cDNA cloning of human N-Oct 3, a nervous-system specific POU domain transcription factor binding to the octamer DNA motif

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ABSTRACT

Octamer transcription factors (Oct or OTF) are a subset of the POU family of transcription factors which regulate transcription of cellular and viral genes by binding to the octamer sequence motif ATGCAAAAT. Neurons and astroglial cells harbour, in addition to the ubiquitous Oct 1 factor, at least four specific factors termed N-Oct 2,3,4 and 5. Here we report the cloning of a human brain-derived cDNA that encodes the N-Oct 3 protein (443 aa) which is the human counterpart of the murine brain-2 gene product. Extracts from mammalian cells transfected with an N-Oct 3 expression vector yield three octamer DNA binding complexes in the electrophoretic mobility shift assay (EMSA): N-Oct 3 and two smaller complexes comigrating with the N-Oct 5A and 5B proteins of brain extracts. We present data suggesting that the N-Oct 5A and 5B proteins are generated by alternative translation initiation at internal AUG residues which are located before the POU domain. In contrast to the putative N-Oct 5 proteins, which are transcriptionally inert, the N-Oct 3 protein activates transcription from a reporter gene promoter with an octamer sequence, when transiently expressed in HeLa cells.

INTRODUCTION

Transcription factors are proteins that control gene expression via specific binding to regulatory DNA sequences in gene promoters and enhancers. These proteins interact with additional transcription factors, and/or other ubiquitous components of the transcription machinery to direct RNA synthesis. The transcription proteins that bind to the octameric DNA sequence ATGCAAAAT (or its complement ATTTGCAT) are called Oct factors. The octamer sequence was first recognized in the histone H2B gene and in the heavy and kappa light chain immunoglobulin genes and later identified as a regulatory sequence motif for many other cellular and viral genes (reviewed in [11]). For example, a simple promoter composed of such an octameric sequence next to a TATA-box functions preferentially in B lymphocytes [2][3]. The observation that a single DNA motif allowed ubiquitous as well as cell type-specific gene activation became explainable by the findings and subsequent characterization of a number of (cell type-specific) Octamer binding proteins. The Oct-1 protein is detectable in virtually all cells and regulates generally active genes that contain an octamer sequence motif [4]. The Oct-2A and Oct-2B proteins are abundant in B lymphocytes and macrophages [5][6]. The Oct-2 proteins are thought to be effectors of octamer-dependent immunoglobulin gene transcription and certain cytokine genes [7]. The Oct-4 (also referred as to Oct-3 or NF-A3 protein) and the Oct-6 protein are transcriptional regulators in embryonic stem cells and tissues (reviewed in [8]). The latter has also been identified in proliferating Schwann cells where it has been termed SCIP [9]. The human or murine cDNAs encoding Oct-1, Oct-2A, Oct-2B, Oct-3/4, and Oct-6 have now been cloned (reviewed in [8]).

A common feature of Oct proteins is a conserved DNA-binding domain referred to as the POU domain [10]. Using polymerase chain reaction (PCR), Rosenfeld and colleagues isolated clones of POU domains from rat and human brain which they termed Tst-1 (identical with Oct-6; SCIP), brn-1, brn-2, and brn-3 [11]. They also mapped the distribution of the respective transcripts in both adult and developing brain by in situ hybridization. The brn-1 and brn-2 POU domain RNAs are highly expressed at the neural tube stage during forebrain development and are widely present in the adult brain. Therefore, they are thought to be important regulators of brain development (reviewed in [12]). However, it was not known at that time whether the cloned cDNA segments termed brn-1, 2 and -3 were octamer-binding proteins. Electrophoretic mobility shift assays (EMSA) with extracts of mouse brain [13] glia and neurons [14] have revealed a complex pattern of at least four nervous tissue-specific Oct (N-Oct) DNA-binding proteins which were termed by us N-Oct 2, N-Oct 3, N-Oct 4 and N-Oct 5A/5B. Here we report the cloning of the human N-Oct 3 cDNA. The cloned human N-Oct 3 appears to

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be the counterpart of the murine brain-2 factor recently cloned by others [15]. We show that expression of the cloned human N-Oct 3 cDNA in mammalian cells gives rise to three proteins: N-Oct 3 and the two putative N-Oct 5A and 5B proteins which have different transcriptional activities.

MATERIALS AND METHODS

Cloning and sequencing

Approximately \(5 \times 10^5\) plaques of a human fetal brain ZAP II cDNA library (Stratagene; Catalog Nr. 936206) were transferred in duplicates to Plaquescreen Filters (DuPont) and then screened with a randomly primed mixture of \(5 \times 10^6\) cpm of radiolabelled POU domain DNA fragments of the oct-2 cDNA (pos. 586–1077) and the oct-6 cDNA (pos. 706–1232; generously provided by Dr. Hans Schöler, Heidelberg) according to standard procedures described in detail in chapter 6.3 in [16]. Phages from plaques that gave rise to identical signals on both filters were purified by another round of plating and screening and were then subcloned to Bluescript plasmids according to the manufacturer’s (Stratagene) instructions. The cDNAs which encoded octamer DNA binding proteins were identified by in vitro transcription of the cDNA inserts and subsequent in vitro translation followed by EMSA as described in [17]. The reticulocyte lysate was from Amersham. For enzymatic sequencing of both strands of the N-Oct 3 cDNA the Sequenase-kit (USB) was used with ‘universal’ and ‘reverse’ primers matching to the flanks of the plasmid polylinker. Several deletion mutants were prepared by exploiting singular restriction sites in the cDNA to enable progressive sequencing. Gaps were resolved by using internal primers and ambiguities were rectified by chemical sequencing. For compilation and analysis of sequence data we used the MacVector (IBI) and GCG software [18] (supplied by ETH Zürich). The nucleotide sequence has been deposited at the EMBL sequence data library under the accession number Z11933.

Northern blot

The Northern blot filter with 2 \(\mu g\) poly A + RNA isolated from various human organs was purchased from Clontech. The filter was hybridized overnight with a randomly primed mixture of \(5 \times 10^6\) cpm of radiolabelled POU DNA fragments in Hanks for 3 min and then incubated for 30 hr in fresh DME-Medium containing 10% FCS at 37°C with 5% CO\(_2\). The RNA and proteins were then prepared as described [20]. The RNase protection assay with 10\(\mu\)g RNA was exactly done as described in [7].

RESULTS

Cloning and sequence analysis of N-Oct 3

To isolate N-Oct cDNAs we screened a human fetal brain cDNA library with radiolabelled POU DNA fragments of the oct-2 [7] and oct-6 cDNAs [21]. These probes were chosen on the basis of successful sequences within the POU domain of octamer DNA binding proteins [8]. We identified several cDNAs which, when transcribed and translated in vitro, produced proteins that bound specifically to the octamer sequence in the EMSA (data not shown). Four cDNAs yielded a protein which comigrated in the EMSA with the N-Oct 3 protein of a human brain extract. These cDNAs showed an identical restriction pattern in the coding region but varied in length between 1.95 – 4 kb (data not shown). The cDNA of 1.95 kb which was longest 5’ to the ORF was chosen for sequencing. The predicted open reading frame (ORF) encoded a protein of 443 amino acids with a \(M_r\) of 47 kDa

Transfection and RNase protection assay

HeLa cells (mycoplasma-free) were cultured in DMEM with 5% foetal calf serum (GIBCO) and antibiotics. These cells were transfected by the CaPO\(_4\)-DNA precipitate method [16] with 10 \(\mu\)g test gene plasmid, 1 \(\mu\)g reference gene plasmid and the indicated amount of either trans-activator plasmid or herring sperm DNA overnight. Cells were then shocked with 25% DMSO in Hanks solution for 3 min and then incubated for 30 hours in fresh DME-Medium containing 10% FCS at 37°C with 5% CO\(_2\). The RNA and proteins were then prepared as described [20]. The RNase protection assay with 10\(\mu\)g RNA was exactly done as described in [7].

Analysis of N-Oct 3 protein by EMSA

The EMSA and the DNA probes used were performed exactly as described in [14]. For proteolytic clipping assays 0.4 U of ArgC protease (Boehringer Mannheim) was used. The cDNAs were subcloned from the Bluescript vectors into the expression vector pEVRFO [19] by standard procedures. COS 7 cells (2 \(\times 10^6\)) were transfected with 1 \(\mu\)g DNA and 500 \(\mu\)g DEAE-Dextran (Pharmacia) in 1 ml Hanks-balanced salt solution (Kantonsapotheke Zürich) for 30 min, shocked in 25% DMSO solution in Hanks for 3 min and then incubated for 30 hr in fresh DME-Medium containing 10% FCS. Miniextracts were prepared as described [20]. Buffers A and C contained 1mM DTT, 1mM PMSF, 1% aprotinin; 2 \(\mu\)g/ml Leupeptin as protease inhibitors.

![Figure 1](image-url)
N-Oct 3 expression in the brain

A Northern blot experiment using a radioactive N-Oct-3 probe detected a single band corresponding to a 5 kb RNA transcript in human brain (Figure 2, lane 2) but not in human heart, lung, pancreas, liver, spleen and skeletal muscle (lanes 1, 3, 4, 5, 6, 7, 8, respectively), indicating specific expression in the nervous system. The same size and the same tissue specificity was reported recently for the mouse brain-2 mRNA [15]. The human brn-2 POU fragment cloned by He et al. was reported to hybridize to a 7 kb transcript from rat brain [11]. The discrepancy in the size of this transcript compared to the 5 kb transcript identified here might be explained by a difference between species.

Expression of N-Oct 3 yields three proteins

To further characterize the authenticity of the cloned cDNA, it was subcloned into an expression vector (Figure 3b; plasmid III) and transfected into COS 7 cells which are devoid of N-Oct proteins. The extracts of the transfected cells showed only weak expression of the cDNA product when tested by EMSA (Figure 3a, lane 5). RNA instability may have caused the low level expression of protein, so we generated a deletion mutant that eliminated 0.5 kb of 3′ untranslated sequences of the cDNA (Figure 3b, plasmid IV). When this shortened cDNA was transfected into COS 7 cells, we observed high level expression, as shown by a complex comigrating with the N-Oct 3 protein and, surprisingly, an additional double band complex comigrating with the two N-Oct 5 complexes of the brain extract (Figure 3a, lane 6). We conclude that a potent RNA destabilizing determinant appears to be located in the 3′ untranslated region. Three Octamer-DNA binding complexes, N-Oct 3 and two additional proteins co-migrating with brain N-Oct-5A and -5B, were generated when the cDNA transcript was translated in vivo but not in vitro. This observation might be explained by the cDNA sequence which contains two internal ATG triplets encoding methionine residues which are in frame with the adjacent POU domain (Figure 1). We hypothesised therefore that the N-Oct 5 complex is generated by alternative translation initiation. The internal ATG triplets were indeed used for translation initiation in a 5′ deletion mutant, where 650 bp including the first ATG codon used for N-Oct 3 translation are missing. In vitro transcription/translation of this mutant (Figure 3b, plasmid II) yielded an EMSA band corresponding to the lower, stronger, band of the brain N-Oct 5 complex (Figure 3a, lane 1 and 4) indicating that the second available AUG was preferentially used for in vitro translation initiation. The EMSA of extracts of COS cells transfected with this deletion mutant (Figure 3b, plasmid V) showed that the protein in the lower complex (N-Oct 5B) was expressed at high levels and that the peptides complex at low levels (N-Oct 5A), which is also the pattern seen in brain extracts (Figure 3a, lane 7 and 1). We also considered the possibility that the upper (weaker) band (N-Oct 5A) seen in vivo reflected a posttranslational modification of the faster complex (N-Oct 5B).

However, when cells were transfected with the longer N-Oct 3 cDNA (Figure 3a, lane 6, Fig. 3b plasmid IV) there was an inverse intensity of the two N-Oct 5 bands. This effect may be due to a different RNA secondary structure affecting the accessibility of the internal AUG codons. Taken together, it appears that N-Oct 5A and 5B are generated by alternative translation initiation of the N-Oct 3 mRNA, rather than by alternative splicing or posttranslational modification such as a specific proteolytic processing (see Discussion).
In vitro In vivo protedytic translation expression competition dipping

Figure 3. A. Identification of recombinant N-Oct 3 and N-Oct 5 proteins by EMSA. In lane 1 the extract of a human brain tumor indicates the position of migration in the EMSA of previously described N-Oct proteins. Lane 2: unprogrammed reticulocyte lysate; lane 3: lysate programmed with RNA of the N-Oct 3 clone (in vitro transcribed from Asp 718 linearized plasmid I). Lane 4, lysate programmed with RNA of the Smal 5' deletion mutant (Fig. 3B; plasmid II). Lane 5: extract of COS 7 cells that were transfected with the full length N-Oct 3 cDNA (Fig. 3B: plasmid III). Lane 6: extract of COS 7 cells transfected with plasmid IV where untranslated sequences 3' of the Xho 1 site were removed. Lane 7: extract of COS 7 cells transfected with plasmid V, the Smal 5' deletion mutant. The additional band migrating between N-Oct 3 and 5 was also observed in brain extracts after longer exposure, perhaps from binding of a cofactor to the N-Oct 5 complex rather than a dimer of N-Oct 5 which is expected to migrate slower. Lanes 8—10: competition experiment; extracts of N-Oct 3 transfected COS 7 cells were incubated before EMSA with no specific competitor DNA (lane 8), with a 250-fold molar excess of wild type unlabelled octamer probe (lane 9) or with the same amount of a mutated octamer probe (lane 10). Lanes 11-14: proteolytic dipping EMSA: unproteolized brain tumor extract (lane 9) or with the same amount of a mutated octamer probe (lane 10). Lanes 11—14: proteolytic dipping EMSA: unproteolized brain tumor extract (lane 11) and ArgC proteolysed brain extract (lane 12) is compared with the pattern of N-Oct 3 transfected COS 7 extract (lane 13 no ArgC, lane 14 with ArgC). B. Plasmids used in this experiment: a) expression vector with oct-2 cDNA; b) expression vector with N-Oct 3 cDNA (identical with plasmid IV, Fig. 3B); c) expression vector with N-Oct 5 cDNAs (identical with plasmid V, Fig. 3B); d) β-globin test gene plasmid with the octamer site (symbolized by 8) in 5 bp distance to the TATA-box (symbolized by T) and downstream located SV 40 enhancer; e) the same plasmid but the octamer site is mutated from ATGCAAAT to CTGAACAT; f) truncated β-globin reference gene; g) radioactive antisense RNA probe spanning positions -37 to +199 of plasmids d and e. Plasmids a, d, e, f and probe g were described in detail in [7]. B Autoradiograph of an RNAase protection assay with RNA of transfected HeLa cells and C. autoradiograph of an EMSA performed with nuclear extracts of these transfected cells to monitor the amount of expressed proteins. lane 1: cells transfected with DNAs d, f; lane 2: cells transfected with DNAs d, f and 5 μg DNA a; lane 3: cells transfected with DNAs d, f and 10 μg DNA a; lane 4: cells transfected with DNAs d, f and 5 μg DNA b; lane 5: cells transfected with DNAs d, f and 10 μg DNA b; lane 6: cells transfected with DNAs d, f and 5 μg DNA c; lane 7: cells transfected with DNAs d, f and 10 μg DNA c; lane 8: cells transfected with DNAs d, f and 5 μg DNA b and 5 μg DNA c; lane 9: cells transfected with DNAs d, f and 5 μg DNA b and 10 μg DNA c; lane 10: cells transfected with DNAs d, f and 5 μg DNA b and 10 μg DNA c; lane 11: cells transfected with DNAs e, f and 5 μg DNA b; lane 12: cells transfected with DNAs e, f and 5 μg DNA b and 10 μg DNA c; lane 13: cells transfected with DNAs e, f and 5 μg DNA c; lane 14: cells transfected with DNAs e, f and 5 μg DNA c and 5 μg DNA c. M: molecular weight size marker; test: correctly initiated test gene transcripts; ref: reference gene transcripts.

We next analyzed the octamer DNA binding specificity of the recombinant proteins produced in transfected COS 7 cells. When an excess of unlabelled octamer competitor fragment was present in the reaction mixture of N-Oct-3 proteins and labelled octamer probe, both binding of N-Oct-3 and N-Oct 5A/5B was strongly reduced (Figure 3, lane 9) compared to no competitor (lane 8) or mutated octamer DNA as a competitor (lane 10). To see
whether the N-Oct 3 complex seen in brain extracts and the cloned protein were identical, we performed a comparative proteolytic clipping EMSA [5]. Extracts of COS 7 cells transfected with N-Oct-3 cDNA and of brain tumor were treated with ArgC before performing EMSA. The protease preferentially degraded the N-Oct 3 protein in both extracts in a similar, if not identical way, leading to the occurrence of two new protein bands (Figure 3, lane 11–14). We conclude therefore, that the recombinant protein corresponds to the brain-derived N-Oct 3 protein.

**N-Oct 3 activates an octamer-containing promoter**

In order to assess the functions of the N-Oct 3 and N-Oct-5 proteins in gene transcription, we performed cotransfection experiments in HeLa cells. As a test gene we used a β-globin gene whose promoter consists solely of an octamer sequence and a TATA box. We supplied in trans different octamer-binding proteins by cotransfection of the respective expression plasmid.

It was previously shown that the transcription of this test gene critically depends on quantity and quality of ectopically expressed octamer binding proteins [7]. Addition of expression vector containing the B-cell derived oct-2 cDNA led to transcriptional activation (Fig. 4b, lane 2 and 3, for amount of Oct-2 protein see the respective lanes in Fig. 4c). Expression of the N-Oct 3 cDNA (Fig. 4b, lane 4 and 5) also activated transcription, though to a lesser extent than Oct-2. Transfection of the N-Oct 5 expression plasmids however, had only marginal effects on test gene transcription (Fig. 4 lanes 6, 7). Since N-Oct 5 proteins contain only the POU DNA-binding domain and lack the homopolymeric glutamine and glycine stretches (see Figure 1 and 2), it seemed possible that N-Oct 5 would inhibit N-Oct 3 by competing for DNA-binding. However, N-Oct 5 are transcriptionally inert since cotransfection of N-Oct 3 and N-Oct 5 gave the same test gene expression as N-Oct 3 (Figure 4, lanes 8, 9). Thus, N-Oct 3 acts as a weak transcriptional activator under these assay conditions whereas the N-Oct 5 proteins were inert, neither activating transcription by themselves nor inhibiting N-Oct 3 action.

**DISCUSSION**

In this paper we describe the cloning and functional characterization of the human transcription factor N-Oct 3. We have presented data which suggest that three proteins, N-Oct 3 as well as N-Oct 5A and 5B are translated from the same mRNA by alternative translation initiation; only the largest protein, N-Oct 3 was able to stimulate transcription from an octamer promoter under our assay conditions. We also showed that the N-Oct 3 protein, previously identified by EMSA as the major Oct-factor in cells of the nervous system [14], is identical to bm-2, a partial clone, generated by PCR, comprising solely the POU domain [11]. Recently, the complete cDNA and genomic sequences of the murine brain-2 gene were published [15]. The amino acid sequence of the human N-Oct 3 (brain-2) and the murine brain-2 protein is identical except for two additional amino acid residues in the mouse protein. As discussed below, several lines of evidence suggest that the N-Oct 3 protein plays an important role during brain development.

**The CAG cluster; a hot spot for triplet repeat mutations?**

The glutamine-rich region in the N-Oct 3 protein is encoded mainly by CAG residues. This type of triplet repeat was recently recognized as the molecular basis for an unusual mutation mechanism that leads to amplification of the triplets, which can turn to disease, such as fragile-X-syndrome or myotonic dystrophy (reviewed in [24]). If such mutations should occur in the CAG cluster of the N-Oct 3 gene it might easily lead to loss of function by aberrantly expanding the bona fide transcription activation domain, or to loss of DNA-binding if a frame shift is introduced. It will be of considerable biological and medical interest to experimentally address the question whether the N-Oct 3 gene is sensitive to this mutation mechanism and if so, if a disease is associated with it.

**Three proteins out of one (unstable) transcript**

Efficient N-Oct 3 expression in vivo depends on deletion of a large portion of the 3' untranslated sequence, implying that it is likely to be a determinant of RNA instability. These sequences contain one copy of the AUUUA motif that is known to mediate RNA instability in a number of short-lived cytokine transcripts (reviewed in [25]). The other surprising result was observed when a stable RNA was expressed after removal of the 3' sequences; three proteins were apparently generated. We consider it most likely, that alternative translation initiation is the mechanism for generation of the N-Oct 5 proteins. Which other mechanisms may be causal? The murine counterpart of N-Oct 3, the brain-2 gene does not have introns. At present, the genomic structure of the human N-Oct 3 gene is not known but given the short evolutionary distance between mouse and human, we assume that the human N-Oct 3 gene does not have introns as well. Therefore, we consider alternative splicing as mechanism for the generation of N-Oct 5 proteins unlikely. It is conceivable, though unlikely, that ectopic expression of N-Oct 3 induces expression of another gene(s) that encode(s) the N-Oct 5 proteins. Post-translational proteolytic processing of N-Oct 3 may be possible. However, no consensus sites for proteases are detectable which might generate the N-Oct 5 proteins. Moreover, the inverse intensity of N-Oct 5 bands seen with the different expression constructs argues against the proteolytic processing mechanism but rather for a different transcript structure that affects accessibility of the AUG translation initiation sequences. Alternative translation initiation was also shown to be effective for the RNA of POU proteins pit-1 [26] and Oct-4 [17] where in each case two isoforms were detected. Descombes and Schibler have recently shown that the RNA of the liver transcription factor LAP (liver activatory protein) also translates a smaller protein, termed LIP (liver inhibitory protein) which lacks the transcription activation domain. The LIP protein could efficiently inhibit the action of LAP [27]. This was apparently not the case in the co-transfection experiment with N-Oct 3 and N-Oct 5 expression plasmids (Figure 4). At present, we have no evidence for a heterodimerization among these proteins which might impair DNA-binding. Therefore, N-Oct 5 protein probably can inhibit only when it is in large excess to N-Oct 3. However, under our assay conditions we were not able to achieve a sufficiently high concentration of N-Oct 5 protein in the transfected cells to test this hypothesis. In light of the potential regulatory effects of alternate expression of the three proteins it will be of interest to examine expression patterns during development or in different cell types.

**Sequence specificity and possible target genes**

The N-Oct 3 protein binds specifically to the octameric regulatory motif ATGCACAAT (or its complement ATTTGCGCAT). However, another typical feature of octamer-binding POU-domain factors,
such as Oct-1, Oct-2, Oct-6 and the recently cloned brain-4 factor (identical with N-Oct 4; A. Tobler and E. Schreiber, unpublished results), is that they can also bind to the heptamer sequence CTCATGTA which is naturally located adjacent to the octamer sequence in the immunoglobulin heavy chain promoter [28] [29][30]. We have previously shown that gel-purified N-Oct 3 protein also binds to the heptamer sequence [14]. In addition, many variants of the octamer sequence have been shown to be functional binding sites for Oct-proteins (reviewed in [31]). This remarkable variety in binding sites hampers the identification of target genes. A candidate gene that could be regulated by N-Oct 3 is the human calcitonin/CGRP gene which contains a consensus octamer motif in the promoter. Moreover, this gene is expressed in a number of neuroectoderm-derived cell types that also express N-Oct 3 (E. Schreiber, unpublished observations). Rosenfeld and his colleagues reported that the corticotropin releasing hormone promoter is transcriptionally up-regulated by brain-2 (N-Oct 3) [32]. The recent development of biochemical and genetical target detection assays, if applied to the N-Oct 3 factor, will certainly help to answer this major question. It will also be of interest to investigate whether N-Oct 3 causes the transcriptional repression of the Herpes simplex virus immediate early genes in neuronal cells that was reported to be mediated by an as yet unidentified neuron-specific Oct-protein(s) [33].

N-Oct3 (brain-2), a primordial transcriptional regulator in the neuroectoderm cell lineage?

This work and previous studies have shown that the expression of the N-Oct 3 (brain-2) transcript and protein is confined to cells of the nervous system in which it is the predominant Oct-protein [14][11, 15] [13]. Rosenfeld and colleagues have shown by in situ hybridizations with embryonal and adult rat brain sections that the brn-2 mRNA is abundant during all stages of neurogenesis and is present at a lower level in distinct neuronal subsets in the adult CNS [11]. These data were corroborated by EMSA studies showing a similar abundance and distribution of the N-Oct 3 protein [13][14]. N-Oct 3 expression is not confined to the central nervous system but detectable in most cell derivatives of the neuroectoderm, including neural crest derivatives. For example, we have previously shown N-Oct 3 expression in neuroblastoma cell lines; melanoma cells as neural crest derivatives also contain the N-Oct 3 protein [14][34]. However, N-Oct protein expression is not ubiquitous in all cells of the nervous system; Schwann cells, the glia cells of the peripheral nervous system, lack N-Oct proteins but contain the Oct-6 protein in addition to the Oct-1 protein [35].

The early and abundant expression of N-Oct3 (brain 2) during neural development and the widespread expression in neural- and glial and other neural crest-derived tissues suggest an important role as gene regulator in the nervous system.

The abundance and the functional effects of N-Oct 3 may be regulated by different mechanisms: (i) transcription of N-Oct 3 may be regulated in a temporal and tissue specific manner, (ii) the RNA half life might be controlled by sequences in the 3’ untranslated region as suggested by our findings (iii) a bias in translation in favour of N-Oct 5 proteins may serve to neutralize the effects of the N-Oct 3 protein. The cDNA described here will be instrumental to experimentally address these issues.

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