Research Article

Evidence for posttranscriptional regulation of the multi K homology domain protein vigilin by a small peptide encoded in the 5′ leader sequence

J. Rohwedel a, *, S. Kügler b, T. Engebrecht a, W. Purschke c, P. K. Müller a and C. Kruse a

a Department of Medical Molecular Biology, Medical University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck (Germany), Fax: + 49 451 5003637, e-mail: rohwedel@molbio.mu-luebeck.de

b Department of Neurology, University of Göttingen, 37073 Göttingen (Germany)

c NOXXON Pharma AG, 13355 Berlin (Germany)

Received 7 April 2003; received after revision 12 May 2003; accepted 16 May 2003

Abstract. Vigilin, a K homology (KH) protein has been found in all eukaryotic species studied. It has a unique structure of 14–15 consecutively arranged KH domains which apparently mediate RNA-protein binding. Cloning and sequencing of the mouse vigilin cDNA confirmed that the amino acid sequences of vertebrate vigilins are highly conserved and contain conserved sequence motifs of nuclear import and export sequences. The human and murine vigilin mRNAs carry two alternatively spliced 5′ exons. In the 5′ leader region of one of the splice variants, variant 1A, we found an upstream open reading frame (uORF) highly conserved between mouse and human. Here we present for the first time evidence that a 13 amino acid long peptide encoded by this uORF is an inhibitor of vigilin expression operating on a posttranscriptional level. We propose that the two structurally different 5′ leader sequences of the human vigilin mRNA are involved in the regulation of vigilin biosynthesis.

Key words. Translation; uORF; RNA-protein interaction; KH protein; gene expression.

Proteins of the K homology (KH) domain family are RNA-binding proteins whose KH domain plays a crucial role in RNA-protein interaction [1, 2]. The KH RNA-binding motif was first described in the human heterogeneous nuclear ribonucleoprotein K [3] and was subsequently found in numerous other proteins among which are FMR1 [4], NusA [5], Mer1 [6], PSI [7] and vigilin [8]. Because the latter exhibits an accumulation of KH domains and is ubiquitously expressed in heterotrophic eukaryotic species, it has been proposed to form a separate protein subfamily [9]. Using nuclear magnetic resonance (NMR)-spectroscopy, the structure of an individual vigilin KH domain has been resolved to high resolution [2].

The vigilin sequence was first identified as a cDNA isolated from human cells [10] and from mesenchymal cells of chicken embryos [8]. The full-length protein carries 14 complete plus one degenerated, reiterative RNA-binding domains of the KH type [2, 11]. The observation that vigilin is part of a nuclear as well as a cytoplasmic multi-protein complex containing tRNA [12, 13] suggested that one cellular function of the protein might be associated with tRNA nucleocytoplasmic export. This functional concept was also in line with the observation that cellular levels of vigilin were always highest under conditions of increased protein synthesis [14], an elevated demand for tRNA molecules being presumed to be a prerequisite for stimulated protein output. Accordingly, by nuclear injection of vigilin into human Hep-2 cells, tRNA export from the nucleus could be accelerated [13]. According to our
hypothesis, quiescent cells do not require high levels of vigilin. To cope with such a situation, a mechanism might be favored by which downregulation of vigilin synthesis is achieved without wasting its own RNA. In this way, vigilin translation may be turned on again immediately when there is a demand for high protein synthesis. Genes are regulated at multiple steps such as transcription, post-transcriptional processing or translation by diverse mechanisms [15, 16]. Specifically, in about 10% of eukaryotic mRNAs, AUG codons located upstream of the cognate start codon are apparently involved in translational regulation mainly of genes that play a role in cell growth and maintenance [17, 18]. In some rare cases, such upstream AUGs are associated with an upstream open reading frame (uORF) which represses downstream translation [19]. Structural analysis of the human vigilin gene showed the presence of two additional 5' exons compared to the chicken gene, designated exon 1A and 1B, which are alternatively spliced [20], though their function, if any, has yet to be elucidated. Here, we present the complete coding sequence of the mouse vigilin gene and further corroborate that vigilin is highly conserved from human to yeast. In particular, an uORF located at the 5' end of exon 1A is also conserved between human and mouse. We provide the first evidence that a 13-amino-acid long peptide encoded by this uORF of the vigilin mRNA may interfere with vigilin translation.

Materials and methods

Cloning and sequencing of mouse vigilin cDNAs
Total RNA was isolated from mouse liver using the Rneasy Midi kit (Qiagen) and 500 ng of total RNA was reverse transcribed using oligo-dT primer and SuperScript II reverse transcriptase according to the manufacturer's recommendations (Life Technologies). To amplify the complete vigilin splice variants carrying either exon 1A or 1B, specific oligonucleotide primer sequences were deduced from the published human sequences [20] as follows (designations and sequences of oligonucleotides are given in parentheses followed by the length of the amplified fragment): exon 1A sense primer (primer 1: 5' CAGAC 3', 4331 bp), exon 1B sense primer (primer 2: 5' CCTCGGAGGCTCCGCTTC 3', 4301 bp) and the same antisense primer (primer 3: 5' CCATCTTCTGCATG 3'). The resulting fragments VIG-1A and VIG-1B from the respective nucleotide 1 ends of human vigilin cDNA were cloned in front of the complete human vigilin cDNA consisting of the AUG in exon 2 down to the stop codon in exon 27. To this end the complete human vigilin cDNA was cloned into the HindIII and XhoI sites of the plasmid vector pAD-CMV (Stratagene). One microgram of total human RNA, isolated from the cell line MG63, was reverse transcribed with Superscript Reverse Transcriptase (Life Technologies) according to the manufacturer's recommendations with 10 pmol of vigilin-specific primer E16as (5' CTGATGAGCCTGATCAGAC 3'). Sense primers for the following PCR were 1A-HIII (5' ATCGTAAAGCTTTACCAAGCGTAGCCTCTTCTCTTTTAC 3') for the human vigilin splice variant with exon 1A (VIG-1A) and 1B-HIII (5' ATCGTAAAGCTTTACCAAGCGTAGCCTCTTCTCTTTTAC 3') for the human vigilin splice variant with exon 1B (VIG-1B); the antisense primer for both reactions was E13as (5' CGTGATGAACTTTACCAAGCGTAGCCTCTTCTCTTTTAC 3'). The resulting fragments VIG-1A and VIG-1B were cut with HindIII and BsmBI, and after gel purification cloned into the appropriate sites of the pAD-CMV vector containing the complete human vigilin cDNA. The correct orientation of the fragments was confirmed by sequencing.

Site-directed mutagenesis
To eliminate the first AUG of the uORF in VIG-1A, site-directed mutagenesis was performed on the VIG-1A construct with primers GUG-s (5' CTTCTCTTTTACCAAGTTGACCGCTTGTCTCCCTG 3') and GUG-as (5' CGGACAGCCGCCCTGCTGAAGAGAAAG 3') using the Quickchange kit (Promega) according to the manufacturer's instructions. The result was confirmed by sequencing. The mutant construct will be referred to as VIG-1A-GUG.

Cloning of the β-Gal reporter constructs
For generation of reporter gene constructs, the 5' ends of VIG-1A and VIG-1B from the respective nucleotide 1 down to the AUG in exon 2 were cloned 5' to β-galactosidase into the pAD-βGAL vector. To achieve this, PCR was performed on the VIG-1A-, VIG-1B- and VIG-1A-GUG constructs with sense primers 1AHHIII and 1BHHIII, and with the antisense primer E2as-Kpn (5' ACTGCAGTGACCCTGAGCTTATGACTCACCTAC 3'). This primer contains an artificially introduced KpnI.