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Promoter paper

Cloning and characterization of the human and rabbit NUDEL-oligopeptidase promoters and their negative regulation

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Abstract

NUDEL-oligopeptidase is a cytosolic cysteine peptidase, active towards oligopeptides and involved in the conversion and inactivation of a number of bioactive peptides. This protein interacts with neuronal proteins and is essential for brain development and cortical organization during embryogenesis. In this study, 5′-flanking sequences of the human and rabbit *NUDEL-oligopeptidase* gene were cloned into the pGL3 reporter gene vector and the promoter activity of the full-length fragment and deletions series was measured in transient transfection assays using two different cell lines, namely, C6 rat glioma and NH15 human neuroblastoma. Overall, a very similar pattern of promoter activity was obtained for both rabbit and human *NUDEL-oligopeptidase* promoter sequences, and their respective serial deletion constructs upon transient transfection into these cell lines. The only exception was for the longest rabbit upstream sequence that displayed about 1.8-fold higher luciferase expression upon transfection into NH15 neuronal cells than that observed upon transfection into C6 glioma cells. On the other hand, no significant difference was observed for the human longest sequence. These results are in good agreement with the expression pattern of NUDEL-oligopeptidase in human and rabbit tissues.

Keywords: Gene expression; Central nervous system; Tissue-specificity; Promoter regulation; Transcription factor

The endooligopeptidase A (EOPA) is a thiol-sensitive endopeptidase that cleaves oligopeptides of 7 to 13 amino acids residues. This enzyme was first isolated due to its ability to inactivate bioactive peptides, such as bradykinin and neurotensin, and also to convert opioid oligopeptides into enkephalins after a single peptide bond cleavage [1–3]. The EOPA is responsible for about 70% of the overall peptidase activity found in the rabbit brain cytosol [4].

Meanwhile, totally independent studies on the neuronal dynamics suggested that the role of this cytosolic oligopeptidase might not be restricted only to its proteolytic activity. Efforts to understand the molecular mechanism of brain disorders, such as the lissencephaly and Miller–Dieker syndrome, led several laboratories to screen for Lis1-interacting proteins [5,6]. Lis1 is a key component of the cell machinery involved in the intracellular transport and neuronal migration [7]. Among the Lis1-interacting proteins identified using a yeast two-hybrid system, a protein named NUDEL (Nuclear Distribution Element-Like) appeared as one of the predominant binding partners of Lis1 in the brain [5,6]. Mutated Lis1 found in lissencephalic patients fails to bind to the NUDEL protein [6]. Surprisingly, more recently, NUDEL was also identified as a protein that binds to DISC1, a protein consisting of a globular N-domain and a coiled-coil C-domain, thereby displaying the potential to

Abbreviations: EOPA, endooligopeptidase A; NUDEL, NUclear Distribution Element-Like; *r*NUDEL-oligopeptidase, recombinant NUDEL-oligopeptidase; α-*r*NUDEL-oligopeptidase, recombinant NUDEL-oligopeptidase antibody; Lis1, lissencephaly gene 1 product; DISC1, *Disrupted-In-SChizophrenia* gene product; qf, quenched fluorescence

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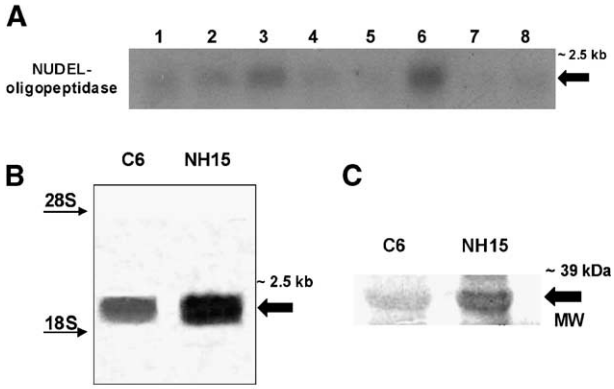
interact with other coiled-coil proteins [8]. The Schizophrenia mutant form of DISC1 also fails to bind NUDEL, indicating that the lack of NUDEL and DISC1 complex formation may contribute to this psychiatric disorder [8,9]. Interestingly, NUDEL and EOPA were found to correspond to the same protein [10]. Therefore, we recently suggested the name NUDEL-oligopeptidase for this thiol-activated oligopeptidase that is part of the cytosolic protein multicomplex involved in neuronal growth, development, motion and plasticity [10,11].

The enzymatic activity of the NUDEL-oligopeptidase was shown to be higher in rat and rabbit brain than in peripheral tissues [4,12], which is consistent with the immunochemical, Western blotting, in situ hybridization and Northern blotting analysis [4,13,14]. Surprisingly, Northern blot analysis of human tissues performed in this work showed a more widespread expression of the NUDEL-oligopeptidase (Fig. 1A). At present, little is known about the structure of the *NUDEL-oligopeptidase* gene and the regulation of its expression. Therefore, we set out to analyze the rabbit and human promoter regions. The mechanism whereby rabbit and human NUDEL-oligopeptidase expression is regulated at the transcriptional level was analyzed using reporter gene assays, demonstrating a tissue-specific regulation of the promoter activity.



D

Cell lineage	NUDEL-oligopeptidase specific activity (mU/mg)
C6	0.9
NH15	2.5

Fig. 1. Enzymatic activity measurement, Northern and Western blot analysis of the NUDEL-oligopeptidase. (A) Northern blot analysis of the human NUDEL-oligopeptidase expression using a membrane with standardized levels of poly(A)$^+$ mRNA from human heart (lane 1), whole brain (lane 2), placenta (lane 3), lung (lane 4), liver (lane 5), skeletal muscle (lane 6), kidney (lane 7) and pancreas (lane 8), hybridized with a ^{32}P-labeled human NUDEL-oligopeptidase cDNA probe. Expression of the NUDEL-oligopeptidase in the C6 and NH15 cells was determined by Northern (B) and Western blot (C) analysis using 30 μg of the total RNA and about 50 μg of protein of the cell extracts, respectively. The oligopeptidase activity of the NUDEL-oligopeptidase product was fluorimetrically assayed at 37 °C, in 50 mM Tris–HCl buffer pH 7.4, using the qf-A^{6}Bk$_{4-9}$ as the substrate (D). One unit of enzyme activity is the amount of enzyme which hydrolyses 1 μmol of the qf-substrate in 1 min.

To evaluate the expression of NUDEL-oligopeptidase mRNA in several human tissues, a membrane obtained from Clontech (Palo Alto, USA) was used for Northern blotting analysis. A single band of about 2.5 kb was observed in various human tissues. Higher levels of NUDEL-oligopeptidase expression were detected in placenta and skeletal muscle, while lower levels were observed in tissues such as heart, brain, lung, liver, kidney and pancreas (Fig. 1A).

Two distinct cell lines were selected to study the transcriptional regulation of the rabbit and human *NUDEL-oligopeptidase* gene, namely, C6 and NH15. Rat C6 glioma cells were selected since they represent a non-neuronal glial cell type and also because they were shown to express the NUDEL-oligopeptidase [15]. The human NH15 neuroblastoma cells were selected due to their neuronal origin. First, the cell lines were analyzed by Northern and Western blotting, as previously described [16], to confirm the expression of the NUDEL-oligopeptidase in these cells. The highest level of NUDEL-oligopeptidase expression was detected in the NH15 cells, while only low levels of expression were observed in the C6 cells (Fig. 1B). Only the data obtained using polyclonal antiserum raised against rat recombinant NUDEL-oligopeptidase is shown (Fig. 1C), since the α-human NUDEL-oligopeptidase showed the same result for these two cell lines (data not shown). In addition, the NUDEL-oligopeptidase-specific activity, measured by fluorimetry [17], showed a clear abundance of this enzyme in the cytosol of the NH15 cells when compared to the C6 cells, namely, 2.5 and 0.9 mU/mg, respectively (Fig. 1D). Taken together, these results confirmed the expression of the NUDEL-oligopeptidase protein in both cell lines, and a higher expression level of this enzyme was confirmed for the NH15 neuronal line compared to the C6 glioma cell line.

To isolate the 5′-flanking region of the rabbit *NUDEL-oligopeptidase* gene, approximately 10^6 phage clones from a rabbit phage genomic library (Stratagene, La Jolla, CA) were screened with a ^{32}P-labeled 5′-terminal fragment (400 bp) of the rabbit NUDEL-oligopeptidase cDNA [4]. Three clones were found with a sequence corresponding to the 5′-flanking region of the rabbit *NUDEL-oligopeptidase* gene, and the longest isolated insert was of about 3.5 kb [GenBank Acc. No. AY566289]. The DNA insert of the respective phage clone was then subcloned into the pBluescript II SK+ vector after digestion with Eco RI (construct named J1b2). The 3.5-kb genomic DNA region obtained comprised a 5′-flanking region of 1,625 bp, an exon corresponding to a 5′-untranslated region of 329 bp, and a partial intron of about 1582 bp. Fig. 2 shows the sequence of a 1111-bp segment of this genomic DNA obtained upon digestion of the J1b2 clone with *Sac*I. This fragment contains about 0.9 kbp of the 5′-flanking sequence, and about 220 bp of the first exon, where potential recognition sites for *trans*-factors such as CP2, SRY, c-Myb, Oct-1, CdxA, GATA, RORa1p, Sp1 and CREB were found after