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# The B-Cell and Neuronal Forms of the Octamer-Binding Protein Oct-2 Differ in DNA-Binding Specificity and Functional Activity

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**B lymphocytes contain an octamer-binding transcription factor, Oct-2, that is absent in most other cell types and plays a critical role in the B-cell-specific transcription of the immunoglobulin genes. A neuronal form of this protein has also been detected in brain and neuronal cell lines by using a DNA mobility shift assay, and an Oct-2 mRNA is observed in these cells by Northern (RNA) blotting and in situ hybridization. We show that the neuronal form of Oct-2 differs from that found in B cells with respect to both DNA-binding specificity and functional activity. In particular, whereas the B-cell protein activates octamer-containing promoters, the neuronal protein inhibits octamer-mediated gene expression. The possible role of the neuronal form of Oct-2 in the regulation of neuronal gene expression and its relationship to B-cell Oct-2 are discussed.**

The octamer motif (consensus sequence, ATGCAAAT) is found in the promoters and enhancers of several different cellular genes such as those encoding the small nuclear RNAs, histone H2B, and the immunoglobulin heavy and light chains (for a review, see reference 7). This motif plays a critical role in determining the pattern of expression of genes which contain it, its deletion or mutation abolishing the B-cell specificity of immunoglobulin gene expression, the cell cycle specificity of histone H2B expression, and the constitutive expression of the small nuclear RNAs (20, 25, 31, 32).

The central importance of this short DNA sequence in the modulation of gene expression has focused attention on the cellular transcription factors that bind to it. Initial studies defined two such proteins. One of these, Oct-1, is expressed in all cell types (30, 34) and plays a critical role in the constitutive expression of the small nuclear RNA genes and the histone H2B gene (8). In contrast, the other protein, Oct-2, is absent in a wide variety of different cell types but is present at high levels in B cells (30, 34) and is of central importance in the B-cell-specific expression of the immunoglobulin genes (27).

More recent studies, however, have detected additional octamer-binding proteins expressed in a limited range of cells such as early embryo (16, 28) and testis (9) cells. In particular, the mouse brain contains at least five additional octamer-binding proteins, some of which are also expressed in the early embryo but are absent in most other adult tissues (28). Moreover, it appears that the Oct-2 protein is not confined to B cells as was originally thought. For example, an octamer-binding protein with a mobility identical to that of Oct-2 has been observed in the brain with use of a DNA mobility shift assay (28), while an RNA hybridizing specifically to Oct-2 probes has been detected in individual neuronal cells in the brain by in situ hybridization (12) and is also detectable in a glioma cell line by Northern (RNA) blotting (33).

Clearly an analysis of the role of these various octamer-binding proteins would be greatly facilitated by the availability of cell lines containing them, which would provide a source of material for functional analysis. We have therefore studied the expression of octamer-binding proteins in the C1300 mouse neuroblastoma cell line (2) and in ND7 cells. The ND7 cell line was derived by fusing C1300 cells with primary sensory neurons, and it retains many of the characteristics of the sensory neuron parent (39).

## MATERIALS AND METHODS

**Cells and viruses.** The ND series of immortalized ganglionic neurons was prepared (39) by fusing the N18TG2 azaguanine-resistant neuroblastoma ($4 \times 10^5$ cells) in serum-free L-15 medium (GIBCO) containing 50% polyethylene glycol 1500 (Kodak) at 37°C for 1.5 min. Cell lines were then selected in L-15 medium containing hypoxanthine, aminopterin, thymidine, 10% fetal calf serum, and 10% bovine endothelial cell conditioned medium. ND cells and BHK-21 cells (clone 13 [19]) routinely were grown in RPMI medium supplemented with 10% fetal calf serum.

**Plasmid DNA.** Oligonucleotides were synthesized on an Applied Biosystems model 381A oligonucleotide synthesizer. The various octamer oligonucleotides shown in Fig. 4 were synthesized with a 5′ GATC overhang at either end and cloned into the *Bam*HI site of pBL2 CAT (17). The Oct-2 expression plasmid was constructed by cloning an *Eco*RI fragment containing the entire Oct-2 coding sequence (23) into the *Eco*RI site of the expression vector pJ4 (22). The Oct-CAT plasmid was a kind gift of Thomas Wirth and David Baltimore (38).

**DNA transfection.** Transfection of plasmid DNA was carried out as described by Gorman (10). Except when indicated otherwise, all transfections were carried out with 10 μg of DNA per $2 \times 10^6$ cells on a 90-mm plate. Twenty-four hours after transfection, cells were harvested for chloramphenicol acetyltransferase (CAT) assays. All transfections included an internal control plasmid in which the myeloproliferative sarcoma virus promoter drives expression of β-galactosidase, to correct for differences in transfection efficiency and for any nonspecific effect of cotransfected plasmids on gene expression.

**CAT assays.** Assays of CAT activity were carried out as

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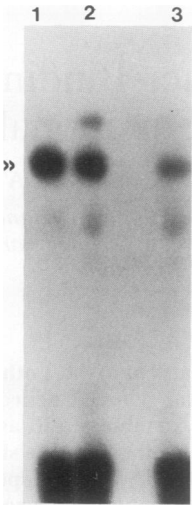


FIG. 1. DNA mobility shift assay using whole-cell extracts (6) from ND7 cells (track 1), B cells (track 2), and C1300 cells (track 3). The position of the Oct-2 band is indicated by the arrowheads.

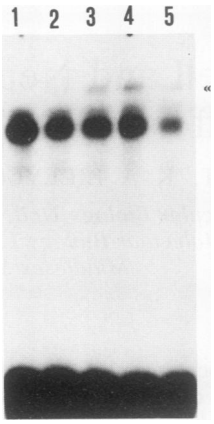


FIG. 2. DNA mobility shift assay using whole-cell extracts from ND7 cells in the absence of a rabbit antiserum known to react with B-cell Oct-2 (track 1), in the presence of 1 μl of antiserum dilutions of 1:8 (track 2), 1:4 (track 3), or 1:2 (track 4), or in the presence of 1 μl of undiluted antiserum (track 5). The arrowheads indicate the supershifted band.

described by Gorman (10), extracts having been equalized for protein content as determined by the method of Bradford (3).

**DNA mobility shift assays.** Oligonucleotides for use in mobility shift assays were labeled following annealing by phosphorylation with [$\gamma$-$^{32}$P]ATP and T4 polynucleotide kinase. Nuclear extracts were made from about $5 \times 10^7$ cells as described by Dignam et al. (6). For binding, 10 fmol of labeled probe was mixed with 1 μl of extract in 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)–5 mM $MgCl_2$–50 mM KCl–0.5 mM dithiothreitol–4% Ficoll–2 μg of poly(dI-dC) in 20 μl. Competitor DNA was added at 100-fold molar excess as indicated. The binding reaction mixture was incubated on ice for 40 min, and DNA-protein complexes were then separated by electrophoresis on a 4% polyacrylamide gel. Gels were prerun for 2 h and then run for 2 to 3 h at 150 V and 4°C.

## RESULTS AND DISCUSSION

We have previously used DNA mobility shift assays to show (13) that both ND7 cells and C1300 cells contain the ubiquitous octamer-binding protein Oct-1 and an additional cell-type-specific octamer-binding protein. The latter produces a faster-migrating complex which was absent in other cell types such as 3T3 cells. Like Oct-1, this protein bound specifically to the consensus octamer motif ATGCAAAT but not to a mutant octamer motif ATAATAAT (13), which has previously been shown not to bind octamer-binding proteins such as Oct-1 and Oct-2 (15). In these experiments, this protein appeared to have a mobility similar to that observed by others for the B-cell-specific octamer-binding protein Oct-2. We therefore prepared nuclear extracts (6) from C1300 cells, ND7 cells, and Oct-2-containing Daudi B cells and used these in a DNA mobility shift assay with a labeled oligonucleotide containing a high-affinity octamer motif (AT GCAAATGATAT) (13). In these experiments (Fig. 1), the neuronal-specific complex observed in C1300 and ND7 cells had a mobility identical to that of the B-cell-specific complex produced by the Oct-2 protein. The neuronal protein therefore produces a complex identical in mobility both to B-cell Oct-2 and to the protein defined as Oct-2 in brain extracts by Scholer et al. (28) on the basis of its being identical in mobility to B-cell Oct-2. Moreover, it is distinct from the other octamer-binding proteins detected in the brain, which are of higher mobility than Oct-1 or Oct-2 (28).

To further define the relationship of this protein to B-cell Oct-2, we investigated its binding to a rabbit antiserum that reacts with the B-cell Oct-2 protein (35). As shown in Fig. 2, when this antibody was added to a DNA mobility shift assay using ND7 cell extract, the antibody was able to bind to the Oct-2–DNA complex and produce a supershifted complex of reduced mobility. Moreover, higher concentrations of the antibody produced a decrease in the intensity of both the Oct-2–DNA complex and the supershifted complex, indicating that at high concentrations, antibodies in the serum directed against the DNA-binding domain of Oct-2 were capable of inhibiting DNA binding. This production of a supershift with subsequent inhibition exactly parallels the interaction of this serum with B-cell Oct-2 (35) and was specific to the immune serum, preimmune serum having no effect on Oct-2 binding at similar concentrations (data not shown). Hence, these experiments provide further evidence for the close relationship of our neuronal-specific protein and B cell Oct-2.

To determine whether an Oct-2 mRNA could be detected in our cells, we used Northern blotting with a cDNA clone containing the coding sequence of lymphocyte Oct-2 (5). In these experiments (Fig. 3), two mRNAs hybridizing to the Oct-2 probe were detected in the neuronal cells at levels similar to those observed in B cells. One of these mRNAs was similar in size (6 kb) to the predominant transcript observed both by ourselves and by others in B cells, while the other was larger (7.5 kb) than the predominant transcript that we detected in B cells, although a B-cell transcript of this size has been detected by others (23, 33). In contrast, no mRNA hybridized to the Oct-2 probe in mRNA prepared from 3T3 cells, which contain only Oct-1, indicating that the Oct-1 mRNA does not hybridize to the Oct-2 probe under the stringent hybridization conditions that we used (washing at 0.1× SSC at 70°C). Similar results were obtained with another Oct-2 cDNA obtained from a different source (23).

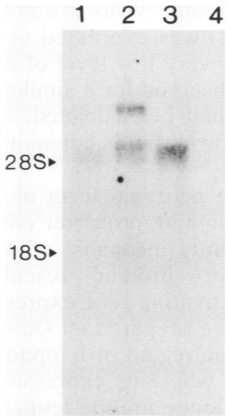


FIG. 3. Northern blot of mRNA prepared from whole brain (track 1), ND7 cells (track 2), B cells (track 3), and 3T3 fibroblasts (track 4) hybridized with an Oct-2 cDNA probe (5). Northern blotting and hybridization were carried out as previously described (24). Arrowheads indicate the positions of rRNA markers.

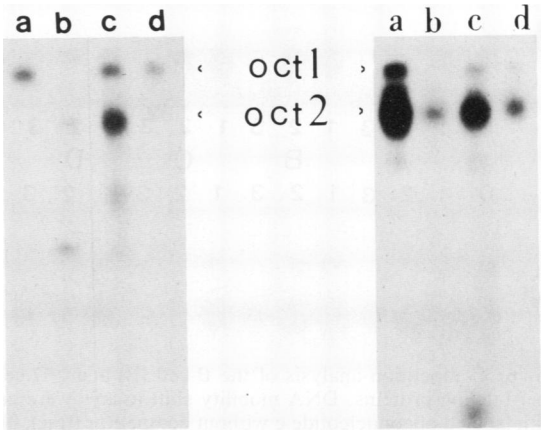


FIG. 5. DNA mobility shift assay using labeled oligonucleotides a to d and extracts prepared from ND7 cells (A) and Daudi B cells (B). Arrowheads indicate the positions of Oct-1 and Oct-2.

All samples contained similar levels of mRNA hybridizing to an actin cDNA clone. Hence, our neuronal cells contain transcripts which cross-hybridize to the Oct-2 probe under stringent hybridization conditions and are therefore likely to be derived from the same gene as that encoding B-cell Oct-2 or one very closely related to it.

Having established that our cells contained the neuronal form of Oct-2 on the basis of both criteria used by others, namely, identical mobility in a band shift assay (28) and cross-hybridization to Oct-2 probes (12), as well as reactivity with an anti-Oct-2 antiserum, we compared the properties of the B-cell and neuronal forms of the protein. Initially we compared the binding of these proteins to various octamer oligonucleotides in a DNA mobility shift assay. The oligonucleotides used (Fig. 4) were the consensus octamer motif that we previously used (oligonucleotide d), an oligonucleotide (b) containing the octamer-related TAATGARAT motif found in the herpes simplex virus (HSV) immediate-early promoters (37), and two overlapping octamer-TAATGA RAT motifs (oligonucleotides a and c). In these experiments (Fig. 5), binding of the B-cell Oct-2 protein was readily detectable with all of the different oligonucleotides. Moreover, although the level of Oct-2 binding predominated over Oct-1 binding on all oligonucleotides tested, the relative binding of Oct-2 to each oligonucleotide generally paralleled that of Oct-1 with oligonucleotides, which bound Oct-2 most strongly also binding the highest levels of Oct-1. Hence, Oct-1 and the B-cell form of Oct-2 appear to exhibit a similar

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Octamer consensus | A | T | G | C | A | A | A | T | N | A | | |
| HSV IE consensus | R | Y | G | N | T | A | A | T | G | A | R | A | T |
| A | A | T | G | C | T | A | A | T | G | A | G | A | T |
| B | G | C | G | G | T | A | A | T | G | A | G | A | T |
| C | A | T | G | C | T | A | A | T | G | A | T | A | T |
| D | A | T | G | C | A | A | A | T | A | A | | | |
| M | A | T | A | A | T | A | A | T | A | A | | | |

FIG. 4. Consensus DNA sequence comparison of the cellular octamer motif (7) and the HSV TAATGARAT motif (36) with the four functional octamer oligonucleotides used in this study (a to d) and a mutant octamer oligonucleotide (M), which does not bind Oct-1 or Oct-2 (13, 15).

pattern of sequence-specific DNA binding. In contrast, the neuronal protein demonstrated strong sequence specificity which was different from that of Oct-1. For example, it bound with high affinity to one of the overlapping octamer/TAATGARAT motifs (oligonucleotide c) which is found in the HSV immediate-early gene 1 promoter (25), but a single base change at base 11 of this motif to produce a perfect octamer/TAATGARAT element (oligonucleotide a) virtually abolished binding. In contrast, Oct-1 exhibited similar levels of binding to oligonucleotides a and c, indicating that its binding was unaffected by this base change. It was also of interest that significant binding of the neuronal Oct-2 protein was observed to a simple TAATGARAT motif (oligonucleotide b; Fig. 5). As expected from previous studies (1, 14), Oct-1 bound only weakly to this motif in the absence of the HSV virion protein Vmw65. All assays were carried out with probes labeled to identical specific activities and in conditions of DNA excess so that the differences in binding activity observed reflected real differences in the affinities of Oct-1 and Oct-2 for the different probes. Thus, binding of the neuronal protein predominates over Oct-1 binding to two of the four oligonucleotides tested (b and c), although the level of binding to these two oligonucleotides differs greatly.

The difference in sequence specificity between B-cell and neuronal Oct-2 observed in these binding experiments was confirmed in competition experiments in which oligonucleotide a competed slightly more strongly than oligonucleotide c for binding of B-cell Oct-2, whereas in the case of the neuronal protein, oligonucleotide c competed much more strongly than did oligonucleotide a (Fig. 6). Hence, a single nucleotide change can increase the binding affinity of the B-cell protein while dramatically decreasing the binding affinity of the neuronal protein.

These studies establish that B-cell and neuronal Oct-2 differ in sequence specificity for different octamer oligonucleotides. Interestingly, Scholer et al. (28) showed that B-cell and brain-derived Oct-2 differ in the influence of bases flanking the octamer on the extent of binding observed as well as in the thermal stability of protein-DNA binding. Our studies extend these observations and show for the first time differences between the two proteins in binding to naturally occurring octamer sequences.

Having established that the neuronal Oct-2 protein differs

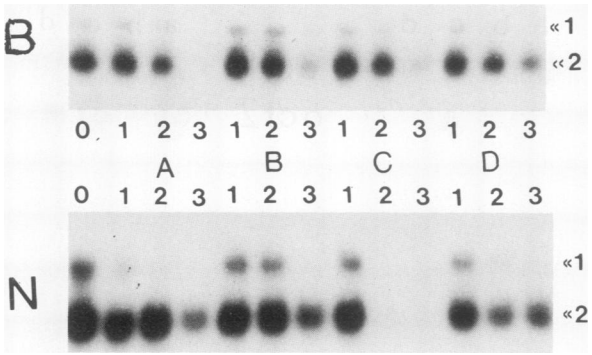


FIG. 6. Competition analysis of the B-cell (B) and ND cell (N) octamer-binding proteins. DNA mobility shift assays were carried out with labeled oligonucleotide c without competitor (track 0) or in the presence of 1-fold (tracks 1), 10-fold (tracks 2) or 100-fold (tracks 3) excess of unlabeled oligonucleotides a to d (A to D). The arrowheads indicate the positions of Oct-1 (1) and Oct-2 (2).

from the B-cell protein in sequence specificity, we investigated whether it also differed functionally in the ability to activate the expression of octamer-containing promoters. In particular, Oct-2, unlike Oct-1, is able to activate a simple promoter containing only an octamer motif and a TATA box of the type found in the immunoglobulin genes (20). In contrast, Oct-1 can activate gene expression via the octamer motif only in conjunction with other transcription factors binding to their corresponding sites in the DNA (for a review, see reference 26). Hence, a simple octamer, TATA box promoter, is expressed at high levels in Oct-2-containing B cells but not in 3T3 cells which contain only Oct-1 (38). To determine whether the Oct-2 in our cells would also activate a promoter of this type, we used a construct (Oct-CAT; a kind gift of Thomas Wirth and David Baltimore) in which a synthetic consensus octamer element has been placed upstream of a simple promoter containing only the TATA box and transcription start site of the c-*fos* promoter. As expected, the Oct-CAT construct was expressed in Daudi B cells at a very high level (90% conversion of chloramphenicol to the acetylated form in the experiment shown in Fig. 7a). In contrast, virtually no expression was detected when this construct was introduced into ND cells or C1300 cells (0.5% conversion in each case), although a construct in

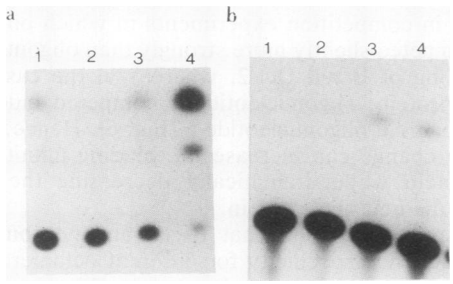


FIG. 7. (a) Assay of CAT activity following transfection of ND7 cells (tracks 1 and 2) or Daudi B cells (tracks 3 and 4) with an RSV-CAT construct (tracks 1 and 3) or the Oct-CAT construct (tracks 2 and 4). (b) Assay of CAT activity following transfection of the Oct-CAT construct into BHK-21 cells (tracks 1 and 3) or ND7 cells (tracks 2 and 4) with either 5 μg of pJ4 vector (tracks 1 and 2) or a similar amount of a pJ4-based construct expressing Oct-2 (tracks 3 and 4).

which the Rous sarcoma virus promoter drives the CAT gene (RSV-CAT; 11) was expressed at similar levels in all cell types. A similar very low level of expression in B cells and ND cells was observed for a similar construct in which the octamer motif had been deleted, confirming that this effect was specific for plasmids containing an octamer motif (data not shown).

The failure of the neuronal form of Oct-2 to activate a simple octamer-containing promoter could occur either because it was inherently incapable of doing so or because some other inhibitory protein present in neuronal cells prevented it from activating gene expression. To distinguish these possibilities, we prepared an Oct-2 expression vector by cloning the coding region of lymphocyte Oct-2 (23) into the vector pJ4 (22), where its expression will be driven by the constitutive Moloney murine leukemia virus promoter. When Oct-CAT was cotransfected with this expression vector into ND cells, its expression was activated (Fig. 7b; see below) in a manner similar to that observed in non-Oct-2-expressing BHK-21 cells (19) following cotransfection of an octamer-containing promoter and an Oct-2 expression vector (Fig. 7b; 23). This effect was dependent on the octamer motif, since the Oct-2 expression vector did not activate a similar plasmid from which the octamer motif had been deleted. Ratios of CAT activity (averages of three replicate experiments) in the presence or absence of cotransfected Oct-2 expression vector for plasmids either containing or lacking Oct-CAT were 2.6 or 0.9, respectively, for BHK cells and 4.0 or 1.0, respectively, for ND cells. Hence, the lymphocyte form of Oct-2 is capable of activating a simple octamer promoter in neuronal cells, indicating that the failure of the neuronal form of Oct-2 to do so is not likely to be due to the presence of another inhibitory factor. Thus, unlike B-cell Oct-2, neuronal Oct-2 resembles Oct-1 in its inability to activate a simple octamer-containing promoter.

To investigate further the role of neuronal Oct-2 in octamer-mediated gene expression, we prepared a series of constructs in which the octamer-containing oligonucleotides shown in Fig. 4 were cloned upstream of the HSV thymidine kinase (*tk*) promoter in the vector pBL2 CAT (18). Unlike the Oct-CAT promoter, the *tk* promoter in pBL2 CAT contains binding sites for several other transcription factors such as Sp1 and the CAAT box-binding factors. As expected, when constructs containing any of the four functional octamer motifs a to d were introduced into BHK cells (19) which contain only Oct-1, the level of CAT activity was higher than that obtained with the pBL2 CAT vector containing oligonucleotide M (ATAATAATAA), which both we (13) and others (15) have previously shown to be a nonfunctional octamer motif which does not bind either Oct-1 or the B-cell and neuronal forms of Oct-2 (Fig. 8). Hence, these constructs can be activated by Oct-1 alone. A greatly increased level of CAT activity was observed from all constructs when they were introduced into Daudi B cells, indicating that they can be activated even more strongly in cells containing Oct-1 and the lymphocyte form of Oct-2 (Fig. 8). In contrast, when these constructs were introduced into ND7 cells, only those containing oligonucleotides a and d were expressed at levels higher than was the construct containing the mutant oligonucleotide M, whereas the constructs containing oligonucleotides b and c were not activated and in fact were expressed at a lower level than the mutant oligonucleotide-containing plasmid (Fig. 8). Interestingly, the level of expression observed does not correlate with the absolute amount of neuronal Oct-2 bound by these oligonucleotides (Fig. 5), since oligonucleotides b and d bind

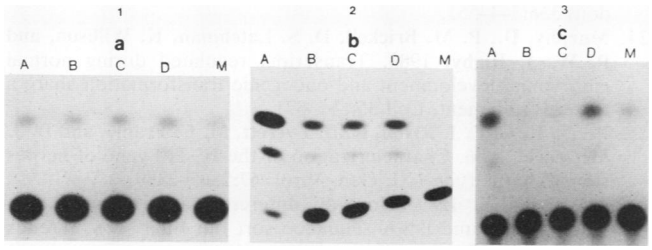


FIG. 8. Assay of CAT activity following transfection of pBL2 CAT vector containing one of oligonucleotides a to d inserted (tracks A to D) or with a mutant oligonucleotide inserted which does not bind Oct-1 or Oct-2 in either its B-cell or neuronal form (13, 15; track M). Constructs were transfected into Oct-1-containing BHK-21 cells (19) (a) or into Oct-2 containing Daudi B cells (b) or ND7 cells (c). Panel a illustrates the results of a typical experiment; panel b illustrates the result of averaging three replicate experiments normalized for the activity of an internal control plasmid.

similar levels of Oct-2, but only a construct containing oligonucleotide d is activated whereas one containing oligonucleotide b is repressed. Similarly, gene activity does not correlate with the level of Oct-1 bound by the different oligonucleotides, similar levels being bound by oligonucleotides a and c, which behave differently in functional assays. Moreover, oligonucleotides b and c, which both repress in functional assays, bind different levels of Oct-1. Rather, the activity observed is dependent on the relative levels of Oct-1 and neuronal Oct-2 that are bound. Thus, binding of Oct-1 predominates over binding of Oct-2 on the two oligonucleotides (a and d) which can activate the *tk* promoter, whereas binding of Oct-2 predominates over binding of Oct-1 on the two oligonucleotides (b and c) which cannot do so.

These data suggest that neuronal Oct-2, in addition to being unable to activate simple octamer-containing promoters, can interfere with the ability of Oct-1 to activate gene expression from promoters which contain the octamer motif in association with other transcription factor binding sites. Such an effect is dependent on the ability of neuronal Oct-2 to bind to individual octamer motifs with higher affinity than Oct-1 and is presumably therefore dependent on the blocking of Oct-1 binding. In addition, the fact that expression from the *tk* promoter is actually reduced by the addition of oligonucleotides which bind neuronal Oct-2 strongly compared with the addition of the nonbinding oligonucleotide M suggests that the factor may also have an inhibitory effect that is independent of Oct-1. This could occur by neuronal Oct-2 either acting directly as a repressor or by inhibiting the binding of other activating factors to adjacent or overlapping sites.

These findings indicate that the primary role of the neuronal form of Oct-2 may be to inhibit octamer-mediated gene expression either directly or by preventing Oct-1-mediated gene activation. A similar role for an octamer-binding protein (NF-A3) that is expressed specifically in embryonal carcinoma cells has also been proposed (16). It is also possible, however, that the neuronal Oct-2 protein is able to activate gene expression via the octamer motif in promoters different from those used here as a result of either the sequence of the octamer or its position relative to other promoter elements. This is particularly so since the effect of the octamer in our experiments is being analyzed in the abnormal context of the *tk* promoter. In other contexts, the neuronal form of Oct-2 may play a positive role in the neuronal-specific expression of some octamer-containing promoters (see, for example, reference 18).

Whatever the case, it is clear that the neuronal form of Oct-2 differs from that present in B cells with respect to both sequence specificity and gene activation ability. The two proteins do show some similarities, however. In addition to their similarity in size and antibody reactivity, both are capable of repressing expression from the HSV immediate-early promoter. We previously showed that the presence of the Oct-2 protein in C1300 cells and ND cells was associated with the repression of HSV immediate-early gene promoters following infection (13, 36), and we have recently shown directly that this repression can be produced by cotransfection of the lymphocyte Oct-2 expression vector and an HSV immediate-early promoter–CAT construct (16a). Moreover, hybridization of lymphocyte Oct-2 cDNA readily detects an mRNA in neuronal cells but not in most other cell types, both by Northern blotting (Fig. 3) and by in situ hybridization (12), indicating that these cells contain an mRNA species more similar to the lymphocyte Oct-2 mRNA than to the mRNA encoding Oct-1.

Interestingly, however, our studies demonstrating functional differences between the neuronal and B-cell forms of Oct-2 are paralleled by recent studies using proteolytic clipping band shift assays, which have demonstrated structural differences between the two proteins (29). Clearly, further studies involving the cDNA cloning of neuronal Oct-2 will be required to determine whether it is encoded by a gene distinct from but closely related to that encoding lymphocyte Oct-2 or whether the differences in properties of the two proteins are brought about by differential splicing or posttranslational modification of the products of a single gene.

## ACKNOWLEDGMENTS

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