Research

Genomic structure of the gene for mouse germ cell nuclear factor (GCNF)

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Abstract

Background: The germ cell nuclear factor (GCNF, also known as retinoid acid receptor-related testis-associated receptor, neuronal cell nuclear receptor or NR6A1) is an orphan receptor in the nuclear receptor superfamily found in mammals, amphibians and fish. The mouse *Gcnf* gene is expressed in the placenta and the developing nervous system and germ cells, and responds to retinoic acid.

Results: We have defined the intron-exon structure of the mouse *Gcnf* gene and found that it contains 11 exons. Exons 1-4 encode the 75 amino acid amino-terminal domain and exon 4 also encodes the core DNA-binding domain. The carboxy-terminal extension is encoded by exon 5, exons 6 and 7 encode the hinge region, and exons 7-11 encode the putative ligand-binding domain. Unusually, the two zinc-finger motifs in the DNA-binding domain are encoded by separate exons.

Conclusions: The protein-coding region of GCNF is contained in 11 exons. The genomic structure of this nuclear receptor gene will be useful for further studies.

Background

The germ cell nuclear factor (GCNF, NR6A1) is a member of the nuclear receptor superfamily [1,2]. Originally isolated from mouse cDNA libraries, homologs of GCNF have been identified in humans, frogs and fish [3-6]. As no ligand has been identified, GCNF is designated an orphan receptor. Also known as RTR (retinoid acid receptor-related testis-associated receptor) or NCNF (neuronal cell nuclear receptor), evolutionary studies have defined GCNF as the only known member of a sixth subfamily of nuclear receptors [7-9]. The mouse *Gcnf* gene is highly expressed in the developing nervous system, in the labyrinthine layer of the placenta and in the developing germ cells [8,10-12]. Two transcripts of approximately 7.5 kb and 2.4 kb are present in testis, but only the larger transcript is found in somatic cells. Hybridization experiments reveal that the size difference is at least partially due to the use of different polyadenylation sites [13]. Interestingly, GCNF expression is transiently up-regulated and later down-regulated again when embryonal carcinoma cells are triggered to differentiate by retinoic acid [14-16].

Results and discussion

We have isolated genomic clones encompassing the mouse *Gcnf* gene, and have defined the intron-exon structure of the gene. Sequence analysis reveals that the coding region of *Gcnf* comprises 11 exons and 10 introns (Table 1). A bacteriophage lambda library and a cosmid library of genomic DNA of the mouse 129 strain were screened with the full-length *Gcnf* cDNA. The DNA from colonies that hybridized was cloned into pBluescript (SK) for further sequence analysis. Exons 3 and 4 were identified from bacteriophage subclones,

Table 1

Organization of the mouse *Gcnf* gene.

Exon number	Exon size (bp)	cDNA position*	5′ splice donor	Intron number	Intron size (kbp)	3′ splice acceptor
1	>344	1-344	**CCGCGCAACGgtgggta**	1	ND	**ctattgttctctctttagGTTTCT**
2	42	345-386	**CCAGGCACTAgtaagttc**	2	>12	**gttctttttgctttgcagATGGAG**
3	45	387-431	**CATATACCTGgtaagtgg**	3	ND	**tgacttatccatgtttagTTTCCG**
4	243	432-674	**AACAGGAAGGgtgagttg**	4	>12	**gtctacatttccttctagCTATCA**
5	56	675-730	**ACCAGTCCAGgtgagtcc**	5	ND	**atccatttcttgccaaagATATCA**
6	155	731-885	**TATCATCCAGgtgagcta**	6	ND	**tgaagtttttctctccagTAGGTC**
7	228	886-1113	**TTGAAGATGGgtgagtta**	7	1.238	**tcctgtccctgccccccagGTATGC**
8	255	1114-1368	**AACTCCACAGgtgagagc**	8	ND	**cctgtatctgttctccagATTTAG**
9	122	1369-1490	**CTGAATCAAGgtgagtag**	9	1.408	**ttttgtttttgttttcagATATCA**
10	153	1491-1643	**TACATCGCAGgtaatatt**	10	1.567	**tctcttccctttacctagGCAAGA**
11	>869	1644				

Lower-case letters are used for the intron sequence and capital letters for the exon sequence. The GenBank accession numbers for the exons and the flanking sequences are AF254575S1-AF254575S8. *Relative to GenBank entry MMU09563.

and exons 6-11 were identified in cosmid-derived subclones. Additional intron-exon boundaries and the 5′-untranslated region (5′-UTR) were identified by genome walking analysis following the manufacturer's instructions (Clontech). DNA sequencing was performed on an ABI 377-sequencer using the dye terminator protocol (Perkin Elmer) and on a DNA sequencer model 400 (Li-Cor). The DNA sequences were processed using the Wisconsin Package Version 10.0 of the Genetics Computer Group (GCG), Madison, Wisconsin.

All intron-exon junctions obeyed the GT/AG rule ([17] and Table 1). The location of the intron-exon junctions relative to the peptide sequence is shown in Figure 1. The translational start and stop codons are on exons 1 and 11, respectively. Exon 1 contains the 244 bp untranslated sequence at the 5′ end of the cDNA and codes for the first 33 amino acids (Figure 2). This cDNA, isolated by Hirose *et al.* ([7]; GenBank entry MMU09563), starts with an *Eco*RI site that is present

```
MERDERPPSG GGGGGGSAGF LEPPAALPPP PRNGFCQDEL AELDPGTNGE
TDSLTLGQGH IFVSVPDDRA EQRTCLICGD RATGLHYGII SCEGCKGFFK
RSICNKRVYR CSRDKNCVMS RKQRNRCQYC RLLKCLQMGM NRKTAIREDGM
PGGRNKSIGP VQISEEEIER IMSGQEFEEE ANHWSNHGDS DHSSPGNRAS
ESNQPSPGST LSSSSRSVELN GFMAFRDQYM GMSVPPHYQY IPHLFSYSGH
SPLLPPQARS LDPQSYSLIH QLMSAEDLEP LGTPMLIEDGTYAVTQAELFA
LLCRLADELL FRQIAWIKKL PFFCELSIKD YTCLLSSTWQ ELILLSSLTV
YSKQIFGELA DVTAKYSPSD EELHRTFSDEG MEVIERLIYL YHKFHQLKVS
NEEYACMKAI NFLNQDIRGL TSASQLEQLN KRYWYICQDF TEYKYTHQFN
RFPDLMMCLP EIRYIATGKMV NVPLEQLPLL FKVVLHSCKT STVKE
```

Figure 1
The location of the different exons in the GCNF amino-acid sequence. The core DNA-binding domain is underlined.

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TTGGGTTCTC CCTACTTAGG TCTTCCTGTT TTTTTTCCAT CACCCCTTTA TTTGGTAGAG
TCCCGTGTGG GCAGCCTCGT TGGGAGGACT ACATTTCCCA GAATTCCTCA CGGGCATGTG
CGTGGCAGCG GCGCGTGACG TCAGAGGAGG GAGCTGGCCA GTGCTGAGGG GGCGCGGCGC
GGAGGGGCGC GGAGCCGGGC GGCTCAGGGG CCCAGAGAGT GCGGCGGCCG AGAGCCTGCC
GGCCCCTGAC AGCCCCCTCC CCCCGTGGAA GACCAGGACG ACGACTACGA AGGCGCAAGT
CATGGCGGAG CAGCGAAGCC CGAGAGGGCC CTGAGCACCG CCGCATGGAG CGGGACGAAC
GGCCACCTAG CGGAGGGGGA GGCGGCGGGG GCTCGGCGGG GTTCCTGGAG CCGCCCGCCG
CGCTCCCTCC GCCGCCGCGC AACG
```

Figure 2
Sequence of exon 1 of *Gcnf*. The location of the *Eco*RI site (GAATTC) marking the 5′-end of the *Gcnf* cDNA (GenBank entry MMU09563) and the putative translational start codon (ATG) are underlined.

in the genomic DNA. The T at position 174 is a G in our genomic isolate, which could represent a genomic variant. As no promoter has been identified for *Gcnf*, the sequence preceding the *Eco*RI site may contain promoter elements. It is also possible, however, that the promoter precedes a not-yet-identified additional exon in the 5′-UTR of *Gcnf*.

The amino-terminal domain of 75 amino acids is encoded by exons 1-4. Exon 4 also codes for the core DNA-binding domain (DBD) of 66 amino acids and for three additional amino acids (Figure 1). The DBD consists of two zinc-finger motifs that are encoded by separate exons in most vertebrate nuclear receptor genes, except for those of the COUP transcription factor subfamily. Evolutionary studies do not provide further evidence that these receptors are closely related to GCNF. A further domain important for DNA binding and for homodimeric interactions, and known as the DBD carboxy-terminal extension, is encoded by the 56 bp of exon 5. The sizes of intron 2 and intron 4 were determined by

PCR amplification of mouse genomic DNA. Exons 6 and 7 code for the hinge region, whereas exons 7-11 code for the putative ligand-binding domain. A variant of the typical AUAAA polyadenylation signal (AGUAAA) and the cleavage site that is used in the testis are part of the eleventh exon [13].

Conclusions

The protein-coding region of GCNF is contained in 11 exons. Additional studies will be required to define the regulatory/promoter region. We think the genomic structure of this first, and at present only, member of the sixth subfamily of nuclear receptors will be useful for further studies of this unique receptor.

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