The transcription of the human fructose-bisphosphate aldolase C gene is activated by nerve-growth-factor-induced B factor in human neuroblastoma cells*

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A DNA region located at around −200 bp in the 5′ flanking region (region D) of the human brain-type fructose-bisphosphate aldolase (aldolase C) gene has been analysed. We show by transient transfection assay and electrophoretic-mobility-shift assay (EMSA) that the binding of transcriptional activators to region D is much more efficient (80% versus 30%) in human neuroblastoma cells (SKNBE) than in the non-neuronal cell line A1251, which contains low levels of aldolase C mRNA. The sequence of region D, CAAGGTCA, is very similar to the AAAGGTCA motif present in the mouse steroid 21-hydroxylase gene; the latter motif binds nerve-growth-factor-induced B factor (NGFI-B), which is a member of the thyroid/steroid/retinoid nuclear receptor gene family. Competition experiments in EMSA and antibody-directed supershift experiments showed that NGFI-B is involved in the binding to region D of the human aldolase C gene. Furthermore, the regulation of the aldolase C gene (which is the second known target of NGFI-B) expression during development parallels that of NGFI-B.

INTRODUCTION

Fructose-bisphosphate aldolase (aldolase) is a glycolytic enzyme that exists in three forms: aldolase A (muscle-type), aldolase B (liver-type) and aldolase C (brain-type) [1,2]. Aldolase C increases during the development of mammalian foetal brain, and is expressed at high levels in adult brain; very low levels of aldolase C have been found in some non-nervous tissues [3]. The protein is localized principally in the Purkinje cells of the cerebellum and in the bipolar cells of the human retina [4,5]. Substantial levels of aldolase C mRNA have been found in the hippocampus, thalamic and amygdaloid nuclei, and lower levels in the cortex, striatum and olfactory nuclei [6,7]. Consistent with the widespread localization in various areas of the brain, the aldolase C gene is a neuronal gene that contains a housekeeping-like promoter [8–11]. The promoter region of this gene, including the human gene, lacks the canonical TATA and CAAT boxes and contains multiple transcription start sites [7,12,13]. We have demonstrated previously that a region of about 80 bp (−37 to −114 bp) in the promoter region of the human gene is necessary for relevant expression in a human neuroblastoma cell line (SKNBE), and that another region, from −420 to −164 bp, is also needed for full and correct expression of this gene in SKNBE cells [14].

We report a detailed analysis of the region from −211 to −184 bp that contains a consensus DNA binding sequence for a member of the thyroid/steroid/retinoid nuclear receptor gene family, the transcriptional activator nerve-growth-factor-induced B factor (NGFI-B). A series of experiments strongly suggests that NGFI-B is specifically involved in this binding.

MATERIALS AND METHODS

Plasmid constructs

Plasmids Δ−420-chloramphenicol acetyltransferase (CAT), Δ−164-CAT and Δ−86-CAT were made as indicated in Buono et al. [14]. The Δ−208-CAT plasmid (see Figure 1) was obtained by 30 cycles of PCR amplification using the segment of oligonucleotide L1 (see Figure 2) from −184 to −211 bp (+1 being the major transcription start site) and the oligonucleotide from +28 to +10 bp of the promoter region. The latter region bears the sequence 5′-TGAGGCTGCAGCCCTGGC-3′ and contains a *Pst*I site (underlined). The plasmid Δ−208*-CAT (see Figure 1), containing a point mutation in area D, was constructed using the oligonucleotide L1. mut.1 (see Figure 2) and oligonucleotide *Pst*I as described above. The template was a DNA clone containing 420 bp of the promoter region (200 ng), and the reaction was carried out in 1 × buffer containing 0.2 mM dNTPs, 50 pmol of the oligonucleotide primers described above, and 5 units of *Taq*I polymerase (Perkin Elmer, Vaterstetten, Germany). The PCR products were purified from agarose gel, flush-ended by Klenow polymerase (Boehringer, Mannheim, Germany), digested by *Pst*I and then cloned in *Sma*I/*Pst*I sites, upstream from the CAT reporter gene in the pEMBL-8-CAT expression vector [15]. The inserted clones were checked by sequence analysis with the chain termination method [16]. Oligonucleotides were synthesized on the Applied Biosystems (Milan, Italy) 951 synthesizer.

Cell culture, TTA and CAT assays

SKNBE cells, kindly provided by Dr. E. Boncinelli (Istituto Scientifico San Raffaele, Milan, Italy) and human kidney carcinoma (A1251) cells, kindly provided by Dr. C. Pietropaolo (Università di Napoli 'Federico II', Naples, Italy) were cultured in Dulbecco's modification of Eagle's medium (Life Technologies, Gaithersburg, MD, U.S.A.) supplemented with 10% (v/v) foetal-calf serum (PAA, Linz, Austria). The cells were plated at a density of about 200000/60-mm Petri dish 16 h before transfection. Cultures were cotransfected by the calcium

Abbreviations used: aldolase, fructose-bisphosphate aldolase (EC 4.1.2.13); EMSA, electrophoretic mobility shift assay; TTA, transient transfection assay; CAT, chloramphenicol acetyl transferase; DTT, dithiothreitol; NGFI-B, nerve-growth-factor-induced B factor; A1251, human kidney carcinoma cells; SKNBE, human neuroblastoma cells; 21-OH-ase, steroid 21-hydroxylase (EC 1.14.99.10).

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* This paper is dedicated to the memory of one of the authors, Dr. Lisa de Conciliis, who recently passed away, and who contributed actively to this study.

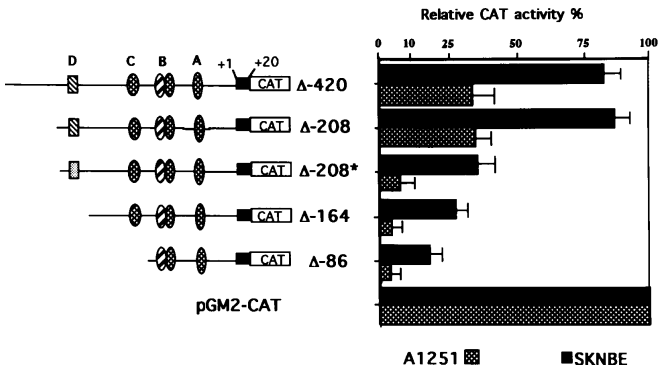

Figure 1 Transient transfection assays (TTAs) in human cell lines driven by human aldolase C gene promoter regions

Chimaeric constructs containing 420 bp, 208 bp, 164 bp or 86 bp of the promoter region fused to the CAT reporter gene were transfected into SKNBE and A1251 cells. Δ—208* contains point mutations in the core region of the binding area D, obtained using oligonucleotide L1 (see Figure 2). The solid and hatched bars show the percentage of CAT activity in SKNBE and A1251 cells respectively. The values are the means ± 2 S.D. of three different transfection experiments; relative CAT activity was normalized to the pGM2-CAT construct, which was arbitrarily assigned 100% activity. All assays were also normalized to identical luciferase activity. The protected areas A, B, C and D were identified by footprinting experiments in SKNBE cells [14] and their localization within the promoter region are shown.

-211 -184
area D

5' TGAACG	**CAAGGTCA**TGGCCAGGCTCC	3'	Oligonucleotide L1
5' TGAAGC	**AAAGGTCAGAG**	3'	21-OH-ase (-79/-62)
5'	**GAAGGTCA**TGACCTG	3'	Oligonucleotide NGFI-B
5' TGAACG	**CAA<u>CTT</u>C<u>T</u>**TGGCCAGGCTCC	3'	Oligonucleotide L1.mut.1
5' TGAACG	**CA<u>TCTG</u>CA<u>GTTT</u>C**AGGCTCC	3'	Oligonucleotide L1.mut.2

Figure 2 The sequence of the human promoter region (area D) of the aldolase C gene compared with other DNA target elements

The nucleotide sequence from —211 bp to —184 bp (oligonucleotide L1) of the human aldolase C gene, which contains the protected area D, is shown in the first line. The consensus segments present in oligonucleotide L1, in the NGFI-B oligonucleotide [25], and in the mouse steroid 21-hydroxylase (21-OH-ase) gene [26] are shown in bold type. The mutations in the DNA-binding site of NGFI-B used to create the mutated oligonucleotides, oligonucleotide L1. mut.1 and oligonucleotide L1. mut.2 are underlined.

phosphate precipitation method [17] using 10 μg of test plasmid and 1 μg of a vector carrying the luciferase gene under the control of a Simian virus 40 enhancerless promoter element [18], as internal standard for transfection efficiency. SKNBE and A1251 cells were exposed to 15% (v/v) glycerol in Hepes-buffered saline solution for 1 min, 2.5 h after transfection, and then re-fed with growth medium. Cellular extracts were prepared 32 h after transfection by several cycles of freeze–thawing. Extracts were centrifuged at 14 000 ***g*** for 15 min at 4 °C and the protein concentration determined by Bio-Rad assay. Luciferase activity was measured as described in Buono et al. [14]. Relative CAT activity was obtained by taking as 100% the acetylation value of the pGEM2-CAT vector (kindly provided by Dr. G. Morrone, Università degli Studi di Reggio Calabria, Italy) containing the Rous sarcoma virus promoter elements, which shows similar acetylation values in these two cell lines. Each transfection experiment was performed three times and gave reproducible results.

Northern blot analysis

RNA was isolated from the SKNBE and A1251 cell lines using the phenol/chloroform method [19]. Total RNA from SKNBE and A1251 cell lines (20 μg) were loaded on to a 1.5% formaldehyde gel and, after electrophoresis, transferred to a nitrocellulose membrane (Nytran; Schleicher and Schuell, Dassel, Germany). Filters were incubated at 80 °C for 2 h under vacuum, hybridized for 16 h at 65 °C in 1× Church buffer [7% (w/v) SDS/0.5 M NaH_2PO_4/1 mM EDTA]. The probe used was a 1600-bp fragment of the 3′-region of the cloned gene which included the 3′ non-coding region. A labelled oligonucleotide containing a complementary sequence of human 28S RNA was used to normalize this assay. The fragments were end-labelled according to a random priming kit procedure (Promega Corporation, Madison, WI, U.S.A.) with Klenow polymerase and [α-^{32}P]dATP nucleotide, specific activity 1×10^8 c.p.m.

Nuclear extracts, gel-retardation and supershift assays

For the electrophoretic mobility shift assay (EMSA) experiments, nuclear extracts were obtained as described by Dignam et al. [20] with slight modifications. Briefly, SKNBE and A1251 cells (at 3×10^6 cells and 2×10^6 cells/100-mm Petri dish respectively) were washed twice with cold PBS solution and pelleted by centrifugation at 1500 ***g*** for 5 min at 4 °C. The pellets were gently resuspended in solution I [10 mM Hepes, pH 7.9/10 mM KCl/1.5 mM $MgCl_2$/0.1 mM EGTA/0.5 mM PMSF/0.1 mM dithiothreitol (DTT)] and the cells were broken by passing ten times through a 25-gauge needle attached to a 1 ml syringe. The nuclear pellet was obtained by centrifugation for 30 s at 4 °C in an Eppendorf centrifuge, and resuspended in solution II, which was similar to solution I except that it contained 400 mM NaCl and 5% (v/v) glycerol and no KCl. The pellet was left on ice for 30 min at 4 °C in solution II and then centrifuged at 14000 ***g*** at 4 °C in an Eppendorf centrifuge for 45 min. The supernatant containing the nuclear extract was divided into portions and was stored at −80 °C. Probes used for EMSA experiments were prepared by labelling 3 pmol of single-stranded oligonucleotides, using 5 units of T_4 polynucleotide kinase (Boehringer) and 40 μCi of [γ-^{32}P]dATP (Amersham International, Little Chalfont, Bucks., U.K.) at 37 °C for 30 min. The labelled probes were purified by Sephadex-G50 column chromatography (Pharmacia Biotech, Piscataway, NJ, U.S.A.) and annealed to complementary strands. A sample containing 40000–50000 c.p.m. of the purified double-stranded oligonucleotides was used for each reaction. Gel-shift assays were performed as described elsewhere [21] with 3 μg poly(dI/dC) per sample as non-specific competitor. The DNA–protein complexes were separated from the unbound DNA probe by non-denaturing PAGE (4% gel) at 4 °C, in 0.5% Tris/borate EDTA buffer (44 mM Tris/HCl, 44 mM boric acid, 12.5 mM EDTA, pH 7.5). For antibody supershift analysis, 5 μl and 8 μl of anti-NGFI-B monoclonal antibody (2E1) [22] (kindly provided by Dr. J. Milbrandt, Washington University School of Medicine, St Louis, MO, U.S.A.), and 8 μl of anti human aldolase A (1A2) [23] (kindly provided by Dr. K. Hori, National Cardiovascular Research Institute, Osaka, Japan), as irrelevant monoclonal antibody, were incubated in the same binding buffer used for the gel-shift assay. The binding reaction was carried out at 25 °C for 30 min, the same conditions as used in the EMSA experiments. The probe was added to the mixture and the reaction was continued for a further 30 min at room temperature. The extract–antibody mixture was then used for EMSA experiments. DNA–protein complexes were separated by PAGE (4% gel) under non-denaturing conditions, as described above.

Southwestern analyses

A portion (50 μg) of the nuclear protein extract from SKNBE cells, obtained as described above, was mixed with three volumes of reducing buffer [100 mM Tris/HCl, pH 6.8, 20% (v/v) glycerol, 4% SDS, 200 mM DTT and 0.2% (w/v) Bromophenol Blue]. Samples were denatured for 2 min at 95 °C and proteins were separated by SDS/PAGE (10% gel) at 10 V/cm for 2 h, with 25 mM Tris/250 mM glycine, pH 8.3, and 0.1% SDS as running buffer. The separated proteins were transferred on to a nitrocellulose filter (Amersham International) by electroblotting in transfer buffer (25 mM Tris, 250 mM glycine, 20% methanol, pH 8.3). After 12 h, the blotted filter was rinsed in PBS and blocked in binding buffer [50 mM Hepes, pH 7.9/50 mM KCl/1 mM EDTA/1 mM DTT, containing 5 μg/ml of sonicated salmon-sperm DNA and 5% (w/v) of low-fat dried milk] for 2 h at room temperature. The filter was washed twice for 15 min in binding buffer without dried milk. The binding reaction was carried out in the same binding buffer containing 0.25% (w/v) low-fat dried milk (2 ml/10-cm^2 strip) in the presence of [γ-^{32}P]dATP-end-labelled oligonucleotide L1 or of oligonucleotide 21-OH-ase (see Figure 2) at a specific activity of 1×10^9 c.p.m./μg for 1 h at room temperature. The filter was washed twice with a large excess of binding buffer without milk for 10 min, dried and autoradiographed. A non-radiolabelled molecular-mass marker (Rainbow; Amersham International) was used.

RESULTS

Region D of the aldolase C promoter binds a transcriptional activator

We have shown previously that a segment of 400 bp of the human aldolase C gene promoter upstream from the major initiation start site is sufficient to confer full transcriptional activity in SKNBE cells [14]. To localize the binding sites for the possible transcriptional activators, chimaeric constructs containing 420, 208, 164 and 86 bp of the promoter region fused to the CAT reporter gene (see Figure 1) were transfected into the SKNBE and A1251 cells. As shown in Figure 3, A1251 cells contained very low levels of aldolase C mRNA when compared with the SKNBE cells. CAT activity was also lower in A1251 than in SKNBE cells with all constructs used (Figure 1). Construct $\Delta-208$, which resulted from the removal of 200 bp from the $\Delta-420$ construct, showed the same activity as clone $\Delta-420$ in both cell lines (Figure 1), indicating that the region from -420 to -208 bp cannot drive the expression of the gene.

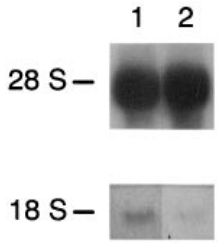

Figure 3 Expression of the aldolase C gene in different human cell lines

A Northern blot of 20 μg of total mRNA from SKNBE (lane 1) and A1251 (lane 2) human cell lines was performed (18S). The probe was a fragment of 1600 bp containing the 3′ untranslated region of the aldolase C gene, which is the most specific DNA segment of the various aldolase genes. An oligonucleotide containing a sequence complementary to human 28S mRNA was used to normalize this assay. The same exposure was used for both preparations.

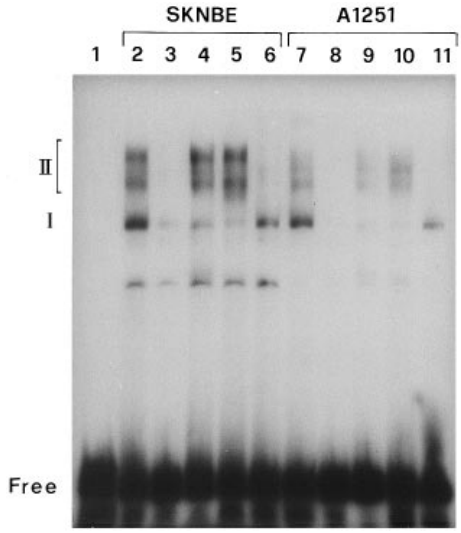

Figure 4 EMSA at the protected area D of the human aldolase C gene

Labelled oligonucleotide L1 was incubated with 6 μg of SKNBE (lanes 2–6) and A1251 (lanes 7–11) cell nuclear extracts. Competitors were added to the reaction medium at a 100-fold molar excess, before the addition of the extracts. Lane 1, no extract; lanes 2 and 7, no competitors; lanes 3 and 8 contained a 100-fold molar excess of unlabelled oligonucleotide L1 as specific competitor. Oligonucleotides L1 mut.1 and L1 mut.2 were used as competitors in lanes 4 and 9, and in lanes 5 and 10 respectively. In lanes 6 and 11, an oligonucleotide containing the core binding site for NGFI-B was used as competitor. DNA–protein complexes are indicated as I and II. Free, indicates unbound labelled oligonucleotide < 1.

On the contrary, the removal of a further 44 bp (clone $\Delta-164$) greatly decreased the activity in SKNBE and in A1251 cells (the activity in A1251 cells was always $\leqslant 30\%$ of that of SKNBE cells). This suggests that the region in which the putative transcriptional activators bind is between -208 and -164 bp in the promoter region. Previously [14], we have mapped by footprinting analysis the protected area D in this region of SKNBE cells (see Figure 1). This element AAGGTCA, depicted in bold face in the first line of Figure 2, is identical with the core recognition element AAGGTCA of oligonucleotide NGFI-B (also in bold face, third line of Figure 2) which binds the zinc-finger transcriptional activator, i.e., protein NGFI-B [24,25]. We next determined if the lack of binding to area D was responsible for the remarkable decrease in the CAT acetylation value observed when the $\Delta-208$ construct was reduced to the $\Delta-164$ construct in both cell lines. For this we used oligonucleotide mut.1 (see Figure 2) to generate the mutated $\Delta-208$*-CAT construct containing mutations in the consensus binding site of oligonucleotide NGFI-B. The CAT activity of clone $\Delta-208$* was similar to that of clone $\Delta-164$, thus indicating that the integrity of the sequence at region D is required for the optimal expression of construct $\Delta-208$ in both SKNBE and A1251 cells (the expression is invariably much higher, from 3–5 fold, in SKNBE cells).

NGFI-B binds at the region from -211 to -184 bp of the aldolase C gene promoter

A 25-bp oligonucleotide spanning from -211 to -184 bp (Figure 2) of the promoter region of the human aldolase C gene, which contains the protected area D, was used as probe in the EMSA experiments conducted on nuclear extracts from SKNBE and A1251 cells. Two retarded complexes (I and II) were observed using SKNBE and A1251 nuclear extracts respectively (Figure 4,

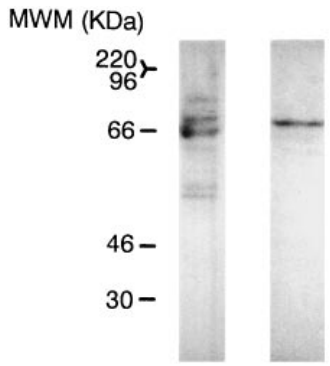

Figure 5 Southwestern analyses of the protected area D of the human aldolase C gene using nuclear proteins from SKNBE cells

SKNBE nuclear extract (50 μg) were separated by SDS/PAGE (10% gel). Oligonucleotide L1 (left) and oligonucleotide 21-OH-ase (right) were used as probes. Molecular-mass markers [MWM (KDa)] are shown on the left.

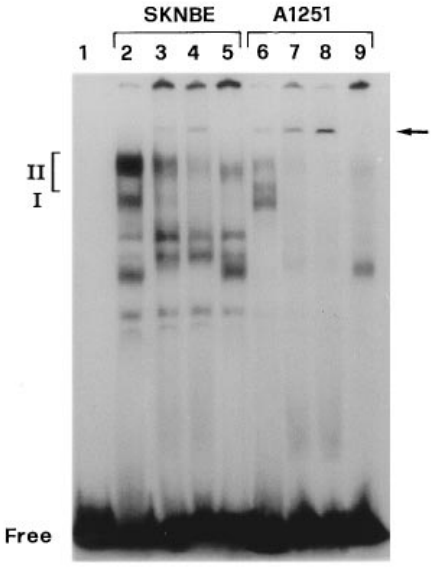

Figure 6 Supershift experiment in the presence of anti-NGFI-B monoclonal antibody

SKNBE and A1251 nuclear extracts (8 μg) were incubated with oligonucleotide L1 as probe (lanes 2 and 6), and specific complexes I and II appear. A supershifted band (arrow) is present in lanes 3 and 7 and in lanes 4 and 8, which contained 5 μl and 8 μl of monoclonal antibody anti-NGFI-B [22] respectively. No supershifted band is present in lanes 5 and 9, which contained 8 μl of the irrelevant monoclonal antibody, anti-human aldolase A (1A2) [23]. No extract was added to lane 1. Free, indicates unbound labelled oligonucleotide < 1.

lanes 2 and 7). These DNA–protein complexes disappeared when a 100-fold molar excess of the same unlabelled oligonucleotide (oligonucleotide L1) was added (Figure 4, lanes 3 and 8). Complex II (Figure 4, lane 2) was also inhibited by a 100-fold molar excess of oligonucleotide NGFI-B (see Figure 2), which contains the recognition site for NGFI-B (Figure 4, lanes 6 and 11), but not by a 100-fold molar excess of oligonucleotides containing point mutations in the NGFI-B DNA binding motif [oligonucleotide L1 mut.1 and oligonucleotide L1 mut.2 (see Figure 2), shown in Figure 4, lanes 4 and 9, and lanes 5 and 10 respectively]. Complex I (Figure 4, lane 2) was inhibited by oligonucleotide L1 (Figure 4, lanes 3 and 8) and by oligonucleotides L1 mut.1 and L1 mut.2 (Figure 4, lanes 4, 9 and lanes 5, 10 respectively), but not equally well by oligonucleotide NGFI-B (lanes 6 and 11). This might suggest the existence of a different factor that binds to longer oligonucleotides (i.e. L1 or L1 mut.1 or L1 mut.2). Furthermore, the sequence of oligonucleotide L1 is very similar to that of the segment of the mouse 21-OH-ase gene from -79 bp to -62 bp, which contains the AAAGGTCA motif and also binds the NGFI-B protein [26]. This oligonucleotide (Figure 2, 21-OH-ase) specifically competed with the DNA–protein complex obtained with the SKNBE nuclear extract when used as competitor in gel-retardation experiments with oligonucleotide L1 as probe (results not shown). These results suggest also that NGFI-B might be involved in this binding and thus be responsible for the DNA–protein complex. Again, it is noteworthy that the intensity of the retarded bands was much higher in SKNBE than in the A1251 cell line (Figure 4, compare lanes 2, 4 and 5 with lanes 7, 9 and 10 respectively). This result is in agreement with the transient transfection data obtained using the $\Delta-208$-CAT construct in the SKNBE and A1251 cells (80% and 30% respectively) and strongly suggests that the NGFI-B protein plays a functional role in the activation of the human aldolase C gene in these two cell lines.

Figure 5 shows the results of a Southwestern experiment in which the nuclear extract from SKNBE cells, after SDS/PAGE, was hybridized with oligonucleotide L1 (left) and with oligonucleotide 21-OH-ase (right). With the 21-OH-ase probe there was a single sharp band between 66 and 96 kDa, while with oligonucleotide L1 there was a series of bands in the area of the single sharp band observed with oligonucleotide 21-OH-ase. This finding indicates that the molecular mass of the binding factor is comparable with that reported for NGFI-B (between 66 and 88 kDa) [22], and supports the premise that the longer oligonucleotide L1 binds other protein factors besides NGFI-B.

To verify that NGFI-B binds to the D region of the aldolase C promoter, a supershift experiment was conducted with the monoclonal antibody anti-NGFI-B (2E1) [22], using nuclear extracts from SKNBE and A1251 cells respectively, and the oligonucleotide L1 as probe (Figure 6). Two slowly migrating DNA–protein complexes appeared that differed in intensity depending on the cell line [compare the bands in lane 2 (SKNBE cells) with the bands in lane 6 (A1251)]. The greater intensity of the supershifted band in the lanes with the larger amount of monoclonal antibody (lanes 4 and 8 versus lanes 3 and 7) excludes the possibility that this band is an artifact resulting from the addition of anti-NGFI-B monoclonal antibody to the binding mixture. Furthermore, no supershifted band was observed when an irrelevant monoclonal antibody, anti-human aldolase A (1A2) [23], was added to the binding mixture (Figure 6, lanes 5 and 9). Taken together, these results seem to leave little doubt that NGFI-B is indeed involved in the binding to area D of the aldolase C promoter region.

DISCUSSION

The regulation of the expression of the human brain aldolase C gene was studied in the human neuroblastoma cell line SKNBE and, for comparison, in the human kidney carcinoma cell line A1251. In an earlier study using the SKNBE system, we characterized four *cis*-elements within the 5′-flanking region of this gene that are involved in the regulation of transcription [14]. Three of these elements bind Sp1 or related factors and are located proximal to the major initiation transcriptional site, whereas the fourth element (area D of Figure 1) is located

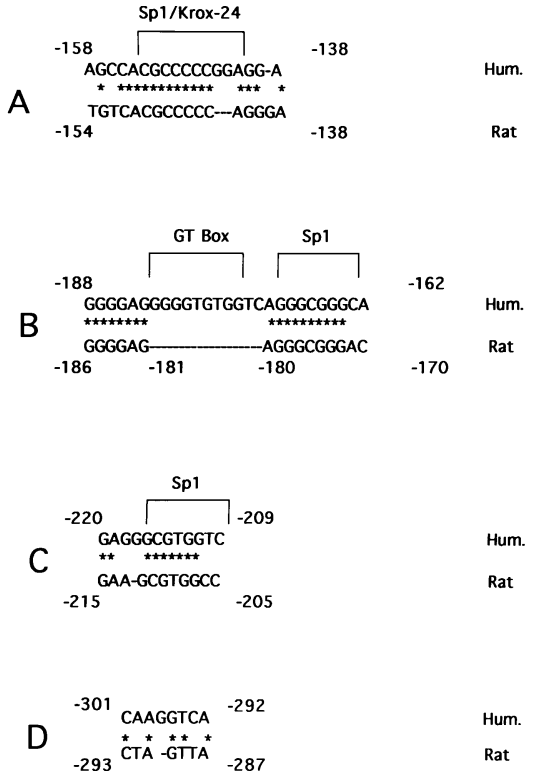

Figure 7 Sequence similarity between the promoter regions of human and rat aldolase C genes

Alignment of sequence similarity between human (Hum.) protected areas A, B, C and D of Figure 1 and the same regions of the rat aldolase C gene promoter. The nucleotide numbering along the promoter regions is according to the rat gene [12] starting from the ATG translation initiation site.

upstream and contains half of the palindromic motif known as an oestrogen-responsive element. Another research group working with the rat system found Sp1 and Krox20/Krox24 binding sites at the same location as our regions A and B (see Figure 1) and suggested that the B element could be essential for the brain-specific expression of the rat gene [27]. More recently, they also showed that another region located very close to the initiation transcription site is essential for the brain-specific expression in the same species [28].

The results reported in the present paper show that the promoter region containing areas A and B is sufficient for neuronal-specific gene expression in humans also (see $\Delta-86$ construct in Figure 1). The protected regions A and B in humans are 90% similar to the respective segments described for the rat aldolase C gene (see Figure 7, where the numbering corresponds to that used in [12] and starts from the ATG translation initiation point). However, as shown in Figure 7, the B element in humans contains a GT box of the type described by Hagen et al. [29] and by Kingsley and Winoto [30], which is lacking in the rat gene [27,28]. This difference does not appear to have any particular physiological meaning.

Using the human neuronal cell line, SKNBE, we found that the region from -208 bp to -184 bp, which contains area D, activates the transcription of chimaeric aldolase C constructs by 2.5-fold with respect to constructs that lack this DNA segment or in which it is mutated. It is interesting to observe that the region from -208 bp to -184 bp, containing the NGFI-B DNA binding motif, was located in a region with only 57% sequence similarity to that of the rat aldolase C gene [12] (Figure 7D). Furthermore, using sequence comparison, we did not find the AAGGTCA motif in the published 5′-untranslated region (900 bp) of the rat aldolase C gene. This suggests that transcriptional regulation of the aldolase C gene is different in humans when compared with rat brain tissues. In fact, although we cannot exclude the presence of a regulating region more upstream, in the human system the D element seems to be a specific target of the NGFI-B factor and is able to activate aldolase C transcription in neuroblastoma cells.

Human aldolase C is the second gene, after mouse 21-OH-ase, that has been shown to be activated by NGFI-B; this observation enlarges the physiological role of NGFI-B.

NGFI-B belongs to a family of transcriptional factors which includes NGFI-A, Krox20 and Krox24, known to have 'early responsive' genes [22,31]. An interesting feature of the NGFI-B gene is that it begins to be expressed at the embryonal 15 stage of rat brain development; it increases gradually during the growth of the foetal rat brain and during the post-natal period up to the adult stage. Furthermore, NGFI-B is expressed at low levels in many neonatal and post-natal rat tissues other than brain [32]. The expression of the rat aldolase C gene in the developing rat brain [3] parallels the expression of the NGFI-B gene. Therefore, these observations also suggest that NGFI-B might be involved in the expression of brain-specific aldolase C and that the two proteins follow the same pattern of expression during brain development.

In conclusion, the results reported in the present paper indicate that NGFI-B interacts with the promoter region of the brain-specific aldolase C isoenzyme in a human cell line, at the level of a DNA *cis*-region which is located about 200 bp upstream from the main site of the initiation of transcription. Taken together, the results suggest that this binding could be responsible for the strong activation of transcription of the aldolase C gene. The correlation between the modulation of expression of this enzyme and NGFI-B in brain tissue may provide some insights into the function of the aldolase C gene in this tissue.

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