Transcriptional regulation of MHC class I gene expression in rat oligodendrocytes

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MHC class I molecules are normally expressed at very low levels in the brain and their up-regulation in response to cytokines and viral infections has been associated with a number of neurological disorders. Here we demonstrate that the down-regulation of surface class I molecules in differentiated primary rat oligodendrocytes was accompanied by reduced steady-state levels of class I heavy-chain mRNA. Transient expression assays were performed in oligodendrocytes and fibroblasts, using a mouse H-2K^b class I promoter chloramphenicol acetyltransferase plasmid termed pH2KCAT (which contained 5′-flanking sequences from -2033 to $+5$ bp of the H-2K^b gene relative to the transcriptional start site at $+1$ bp). These assays showed that H-2K^b promoter activity was reduced in oligodendrocytes but not in class I-expressing fibroblasts. H-2K^b promoter activity was up-regulated in oligodendrocytes co-transfected with a plasmid expression vector encoding the transcriptional activator *tax* of human T-cell leukaemia virus type I, showing that down-regulation of promoter activity was reversible. Deletion mutant analysis of the H-2K^b promoter revealed the presence of negative regulatory elements that were functional in oligodendrocytes at -1.61 to -1.07 kb and -242 to -190 bp. Deletion of sequences in pH2KCAT encompassing the downstream element totally abolished promoter activity in both oligodendrocytes and fibroblasts, whereas a deletion within the upstream negative regulatory element increased promoter activity specifically in oligodendrocytes. The upstream negative regulatory element also down-regulated a linked heterologous herpes simplex virus thymidine kinase promoter in oligodendrocytes, but not in fibroblasts. Gel retardation assays using overlapping DNA probes that spanned the entire -1.61 to -1.07 kb region revealed the presence of a number of DNA-binding activities that were present in oligodendrocyte, but not in fibroblast nuclear extracts.

INTRODUCTION

MHC class I molecules (H-2K, D and L in the mouse and HLA-A, -B and -C in humans) are highly polymorphic glycoproteins that are expressed on the surface of most nucleated cells. They participate in the presentation of viral and tumour cell-derived peptide molecules to the T-cell receptor of cytotoxic T lymphocytes (CTLs) and in the mediation of allograft rejection. Elimination of virally-infected cells by CTLs requires recognition of processed viral molecules on the surface of the target cell in association with MHC class I molecules [1,2]. The brain has been reported to express considerably lower levels of class I molecules compared with other tissues [3], and this has been associated with the lack of CTL-mediated responses and viral persistence in the central nervous system [4,5]. Extensive immunocytochemical studies have demonstrated that class I molecules are hardly detectable in brain sections from newborn mice [6–8]. Similarly in the human brain, *in situ* expression of class I molecules is confined to endothelial cells in the normal central nervous system [9], but is significantly up-regulated during viral infections [8,10–12] and other pathological conditions such as multiple sclerosis [7]. Surprisingly, some neural cell types spontaneously express class I molecules upon their cultivation *in vitro* [6,13], an effect attributed to the culture techniques.

Oligodendrocytes are the myelinating cells of the central nervous system. They produce and maintain the myelin that surrounds neuronal axons and have been suggested to be possible targets for immune attack in demyelinating diseases [10,14]. Oligodendrocytes maintain low levels of surface class I molecules in culture [6,15,16]. Class I expression is up-regulated in response to cytokines and during some viral infections [8,10]. There is significant augmentation of surface class I molecules following treatment of oligodendrocytes with interferon-γ, IFN-γ [16–19], or interleukin 2 [20]. Class I expression can also be detected on cultured oligodendrocytes following intracerebral injection of IFN-γ into the brains of neonatal mice *in vivo* [17]. Modulation of weak class I expression on human oligodendrocytes has also been demonstrated in response to tumour necrosis factor-α [9]. Factors secreted from activated T cells can also induce expression of class I molecules on oligodendrocytes in culture [21]. The potential for up-regulation of class I expression on oligodendrocytes is of particular importance. Oligodendrocytes expressing class I molecules in culture are susceptible to cytolysis by class I-restricted activated T lymphocytes [14]. Cell-type-specific expression of class I molecules under the control of the myelin basic protein promoter in oligodendrocytes of transgenic mice resulted in shortage of myelinating oligodendrocytes and demyelination, although this did not involve immune reactions or infiltration of T lymphocytes [22,23].

Little is known about the molecular aspects of down-regulation of MHC class I expression in the central nervous system or the mechanism by which these molecules are induced during viral infections and autoimmunity. This study has been focused on the molecular mechanisms that mediate the down-regulation of class I molecules in cultured oligodendrocytes isolated from neonatal rat brain. We show that mature myelin basic protein (MBP)-positive oligodendrocytes expressed low levels of surface class I molecules and reduced steady-state levels of class I mRNA

Abbreviations used: CAT, chloramphenicol acetyltransferase; CRE, class I regulatory element; CTL, cytotoxic T lymphocyte; RSV, Rous sarcoma virus; HTLV-1, human T cell leukaemia virus type 1; IFN-γ, interferon-γ; MBP, myelin basic protein; NRE, negative regulatory element; tk, thymidine kinase.

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compared to 3Y1 fibroblasts and primary astrocytes that constitutively expressed surface class I molecules. We have carried out detailed deletion analysis of the 5′-flanking region of the H-2K^b class I gene and have identified regions that have negative regulatory properties specifically in oligodendrocytes. Furthermore, we have identified DNA-binding proteins that interact with domains of the negative regulatory regions and thus may be candidate oligodendrocyte transcriptional regulators.

EXPERIMENTAL

Cell culture

Primary cell cultures of differentiated oligodendrocytes and astrocytes were prepared from cerebral hemispheres of Wistar rats as previously described [24]. Cells were plated out on poly-(D-lysine)-treated plates (10^6 cells per 60 mm plate) for use in DNA-transfection experiments. Cells were also grown at the same density on coverslips in 35 mm dishes for immunofluorescent-antibody staining. After 18 days in culture, cells were fixed with acetone/methanol (1:1, v/v), incubated with a monoclonal antibody against MBP (kindly supplied by Dr. R. Reynolds, Charing Cross and Westminster Hospital Medical School, London, U.K.), followed by FITC-conjugated secondary antibody and viewed under ultraviolet epifluorescent optics in a Leitz Dialux II microscope. Rat 3Y1 fibroblasts were kindly provided by Professor K. Fujinaga, Cancer Research Institute, Sapporo Medical University, Sapporo, Japan.

Plasmids

The following plasmids were used: (i) pBLCAT3 is a chloramphenicol acetyltransferase (CAT) reporter gene vector that lacks promoter elements, while pBLCAT2 is a basal herpes simplex virus thymidine kinase (tk) promoter linked to the CAT gene [25]; (ii) plasmid H2KCAT consists of the entire -2033 to $+5$ bp promoter region (relative to the transcriptional start site at $+1$ bp) of the mouse H-2K^b gene fused directly upstream of the CAT reporter gene in pBLCAT3 [26]. Plasmids p($-1070/+5$)CAT, p($-670/+5$)CAT, p($-370/+5$)CAT and p($-53/+5$)CAT were constructed by digesting pH2KCAT with *Hin*dIII (which cuts in the polylinker of pBLCAT3) and *Stu*I, *Asp*I, *Xba*I and *Sac*I respectively, followed by repair synthesis of DNA ends with the Klenow fragment of DNA polymerase I and blunt-end ligation. Plasmids p($-2.0/-1.61$)tkCAT and p($-1.61/-1.07$)tkCAT contained restriction endonuclease fragments of the 5′-flanking region of the H-2K^b gene cloned in pBLCAT2 [26]; (iii) plasmid p242H2KCAT consists of the -242 to $+5$ bp sequence of the mouse H-2K^b gene fused directly upstream of the CAT reporter gene [27]; (iv) plasmid p190H2KCAT consists of the -190 to $+5$ bp sequence of the H-2K^b gene fused directly upstream of the CAT reporter gene [27]; (v) plasmid pH2KCAT DelR2 carries a deletion from -201 to -185 bp and was generated by PCR mutagenesis using a four-primer method [28]; (vi) plasmid pH2KCAT Del1 was made by deleting the region between -1.49 to -1.18 kb of the H-2K^b promoter between two *Bcl*I sites [26]; (vii) plasmid pRSVCAT contains a 524 bp fragment of the Rous sarcoma virus (RSV) long-terminal repeat promoter element fused directly upstream of the CAT reporter gene [29]; (viii) plasmid pMBPCAT contains the -1350 to $+70$ region of the mouse MBP gene [24]; (ix) plasmid pJFE14-*tax* is an expression vector of the *tax* protein of human T cell leukaemia virus type 1 (HTLV-1), kindly donated by Professor C. Bangham, Imperial College of Science, Technology and Medicine, London, U.K.; (x) plasmid pMBP-1 is a rat MBP cDNA clone [30]; (xi) plasmid pPst8-8 is a mouse class I genomic clone containing the first three exons of the H-2K^b gene [31]; and (xii) pAL41 is a partial cDNA clone for mouse β-actin [32]. Cloned inserts were excised by *Eco*RI digestion from pMBP-1 and *Pst*I digestion from pPst8-8 and pAL41. The DNA inserts were purified by agarose-gel electrophoresis and labelled *in vitro* using [α-^{32}P]dCTP (3000 Ci·mmol^{-1}; ICN, Oxfordshire, U.K.) and a random hexamer priming kit (Pharmacia, Herts, U.K.) for use in Northern-blot analysis.

Northern blotting

Cytoplasmic RNA (30 μg) was separated by electrophoresis on 1.5 % agarose gel containing 2.2 M formaldehyde and subjected to Northern-blot analysis as previously described [33]. Quantification was performed on a Fuji BAS1000 phosphorimager.

DNA transfection and CAT assays

Transfection of DNA was performed by the calcium phosphate method as previously described [29], using 10 μg of DNA/60 mm dish of cells. Oligodendrocytes were transfected after 18 days in culture using a transfection kit (Pharmacia) to improve efficiency. Cells were harvested 48 h after transfection, cell lysates were prepared and CAT assays were performed, as described previously [29], using equivalent amounts of cell protein as determined using a dye-binding assay (Bio-Rad, Herts, U.K.). Quantification of CAT assays was performed using a Fuji BAS1000 phosphorimager. Differences in DNA uptake into cells between different plasmids were estimated by measuring the amount of plasmid DNA in the transfected cells by a dot-hybridization assay of plasmid DNA isolated from transfected cells using a modification of Hirt's method for the isolation of low-molecular-mass DNA from virus-infected cells [34]. Equivalent numbers of cells transfected with the CAT constructs, or untransfected cells as controls, were lysed with SDS buffer [0.6 % SDS/10 mM Tris/HCl (pH 7.5)/10 mM EDTA] and left for 18 h in 1 M NaCl at 4 °C. Following centrifugation in a bench centrifuge at 1000***g*** for 20 min at 4 °C, the DNA was recovered from the supernatant fraction by ethanol precipitation and resuspended in TE buffer [10 mM Tris/HCl (pH 7.5)/1 mM EDTA]. Serial dilutions of the plasmid DNA were spotted on to nylon membranes and hybridization was performed as previously described [33] using ^{32}P-labelled pBLCAT3 DNA as probe, labelled as described in the Plasmids section. Quantification was performed on a Fuji BAS1000 phosphorimager. The DNA uptake among individual constructs did not vary by more than 10 %. This was confirmed in co-transfection assays with a β-galactosidase expression plasmid, performed as previously described [26].

Gel retardation analysis

DNA probes were either directly synthesized (Pharmacia) or amplified by PCR using Vent DNA polymerase (New England Biolabs, Hitchin, Herts, U.K.) as previously described [26]. Nuclear extracts were prepared as previously described [26] including the proteinase inhibitors (obtained from Boehringer Mannheim, Lewes, East Sussex, U.K.) PMSF (0.5 mM), aprotinin (30 μg/ml) and leupeptin (10 μg/ml) in all solutions. Binding assays were performed and separated on native polyacrylamide gels as previously described [26]. For competition assays a 100-fold excess of unlabelled probe over labelled DNA was added.

Flow cytometry

Intact cells were incubated with the monoclonal antibody OX18 (which recognizes a monomorphic determinant on rat class I heavy chains), obtained from Serotec, Kidlington, Oxford, U.K. or the control antibody TB5 (a mouse monoclonal antibody that recognizes an adenovirus structural protein epitope; G. E. Blair, unpublished work) followed by FITC-conjugated anti-mouse immunoglobulin secondary antibody (1:100 dilution; Sigma, Poole, Dorset, U.K.). All incubations were carried out at room temperature for 20 min. The cells were then washed with PBS containing 1% foetal calf serum and analyzed on a FACScan flow cytometer (Becton Dickinson, Oxford, U.K.).

RESULTS

Reduced levels of surface class I molecules and steady-state mRNA levels in oligodendrocytes

The expression of MHC class I molecules on the surface of oligodendrocytes was analyzed by flow cytometry on the 18th day of culture *in vitro*. The cultures were characterized by immunofluorescent-antibody staining using cell-specific markers as previously described [24]. More than 80% of the cells in the oligodendrocyte-enriched cultures reacted with an antibody against MBP, which is specifically expressed in oligodendrocytes. The presence of contaminating astrocytes in the cultures was detected using a monoclonal antibody against glial acidic fibrillary protein, against which less than 15% of the cells reacted (results not shown). To determine the surface levels of MHC class I molecules, primary oligodendrocytes, astrocytes and 3Y1 fibroblasts were reacted with the mouse anti-rat class I monoclonal antibody OX18, which recognizes a monomorphic determinant on the heavy chain of MHC class I molecules. As control, cells were reacted with TB5, a mouse monoclonal antibody that recognizes an adenovirus structural protein epitope. Antibody-bearing cells were detected with FITC-conjugated anti-mouse immunoglobulins and analyzed by flow cytometry. Oligodendrocytes expressed approximately 18–20-fold lower levels of class I molecules on their surface compared with 3Y1 fibroblasts and astrocytes (Figure 1). Class I expression on the surface of oligodendrocytes was elevated by 10-fold when the cells were treated with IFN-γ, compared with an approximate 2-fold increase in class I expression on the surface of astrocytes and 3Y1 fibroblasts following the same treatment (Figure 1).

To define the level of control on down-regulation of MHC class I expression in oligodendrocytes, the steady-state levels of

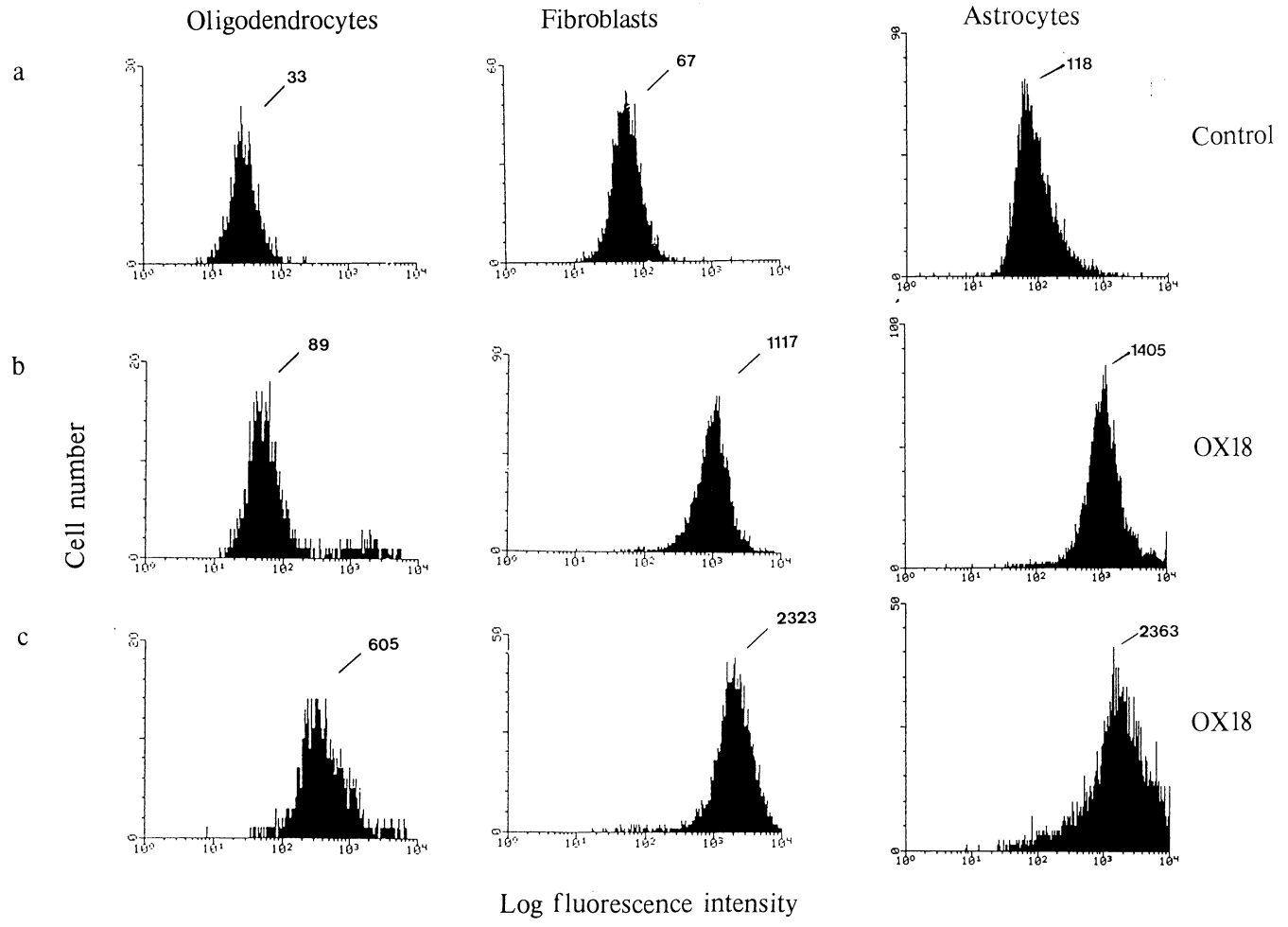

Figure 1 MHC class I molecules are expressed at low levels on the surface of primary rat oligodendrocytes

Primary oligodendrocytes and astrocytes isolated from newborn rats and maintained in culture for 18 days, and 3Y1 fibroblasts were incubated with the negative control antibody TB5 (a) or the monoclonal antibody OX18 followed by FITC-conjugated anti-mouse immunoglobulin secondary antibody (b) and (c). Cells were treated with medium alone (a and b) or with 1000 U/ml of IFN-γ for 18 h (c). The mean fluorescence intensity of 150000 cells is shown.

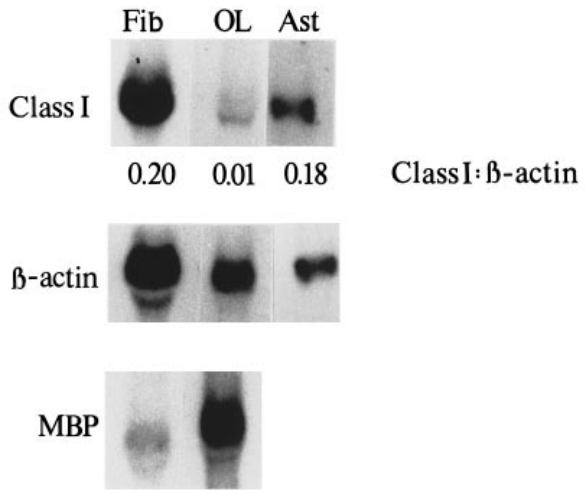

Figure 2 Primary rat oligodendrocytes express reduced steady-state levels of class I mRNA

Cytoplasmic RNA (30 μg), isolated from primary oligodendrocytes (OL) astrocytes (Ast) and 3Y1 fibroblasts (Fib), was separated by formaldehyde/agarose electrophoresis, transferred to nitrocellulose and hybridized with a ^{32}P-labelled cloned H-2K^b DNA probe (upper panel). The same blot was stripped and hybridized successively with radiolabelled β-actin cDNA (middle panel) and finally, MBP cDNA probes (lower panel).

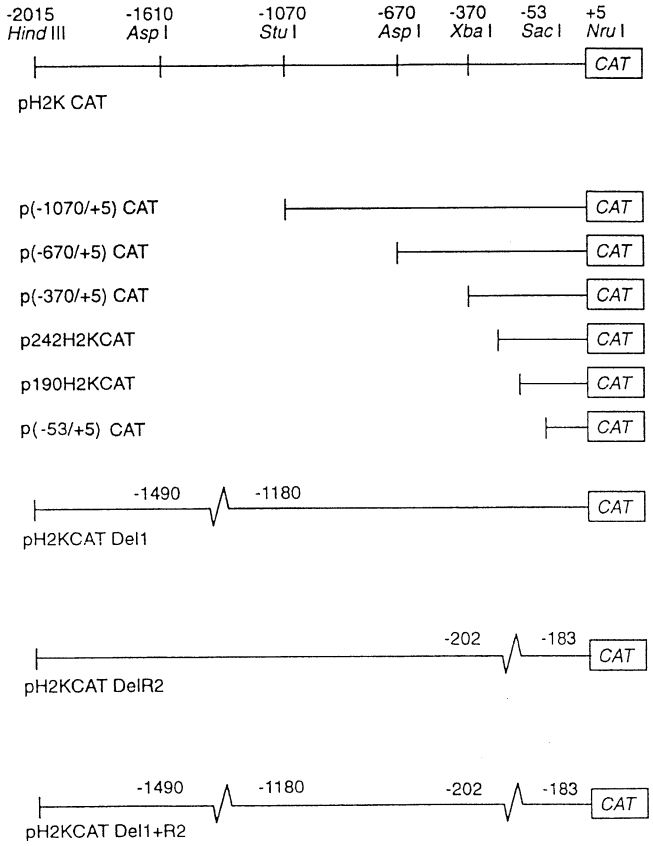

Figure 3 Structural organization of plasmids used in CAT assays and the proximal promoter region of the H-2K^b gene

The 2 kb 5′-flanking region of the H-2K^b gene was fused directly to the promoterless reporter gene vector pBLCAT3 to generate the plasmid pH2KCAT [26]. The 5′ deletion mutants were constructed by digesting pH2KCAT with *Hin*dIII, which cuts at the polylinker site of pBLCAT3, and the restriction enzymes *Stu*I (−1070), *Asp*I (−670) and *Xba*I (−370), followed by blunt-end ligation into the pBLCAT3 vector. The sites of deletions introduced into pH2KCAT by directed mutagenesis are shown.

class I mRNA were analyzed and compared with those expressed in 3Y1 fibroblasts. Total RNA isolated from oligodendrocytes and 3Y1 fibroblasts was subjected to Northern blotting using an H-2K^b gene probe known to hybridize to all MHC class I genes [35]. The probe hybridized to an approximately 1.8 kb species that was present at much lower levels in oligodendrocytes compared with astrocytes and 3Y1 fibroblasts (Figure 2). The relative ratio of class I mRNA to β-actin mRNA was 18–20-fold lower in oligodendrocytes compared with that in astrocytes or 3Y1 fibroblasts. There was therefore good correlation between surface class I protein levels and steady-state class I mRNA levels in oligodendrocytes, astrocytes and 3Y1 fibroblasts. Hybridization was also carried out using an MBP cDNA probe. The labelled MBP probe hybridized strongly to an approx. 2.3 kb species in RNA isolated from oligodendrocytes [30], confirming that the primary cultures used in the study were greatly enriched in oligodendrocytes. Since most changes in levels of mRNAs in mammalian cells result from altered promoter activity, we investigated whether a class I promoter was specifically down-regulated in oligodendrocytes.

A distal negative regulatory element (NRE) mediates reduced class I promoter activity in oligodendrocytes

The 2 kb 5′ flanking region of the murine H-2K^b gene was cloned into the pBLCAT3 reporter gene vector to generate the plasmid pH2KCAT (Figure 3; [26]). The construct was used in transient expression assays in oligodendrocytes 18 days after their isolation, at which time MBP expression has been shown to be at its highest level [24]. As control, parallel transfections were performed with the constructs pBLCAT3 (which contains no promoter elements and acts as a negative control), pRSVCAT (which contains the RSV LTR (long terminal repeat) and is highly active in most cell types) and pMBPCAT (which is expressed mainly in oligodendrocytes) (Figure 4). The CAT activities, normalized for differences in the DNA uptake between oligodendrocytes and 3Y1 cells, are shown in Table 1. The H-2K^b class I promoter drove approximately 15-fold lower levels of CAT expression in oligodendrocytes compared with 3Y1 fibroblasts. It could therefore be postulated that the down-regulation of MHC class I expression in oligodendrocytes took place at the level of initiation of transcription.

To examine the inducibility of the H-2K^b promoter in oligodendrocytes, an HTLV-1 *tax* gene expression plasmid (pJFE14-*tax*) was used in co-transfection experiments with pH2KCAT in primary oligodendrocytes and 3Y1 fibroblasts. The transcriptional activator *tax* of HTLV-1 has previously been shown to up-regulate the activity of the H-2K^b promoter and increase the surface levels of class I molecules in the C6 glioma cell line [36]. There was a significant, approx. 5-fold increase in the CAT activity driven by the entire H-2K^b promoter in co-transfection assays with pJFE14-*tax* in oligodendrocytes, but no increased CAT expression was detected in 3Y1 fibroblasts. This indicates that down-regulation of H-2K^b promoter activity in oligodendrocytes is reversible and can be activated by a transcriptional activator such as the *tax* gene product.

To define the target site of down-regulation of the class I promoter in oligodendrocytes, a series of 5′ deletion mutants of the promoter region was generated using the restriction sites

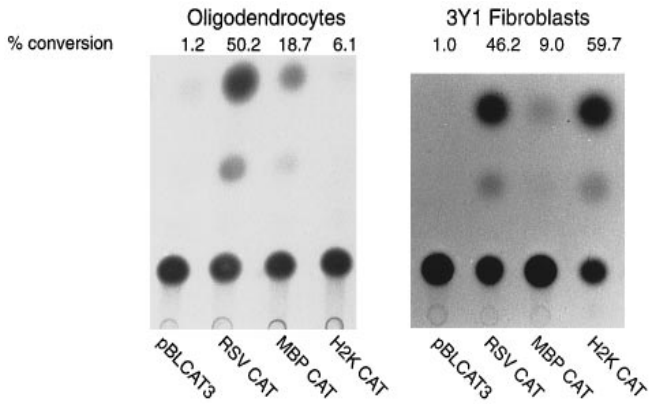

Figure 4 Reduced activity of the H-2K^b promoter/enhancer region in oligodendrocytes

The construct pH2KCAT, which contains the *Hin*dIII/*Nru*I fragment of the 5′-flanking region of the H-2K^b gene fused directly to the CAT reporter gene (see Figure 3), was transfected into primary oligodendrocytes and 3Y1 fibroblasts. Plasmids pBLCAT3, pRSVCAT and pMBPCAT were transfected into both cell types as controls. Cells (grown in 60 mm plates) were transfected with 10 μg of plasmid DNA. Cell extracts from oligodendrocytes (30 μg) and 3Y1 cells (40 μg) were used in CAT assays. The CAT activity was measured 36 h after transfection and was expressed as percentage conversion of [^{14}C]chloramphenicol to its acetylated derivatives.

Table 1 The down-regulation of the H-2K^b promoter in oligodendrocytes is mediated by NREs

The plasmid H2KCAT containing the entire H-2K^b promoter and 5′ deletion mutants cloned directly upstream of the CAT gene were transfected into primary oligodendrocytes and 3Y1 fibroblasts. Cells were harvested and the CAT activity was assayed as described in Experimental and quantified from the TLC plates on a Fuji BAS1000 phosphorimager. The CAT activities are expressed as percentage conversion of [^{14}C]chloramphenicol to its acetylated derivatives which has been normalized for differences in the DNA uptake between the two cell types as described in Experimental. Differences in uptake among individual constructs were estimated using the same method and those did not vary by more than 10%. The values are means of three independent experiments using two different plasmid preparations with variations in parentheses. ND, not determined.

	% CAT conversion	
Plasmid	Oligodendrocytes	3Y1 Fibroblasts
pH2KCAT	6.1 (±0.9)	88.7 (±1.4)
pH2KCAT + 4 μg pJFE14-*tax*	28.2 (±1.2)	93.9 (±2.9)
p(−1070/+5)CAT	20.4 (±4.5)	63.5 (±12.2)
p(−670/+5)CAT	4.1 (±3.1)	79.6 (±3.8)
p(−370/+5)CAT	5.2 (±0.3)	35.4 (±7.6)
p242H2KCAT	3.0 (±1.8)	31.5 (±6.4)
p190H2KCAT	10.2 (±0.7)	21.8 (±3.5)
p(−53/+5)CAT	1.3 (±0.1)	ND
pBLCAT3	0.6 (±0.1)	0.8 (±0.1)
pH2KCAT Del R2	1.0 (±0.8)	0.8 (±0.1)

shown in Figure 3. The constructs were used in transient expression assays in oligodendrocytes and 3Y1 fibroblasts (Table 1). A distal NRE unique to oligodendrocytes was identified between −2.0 and −1.07 kb relative to the transcriptional initiation site, located upstream of a positive regulatory element (−1.07 to −0.67 kb), which was also unique to oligodendrocytes. When cloned upstream of the CAT gene in the plasmid p(−2.0/−1.07)tkCAT, this NRE down-regulated the heterologous tk basal promoter (Table 2).

Table 2 The −1.61 to −1.07 kb region of the H-2K^b promoter acts as an NRE specifically in oligodendrocytes

CAT plasmids containing the indicated fragments of the H-2K^b promoter cloned upstream of the tk promoter were transfected into primary oligodendrocytes and 3Y1 fibroblasts, and the CAT activity was assayed as described in Experimental. The CAT activities are expressed as relative activity compared with the CAT activity of pBLCAT2 or pRSVCAT as indicated. Differences in uptake between plasmids were estimated as described in Experimental and those did not vary by more than 10%. The values are means of three independent experiments using two different plasmid preparations with variations in parentheses. In a representative experiment the percentage conversion of [^{14}C]chloramphenicol for pBLCAT2 was 16.0% in oligodendrocytes and 42.0% in 3Y1 cells.

Plasmid	Oligodendrocytes	3Y1 Fibroblasts
Relative CAT expression (% of pBLCAT2)		
p(−2.0/−0.67) tkCAT	47.0 (±2.5)	89.6 (±6.6)
p(−2.0/−1.07) tkCAT	54.1 (±4.1)	91.4 (±7.1)
p(−2.0/−1.61) tkCAT	93.4 (±7.6)	104.4 (±9.4)
p(−1.61/1.07) tkCAT	49.3 (±3.5)	98.4 (±6.3)
Relative CAT expression (% of RSVCAT)		
pH2KCAT	7.2 (±1.2)	88.9 (±2.2)
pH2KCAT Del 1	18.3 (±4.3)	88.7 (±3.8)
pH2KCAT Del R2	1.0 (±0.8)	0.8 (±0.1)
pH2KCAT Del1 + R2	0.8 (±0.1)	1.0 (±0.1)

To delineate the NRE further, the plasmids p(−2.0/−1.61)tkCAT and p(−1.61/−1.07)tkCAT (which divide the putative down-regulatory sequence into two similar-size fragments) were used in transient expression assays in oligodendrocytes and 3Y1 cells. The −1.61 kb/−1.07 kb sequence significantly down-regulated CAT expression from the basal tk promoter in oligodendrocytes, but not in 3Y1 cells (Table 2). It was therefore concluded that the oligodendrocyte-specific NRE was located between −1.61 and −1.07 kb relative to the transcriptional start site. It was postulated that the NRE is dominant over the positive regulatory element located between −1.07 and −0.6 kb, since the sequence between −2.0 and −0.6 kb in the plasmid p(−2.0/−0.6)tkCAT also down-regulated the tk promoter in transient expression assays (Table 2). In a complementary approach, transfection assays were performed with a deletion construct, pH2KCAT Del1, lacking part of the −1.61 to −1.07 sequence (from −1.49 to −1.18 kb between two *Bcl*I restriction sites). This deletion up-regulated promoter activity by approximately 2–3-fold in oligodendrocytes, but not in 3Y1 cells (Table 2), confirming that sequences within the −1.6 kb/−1.07 kb region may actively repress the MHC class I promoter specifically in oligodendrocytes.

The class I regulatory element (CRE) II domain of the proximal CRE element also modulates class I promoter activity in oligodendrocytes and 3Y1 fibroblasts

The construct p242H2KCAT containing the major CRE, which has been previously described as a major regulatory element in tissues that constitutively express class I molecules [37,38], also drove considerably lower levels of CAT expression in oligodendrocytes compared with 3Y1 fibroblasts (Table 1). Plasmid p190H2KCAT in which region II of the CRE (−201/−184 bp) is disrupted drove significantly higher levels of CAT activity in oligodendrocytes compared with plasmid p242H2KCAT in which the entire CRE is intact (Table 1). The results suggested that region II might act as an NRE in oligodendrocytes; alternatively there could be an NRE between −242 and −190 bp

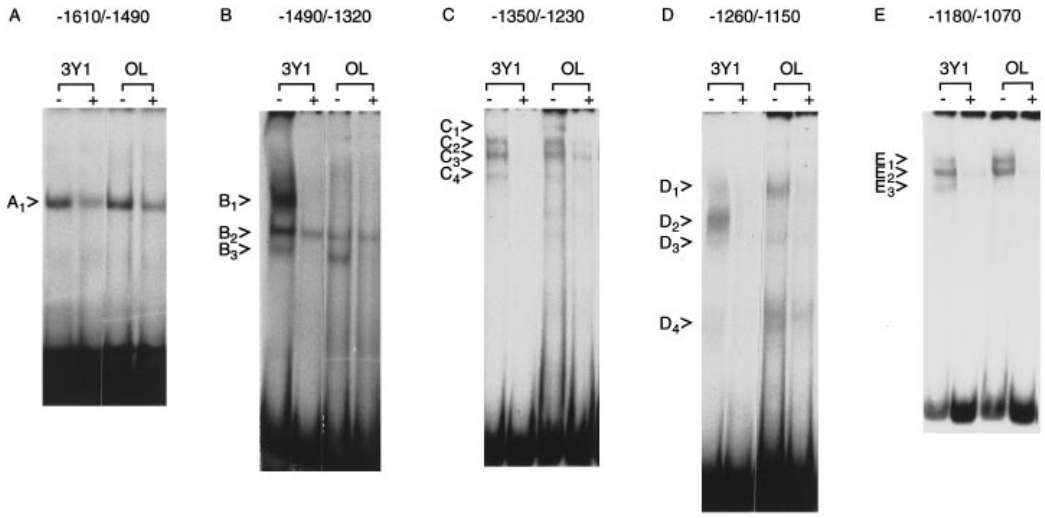

Figure 5 Identification of nuclear factors interacting with the −1. 61 to −1. 07 kb region of the H-2K^b promoter in oligodendrocyte and 3Y1 nuclear extracts

Overlapping DNA fragments spanning the −1.61/−1.07 down-regulatory region of the H-2K^b promoter were generated by PCR, radioactively labelled and used in gel-retardation assays as described in Experimental with oligodendrocyte (OL) and 3Y1 nuclear extracts. Competition reactions (+) were carried out by preincubation of extracts with a 100-fold molar excess of unlabelled fragment. Protein–DNA complexes were separated on 5% native polyacrylamide gels. Arrows denote the position of specific protein–DNA complexes.

relative to the transcriptional initiation site, or plasmid sequences upstream of the H-2K^b promoter sequence might influence transcription. To determine whether region II of the CRE had negative regulatory properties in oligodendrocytes, the mutant construct pH2KCAT DelR2 (which carries a deletion located at −201 to −184 bp in the 2 kb 5′-flanking region) was used in transfections. Interestingly, this deletion completely abolished promoter activity in both oligodendrocytes and 3Y1 cells (Table 2). Deletion of the −201/−184 bp and the −1.49/−1.18 kb sequences in a single double-mutant promoter construct (pH2KCAT Del1 + R2) had the same effect (Table 2). It could be concluded that region II of the CRE was essential for the initiation of transcription from the H-2K^b promoter in both oligodendrocytes and 3Y1 fibroblasts.

Distinct nuclear proteins interact with the upstream NRE of the class I promoter in oligodendrocytes and 3Y1 fibroblasts

Since the −1.07 to −1.61 kb region had negative transcriptional regulatory properties in oligodendrocytes but not in 3Y1 fibroblasts, protein–DNA interactions were analyzed using five overlapping PCR fragments that spanned the entire region (Figure 5). Some protein–DNA complexes were present at much higher levels using 3Y1, compared with oligodendrocyte nuclear extracts, for example B_1, D_2 and E_3. Other complexes were found only when using oligodendrocyte nuclear extracts, for example C_1, D_1 and D_4. Formation of all these complexes was abolished in the presence of excess homologous unlabelled competitor DNA fragment, but they were unaffected by unrelated 100 bp fragments (results not shown).

DISCUSSION

A number of studies have demonstrated that MHC class I molecules are differentially expressed among different cell types of the central nervous system, including astrocytes, oligodendrocytes and neurons [6,9,16]. It has been suggested that the levels of class I expression are related to the susceptibility or the resistance to lysis of neural cells by cytotoxic T lymphocytes [13,39,40]. The present study has focused on the molecular mechanisms that control MHC class I expression in oligodendrocytes. The findings suggest that the down-regulation of MHC class I expression in these cells is mainly under transcriptional control, since the reduced surface levels of class I molecules corresponded to extremely low steady-state levels of class I mRNA and significantly reduced transcriptional activity driven by the H-2K^b promoter. Although low levels of astrocytes and fibroblasts were found to be present in primary oligodendrocyte cultures, it is extremely unlikely that these contaminating cell types contributed to the lowered level of H-2K^b promoter activity detected in primary oligodendrocyte cultures, since both astrocytes and fibroblasts contained high levels of class I proteins and mRNAs. H-2K^b promoter activity was up-regulated by several fold in co-transfection assays with an HTLV-1 *tax* expression plasmid in oligodendrocytes, showing that transcriptional repression is an active process that can be abrogated by the presence of a viral transcriptional activator.

Deletion analysis of the 5′-flanking region of the H-2K^b gene revealed the presence of two regions that had negative regulatory properties in oligodendrocytes. One down-regulatory sequence was located within the previously defined CRE. The CRE was initially identified as a positive regulatory element in fibroblasts [37] but later studies showed that it may control the negative regulation of MHC class I genes during early embryonic development [41,42]. In this study, the construct p242H2KCAT containing the entire CRE and interferon consensus sequence had very low promoter activity in oligodendrocytes. Deletion of a sequence containing region II of the CRE resulted in up-regulation of transcriptional activation mediated by p190CAT in oligodendrocytes, but not in 3Y1 fibroblasts, suggesting that the CRE II acts as a negative regulatory element specifically in oligodendrocytes. Surprisingly, deletion of CRE II in the context of the entire H-2K^b promoter (in pH2KCAT Del2) did not up-regulate, but completely abolished transcriptional activity both in oligodendrocytes and 3Y1 fibroblasts. Similarly, in cells transformed by the highly oncogenic adenovirus 12, where the CRE II has also been shown to act as an NRE [43], it has been

demonstrated that introducing point mutations within the CRE II domain abolishes promoter activity driven by the 1.4 kb 5′-flanking region of the H-2L^d gene [43,44].

An upstream regulatory element, located between −1.61 and −1.07 kb relative to the transcriptional start site, actively repressed promoter activity specifically in oligodendrocytes. This element down-regulated transcription from both the class I promoter as well as the heterologous tk promoter in oligodendrocytes, but not in 3Y1 fibroblasts. Deletion of a 260 bp sequence from −1.49 to −1.18 kb relative to the transcriptional start site significantly increased the activity of the H-2K^b promoter, suggesting that sequences within the putative down-regulatory region are important in maintaining low promoter activity in oligodendrocytes. It will now be important to use directed mutagenesis techniques to identify functionally important regulatory elements within the −1.61 to −1.07 bp region that modulate class I promoter activity in oligodendrocytes. Interestingly, the −1.49 to −1.18 bp deletion has been shown to up-regulate promoter activity in adenovirus 12-transformed cells [26], suggesting that common regulatory elements in the H-2K^b promoter are involved in cell-type-specific regulation of expression in oligodendrocytes and are also targets for class I transcriptional repression by adenovirus 12-transforming gene products.

Computer analysis of the −1.61 to −1.07 kb region revealed sequence similarities with protein-binding consensus sequences and *cis*-acting elements that regulate the early promoter region of polyoma viruses. We identified a perfect match to the 5′-GGGXGGPuPu-3′ element [45] of the early promoter region of the neurotropic human polyoma virus JC at −1289/−1282 bp in the H-2K^b promoter. The early promoter region of the neurotropic human polyoma virus is known to direct high levels of transcription specifically in glial cells [46,47], however, the protein factors that mediate its specificity have not been characterized. We also identified putative binding sites for the polyoma virus enhancer A binding protein 3 (PEA3; [48]) at −1489/−1484 bp in the H-2K^b promoter (AGGAAG) and the polyoma enhancer B binding protein 1 (PEB1; [49]) at −1434/−1429 bp (AGAGGG) in the H-2K^b promoter. We detected significant differences between oligodendrocytes and 3Y1 fibroblasts in the binding of protein factors to DNA sequences spanning the NRE in gel-retardation assays. It is possible that oligodendrocyte-specific nuclear factors interact within the NRE and actively repress class I expression in oligodendrocytes. Future experiments will focus on mutational analysis of the NRE and the molecular characterization of binding factors that interact with the region to modulate the activity of the MHC class I promoter in oligodendrocytes.

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