaatattttaaccaaaatcgccc-3'); construct U–2, with primers 3U(5'-aaaggtaccccggggcggggattagctcggat-3')–2U; and construct U–3, with primers 4U(5'-aaaggtacctgaagaaagtgcggagcaca-3')–2U. The reaction products were sequenced, digested with *Kpn*I restriction endonuclease and cloned into the pGL3–enhancer and pGL3–basic vectors (Promega) digested with *Kpn*I and *Sma*I enzymes. Due to this procedure, 3'-termini of both U–containing and L–containing fragments were identical, whereas 5'-termini differed in length.

2.3. Transient transfection

Transfection of HEK293 cells was carried out by calcium phosphate method using 0.5 μg reporter plasmid and 0.3 μg pCMVlacZ (Clontech). Raji cells were transfected using electroporation: 1.1 kV, 40 μs; and dual pulse with the interval of 2 min (2×10^6 cells; 0.4–mm cuvette; gap volume 145 μl). Cells in both cases were incubated for 48 h in CO_2 incubator, trypsinized, pelleted by short centrifugation, and the supernatant was discarded. Cells were then suspended in lysis buffer (Promega) as described by the manufacturer. Luciferase activity was measured as described by the manufacturer (Promega). A relative luciferase activity for each point was normalized to β-galactosidase activity. All values are the means calculated from the results of at least three independent experiments.

3. Results and discussion

3.1. Genomic organization of the oct-1 5'-terminal region

Human oct-1 locus (OTF-1 locus) spans the region of about 200 kb which contains at least 21 exons. The distance between exons 1U and 1L constitutes 100 kb, almost a half of the whole locus (Fig. 1A). To study the role of human 1U and 1L flanking regions in transcription control, these regions were cloned in pGL3–vectors. As seen from Fig. 1B, the region around exon 1U in mouse and man appeared to be extremely GC-reach. The GC-content usually constituted 60–70%, reaching 90% in some 100 bp DNA stretches. This area contained at least 25 sites for the Sp1 factor and other transcription factors, recognizing GC-enriched targets. This pattern is typical of promoters of constitutive genes, i.e. the genes expressed in all cell types. The region around exon 1L is AT-rich (Fig. 1B) and contains numerous TAAT-core sites for homeoproteins, CCAAT-binding proteins, and two octamer sites ATGCAAAT which are recognized with high affinity by Oct-1, Oct-2, and other POU proteins. Almost all these sites (including octamers) were found both in human and mouse oct-1 gene, pointing to their possible involvement in the oct-1 gene transcription regulation.

3.2. Regulation of oct-1 gene expression in non-lymphoid cells

In non-lymphoid HEK293 cells, promoter activity of 1U region was much higher, when compared with the 1L promoter region. The U–3 construct activity was 50 times higher than that of control “empty” vector (Fig. 2A). Elongation of this sequence (constructs U–1 and U–2) resulted in the 2.5 times decrease of the promoter activity. For comparison, promoter activity of the regions upstream of exon 1L appeared to exceed only five times that of the empty vector. The Oct-1 binding sites located in this region did not influence the promoter activity in HEK293 cells. It is interesting to note that the investigated promoter fragments were weakly affected by the enhancer in pGL3 enhancer constructs (Fig. 2A).

3.3. Regulation of oct-1 gene transcription in lymphoid Raji cells

Transfection of our constructs in Raji cells revealed another pattern of activity. Promoter activity of the regions L–1, L–2 and L–3 in the absence of the enhancer was relatively low, while the presence of the latter resulted in a remarkable increase of the activity up to three to eightfold. Our data indicated that transcription from L promoter was tissue-specific and dependent on external enhancer (Fig. 2B). With respect to transcription activity in lymphoid Raji cells, 1L–constructs may be roughly arranged in the following order: L–1 < L–3 < L–2. This may be explained as follows: though L–1 construct contains promoter and is activated by an external enhancer, this activation is not complete due to the presence of the silencer in the 5'-terminal region. In the presence of enhancer, the highest level of activity was observed for the L–2 construct. This construct contains a proximal octamer site which may associate with TBP which is, in turn, essential for the interaction with enhancer proteins [9]. Without the enhancer, the level of the promoter activity of the U–1 con-

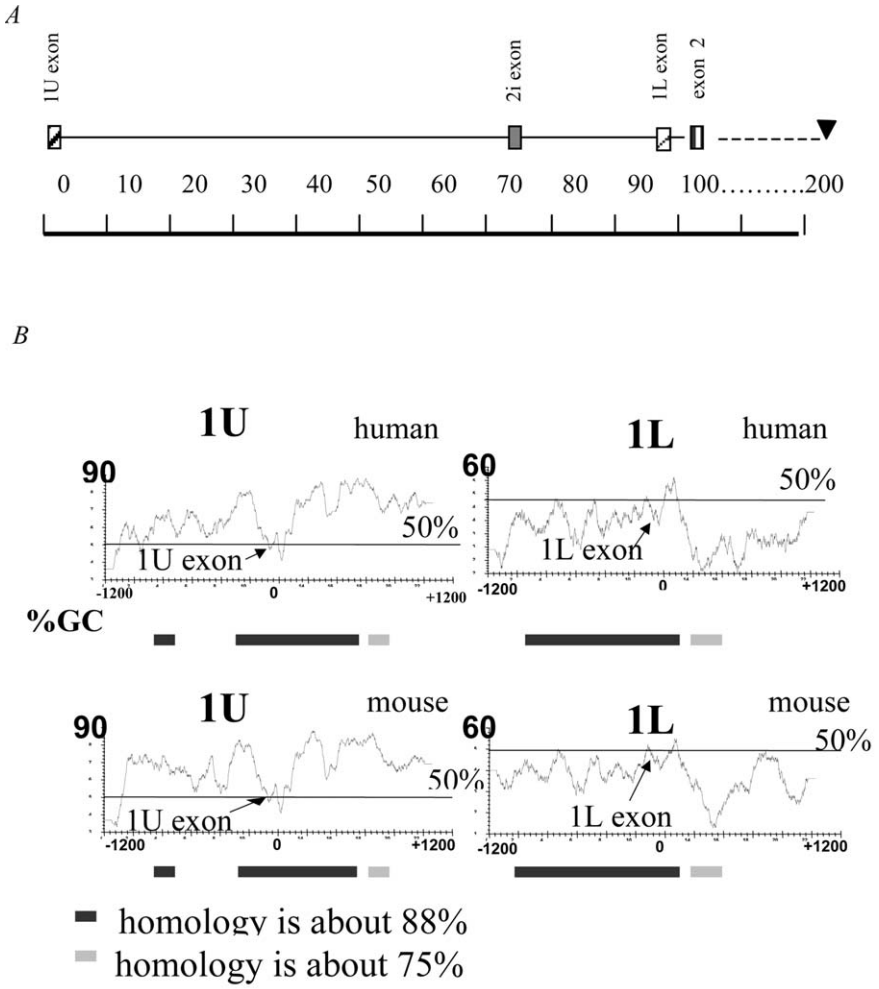

Fig. 1. Organization of 5'-region of human OTF-1 locus. (**A**) Scheme of exon location in 5'-region of human OTF-1 locus. (**B**) Analysis of DNA regions surrounding human and mouse 1U and 1L exons: the levels of GC-content and human/mouse homology are shown by black and open bars. Location of 1U and IL exons is indicated by arrows. The level of GC-content was established using the Gene Runner software with steps of 100 bp. – indicates the position of the last exon of gene oct-1.

struct only slightly exceeds that of the empty vector, while the enhancer insertion into the construct results in the tenfold increase of the promoter activity. The activity of the smallest U–3 construct is the same in both vectors, i.e. the enhancer had a negligible effect on activity. When compared with the data obtained in HEK293 cells, this result allows us to suggest that 1U region between –600 and –800 kb exhibits a silencer activity that can be completely overcome by enhancer in lymphoid Raji cells.

An especially intriguing and important result is that L–promoter activity in lymphoid cells in the presence of enhancer is comparable (for L–2 is higher) with that of U promoter responsible for the synthesis of ubiquitous Oct-1 isoforms. These results suggest that oct-1 gene may be expressed in lymphoid cells in a tissue-specific manner and provide an explanation for selective expression of L–containing oct-1 mRNA isoforms in lymphoid tissues as was shown by us earlier [12].

The identical binding properties but different distribution of Oct-1 and Oct-2 led to initial idea that the ubiquitously expressed Oct-1 regulates the expression of octamer-dependent housekeeping genes, whereas Oct-2 regulates the expression of Ig genes through functional octamer elements in their promoters [15]. This model was further corrected in later work. It was shown that B cell-specific transcription of Ig genes is induced by interaction of Oct-1 or Oct-2 with the co-activator OCA-B [16–18]. As was shown in [19], Oct-1 is the most potent activator of Ig promoters in conjunction with cognate Ig gene enhancers in B cells. Further it was shown [20] that in the absence of Oct-2 and OCA-B, B cells develop normally to the membrane Ig M–positive stage, and Ig gene transcription is essentially unaffected. These factors are essential for germinate center formation. We suppose that an activity of both oct-1 promoters in B cells may be essential for the high level expression of Ig genes.

Acknowledgements

We appreciate Dr. Egor Egorov for providing the cuvettes for electroporation. This work was supported by Russian

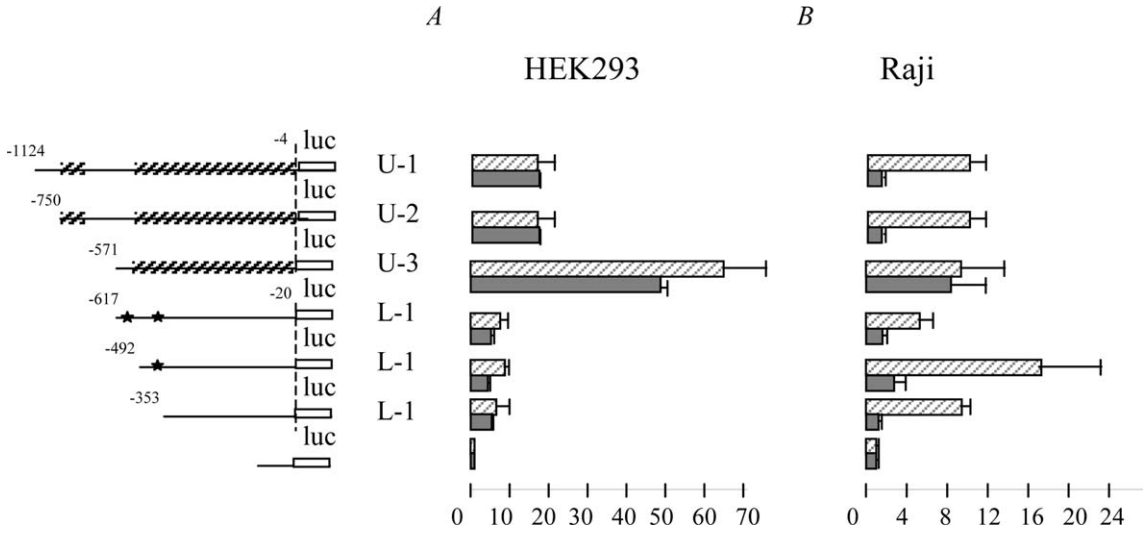

Fig. 2. Regulation of transcription by 5'-flanking regions of exons 1U and 1L in HEK293 (**A**) and Raji cells (**B**). The investigated DNA fragments containing reporter luc-gene are shown at the left. Human/mouse homology is shown by striped regions. Octamer sites are indicated by asterisks. Promoter activity of the constructs cloned into the pGL3–enhancer vector (striped bars) and pGL3–basic vector (dark bars) is shown as relative luciferase activity.

Foundation for Basic Research (grants 02-04-49129 and 02-04-6752) and Russian State Program "Studies and Projects in Priority Directions in Science and Technology".

References

[1] C. Fletcher, N. Heintz, R.G. Roeder, Purification and characterization of OTF-1, a transcription factor regulating cell cycle expression of a human histone H2b gene, Cell 51 (1987) 773–781.

[2] C. Scheidereit, A. Heguy, R.G. Roeder, Identification and purification of a human lymphoid-specific octamer-binding protein (OTF-2) that activates an immunoglobulin promoter in vivo, Cell 51 (1987) 783–793.

[3] A.G. Stepchenko, N.N. Luchina, O.L. Polyanovskii, A protein factor interacting with the promoter region of the immunoglobulin [kappa] genes, Dokl. Akad. Nauk. SSSR 303 (1988) 1008–1010.

[4] W. Herr, R.A. Sturm, R.G. Clerc, L.M. Corcoran, D. Baltimore, P.A. Sharp, H.A. Ingraham, M.G. Rosenfeld, G. Finny, G. Ruvkun, H.R. Horvitz, The POU domain: a large conserved region in the mammalian pit-1, oct-1, oct-2, and *unc-86* gene products, Genes Dev. 2 (1988) 1513–1516.

[5] F.C. Falkner, H.G. Zachau, Correct transcription of the immunoglobulin [kappa] gene requires an upstream fragment containing conserved sequence elements, Nature 310 (1984) 71–74.

[6] W. Herr, M. Cleary, The POU domain: versatility in transcriptional regulation by a flexible two-in-one DNA-binding domain, Genes Dev. 9 (1995) 1679–1693.

[7] A.G. Stepchenko, O.L. Polyanovskii, Interaction of Oct proteins with DNA, Mol. Biol. (Moscow) 30 (1996) 503–513.

[8] K. Phillips, B. Luisi, The virtuoso versatility: POU proteins that flex to fit, J. Mol. Biol. 302 (2000) 1023–1039.

[9] E. Bertolino, H. Singh, POU/TBP cooperativity: a mechanism for enhancer action from a distance, Mol. Cell 10 (2002) 397–407.

[10] M. Tanaka, J.-S. Lai, W. Herr, Promoter-selective activation domains in Oct-1 and Oct-2: direct differential activation of an snRNA and mRNA promoter, Cell 68 (1992) 755–767.

[11] M. Kim, L.A. Lesoon-Wood, B.D. Weintraub, J.H. Chung, A soluble transcription factor, Oct-1, is also found in the insoluble nuclear matrix and possesses silencing activity in its alanine-rich domain, Mol. Cell. Biol. 16 (1996) 4366–4377.

[12] E.V. Pankratova, I.E. Deyev, S.V. Zhenilo, O.L. Polanovsky, Tissue-specific isoforms of the ubiquitous transcription factor Oct-1, Mol. Gen. Genet. 266 (2001) 239–245.

[13] A.G. Stepchenko, The nucleotide sequence of mouse Oct-1 cDNA, Nucleic Acids Res. 20 (1992) 1419.

[14] I.E. Deyev, S.V. Zhenilo, O.L. Polanovsky, Spliced oct-1 mRNA isoforms with untranslated exons and a deletion from the region coding for the POU-specific domain, Mol. Biol. (Moscow) 37 (2003) 139–146.

[15] W. Schaffner, How do different transcription factors binding the same DNA sequence sort out their job?, Trends Genet. 5 (1989) 37–39.

[16] M. Strubin, J.W. Newell, P. Matthias, OBF-1, a novel B cell specific coactivator that stimulates immunoglobulin promoter activity through association with octamer-binding proteins, Cell 80 (1995) 497–506.

[17] M. Gstaiger, L. Knopfel, O. Georgiev, W. Schaffner, G.M. Hovens, A B–cell coactivator of octamer-binding transcription factors, Nature 373 (1995) 360–362.

[18] Y. Luo, R.G. Roeder, Cloning, functional characterization, and mechanism of action of B–cell specific transcriptional coactivator OCF-B, Mol. Cell. Biol. 15 (1995) 4115–4124.

[19] P.C. Shach, E. Bertolini, H. Singh, Using altered specificity Oct-1 and Oct-2 mutants to analyze the regulation of immunoglobulin gene transcription, EMBO J. 16 (1997) 7105–7117.

[20] K. Schubart, S. Massa, D. Schubart, L.M. Corcoran, A.G. Rolink, P. Matthias, B cell development and immunoglobulin gene transcription in the absence of Oct-2 and OBF-1, Nature Immunol. 2 (2000) 69–74.