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Cell differentiation in vitro and the expression of Oct-2 protein and *oct*-2 RNA

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Abstract

Expression of the *oct*-2 gene was studied in mouse tissues and during in vitro differentiation of embryocarcinoma PCC4, mouse neuroblastoma Neuro2A and NB41A3 cells in the presence of retinoic acid (RA) or 1% DMSO. *oct*-2 mRNA is characterized by a complex pattern and exists in both poly(A)$^+$ and poly(A)$^-$ forms. *oct*-2 mRNA was found in many cell lines, whereas Oct-2 protein was detected only in some of these cells. *oct*-2 expression also changed during cell differentiation. The cell differentiation is likely to be controlled not only at the gene transcription level, but also at the level of regulation of nuclear transcription factor activity. Such a regulatory mechanism would provide for a finer regulation of cell differentiation.

Key words: Induction of differentiation; Oct-factor; Neuroblastoma; Embryocarcinoma; POU protein

1. Introduction

About ten proteins have been identified that bind to the octamer sequence (ATTTGCAT) by gel retardation assay. cDNAs for some of these proteins were obtained and sequenced [1–10].

Analysis of *oct* expression during mammalian ontogenesis has shown that the Oct proteins are expressed in various tissues and organs during both embryogenesis and the postnatal period. Their expression appears at very early stages of embryogenesis [11–15].

In order to determine whether the expression of active Oct-2 protein and *oct*-2 mRNA depends on the stage of cell differentiation, we have chosen as a model system the in vitro differentiation of two neuroblastoma cell lines, NB41A3 (blasts) and Neuro2A (more differentiated cells with well-defined axons and dendrites), as well as the embryonic carcinoma PCC4 cell line. Retinoic acid (RA) and DMSO were used as the differentiation inducers. RA was shown to be an endogenous inducer of differentiation. Its influence on the genome is mediated by nuclear receptors whereas the target of DMSO is still unknown [16].

It is known that differentiation of neuroblastoma cells to more mature neuron precursors is induced by cultivation in the presence of 1% DMSO. Differentiation of PCC4 embryonic carcinoma cells caused by 1% DMSO results in derivatives of all three embryo sheet leaves. RA stimulates differentiation of PCC4 cells mainly to the neuron precursors [16].

2. Materials and methods

2.1. The in vitro induction of differentiation

The induction of differentiation was carried out as described [16]. Two different ways of induction were used: cultivation for 6 days in the presence of 250 nM RA or 1% DMSO.

2.2. DNA probe

A double-stranded oligonucleotide, containing the octa-sequence, was used as a probe for protein binding (OCTA-26): CCGGAAGCTGATTTGCATGTGCTGCC.

2.3. Preparation of whole-cell extracts

The lysates were prepared according to the earlier described scheme [17].

2.4. Gel retardation assay

Gel retardation assay was performed as described previously [18].

2.5. Preparation of total and poly(A)$^+$ RNA

This was carried out using 5 M guanidine thiocyanate as described previously [19]. Poly(A)$^+$ was obtained from oligo(dT) columns.

2.6. Preparation of cryohistoblots from the mouse embryos

The day of the vaginal plug appearance was taken as the zero day of pregnancy. The cryohistoblots were obtained as described previously [20].

2.7. Northern blot hybridization and hybridization of cryohistoblots

Earlier we obtained *oct*-2 cDNA [21] which was used for the preparation of a ^{32}P-labeled RNA probe. The probe corresponds to the *Pst–Sma* fragment of *oct*-2 cDNA, and does not contain POU box. The *Pst–Sma* fragment of the *oct*-2 gene is not highly homologous to other POU genes.

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3. Results and discussion

The Northern blot hybridization of the myeloma NS/O cell RNA with the RNA probe revealed *oct*-2 mRNAs of 13, 8, 6, 3.6 and 2.3 kb (Figs. 1 and 2A). *oct*-2 mRNA is present in the NS/O cells in both the poly(A)$^+$ and the poly(A)$^-$ fractions. Only poly(A)$^+$ RNA is present as 13 and 8 kb transcripts (Fig. 2). The gel retardation assay identified active Oct-2 protein in the myeloma NS/O cell line (Fig. 3A).

The *oct*-2 RNA probe hybridization with RNA from Balb/c mice tissues, followed by RNase A treatment, demonstrated the presence of *oct*-2 mRNA in brain (transcripts 8 and 2.3 kb in poly(A)$^+$ RNA and additional bands of 1.7 and 1.3 kb in total brain RNA). The high molecular weight *oct*-2 mRNA was not found in liver but present were *oct*-2 mRNA transcripts of 2.3, 1.5 and 1.3 kb. Transcripts of 2.3, 1.7 and 1.3 kb were found in kidney. Mainly mRNA of 1.3 kb was observed in poly(A)$^+$ RNA from kidney (Fig. 1).

Northern blot hybridization of the same RNA probe with RNA from in vitro-differentiated and undifferentiated cell lines of embryocarcinoma PCC4, neuroblastomas NB41A3 and Neuro2A revealed the expression pattern of the *oct*-2 gene (Fig. 2).

We have shown that active Oct-2 proteins are present in low numbers in the differentiated neuroblastoma cell line. NB41A3 and five bands of *oct*-2 mRNA (13, 6, 1.9, 1.7 and 1.3 kb) were detected in these cells. After DMSO treatment all the RNA bands were detected but Oct-2 protein disappeared (Fig. 3A).

In the more differentiated Neuro2A cell line, active Oct-2 protein is present in very low amounts. Six *oct*-2 mRNA bands (13, 6, 2.3, 1.9, 1.7 and 1.3 kb) were detected in these cells. After DMSO treatment the amount of Oct-2 protein remained unaffected (Fig. 3A). RA caused a significant decrease in the 13 kb transcript (Fig. 2B) and stimulated the expression of Oct-2 and Oct-1 proteins. Evidently, under the experimental conditions

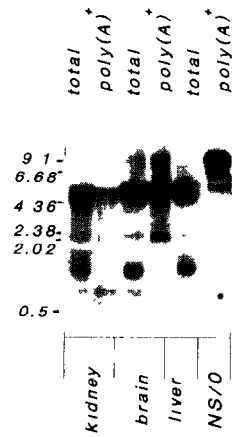

Fig. 1. Northern blot hybridization of RNA from the mouse myeloma cell line, NS/O, and from mouse Balb/c tissues. Hybridization was carried out with the ^{32}P-labeled RNA probe. The positions of markers are shown on the left.

used, dedifferentiation of Neuro2A cells begins, because RA causes some alterations in cellular morphology and the cells resemble neuroblasts.

Both Oct-2 protein and *oct*-2 mRNA are absent in undifferentiated embryocarcinoma PCC4 cells. The differentiation caused by both RA and DMSO leads to the appearance of *oct*-2 mRNA transcripts (6, 2.3, 1.9, 1.7 and 1.3 kb) (Fig. 2B) and active Oct-2 protein (Fig. 3B).

The 4.5 kb band is characteristic of all cell types. We assume that this is due to cross-hybridization with ribosomal 28 S RNA, because *oct*-2 RNA and ribosomal 28 S RNA contain extended GC-rich regions in which an essential homology is observed.

To find out what level of the *oct*-2 mRNA expression in the above-described differentiation systems in vitro corresponds to those proceeding during differentiation in vivo, we have carried out a series of hybridizations on mouse embryo histoblots, beginning from the sixth day of embryonic development to the third postnatal day. At day 6 (this stage corresponds to undifferentiated PCC4

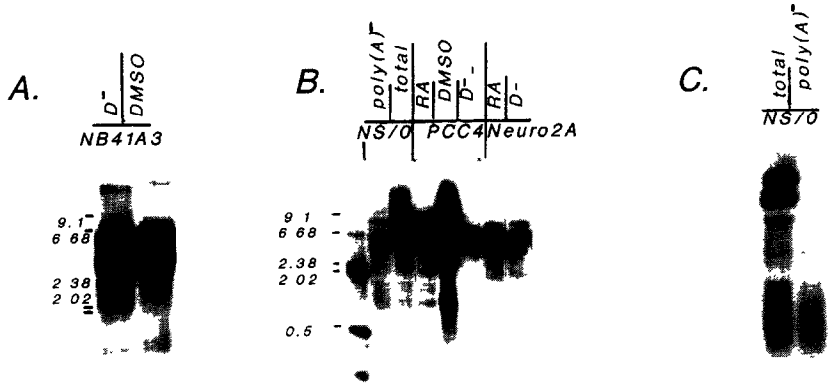

Fig. 2. Northern blot hybridization of RNA from undifferentiated and in vitro-differentiated cell lines (A,B). Cell lines are indicated above the picture. D, undifferentiated cells; DMSO, DMSO-induced differentiation; RA, RA-induced differentiation. To check the purity of poly(A)$^-$ RNA, the filter was hybridized with a foreign cDNA probe obtained from MS/O cells (C).

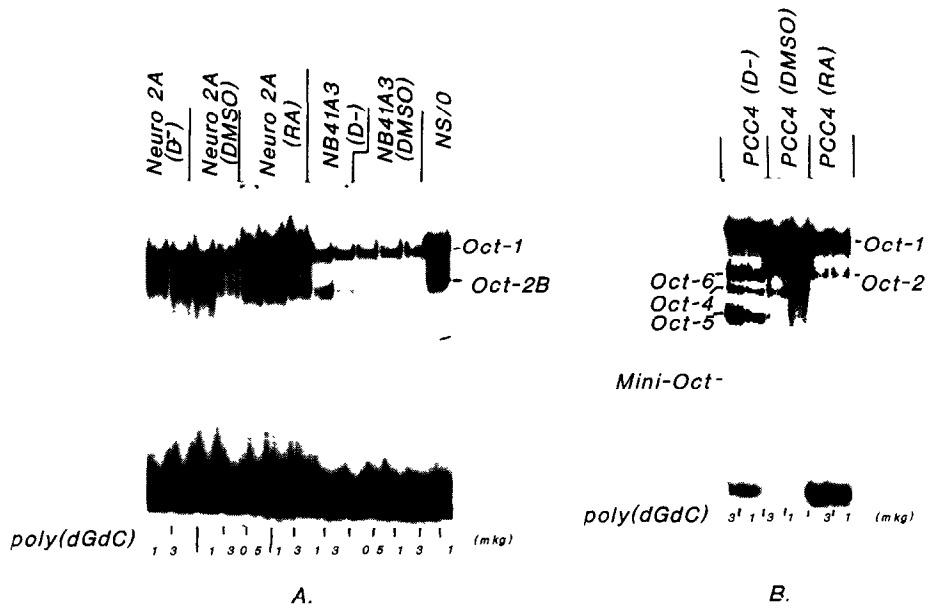

Fig. 3. Gel retardation assay with the octanucleotide probe, OCTA-26. Cell lines are indicated above the picture. The observed complexes are named according to the nomenclature of Scholer [30].

cells), the *oct*-2 RNA expression was not observed on histoblots: it was first detected on day 8. On day 10 a strong hybridization in the neural tube was observed (data not shown) and on days 14–17 a very powerful expression was found in central nervous system, as well as in liver and placenta (Fig. 4). On postnatal day 1 a decrease in *oct*-2 expression begins in central nervous system and liver and on postnatal day 3 it disappears in spinal cord in the tail-to-head direction. By this time the differentiation of neurons is over.

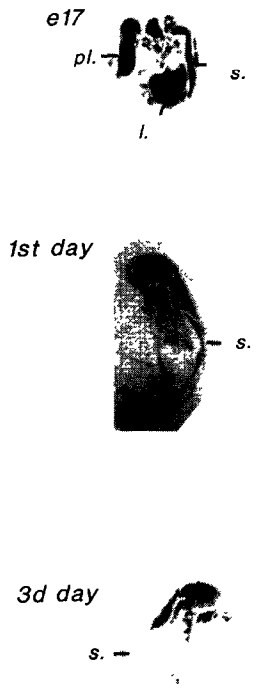

Fig. 4. Expression of *oct*-2 mRNA in embryonic and mature mouse tissues. Hybridization on cryohistoblots of embryos and newborn Balb/c mice at the stages: e17, embryonal day 17; 1st day, postnatal day 1; 3rd day, postnatal day 3; pl., placenta; l., liver; s., spinal cord.

This analysis of *oct*-2 RNA has revealed a very complicated expression pattern in various cells and the existence of a large number of hybridization bands. From 1 to 5 *oct*-2 mRNA bands were found in different cells: such a variety may be the result of alternative splicing [14,22]. Our results show that there must be an additional mechanism of formation of a different molecular weight RNA which involves the specific splitting-off of the poly(A)$^+$ terminus since we found *oct*-2 mRNA in both poly(A)$^+$ and poly(A)$^-$ fractions.

In all the cell lines studied here, *oct*-2 mRNA was present at various stages of differentiation, whereas Oct-2 protein was found only at some stages of differentiation. This may be due to different reasons. First, the Oct-2 protein may exist in the cell in an inactive form, incapable of binding DNA, and, second, there may be a block at the translation level. We suggest that there may be an intracellular mechanism regulating Oct-2 protein activity which allows the quick transformation of active Oct-2 protein into its inactive form. The differentiation is likely to be accompanied by both a complete inactivation of the gene and by a simple inactivation of the protein nuclear transcription factor itself. Such a mechanism would provide for a finer regulation of cell differentiation without making it irreversible at each stage.

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