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Research Article

Gene structure of the murine 2′-5′-oligoadenylate synthetase family

S. Eskildsen [a], R. Hartmann [b], N. O. Kjeldgaard [a] and J. Justesen [a,*]

[a] Department of Molecular and Structural Biology, University of Aarhus, CF Møllers Allé 130, 8000 Aarhus (Denmark), Fax + 45 86196500, e-mail: jj@mbio.aau.dk
[b] Department of Molecular Cardiology, Lerner Research Institute, The Cleveland Clinic Foundation, Ohio (USA)

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Abstract. The 2′-5′-oligoadenylate synthetases (OASs) are members of a family of interferon-induced proteins playing an important role in the antiviral effect of interferons as well as being involved in apoptosis and control of cellular growth. Based on sequence data from the murine BAC clone (RP23-39M18), and a number of EST and IMAGE clones and the Celera Mouse database, we identified twelve *Oas* genes in the mouse genome, all localized to the chromosome 5F region. In contrast to the single *OAS1* gene found in humans, we identified eight closely linked *Oas1* genes in the murine genome, together with the genes of *Oas2* and *Oas3*. Compared to the single *OASL* gene found in humans, two genes of OAS-like proteins, *Oasl1* and *Oasl2*, were identified. All the putative genes seem to be transcribed.
The exon/intron structures of the murine *Oas* genes were found to be identical to those of the human genes.

Key words. Mouse; chromosome 5; oligoadenylate synthetase; Oas; Oasl; *Oas* genes; exon structure.

Introduction

The 2′-5′-oligoadenylate synthetases (OASs) belong to a family of interferon-induced antiviral proteins, which are highly induced by type I interferons and to a lesser extent by type II interferon. The OAS proteins are produced as latent enzymes, which must bind double-stranded RNA (dsRNA) in order to form the enzymatic active complex, producing 2′-5′-oligoadenylates (2-5A). The dsRNA is produced in virus-infected cells either as a part of viral replication or because of symmetric transcription of the viral genome, allowing the OAS enzymes to sense and react to viral infection of a cell. The 2-5A produced by the activated OAS binds to the latent endoribonuclease RNase L leading to the dimerization and activation of the RNase L, followed by degradation of cellular and viral RNA [1]. Activation of the OAS system leads to the shut down of protein biosynthesis and blocking of viral proliferation. The OAS system also plays a role in the induction of apoptosis and regulation of cell growth. This occurs both through direct interaction with the antiapoptotic protein Bcl2 and indirectly through the inhibition of protein biosynthesis [2–5]. Finally, recent data from an NIH lead collaborative effort showed that mutations in the RNase L gene correlate with family forms of prostate cancer, implying a role for the 2-5A system in tumorigenesis [6].
The human genome contains four genes belonging to the *OAS* gene family, *OAS1*, *OAS2, OAS3* and *OASL*. The genes encoding the three OAS proteins, small, medium and large, are situated within a 120-kb segment on 12q24.1 in the order *OAS1*, *OAS3*, *OAS2* (centromeric to telomeric), whereas the *OASL* gene is located 19 megabases towards the telomeric end of chromosome 12 (fig. 1). Several other mammalian cDNAs encoding OAS1 proteins have been characterized (mouse, rat, woodchuck and pig) as well as

* Corresponding author.

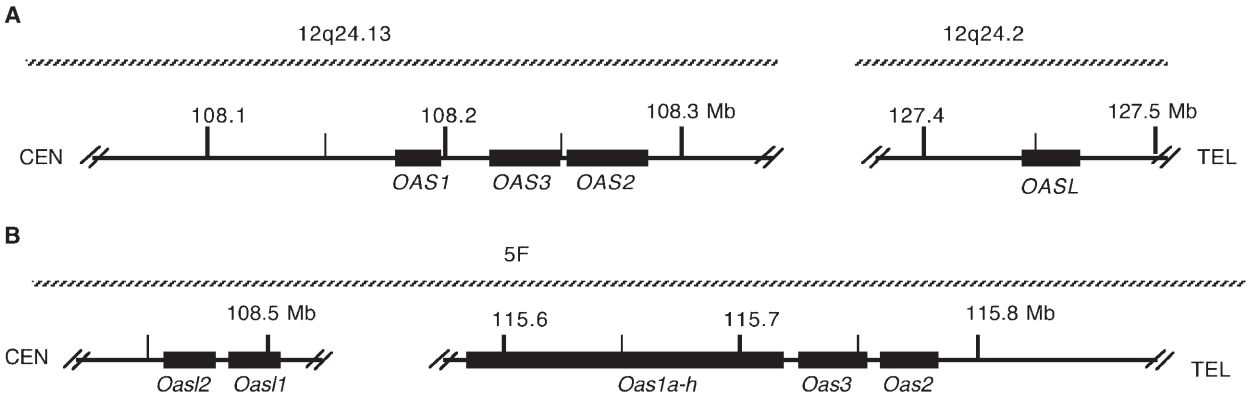

Figure 1. Chromosomal locations of the genes of the OAS family (CEN, centromere; TEL, telomere). (*A*) Human chromosome 12. Positions of the genes in Mb according to *Homo sapiens* Map View, build 28 of 28 December 2001. (*B*) Mouse chromosome 5F. Positions of the genes in Mb according to the Celera Mouse Database, December 2001. All eight *Oas1* genes described in this paper are located within the 150-kb *Oas1* region indicated.

cDNAs of some non-mammalian oligoadenylate synthetases (chicken, and the marine sponge *Geodia cydonium*). Further details about the OASs and their function are given in recent reviews [7–10].

All members of the human *OAS* gene family share the same general build up consisting of a basic exon/intron unit of five translated exons: exons A–E. This unit is found once in the *OAS1* and *OASL* genes, is repeated twice in the *OAS2* gene and three times in the *OAS3* gene. In all *OAS* genes, the terminal exon E is followed by a C-terminal exon (exon T). We have suggested that the exon/intron structure observed in the human *OAS* genes might exist in other species as well [7].

In the mouse, several members of the OAS family have been characterized. In interferon-induced Ehrlich ascites tumor cells, two different size classes of murine OAS mRNAs were identified which, in *Xenopus* oocytes, could be translated into a small- and a large-molecular-weight OAS protein [11]. By cross-hybridization to a human *OAS1* clone, Ichii et al. [12] isolated a cDNA clone (GenBank no. X04958) from interferon-treated mouse L-cells with strong homology to the human *OAS1* clone [12]. Three further murine cDNA clones were isolated, the *L3* from interferon-treated L-929 cells (GenBank no. M33863) [13], the *L2* (GenBank no. X58077) and the *L1* clone (GenBank no. X55982) from JLSV9R cells [14]. The *L3* clone codes for a protein identical to that of the Ichii clone and except for some minor differences in exons C, D and E, *L3* and *L2* are also identical. The cDNA variant, *L1*, is incomplete and only contains the sequences corresponding to exon B and part of exon C. The amino acid sequence of *L1* is sufficiently different from that of *L3* to suggest that it represent another *OAS* gene.

Yet another mouse cDNA clone, termed *Oasl5* (GenBank no. AB067528) coding for a protein homologous with the other OAS1 proteins, but without 2-5A synthetase activity was isolated by Shibata et al. [15].

Two variant murine *OASL* cDNA clones have been described, the p54 clone (GenBank no. AF068835) [16] and the adult male tongue cDNA clone 2310065A10 (GenBank no. AK010034) [Adachi et al. unpublished data]. The open reading frames of these two cDNA clones are largely identical and both have C-terminal sequences with ubiquitin homology.

Recently, the sequences of seven murine cDNA clones (GenBank no. AB067529–AB067535), belonging to the *Oas* gene family, were released [Kakuta et al. unpublished data]. Four of the clones show homology with the Oas1 proteins, and orthologs to the human proteins OASL, OAS2 and OAS3 are each represented by a single cDNA clone (table 1).

The increasing amount of sequence data available for the mouse genome has created the possibility to construct a detailed map of the genes of the *OAS* family in mice, which we found localized to murine chromosome (Chr.) 5F (fig. 1). This search confirmed our earlier suggestion that the exon/intron structure of the *OAS* genes known from the human genome also exists in the mouse. However, here we found a more complex picture, with eight copies of the *Oas1* gene within a 150 kb-region of Chr. 5. All of these *Oas1* genes seem to be actively transcribed upon interferon stimulation, compared to the single *OAS1* gene in humans, which, however, is expressed as four splice variants. In a neighboring 50-kb region, the murine *Oas2* and *Oas3* genes were identified, both with exon/intron structures identical to those of the human genes. Furthermore, two copies of the *Oasl* gene were identified located around 7 Mb centromeric to the *Oas* genes. Knowing the full build-up of both the human and mouse *OAS* gene families will allow us to gain insight into the evolution of multigene families and, eventually, to construct knock-out mice to delin-

Table 1. Summary of the murine *Oas* genes.

Gene	Name*	Identities†	GenBank accession numbers	IMAGE clone‡	EST‡
Oas1a	L3	71%	M33864		
Oas1b	L2	71%	X58077		
Oas1c	L1	60%	X55982, AB067529	AI386448, BF136699	
Oas1d	Oasl5	53%	AB067528,		
Oas1e	Oasl5 hom	52%	AB067531		
Oas1f	Oasl5 hom	53%	AB067532		
Oas1g	Oasl5 hom	52%	AB067530		
Oas1h		57%			
Oas2		61%	AB067535	AW230357	BE281841, BI111250 BI647382
Oas3		65%	AB067534	BC016461, BE283110 BB868908 BI159457, BI158026	BI651424,
Oasl1		74%	AB067533, AY089728	BE136926	
Oasl2	Oasl2	49%	AF068835, AK010034		

* Traditional names of cDNA clones.
† Per cent amino acid identities of murine to human exons A–E.
‡ IMAGE and f EST clones used in identification of the exons.

eate the cellular functions of the OAS proteins which, until now, are rather poorly described.

Materials and methods

Sequencing of the IMAGE clone encoding *Oasl1* cDNA

IMAGE clone 1546490 (GenBank no. BE13926) encoding the *Oasl1* cDNA (The I.M.A.G.E. Consortium) was sequenced by the primer-walking method [17], using the following primers:

1) 5′-AATACGACTCACTATAG-3′ (T7 primer situated in the IMAGE vector)
2) 5′-ACAGAGTACCCAGTAGTCTCA-3′ (409–429)
3) 5′-TCCTCTGTATCTACTGGACCAA-3′ (823–844)
4) 5′-GGCCTGCAGCGTCTGTCCTTTCAG-3′ (1179–1202)

Position numbers (in parentheses) correspond to GenBank accession no. AY089725.
Polymerase chain reactions (PCR) were carried out with 25 cycles of denaturation at 95 °C for 20 s, annealing at 50 °C for 15 s and extension at 60 °C for 60 s. Sequencing was performed on an Applied Biosystem ABI PRISM 3100 Genetic Analyser using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech). The sequence is submitted under GenBank accession number AY089728.

Cell line and extraction of RNA

Mouse L-929 cells (DSMZ: ACC.2) were cultured as monolayers in RPMI 1640 medium (Life Technologies) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Life Technologies). The cells were either untreated, treated with murine interferon-α (500 U/ml) or interferon-γ (100 U/ml) for 24 h and harvested by a rubber policeman in cold PBS. Total RNA was purified from L-929 cells using the Midi RNEasy purification kit (Qiagen) according to the manufacturer's instructions.

RT-PCR for detection of transcription of the *Oas* genes

Reverse transcription was performed on 5 µg of total RNA from untreated or interferon-treated cell extracts using the first-strand cDNA synthesis kit from Amersham Pharmacia Biotech. The primers specific for each gene and the temperatures used as well as the expected size of the PCR products are indicated in table 2. The PCR was carried out with 30 cycles of denaturation at 96 °C for 30 s, annealing at the given temperature for 30 s, and extension at 72 °C for 2 min 30 s.
As a control, a PCR was performed with the same cDNA with primers in the murine *β-actin* gene (GenBank M12481). Ten microliters of the PCR products was loaded on a 1% agarose gel containing ethidium bromide.

Sequence analysis

Several databases have been used in this study: the standard nucleotide-nucleotide BLAST as well as the pairwise BLAST (http://www.ncbi.nlm.nih.gov/BLAST/); the Trace database (http://www.ncbi.nlm.nih.gov./blast/mmtrace.html), the Celera database (http://www.celera.com/) and the ClustalW sequence alignment (http://www.ebi.ac.uk/clustalw/).

Table 2. Primers for *Oas* genes.

Gene	Forward and reverse primer	PCR product (bp)	Temp (°C)
Oas1a	5′-CTGGGTCATGTTAATACTTCC-3′		
	5′-CACTCGGGAACCATCCTTTTTA-3′	525	56.9
Oas1b	5′-TTTGATGTCCTGGGTCATGG-3′		
	5′-CACTCGGGAACCATCATTTTTC-3′	525	57.8
Oas1c	5′-AGTCTTCCCAAAGATGAAATG-3′		
	5′-CTGAGGAGCATCCCGGCCTCG-3′	1125	59.1
Oas1d	5′-CTCTGCAGCATCCAAGCCAGG-3′		
	5′-CGACAAATCCCAGGTGGG-3	1025	59.1
Oas1e	5′-CCAATCTGGAGGCTGGATAAC-3′		
	5′-TACCGATTCCCAGCCATT-3′	1040	59.1
Oas1f	5′-CTCTGGCATCACTTCCCAGGC-3′		
	5′-TCCATAAGTGCTTTCCTCATG-3′	935	56.0
Oas1g	5′-GTTTAAACCAGCCACGTTCCA-3′		
	5′-CGGATTGCTCTCTGCAGCACT-3′	960	57.8
Oas1h	5′-CGGCACACCCCAGGGGCC-3′		
	5′-CTTAGCAGCACCCCAGCCTGT-3′	1040	64.2
Oas2	5′-ATGGGAAACTGGCTGACTGGA-3′		
	5′-TCACTTAGTAACTCCACCCAC-3′	2228	64.2
Oas3	5′-ATGGACCTGTTCCACACGCCA-3′		
	5′-TCACACAGCGGCCTTTACCGG-3′	3416	67.0
Oasl1	5′- ATGGCAGTCGCCCAGGAGCTG -3′		
	5′- GCTGGGCACAAATGGAGCTGC -3′	1535	59.1
Oasl2	5′-ATGGACCCGTTCCCGCACCTG-3′		
	5′-CTAGTCGATTACCCTAATGAG -3′	1526	59.1
β-actin	5′-CATCGTGGGCCGCTCTAGGC -3′		
	5′-GCTTGCTGATCCACATCTGC -3′	980	64.2

The BoxShade server was used to emphasize the conserved amino acids in figures 3, 7 (http://www.ch.embnet.org/software/BOX_form.html).
When searching for homologies with the human *OAS1* gene, we used the sequences of GenBank accession no. D00068.

Results

Below we present a summary of all members of the murine *OAS* gene family including their genomic locations as shown in fig. 1 and table 1. Novel names are suggested for some of the murine *Oas1* and *Oasl* genes.

Identification of the *Oas1* genes

The nucleotide sequences of the four previously described murine Oas1 clones were used in a BLAST search against the BAC clone RP23-39M18 (GenBank no. AC015535.5). This led to the identification of a rather large number of murine *Oas* exons, permitting us to characterize eight members of the *Oas1* gene family of *Mus musculus* termed *Oas1a*, *Oas1b*, *Oas1c*, *Oas1d*, *Oas1e*, *Oas1f*, *Oas1g* and *Oas1h* (table 1, fig. 2).
The latest release of the nucleotide sequence of the BAC clone RP23-39M18 (GenBank no. AC015535.5) with a total of about 270 kb was submitted in December 2000 by the Whitehead Institute/MIT Centre for Genome Research. This sequence release consists of 14 unordered contigs as indicated in fig. 3A. Contig 1: (nt. 1–51,876), contig 12: (nt. 171,141–207,660) and contig 13: (nt. 207,760–270,196) were identified as harboring *Oas* exons highly homologous with the exons A–E of the human *OAS1* gene, localized in exon units (exons A, B, C, D, E) corresponding to the build-up of the human gene (fig. 2).
Using the sequences of the C-terminal exons (exT) of the L2/L3 cDNA clones in BLAST searches revealed the locations of the *Oas1a* and *Oas1b* genes in an area containing two 18-kb repeat sequences (fig. 3A). A BLAST search using exon T of the Oasl5 clone revealed the presence of three homologous terminal exons corresponding to the genes *Oas1d*, *Oas1e* and *Oas1f*. To identify all exons of the *Oas1c* gene, the sequence of the L1 cDNA clones as well as the IMAGE clones BF136699 (exons A–D) and AI386448 (exons D–T) were used in the BLAST search. Similarly, the complete cDNA sequence of the recently released clone (GenBank no. AB067530) [Kakuta et al., unpublished data] corresponding to *Oas1g* was used to locate the corresponding C-terminal exon. The detailed exon/intron structure of the *Oas1* genes (*Oas1a–g*) on the BAC clone is shown in figure 2.
In this manner we identified seven *Oas1* genes, with a complete set of exons (A, B, C, D, E, T) and a single incomplete gene, *Oas1h*, with exon groups (A, B, C, D, E), but without an identified T exon. Furthermore, we identi-

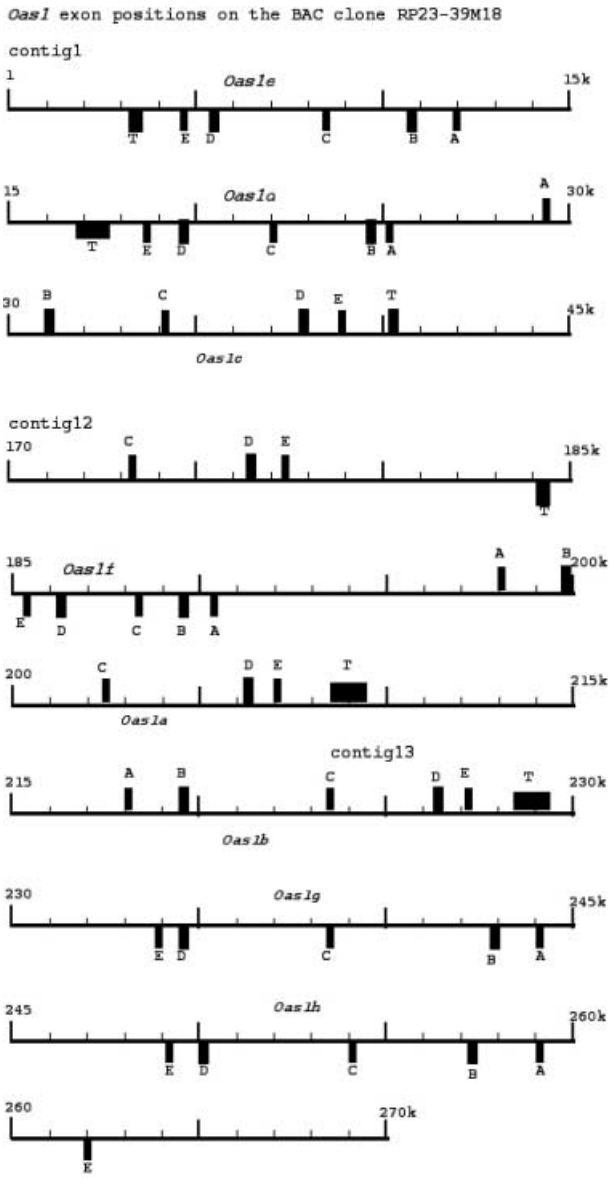

Figure 2. Exon/intron structure of the *Oas1* genes. The exons were identified on the BAC clone RP23-39M18 using the clones indicated in table 1.

fied four isolated exons, a group of exons C, D and E, and a single exon E homolog.

In figure 4, the amino acid sequence coded for by each of the murine *Oas1* exons is shown aligned with the sequences of the human *OAS1* exons. This clearly demonstrates that within each group of exons, there are high degrees of homology between the different *Oas1* genes.

To identify the correct genomic position of the *Oas1* genes, we tried to establish the exact order and orientation of the various contigs of the BAC clone, RP23-39M18.

The relative positions of contigs 12 and 13 (fig. 3 A) seem to be correct, as we identified a duplication of an 18-kb segment of which one copy crosses the boundary between contigs 12 to 13. These duplicated regions (nt 192,019–210,328 and nt. 212,760-230,368) have almost identical nucleotide sequences, and both hold a copy of an *Oas1* gene, corresponding to the *Oas1a* gene and the *Oas1b* gene, respectively.

Search of the Trace database revealed a clone (914652 ml1B-a2396d07.plc) extending beyond the 5′ end of contig 12. The nucleotide sequence of this clone showed that contig 12 is preceded by contig 7 in opposite orientation, as indicated in figure 3 B.

The chromosomal markers D5Mit368, C87484 and C85127 were found to be present in the BAC clone RP23-39M18 locating the *Oas1* genes to murine Chr. 5F (fig. 3 A, B).

The entire *M. musculus* genomic nucleotide sequence is commercially available at the Celera Mouse Genome Database (http://www.celera.com). A BLAST search showed that the *Oas1* genes are to be found on a Chr. 5 scaffold at 115.32–115.82 Mb (GA_x5J8B7W3GVH: 2500001–300000), where they span a 150-kb region around 115.6 Mb. The sequences of the contigs of the RP23-39M18 BAC clone are identical to the genomic sequence except for minor, mostly single nucleotide differences. In the chromosomal sequence, contig 1 appears to be located upstream of contig 13 but in the reverse orientation (fig. 3 B).

The genomic localization of the *Oas1* genes and the few isolated exons is shown in fig 3 B. Of the eight *Oas1* genes, four are transcribed in the centromere to telomere direction and four in the opposite direction (fig. 3 A, B).

Using primers specific for each of the *Oas1* genes in RT-PCR on RNA isolated from interferon-α-treated L929 cells, we have been able to demonstrate the presence of transcription products of seven of the *Oas1* genes (fig. 5). In interferon-γ-treated cells, only the transcripts of *Oas1a*, *Oas1b*, *Oas1c* and *Oas1d* were identified (data not shown). No transcription product of the *Oas1f* gene was observed by the amplification of cDNA from either of the interferon-induced L929 cell cultures. However, a cDNA clone from polyI:polyC-stimulated C57BL/6J ovary cells representing the *Oas1f* gene was recently reported by Kakuta et al. (GenBank AB067532). The rather large number of different *Oas1* cDNA clones so far isolated (table 1), together with our transcription data, indicates that all of the murine *Oas1* genes identified above can be transcribed in murine cells.

Except for the *Oas1h* gene, all of the other *Oas* genes were represented as cDNA clones from the C57BL/6J strain (GenBank no. AB067529–AB067531). However, this does not necessarily mean that all genes are translated into functional proteins. In the case of *Oas1c*, a stop codon is present within the open reading frame of exon D in all published sequences (the BAC clone, the Chr. 5, AI386448 and AB067529), as indicated by as asterisk in figure 4. Whether such a short version of an oligoadenylate synthetase (28.6 kDa) does exist in the mouse is not yet known.

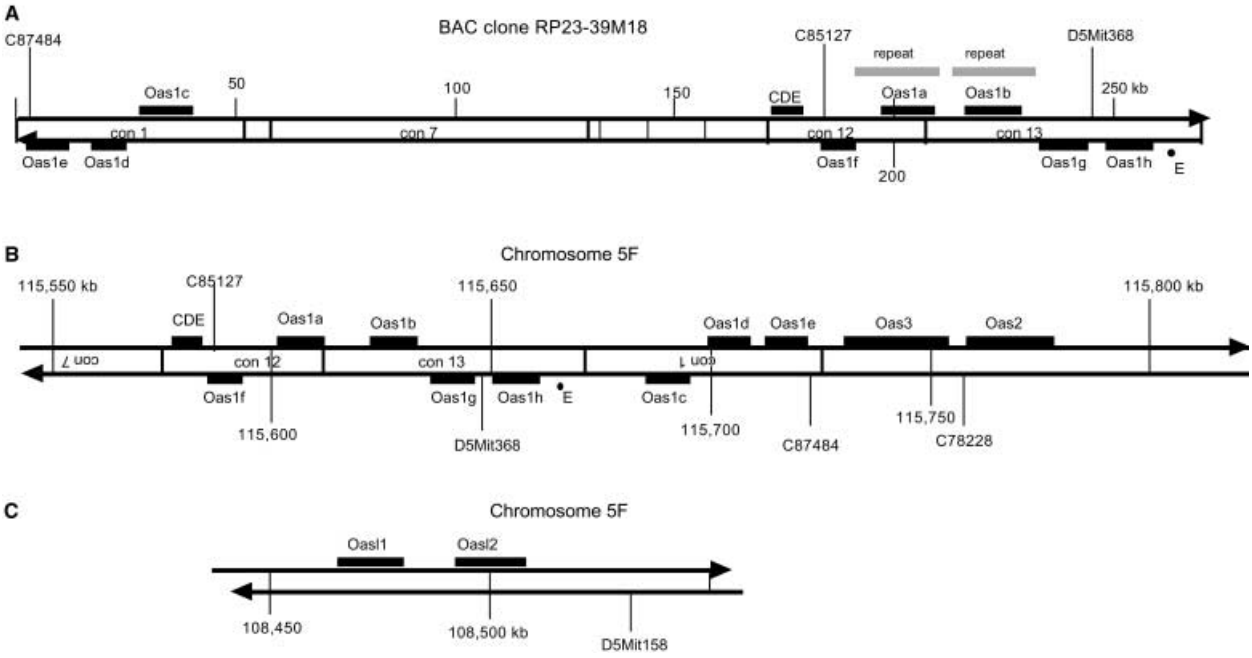

Figure 3. Positions of the murine *Oas* genes on BAC clone RP23-39M18 and mouse Chr. 5F. (*A*) Locations of the *Oas1* genes on the BAC clone RP23-39M18. Arrowheads represent the 3′ ends of each DNA strand with numbering and contigs (con) as indicated in GenBank no. AC015535.5. Only some of the contigs are numbered. Genes with reading frames in the same direction as the annotated nucleotide sequence are indicated by black squares above the lines, genes in the reverse direction are shown below the lines. A group of three exons (C, D, E) are located early in contig 12 and a single E exon is found late in contig 13. The 18-kb repeat regions within contigs 12 and 13 are indicated by gray squares. The positions of the three chromosomal markers C87484, C85127 and D5Mit368 are indicated. (*B*) Locations of the *Oas1*, *Oas2* and *Oas3* genes on Chr. 5F with numbering according to the Celera Mouse Database (scaffold: GA x5J8B7W3GHV: 2500001–3000000). The positions of four of the BAC clone contigs are indicated between the horizontal lines. Compared to the BAC clone, contigs 1 and 7 are present in the reverse direction on the chromosome. The positions of the three chromosomal markers C87484, C85127 and D5Mit368 are indicated. (*C*) Locations of the *Oasl1* and *Oasl2* genes on Chr. 5F with numbering according to the Celera Mouse Database (scaffold: GA x5J8B7W5JQ8:1–500000). The position of the chromosomal marker D5Mit158 is indicated.

Murine *Oas2* and *Oas3* genes

As we were unable to identify putative exon groups corresponding to the larger forms of the *OAS* genes on the BAC clone, we extended our search to the chromosomal sequence. A BLAST search of the human *OAS2* and *OAS3* exon sequences (GenBank M87284, M87434 and AF063613) against the Chr. 5, 115.3–115.8 Mb scaffold, revealed a number of *Oas* exons in the region around 115,750 kb (figs 3B and 6A). Using the standard nucleotide BLAST search of the NCBI *M. musculus* database, we identified a number of IMAGE clones and some ESTs harboring *Oas2* and *Oas3* exons (table 1). As gaps are present in the genomic sequence in this region on Chr. 5, these clones were used to define some of the exons. The exons identified suggest the existence of all sixteen *Oas3* exons ([A3.1, B3.1, C3.1, D3.1, E3.1]; [A3.2, B3.2, C3.2, D3.2, E3.2]; [A3.3, B3.3, C3.3, D3.3, E3.3]; T) in the chromosomal region 115,730–115,755 kb (figs 3B, 6A) and all eleven *Oas2* exons ([A2.1, B2.1, C2.1, D2.1, E2.1]; [A2.2, B2.2, C2.2, D2.2, E2.2]; T) at 115,758–115,776 kb. The detailed exon/intron structure of the *Oas2* and *Oas3* genes is shown in figure 6A.

The amino acid sequences coded for by the predicted exons revealed strong homologies between the human and murine A–E exons with 61% for *Oas2* and *OAS2* and 65% amino acid identities for *Oas3* and *OAS3* (data are available on our home page http://www.mbio.aau.dk/˜jj/welcome.htm).

Two of the *Oas2* exons show abnormalities when compared to the human exons. Exon A2.1 was found to be about 50 amino acids longer than the corresponding human exon A2.1. The existence of such a long N-terminal coding sequence is confirmed by the EST clone (GenBank no. BI647382) and the recently released *Oas2* cDNA clone [Kakuta et al. unpublished data] (GenBank AB067535), both of which have nucleotide sequences of exon A2.1 identical to that predicted from the Chr. 5 sequence.

The C-terminal exon T of *Oas2* has a coding sequence homologous to, but shorter than the exon T of the human p71 *OAS2* splice variant. In this case also, the sequences of AB067535 and the Chr. 5 sequence were found to be identical.

For *Oas3*, two of the exons, exon A3.2 and exon C3.2, are located within sequence gaps in the available Chr. 5 se-

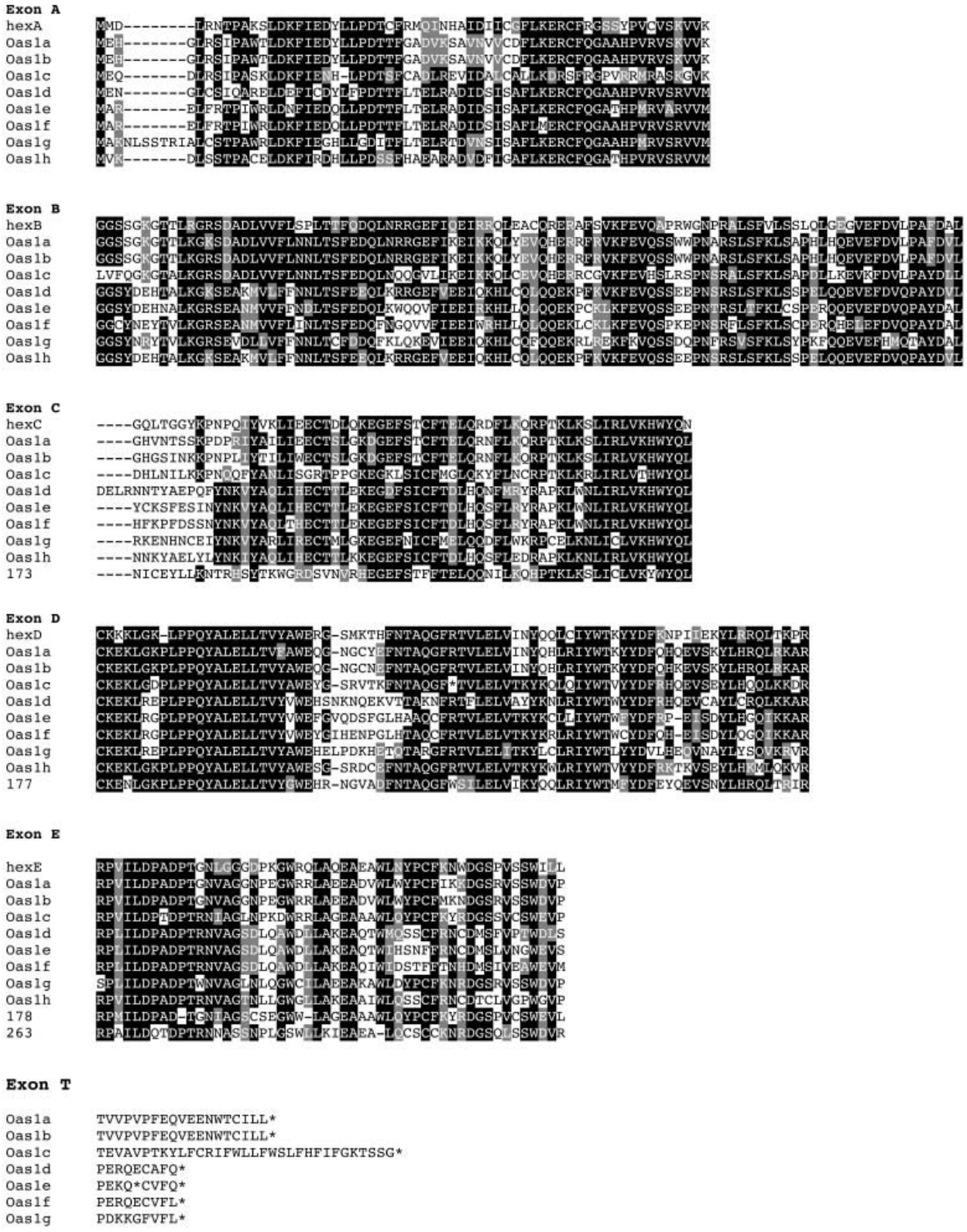

Figure 4. Amino acid alignments of human and mouse OAS1 proteins. Alignment of the amino acid sequences coded for by exons A–T of the human (hex) *OAS1* gene and the murine *Oas1* genes. The stop codon in *Oas1c*, exon D is indicated by *. The four isolated exons (CDE) and (E) are marked according to their positions in kilobases on the BAC clone RP23–39M18. Black boxes indicate conserved amino acids and gray boxes indicate related amino acids. Gaps are indicated by a dash.

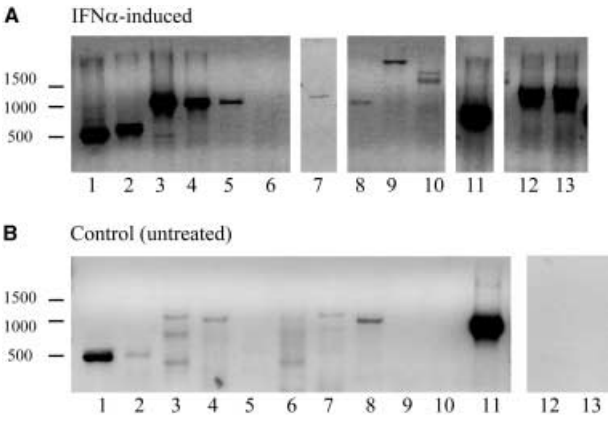

Figure 5. Transcription of the murine *Oas* genes. Products of the PCR reactions on extracts from interferon-induced mouse cells, analyzed by agarose gel electrophoresis. Lanes: 1, *Oas1a*; 2, *Oas1b*; 3, *Oas1c*; 4, *Oas1d*; 5, *Oas1e*; 6, *Oas1f*; 7, *Oas1g*; 8, *Oas1h*; 9, *Oas3*; 10, *Oas2*; 11, actin; 12, *Oasl2*; 13, *Oasl1*. (*A*) Induced by interferon α. (*B*) Uninduced control.

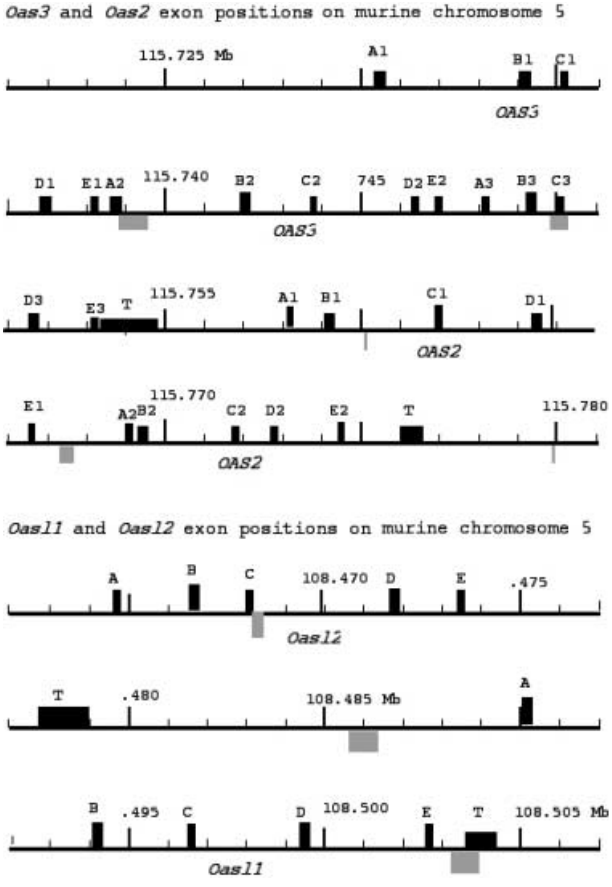

Figure 6. Exon structure of *Oas2, Oas3* and *Oasl* genes. The *Oas2* and *Oas3* exons were identified on the basis of the human *OAS2* and *OAS3* genes and the clones indicated in table 1. The *Oasl1* and *Oasl2* exons were identified on the basis of the human *OASL* gene and the clones indicated in table 1. Exons are indicated by black squares above the lines. Gray squares below the lines represent gaps in the Celera Mouse Database sequences.

quence. Whereas the IMAGE clone, GenBank no. BC016461, codes for exon C3.2, the recently released sequence of the *Oas3* cDNA clone (GenBank no. AB067534) contains both exons [Kakuta et al., unpublished data].

Most of exons A, in human as well as in mouse, code for about 60 amino acids. However, the human exon A3.2 codes for 115 amino acids [7] and in *Oas3*, exon A3.2 is predicted to code for 169 amino acids. Of these amino acids the first 58 and the last 52 amino acids, show homology to human exon A3.2.

In the RT-PCR assay (fig. 5), the presence of transcription products of the *Oas2* and *Oas3* genes were demonstrated in RNA isolated from interferon-α-induced cells, but not from interferon-γ-treated cells.

Murine *Oasl1* and *Oasl2* genes

A murine cDNA clone coding for an OASL p54 protein, with a C-terminal end with ubiquitin homology, was isolated by Tiefenthaler et al. [16] (GenBank no. AF068835). Furthermore, the sequence of an adult male tongue cDNA clone coding for an OASL protein was deposited in July 2000 [Adachi et al., unpublished data] (GenBank no. AK010034). These two clones are identical except for the C-terminal exon. Exon T of the p54 clone appears to have a 17-nucleotide insertion creating an early stop codon in the reading frame (fig. 7). Compared to the sequence of the AK010034 clone, this insertion is soon followed by a 72-nucleotide deletion.

A BLAST search against the Celera Mouse genome database showed that the A–E exons of these two clones are localized to Chr. 5 in the scaffold 108.1–108.6 Mb (GA_x5J8B7W5JQ8: 1–500,000). The genomic sequence is identical to that of the AK010034 clone, with all six exons located in the 108,466–108,480 kb region (fig. 6B). This gene is registered under the name *Oasl2* in the Mouse Genome Database of The Jackson Laboratory (http://www.informatics.jax.org).

The BLAST search also revealed that the untranslated region of exon T of AK010034 contains an 80-nucleotide sequence which is repeated 52 times in + orientation and 45 times in – orientation in the Chr. 5 scaffold 108.1–108.6 Mb. This repeated sequence is highly homologous to the 3′ end of the 130-nucleotide consensus sequence of the B1 family of short interspersed elements (SINE B1), and like these, is flanked by a 28-nucleotide CA-rich sequence [19].

The partial nucleotide sequence of another *Oasl* cDNA clone, IMAGE 1546490, [Marra et al., unpublished data], (GenBank no. BE136926), was submitted on 20 June 2000. We have further determined the nucleotide sequence of this cDNA clone (GenBank no. AY089728) and found it to contain five OAS exons (A–E) and a C-terminal exon (exT) with ubiquitin homology. The amino acid sequence coded for by this clone is homologous with but different to that of the AK010034 clone (fig. 7).

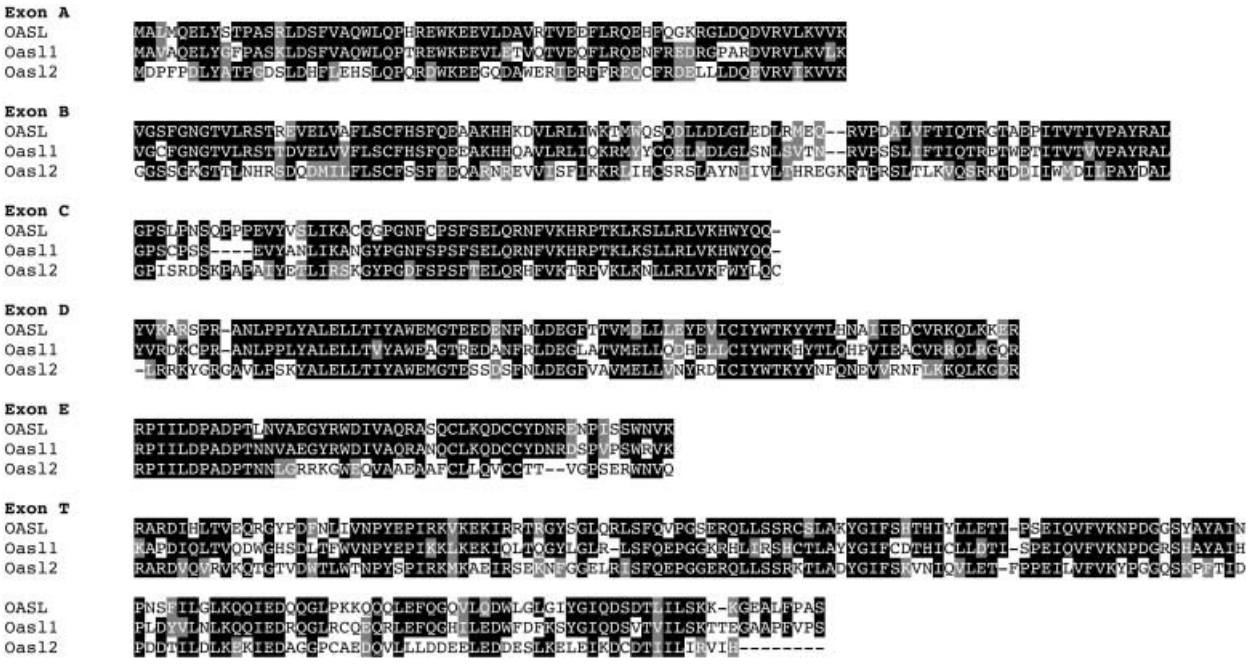

Figure 7. Alignment of the amino acid sequences coded for by exons A–T of the human *OASL* gene and the murine *Oasl1* and *Oasl2* genes. Black boxes indicate conserved amino acids and gray boxes indicate related amino acids. Gaps are indicated by a dash.

The IMAGE clone therefore represents a different *Oasl* gene, which we have termed *Oasl1*, spanning the region 108,490–108,505 kb (figs 3C, 6B).

With the *OASL* genes also, we observed a high degree of amino acid homology of the translated exons of murine *Oasl2* and *Oasl1* genes with that of the human *OASL* gene (fig. 7). For the amino acids in exons A–E, there is 74% identity to human *OASL* in the case of *Oasl1* and only 49% identity in the case of *Oasl2*. This suggests that *Oasl1* is the ancestral gene and that the *Oasl2* gene is duplicated from the *Oasl1* gene.

Recently, the sequence of yet another Oasl1 cDNA clone (GenBank no. AB067533) was released by Kakuta et al. [unpublished data]. This clone is identical to the IMAGE clone 1546490 except for a few nucleotide positions, only two of which create changes in the amino acid sequence. In the RT-PCR assay (fig. 5), the presence of transcription products of the *Oasl1* and *Oasl2* genes were demonstrated in RNA isolated from interferon-α-induced cells. Only the *Oasl2* gene was induced by interferon γ (data not shown).

Discussion

In humans, four classes of interferon-induced OAS proteins have been identified both as proteins and as cDNA clones, and their genes (*OAS1, OAS2, OAS3* and *OASL*) have been localized to Chr. 12 (fig. 1A) [7]. Of these proteins, three forms, the small (p42, p44, p46, p48), the medium (p69, p71) and the large (p100) have oligoadenylate synthetase activity, whereas no activity has been detected for the OASL (p59) protein.

In mice, only the small OAS forms have so far been characterized by their enzymatic activity, although indications for the presence of a larger form exist [11]. Of the small forms, several types have been characterized by cDNA cloning and there are indications for the presence of more than one *Oas1* gene [14, 18]. A murine *Oasl* cDNA clone (GenBank no AF068835) was isolated by Tiefenthaler et al. [16].

Using the sequence of the BAC clone (RP23-29M18), sequences from EST and IMAGE clones, as well as genomic sequence of the Celera Mouse Database, we were able to identify the exon/intron structure of a rather large number of murine *Oas* genes. All murine genes have the exon A–E units as observed in the human genes. This confirms our earlier suggestion of exon conservation during evolution [7]. The positions of all the murine *Oas* and *Oasl* genes on murine Chr. 5 were established. In total, we identified eight *Oas1* genes, of which seven (*Oas1a, Oas1b, Oas1c, Oas1d, Oas1e, Oas1f* and *Oas1g*) have a complete set of exons (A, B, C, D, E, T). Furthermore, we identified a putative *Oas1* gene (*Oas1h*), where a C-terminal exon (exon T) has not yet been identified. All eight *Oas1* genes are localized within a 150-kb region on murine Chr. 5 (figs 1B, 3B).

The homologies of amino acid sequences within all groups of exons A–E of the different *Oas1* genes are shown in figure 4 and the per cent identity between amino

Table 3. Amino acid sequence identities in percent between the exons coded for by the *Oas1* genes.

	Oas1a	*Oas1b*	*Oas1c*	*Oas1d*	*Oas1e*	*Oas1f*	*Oas1g*
Oas1a	–						
Oas1b	96	–					
Oas1c	66	66	–				
Oas1d	61	61	54	–			
Oas1e	56	56	52	77	–		
Oas1f	56	56	53	73	84	–	
Oas1g	58	58	53	64	64	63	–
Oas1h	63	62	58	74	68	65	61

acids in the sequences are given in table 3. The two almost identical genes *Oas1a* and *Oas1b* are clearly duplicates of the same ancestral gene, and are located within an 18-kb repeat sequence (fig. 3A). For the amino acids coded for by exons A–E, both the *Oas1a* and *Oas1b* genes show 71% identity to the exons of the human *OAS1* gene (table 1). As seen by comparison of the amino acid sequences of the murine exons A–E, and the C-terminal exons (fig. 4, table 3), the five genes *Oas1d*, *Oas1e*, *Oas1f, Oas1g* and *Oas1h* are closely related and most likely derived by multiplications of the same ancestral gene. The *Oaslc* gene is more distantly related, but closer to the *Oas1a/b* group than to the rest of the genes (table 3).

This indicates that multiplication of *Oas1*-type genes has occurred in the mouse, and as only a single *OAS1* gene is found in humans, the gene multiplication might have occurred after the divergence of *M. musculus* and *Homo sapiens* during evolution. However, early gene multiplications might have been followed by subsequent loss of all but a single *OAS1* gene in the human.

Our RT-PCR assays (fig. 5) and the cDNA clones of Kakuta et al. [unpublished data] indicate that all eight *Oas1* genes are transcribed. Not known at present is whether all the transcripts are translated into proteins, but several species of oligoadenylate synthetases are found in interferon-induced mouse cells.

In humans, the single *OAS1* gene gives rise to four splice variants (p42, p44, p46 and p48) indicating a possible need for several OASs in both human and murine cells.

We also identified the murine orthologs of the human *OAS2* and *OAS3* genes. As in humans, the mouse genome has a single copy of both the *Oas2* and *Oas3* genes with the same build-up of two and three domains of A–E exons, respectively. At the level of amino acid sequences, most of the murine exons show a high degree of homology with the corresponding exons of the human *OAS2* and *OAS3* genes (table 1). The *Oas3* and *Oas2* genes are found within a 47-kb region immediately downstream of the *Oas1* genes in the telomeric direction, similar to the situation observed in humans (fig. 1) and our RT-PCR assays show that both genes are transcribed.

Besides the already reported cDNA clones corresponding to the *Oasl2* gene, we were able to identify an IMAGE clone coding for yet another *Oasl* protein with a ubiquitin-like C-terminal sequence and to identify the corresponding gene *Oasl1*. Both *Oasl* genes are found on murine Chr. 5F within a 45-kb region around 108.5 Mb (fig. 3C). In humans, only a single *OASL* gene has been identified [20]. Comparisons of the amino acid sequences coded for by the A–E exons of the murine *Oasl* genes with those of the human *OASL* gene show that in the case of *Oasl1* there is 71% identity of amino acids, but only 49% identity in the case of *Oasl2* (table 1, fig. 7).

On murine Chr. 5, the *Oasl1* and *Oasl2* genes are located 7 Mb towards the centromere from the *Oas1*, *Oas3* and *Oas2* genes contrary to the situation in humans, where the *OASL* gene is located 21 Mb telomeric to the *OAS* genes on Chr. 12. This suggests that chromosomal rearrangements might have occurred during evolution.

The basic domain of the five translated exons (A–E), which is found in all of the human *OAS* genes, has now been identified in the *Oas* genes of the mouse. Thus the exon/intron structure of the *OAS* genes appears to have been conserved during evolution, at least among mammals, and all the genes of the OAS protein family likely originated by duplication of a common ancestral gene. This was previously proposed for the human *OAS* genes by Kumar et al. [21] in a model where gene duplications of an ancestral gene was suggested to give rise to the *OAS1* and *OASL* gene types, as well as to the genes of the two larger forms, *OAS2* and *OAS3*. The comparative amino acid sequence analyses demonstrate an identical situation for the murine genes in support of the model of Kumar et al. [21].

In summary, we have demonstrated that the exon/intron structures of all the genes, *OAS1*, *OAS2*, *OAS3* and *OASL* are conserved between mice and humans. Consequently, we expect that this gene structure with units of five exons will be found in other mammals and, possibly, in other animals as well, such as the chicken.

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