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Review

Gene structure and function of the 2′-5′-oligoadenylate synthetase family

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Abstract. 2′-5′-Oligoadenylate synthetase was among the first interferon-induced antiviral enzymes to be discovered. This family of enzymes plays an important role in the mechanisms of action of interferon antiviral activity, but is also involved in other cellular processes such as apoptosis and growth control. We have reviewed the function and genomic structure of this class of at least nine proteins. By studying the recently available data in the human genome database and the human Expressed Sequence Tag database, we have been able to build a comprehensive picture of the 2′-5′-oligoadenylate synthetase gene family and its precise location on chromosome 12. Chromosomal localization as well as the intron/exon structure of all four genes has been established and an overview of the splice variant forms of the 2′-5′-oligoadenylate synthetases arising from expression of the four genes is presented. Alignments of the human 2′-5′-oligoadenylate synthetase sequences with non-human 2′-5′-oligoadenylate synthetase sequences suggest that the exon structure and several amino acid sequence motifs have been conserved during evolution.

Key words. Oligoadenylate synthetase; OAS; OASL; 2-5A; chromosome 12; exon structure; OAS genes.

Introduction

The 2′-5′-oligoadenylate synthetases (OASs) are a family of interferon (IFN)-induced enzymes which, when activated by double-stranded RNA, oligomerize ATP into 2′-5′-linked oligoadenylate (2-5A) ranging from dimers up to 30-mers.

The enzymes, which are induced by both IFN-I and IFN-II, are suggested to play a role in the antiviral effect of IFN (fig. 1) [1–3]. 2-5A binds strongly to a latent endoribonuclease, RNaseL, which then forms dimers and becomes active [4]. In virus-infected cells, this leads to degradation of viral and cellular RNA, and subsequently to a drop in protein synthesis [5–7]. Several recent studies have also proposed a role for OAS in the induction of apoptosis [8–10].

In human cells three types of interferon-induced OAS were recognized by immunoblotting, the small form (p42/p46 OAS), the medium form (p69/p71 OAS) and the large form (p100 OAS) (fig. 2, table 1) [11, 12]. Similar size classes have been identified in the mouse as p42 OAS, p75 OAS and p105 OAS [12, 13]. The small forms of OAS have also been identified in mammals such as rat [14], woodchuck [Zhou et al., accession no. AF082498] and pig [Hartmann, accession no. AJ225090], in chicken [15] and recently in a marine sponge *Geodia cydonium* [16].

Furthermore, we [17] and Rebouillat et al. [18] have identified an IFN-inducible OAS-like protein p59 (OASL) devoid of 2′-5′-oligoadenylate synthesizing ac-

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tivity. Recently, a homologous OASL protein was identified in murine dendritic cells [19].

The cDNAs corresponding to all three human OAS species have been cloned and recombinant protein produced. All three proteins possessed enzymatic activity [20–22]. Both we [17, R. Hartmann and J. Justesen, unpublished data] and others [18] produced recombinant protein of the p59 OASL, and failed to detect any enzymatic activity of this protein, despite its strong homology to the other OAS proteins.

The OAS1 gene has been cloned [20, 23] and shown to have five N-terminal translated exons and two C-terminal exons permitting the formation of different OAS proteins through alternative splicing.

By cytogenetic in situ hybridization, the human OAS1, OAS2 and OAS3 genes have been localized to a 130-kb region on chromosome 12q24.2 [24, 25]. The rapidly increasing amount of sequence information available for the human genome has permitted us to locate exactly the three OAS genes as well as the OASL gene on the 147 Mb of chromosome 12. The three OAS genes are located at the chromosomal region 12q24.1 at 120 Mb and the OASL gene at the chromosomal region 12q24.2 at 129 Mb as measured from the p-telomeric end.

The structure of the OAS gene family will be described in detail (fig. 9) in this review. The five N-terminal exons (A–E) of the OAS1 gene constitute a basal OAS unit which is repeated twice in the OAS2 gene (fig. 3) and three times in the OAS3 gene (fig. 8) in all cases followed by one or two C-terminal exons. The OASL gene comprises one N-terminal OAS unit and a C-terminal exon having homology to ubiquitin (fig. 8) [17].

There is a high degree of amino acid identity between all members of each of the five subgroups of exons (fig. 4, table 3). Furthermore, the splice sites are generally conserved (fig. 7). The conservation of the exon structure suggests a common developmental origin for the OAS family genes.

The published sequences of the cDNA clones of p42/46, p69/71 and p100 are with minor exceptions identical to the genomic sequences derived from a PAC clone RP1-71H24 (from 12q24.1, accession no. AC004551). The OASL gene is found on the BAC clone bK92N15 (from 12q24.2, accession no. Z93097). Therefore, in this review, all amino acid sequences shown are translated from the DNA sequences of the PAC clone RP1-71H24 and the BAC clone bK92N15 yielding the most likely correct amino acid sequences for the proteins in the

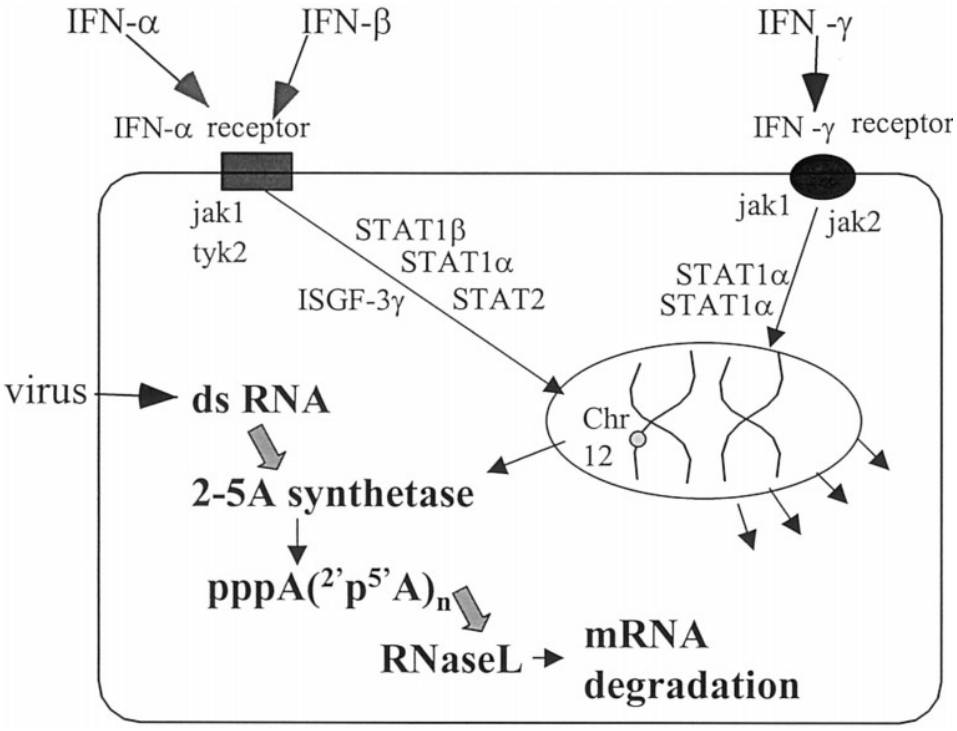

Figure 1. IFN system: the 2′-5′A pathway. IFN, interferon; jak, Janus kinase; tyk, tyrosine kinase; STAT, signal transducer and activator of transcription; ISGF-3, interferon-stimulated gene factor-3; dsRNA, double-stranded RNA; Chr 12, chromosome 12.

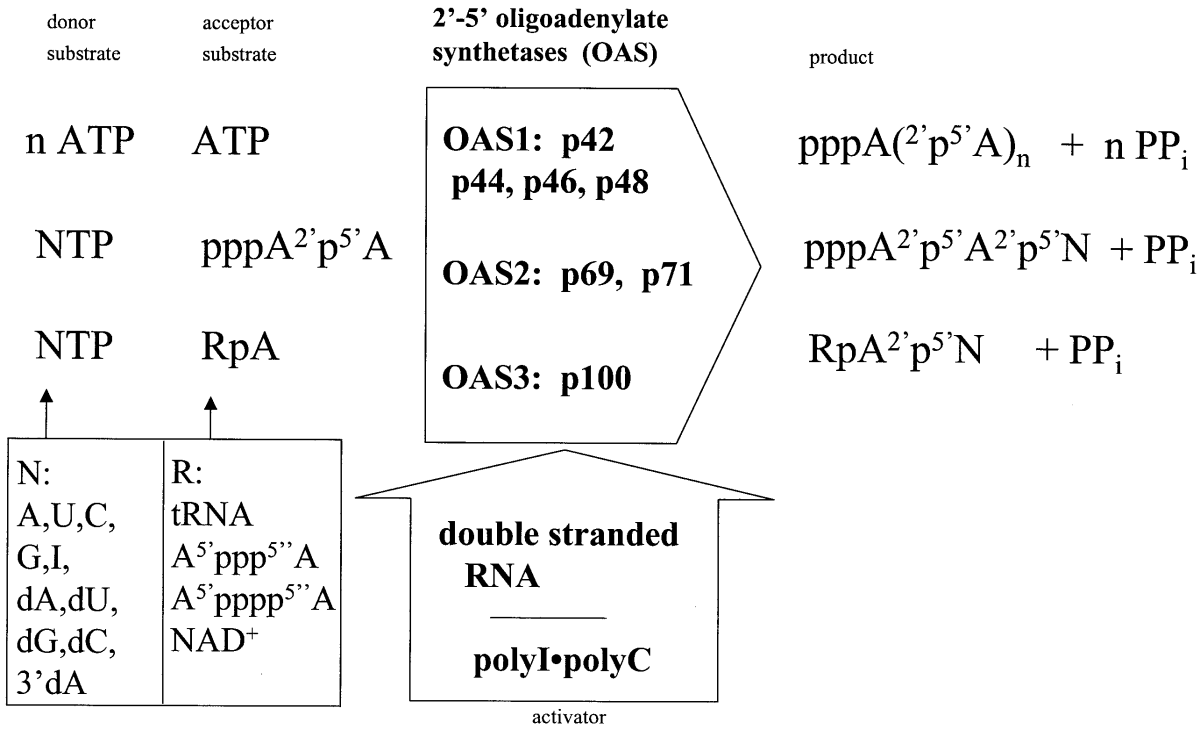

Figure 2. The 2′-5′-nucleotidyl transferase reaction.

OAS family. The sequences presented in this review are available at our website: http://www.mbio.au.dk/~jj/OAS/justGENE.htm. For details about 2-5A synthetase proteins and regulation of IFN induction, the reader is directed to recent reviews by Rebouillat and Hovanessian [22], Sarkar and Sen [26] and Stark et al. [27].

The 2′-5′-oligoadenylate synthetase

Isolation and characterization

In the absence of IFNs the concentration of OAS is rather low in most cells and tissues. IFN-treated cell cultures have therefore in most cases been the starting material for the isolation of OAS proteins.

The 2′-5′-oligoadenylates [pppA($^{2'}$p$^{5'}$A)$_n$] were first described in 1978 as low-molecular-weight inhibitors of protein synthesis [5, 6, 28–30]. A little later, the synthesizing enzyme, OAS (the 2-5A synthetase), was discovered [31] simultaneously with the double-stranded RNA (dsRNA)-dependent protein kinase (PKR) in IFN-treated cell extracts and in rabbit reticulocyte lysates [28, 32, 33].

Dougherty et al. [34] were the first to isolate the large form of OAS from IFN-induced mouse cells by high-salt elution of the ribosomal pellet and subsequent column chromatography. Later, the same group [35] made use of the affinity of OAS towards dsRNA to isolate a homogeneous preparation of human p100 OAS from IFN-treated HeLa cells using a column of matrix-bound polyI · polyC during the purification procedure. Rabbit reticulocytes were used to prepare a homogeneous OAS with an apparent molecular weight of 110 kDa [36]. Revel et al. [37] purified the small form of human OAS and later the same group cloned and sequenced the cDNA of this form [20].

When cDNA clones of the various size forms of OAS became available, expression systems such as *Escherichia coli* and the baculovirus/insect cell system were used as the materials of choice for the preparation of OAS, as reviewed by Sarkar and Sen [26]. This also permitted the production of genetically modified proteins and identification of the various sites of importance for enzyme function.

Chebath et al. [11] prepared an antibody (termed antibody B) against a synthetic 20-amino-acid polypeptide containing the conserved N-terminal amino acids of the human exon E (RPVILDPAD, fig. 4) and showed that this antibody recognizes all molecular forms of OAS. Immunoblots of extracts from a number of cell lines

using antibody B showed that different cell lines produce various amounts of the four different forms of OAS, both in the IFN-induced and uninduced state [12]. Using partially purified 69-kDa or 100-kDa OAS, Hovanessian et al. [12] produced monoclonal antibodies towards each of these forms. The antibodies were used by Marié et al. [38] specifically to purify the medium and the large forms of OAS from cell extracts and to localize the two forms inside IFN-induced cells [39]. The 69-kDa OAS is localized around the nucleus and in certain patterns throughout the cytoplasm, whereas the 100-kDa OAS is found evenly distributed throughout the cytoplasm. Furthermore, the 69-kDa form is myristylated [38]. p100 OAS is present at rather high concentrations in human placenta, where the enzyme is located mainly in the trophoblast [40].

Size fractionation by gel filtration of proteins from IFN-induced cell extracts showed that the large form eluted in fractions corresponding to a 100-kDa protein, the medium form in fractions corresponding to a protein of about 170-kDa and the small form in about the same fractions. This was interpreted to mean that the large form of OAS exists in a monomeric state, the medium form in a dimeric conformation and the small form in a tetrameric conformation [38]. However, the high molecular weights could be due to binding of the small and medium OASs to other cellular components.

Enzymatic properties of OAS

The active OAS enzymes all perform the oligomerization of ATP into 2′-5′-linked oligoadenylate, in the presence of a suitable RNA activator. The α-phosphate of the donor ATP is linked to the 2′-hydroxyl group of the acceptor molecule and pyrophosphate is released according to the equation:

$$\mathrm{nATP} \rightarrow \mathrm{pppA}(^{2'}\mathrm{p}^{5'}\mathrm{A})_{n-1} + (n-1)\mathrm{PP_i}$$

Most of the assays employed [41] make use of chromatographic or electrophoretic methods to determine enzyme activity by the end-point amounts of oligoadenylates formed, either the intact pppA($^{2'}$p$^{5'}$A)n [26, 42, 43] or after phosphatase treatment of the core nucleotides A($^{2'}$p$^{5'}$A)n [13, 41].

We have introduced a rapid pyrophosphate assay of OAS activity [44] where the release of pyrophosphate (PP_i) in the polymerization reaction is continuously monitored through an enzymatic coupling of the PP_i to the reduction of $NADP^+$ to NADPH on a mole-to-mole basis. As NADPH absorbs strongly at 340 nm, OAS activity can be followed continuously either in a photometer or in multiwells in an ELISA reader, or by using the strong fluorescence of the NADPH.

During the first years after the discovery of OAS, a number of papers described the enzymatic properties of some of the isoforms of the enzyme, although it was generally presumed that there was only a single form of the enzyme. Johnston and Torrence [41] have described these thorough studies in a comprehensive review. It is now evident that some of the enzyme preparations did not consist of one pure isoform only.

To perform the enzymatic oligomerization reaction, the protein must have three distinct active sites: a binding site for the acceptor ATP, a binding site for the donor ATP and a site which catalyzes the nucleotidyl transferase reaction. The acceptor and donor sites are distinct even though they can bind the same substrate.

The substrate specificity of OAS can be broadened to use RpA as acceptor and NTP as donor (see fig. 2). The enzyme should thus be defined as a 2′-nucleotidyl transferase [45]. The in vivo existence of 2′-5′-oligonucleotides other than 2-5A, R$^{2'}$pN, has been questioned, although early data [46] have since been affirmed in the case of Ap3A($^{2'}$p$^{5'}$)A [47, 48]. The kinetic constants of

Table 1. Nomenclature of the OAS protein family.

Gene symbol	Review nomenclature	Predicted molecular weight	Alternative names	mRNA (kb)	Mouse OAS forms
OAS1 (4938)	small, p42	41.7	p40 [11], E16 [20], 3–9 [56]	1.6	p42
	p44	41.7, 43.9[a]			
	p46	46.0	p46 [11], E18 [20]	1.8	
	p48	47.5	9–2 [56]	1.8	
OAS2 (4939)	medium, p69	78.8		3.1	p75
	p71	83.4		2.9	
OAS3 (4940)	large, p100	121.3		6.3	p105
OASL (8638)	p59	58.4	p56 [18]	1.9	p59
			p30 [18]		p59

Gene symbol: the numbers in parentheses are the LocusLink numbers from NCBI. Predicted molecular weight: calculated from the translated sequences of the exons in the genomic PAC clone RP1-71H24 and the BAC clone bK92N15.
* Calculated from the translated sequences of the EST clone, IMAGE: 511242.

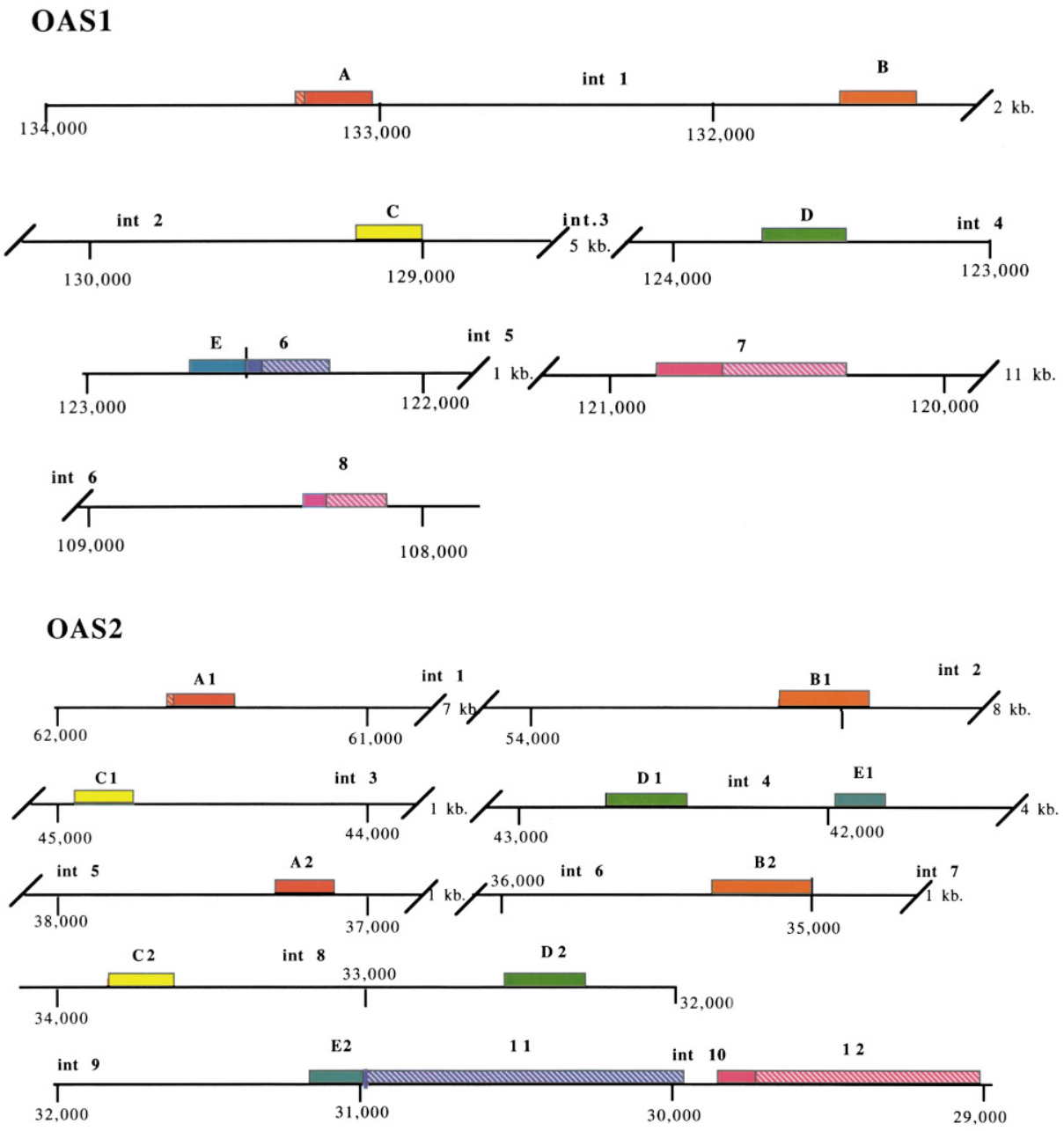

Figure 3. Exon/intron structure of the human OAS1 and OAS 2 genes. Exons are marked by coloured bars, and the non-translated parts of the terminal exons are cross-hatched. For the OAS1 gene, the translated part of exon 6 corresponds to that of the p42 splice variant, for exon 7 to that of p46, and the translated part of exon 8 corresponds to that of the EST clone. For the OAS2 gene, the translated part of exon 11 corresponds to that of the p69 splice variant and for exon 12 to that of p71. The numbers refer to positions on the PAC clone RP1-71H24.

OAS have been determined by end-point assays only and the Km values for ATP range from 1 to 3 mM, which is fairly high compared to other enzymes using ATP as a substrate [26, 41]. Detailed kinetic values for the donor and acceptor site have not been convincingly determined.

Stimulation of enzymatic activity by RNA

The enzymatic activity of all forms of OAS is strongly enhanced by dsRNA. Since no assay exists that is capable of measuring the activity of OAS in the absence of an RNA activator, the actual degree of stimulation is not known, but it is more than 1000-fold. Thus, in practice, OAS is only active in the presence of a suitable RNA activator. The in vivo association of OAS1 protein with viral RNA has been shown [49]. Production of dsRNA engendered by the virus is believed to be the actual trigger of 2-5A synthesis in viral-infected cells. Originally, only dsRNA was thought capable of activating OAS, but recently we have shown that 103-nucleotide, single-stranded aptamers, with no apparent secondary structure, can activate OAS [50]. However, the dsRNA versions of the same aptamers are still five- to tenfold stronger activators.

Most studies of OAS activation by dsRNA have been undertaken using polyI · polyC, but commercially available polynucleotides are rather ill-defined and the molecular-size distribution and double-strandedness might vary between preparations and between companies. However, in vitro, the requirement for the activator polyI · polyC (dsRNA) clearly varies between the different forms of OAS, ranging from less than 1 μg/ml to more than 500 μg/ml to give half-maximum activity. OAS appears to interact with RNA in a nucleotide-sequence-unspecific manner [51], but modifications of the

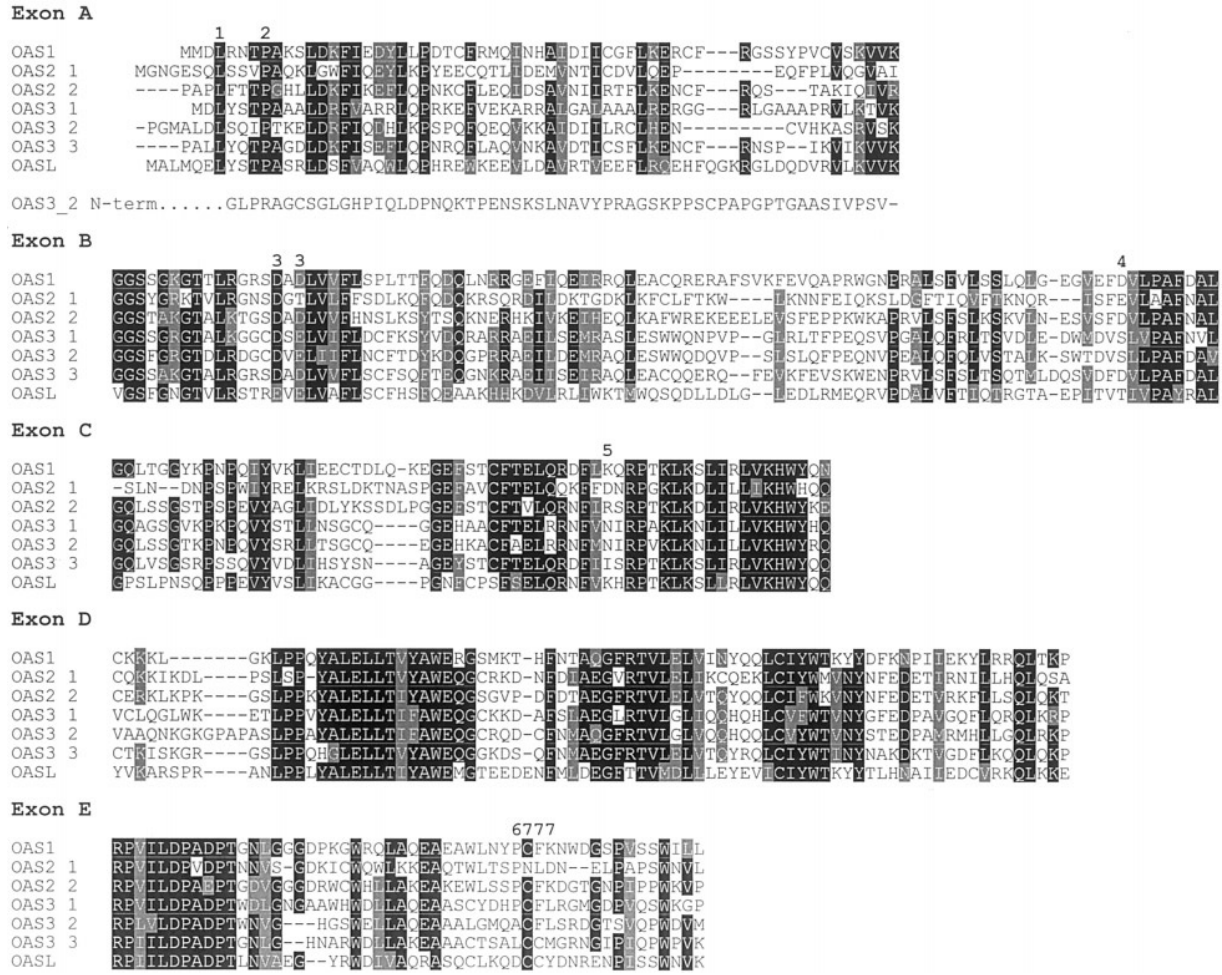

Figure 4. Alignment of the amino acid sequences encoded by the seven groups of exon A–E of the human OAS1, OAS2, OAS3 and OASL genes. The amino acid sequences are all derived by translating the DNA sequences of the PAC clone RP1-71H24 for the OAS genes. The N-terminal 58 amino acids of the long exon A2 of OAS3 are indicated below the exons of the A group. The numbers above the sequences relate to the mutations in table 2. Black boxes indicate positions at which the residues are identical and grey boxes show residues that are similar.

Table 2. Mutations in OAS.

Number	Mutation	OAS form	Activity of protein expressed in *E. coli*	Activity of protein expressed in baculovirus/insect cells
1	L4H	OAS1, exon A	20% of wild type	ND
2	P8Q	OAS1, exon A	inactive	as wild type
3	D74A, D77A	OAS1, exon B	inactive	inactive
3	D408A, D410A	OAS2, exon B2	ND	inactive
4	D481A	OAS2, exonB2	ND	inactive
5	K199H	OAS1, exon C	inactive	as wild type
5	K199R	OAS1, exon C	inactive	as wild type
6	P330A	OAS1, exon E	as wild type	ND
7	C331A	OAS1, exon E	15–29% of wild type	ND
7	F332A	OAS1, exon E	15–29% of wild type	ND
7	K333A	OAS1, exon E	15–29% of wild type	ND
7	C331A, F332A, K333A	OAS1, exon E	inactive	inactive
7	C668A, F669A, K670A	OAS2, exon E2	ND	inactive

Numbers refer to figure 4. ND, not determined.

2′-hydroxyl groups in the RNA abolish the activation [50]. Longer RNA molecules are more potent activators than shorter RNAs [52, 53], but the actual minimum length required for activation of OAS has not yet been defined.

None of the OAS proteins possesses a typical RNA-binding domain and no structural information about this interaction is currently available. Binding of the substrate ATP is not affected by the presence or absence of RNA [54]. Therefore, the most logical assumption is that the RNA activator either interacts directly with the active site of the enzyme or promotes a rearrangement in the active site, leading to the active conformation of the enzyme. One can argue that the extremely low enzymatic activity of OAS in the absence of dsRNA stimulator favours a model where the activating RNA interacts directly with the active site.

Structural elements of the OAS protein

As mentioned above, the OAS enzymes have at least three distinct active sites: an acceptor, a donor and a nucleotidyl transferase site. Since all OAS proteins require RNA for activity, they must also contain at least one RNA-binding site, which might overlap any of the above-mentioned sites.

Several groups have taken a mutational approach to identify the individual active sites of the OAS protein.

The results discussed below are summarized in table 2.

Sarkar et al. [55] studied the effect of replacing the three well-conserved aspartic acid residues in exon B2 (D408, D410 and D481) of the human OAS2 protein (fig. 4). These residues were selected because molecular modelling suggested that they form part of a structure similar to the catalytic domain of DNA polymerase β, where they co-ordinate two Mg^{2+} ions that catalyse the nucleotidyl transferase reaction. Two mutants were produced replacing the aspartic acids by alanine, the D481A and the double-mutant D408A, D410A, which were expressed in a baculovirus system. Both proteins were enzymatically inactive. The analogous mutant in exon B of the human small OAS protein (cDNA clone 9-2), D75A, D77A, was also found to be catalytically inactive when expressed in insect cells. The medium OAS D408A, D410A mutant protein was found to form dimers as efficiently as the wild type and the mutations had only limited effects on the binding affinity of the substrate at either the acceptor or the donor site. A catalytically active site must therefore be located in exon B.

To study the properties of mutant proteins, Ghosh et al. [56] constructed a series of progressively longer deletions in the cDNA clone 9-2 starting from the C-terminal end of the p48 OAS. The cDNA was transcribed and the mRNA translated in vitro in a reticulocyte-protein-synthesizing system. The deleted proteins were tested for activity and dsRNA binding. Whereas deletion of amino acids 344–321 in exon E led to a complete loss of enzymatic activity, the capacity of the synthesized protein to bind polyI · polyC was only lost after deletions reaching amino acids 158–104 in exon B.

This led these authors to search for point mutations to reveal amino acids critical for the catalytic activity of OAS. Mutagenic changes in exon E of the p48 protein replacing any of the three residues in the CFK sequence resulted in an 80% loss of enzymatic activity of the protein expressed in *E. coli* [57]. Substitution of all three residues by alanine (C331A, F332A, K333A) gave a complete loss of activity. This mutant could bind both ATP and dsRNA as efficiently as the wild-type OAS, but was defective in the formation of the tetrameric form of the small OAS. The results were identical whether the proteins were synthesized by *E. coli*, in a rabbit reticulocyte system in vitro or in insect cells.

Gel filtration studies of wild-type p48 OAS indicated that enzymatic activity was only found in the tetrameric form, the monomeric form of the enzyme being inactive [57]. However, we find that the monomeric form of the p42 OAS is fully active both when expressed in insect cells or in *E. coli* [R. Hartmann and J. Justesen, unpublished results]. This might reflect differences between the p48 and the p42 OAS.

The medium-form OAS protein (p69/p71 OAS2) is composed of two structural domains each homologous to the OAS1 protein (fig. 3) and forms homodimers in vitro as well as in vivo [21]. Mutational data and molecular modelling suggests that the p69 protein chain has a single active site in the most C-terminal of the two homologous domains. Replacing the CFK amino acids in exon E2 of the p69 protein (fig. 4) by alanine (C668A, F669A, K670A) abolished its ability to form dimers and the resulting p69 mutant protein was devoid of enzymatic activity [55]. By creating heterodimers containing one p69 chain mutated in the active site (D408A, D410A) and one wild-type p69 chain, the hybrid protein was shown to be enzymatically active with half the catalytic activity of the wild-type homodimer. The OAS2 protein was concluded to form a dimer with two independent active sites. Interestingly, the large OAS protein (p100), which does not oligomerize, has a CCM sequence at the position in the E3 exon analogous to the CFK sequence in OAS1 and OAS2 (fig. 4).

To identify the ATP-binding domain of the 42-kDa protein, Kon and Suhadolnik [54] photoaffinity-labelled recombinant human OAS1 protein with 8-azido-αP^{32}ATP, and saw a chemical modification of the lysine, K199, in the DFLKQ pentapeptide of exon C (fig. 4). When expressed in *E. coli*, site-directed mutagenesis of the OAS1 protein changing the K199 to arginine or histidine resulted in a protein product without enzymatic activity, but still able to bind the azido-ATP and dsRNA. However, K199 is not a conserved amino acid in the C exons of the different OAS molecules (fig. 4), whereas the phenylalanine in the same sequence is highly conserved.

Ghosh et al. [58] used mutant forms of the small human OAS p48 (clone 9-2), where the highly conserved amino acids (LXXXPA) early in exon A were replaced (fig. 4). Replacement of leucine in position 3, (L3H) or alanine in position 8 (A8D), gave a partial loss of enzyme activity when the mutant mRNAs were translated in a rabbit reticulocyte lysate, whereas a P7Q substitution resulted in a complete loss of enzymatic activity. The mutated protein maintained the ATP- and dsRNA-binding capacities of the wild-type OAS1, as the oligomerization properties.

When expressed in a baculovirus/insect cell system, Bandyopadhyay et al. [59] saw that the two substitutions P7Q and K199R had full enzymatic activity. At least in these cases, the expression system seems important for the results, most likely through the effect on protein folding or post-translational modifications.

In summary, to date, only amino acid substitutions of the three aspartic acid residues in exon B and of the CFK sequence in exon E show an indisputable effect on the activity and the molecular structure of the OAS.

Biological functions of the 2′-5′-oligoadenylate system

In 1987, Chebath et al. [1] provided the first direct evidence for the involvement of the 2-5A system in the antiviral effect exhibited by IFNs. Cells harbouring a DNA construct expressing the p42 isoform of OAS1 were shown to have increased resistance towards picornavirus infection. Furthermore, the level of OAS protein expression and the antiviral effect observed were correlated [1].

In 1996, two groups independently constructed transgenic plants expressing cDNA encoding the human p42 isoform of OAS1 and RNase L [2, 3]. Even though they used different plant species (tobacco and tomato), both groups observed a strong increase in the resistance to viral infection in transgenic plants expressing both the OAS1 and RNase L cDNA, whereas plants having only one of the transgenes showed no increase in viral resistance. Following challenge of the transgenic plant with virus, necrotic spots were observed, indicating that infected cells are actually killed by the action of the OAS system.

Recent studies suggest that the OAS system might play a role in induction of apoptosis. Diaz Guerra et al. [9] used recombinant vaccinia virus to express RNase L in mammalian cells. This alone led to some increase in apoptosis, which was strongly enhanced by co-infection with an OAS1 recombinant virus. In cells stably transfected with RNase L cDNA, Castelli et al. [10] found that addition of 2-5A led to apoptosis of those cells, whereas apoptosis was not induced by the addition of an analogue of 2-5A unable to activate RNase L. Similarly, when a dominant-negative RNase L mutant was expressed, apoptosis was also impaired [10]. The mechanism by which apoptosis is induced by the 2-5A system is as yet unknown. It might simply be the result of a low rate of protein synthesis due to RNA degradation, or direct interaction of some component of the 2-5A system with key regulators of apoptosis, or a combination of both. However, a viral infection can likely be curtailed at expendable cost for the host, simply by killing the virus-infected cells early during infection. If this is to be accepted, some regulatory mechanism must also exist, which can prevent a state of shock if the infection should become systemic. A protein capable of inhibiting RNase L has been described, but its effect has not been

tested in connection with 2-5A/RNase L-induced apoptosis [60].

Structure of the human OAS genes

OAS1

From the 17S fraction of RNA isolated from IFN-induced human SV80 cells, Merlin et al. [61] identified a cDNA clone (E1 cDNA), which upon injection into *Xenopus laevis* oocytes produced an active small-form OAS, p42. The cDNA hybridized to three mRNA species of 1.6, 1.8 and 3.6 kb, which were found to be co-induced by IFN in SV80 cells.

Using the E1 cDNA as a probe, several groups isolated cDNA clones of the 1.6- and 1.8-kb mRNA together with genomic clones of the corresponding OAS1 gene [20, 23, 62–64].

Two cDNA clones, one corresponding to the 1.6-kb mRNA coding for the p42 OAS (E16 cDNA, accession no. X02874) and another corresponding to the 1.8-kb mRNA coding for the p46 OAS (E18 cDNA, accession no. X02875) as well as a genomic clone have been isolated and sequenced [20]. The two mRNA species were found to be transcribed from the same gene, OAS1, containing five translated N-terminal exons (here termed exon A–E), coding for 346 amino acids (fig. 3). The two mRNAs were found to be C-terminal splice variants. In the case of the E16 mRNA, no splicing occurs at the end of exon E and translation continues with 18 amino acids to the end of the open reading frame of exon 6 (figs 5, 6). Thus the p42 protein contains 364 amino acids and has a predicted molecular weight of 41.8 kDa. In the case of the E18 mRNA, exon E is spliced to exon 7 coding for the 55 C-terminal amino acids (figs 5, 6) of the p46 protein which in total contains 401 amino acids and has a predicted molecular weight of 46.1 kDa.

The 3.6-kb mRNA which is seen in Northern blots from IFN-induced cells [61] was found to have the same polyadenylation site as the 1.8-kb mRNA and contains unspliced intron sequences [20].

About 11 mRNA transcription initiation sites have been identified within the OAS1 promoter region, most of which are located up to 100 bp upstream of the initiator ATG [65]. The E16 clone [20] represents an additional transcription start site about 520 bp upstream of the ATG initiation codon and the transcript was found to contain two 5′-untranslated exons of 70 and 110 nucleotides (nt) preceding exon A.

The complete deoxynucleotide sequence of the 162,346-bp human genomic PAC clone RP1-71H24 (Human Genome Sequencing Center, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Tex.; accession no. AC004551) was released by GenBank in July 1998. The PAC clone, which is localized to chromosomal region 12q24.1, is indicated in the annotations to contain the OAS1 gene at position 133–120 kb (fig. 9) with the exon/intron boundaries identified as splice consensus sequences (fig. 7). The amino acid sequence predicted by translating the PAC

OAS1

p40, exon 6	VRPPASSLPFIPAPLHEA
p46, exon 7	AESNSTDDETDDPRTYQKYGYIGTHEYPHFSHRPSTLQAASTPQAEEDWTCTIL
p48, exon 7	TQHTPGSIHPTGRRGLDLHHPLNASASWGKGLQCYLDQFLHFQVGLLIQRGQSSSVSWCIIQDRTQVS
EST, exon 8	VNLTLVGRRNYTNN

OAS2

p69, exon 10	VKVI
p71, exon 11	TMQTPGSCGARIHPIVNEMFSSRSHRILNNNSKRNF

OAS3

exon 16	KAA

OASL

exon U	RARDIHLTVEQRGYPDFNLIVNPYEPIRKVKEKIRRTRGYSGLQRLSFQVPGSERQLLSSRCSLAKYG-IFSHTHIYLLETIPSEIQVFVKNPDGGSYAYAINPNSFILGLKQQIEDQQGLPKKQQQLEFQGQVLQD-WLGLGIYGIQDSDTLILSKKKGEALFPAS

Figure 5. The translated amino acid sequences of the C-terminal exons of the members of the human OAS family. The sequences presented are translated from the DNA sequences of the PAC clone RP1-71H24 for OAS proteins and the BAC clone bk92N15 for OASL protein. The EST clone (IMAGE:511242) was sequenced and a translation resulted in a predicted p44 OAS with an exon 8 sequence: NLTLVGRRNYPIISEHAVNLQQTRRASLSYSFQVA.

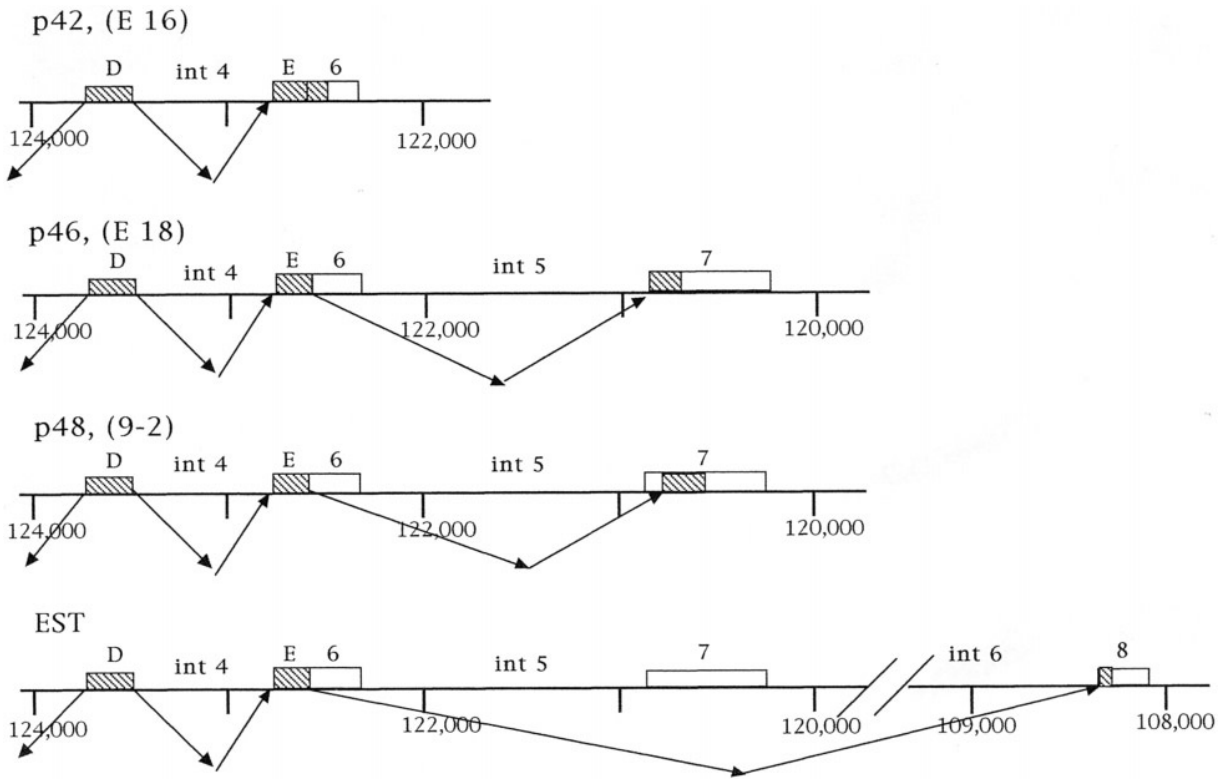

Figure 6. Splice variants of the human OAS1 gene. p42 corresponds to the E16 clone, p46 to the E18 clone, p48 to the clone 9-2 and EST to the clone IMAGE:511242.

clone RP1-71H24 corresponds to that of the E16/E18 cDNA except for some minor differences. The gene is transcribed in the centromer to telomer direction (fig. 9).

Search of the Expressed Sequence Tag (EST) database for homologies to exons A–E of the OAS1 gene revealed a 650-nt cDNA clone from human colon epithelial cells (IMAGE: 511242, accession no. AA085948) which might represent yet another splice variant of OAS1 transcript. The sequence of the 3′ end of this EST clone showed the presence of most of exon D and a complete exon E, and yet another 3′ exon, exon 8 (figs 3, 6). This exon, which is found at position 108 kb on the PAC clone RP1-71H24, codes for 14 C-terminal amino acids (fig. 5), yielding a protein with a predicted molecular weight of 41.5 kDa. Preliminary experiments using RT-PCR with primers in exon D and exon 8 to amplify specific DNA molecules derived from OAS mRNA in HeLa cells have demonstrated the existence of amplified DNA products with a molecular size corresponding to that of the EST clone sequence [J. Justesen, unpublished data]. Sequencing of this amplified DNA revealed that the cellular mRNA most likely has a single nucleotide deletion compared to the genomic sequence, changing the open reading frame of exon 8 to code for a 36-amino acid C terminus corresponding to a protein with a predicted molecular weight of 43.9 kDa (p44).

Ghosh et al. [56] isolated two cDNA clones of the small OAS, the clone 9-2 (accession no. M63850) and the clone 3-9 (accession no. M63849), by screening a cDNA library from murine Ehrlich ascites tumour cells. Whereas other murine cDNA clones have about 70% amino acid sequence homology to the human OAS1 clones [66, 67], the N-terminal sequences of both the 9-2 and 3-9 clones are identical to that of human OAS1. The sequence of clone 3-9 is identical to that of human p42 OAS, whereas clone 9-2 represents a splice variant, where 98 nt were deleted during splicing of exon E to exon 7 (fig. 6) [56]. This deletion of the 5′ end of exon 7 results in a reading frame change, yielding the 68 C-terminal amino acids of the resulting p48 protein (fig. 5). Therefore, the 9-2/3-9 cDNA clones must be human clones. The most likely explanation for the discrepancy seems to be that the murine cDNA preparation used for isolating these two clones was contaminated by human cDNA.

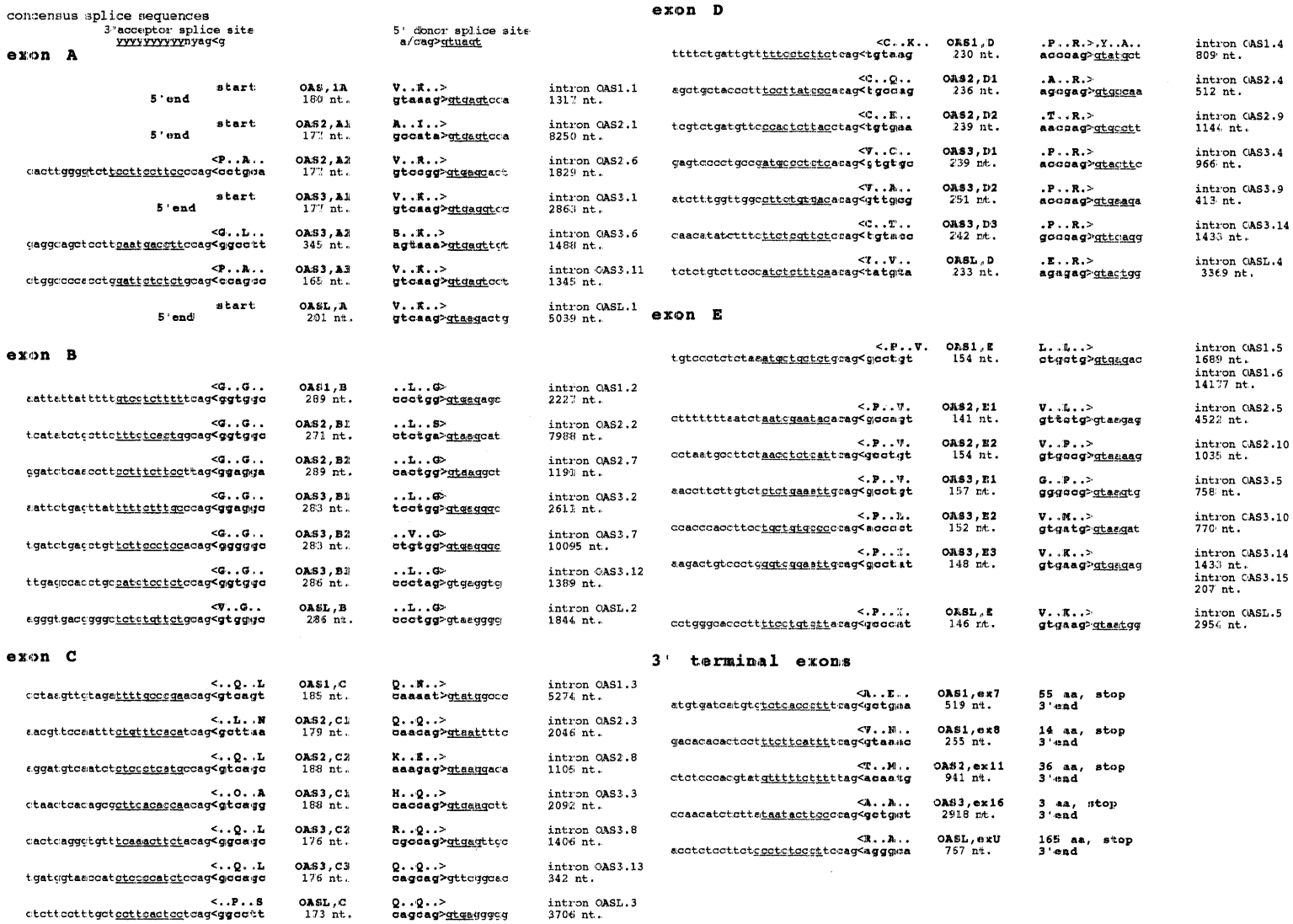

Figure 7. DNA sequences of exon/intron boundaries of the human OAS1, OAS2, OAS3 and OASL genes. The 3′ and 5′ consensus splice sequences are indicated at the top.

OAS2

Clones of cDNA from IFN-treated Daudi cells encoding the p69 and p71 oligoadenylate synthetases have been isolated and sequenced (accession no. M87284 and M87434) [21]. The deduced amino acid sequences of the two proteins with 687 and 727 amino acids, respectively, share the first 683 amino acids and were identified as consisting of two homologous domains combined by a hypothetical linker region. Both domains were reported to have strong homology to the N-terminal 346 amino acids of the p42 OAS, and Marié and Hovanessian [21] suggested that the OAS2 gene coding for the p69 and the p71 OAS was derived by fusion of two ancestral OAS1 genes. Wang and Floyd-Smith [68] mapped the major transcriptional initiation site to 84 bp upstream of the translation start site.

The annotations to the genomic PAC clone, RP1-71H24, indicate that the OAS2 gene is localized to the region 62–29 kb and contains 12 exons (figs 3, 9). Among these exons, two groups of five (exons A1–E1 and exons A2–E2) show homologies to the exons A–E of the OAS1 gene (fig. 3, table 3). As shown in table 3, especially high amino acid identity exists between exons A2–E2 and exons A–E of OAS1. The 21-amino-acid linker region suggested by Marié and Hovanessian [21] to combine two homologous domains of the p69/71 proteins is derived from the C terminus of exon E1 (11 amino acids) and N terminus of exon A2 (10 amino acids). Therefore, no specific linker region exists in the OAS2 gene combining the domains coded by exons A1–E1 and exons A2–E2.

The conservation of the A–E exon structure of the OAS1 gene in two copies in the OAS2 gene is a strong indication that gene duplication has occurred. Furthermore, as seen in figure 7, the reading frames across the splice junctions have been conserved in all of the exons. Since the introns show strong size variation (fig. 7), the duplication is likely to have been a rather early event during evolution, and might be expected to exist in other organisms as well.

As in the case of OAS1, the OAS2 gene is transcribed in the centromer to telomer direction (fig. 9).

The 1170 nt of exon 11 and the 940 nt of exon 12 encode the C termini of p69 and p71, respectively. In analogy to the situation with p42, no splicing occurs at the end of exon E2 in the mRNA coding for the p69 protein and translation continues in exon 11 by only 4 amino acids (fig. 5). In the case of p71 mRNA, exon E2 is spliced to exon 12 where an initial open reading frame encodes the 36 C-terminal amino acids (figs 3, 5).

The amino acid sequence of p69 and p71, predicted from the PAC clone RP1-71H24 OAS2 gene (fig. 4), corresponds to that presented by Marié and Hovanessian [21], except for differences at 11 amino acid positions in exon A1–E2 and an earlier stop codon in exon 12. The translation of the PAC clone OAS2 exons predicts a molecular weight for p69 of 78.6 kDa and for p71 of 82.3 kDa.

OAS3

Recently, the 6276-nt sequence of the p100 OAS mRNA was established by Rebouillat et al. [69]. The cDNA clone was constructed through a combination procedure, where several partial cDNA clones were isolated and sequenced and the final full-length sequence of the p100 OAS mRNA (accession no. AF063613) was constructed by combining overlapping partial cDNA sequences. The derived amino acid sequence of 1087 amino acids of the p100 OAS was found to have three structural domains homologous to p42 OAS.

By fluorescent in situ hybridization analysis, Hovnanian et al. [25] found that the OAS3 gene, like that of OAS1 and OAS2, is located at chromosomal region 12q24.2, and further analysis of a number of PAC and YAC clones showed that all three genes are located within a 130-kb chromosomal region.

Table 3. Size of exons and identities of amino acids between OAS1 and other members of the OAS family.

	Exon A		Exon B		Exon C		Exon D		Exon E	
	aa	Identity	aa	Identity	aa	Identity	aa	Identity	aa	Identity
OAS1	60	–	96	–	61	–	76	–	51	–
OAS2, 1	59	32	90	37	59	49	78	50	47	49
OAS2, 2	58	45	96	49	66	56	79	58	51	55
OAS3, 1	59	36	94	38	59	53	80	48	51	53
OAS3, 2	115	21	94	41	59	56	80	53	49	49
OAS3, 3	60	53	95	68	59	66	80	56	49	47
OASL	67	33	95	26	58	55	81	48	49	45

The amino acid sequences of the six groups of exons A–E of OAS2, OAS3 and OASL were compared to the amino acid sequence of exons A–E of OAS1. The table indicates the number of amino acids (aa) in each exon and the percent amino acid identity to the OAS1 exons, as obtained by the Clustal method.

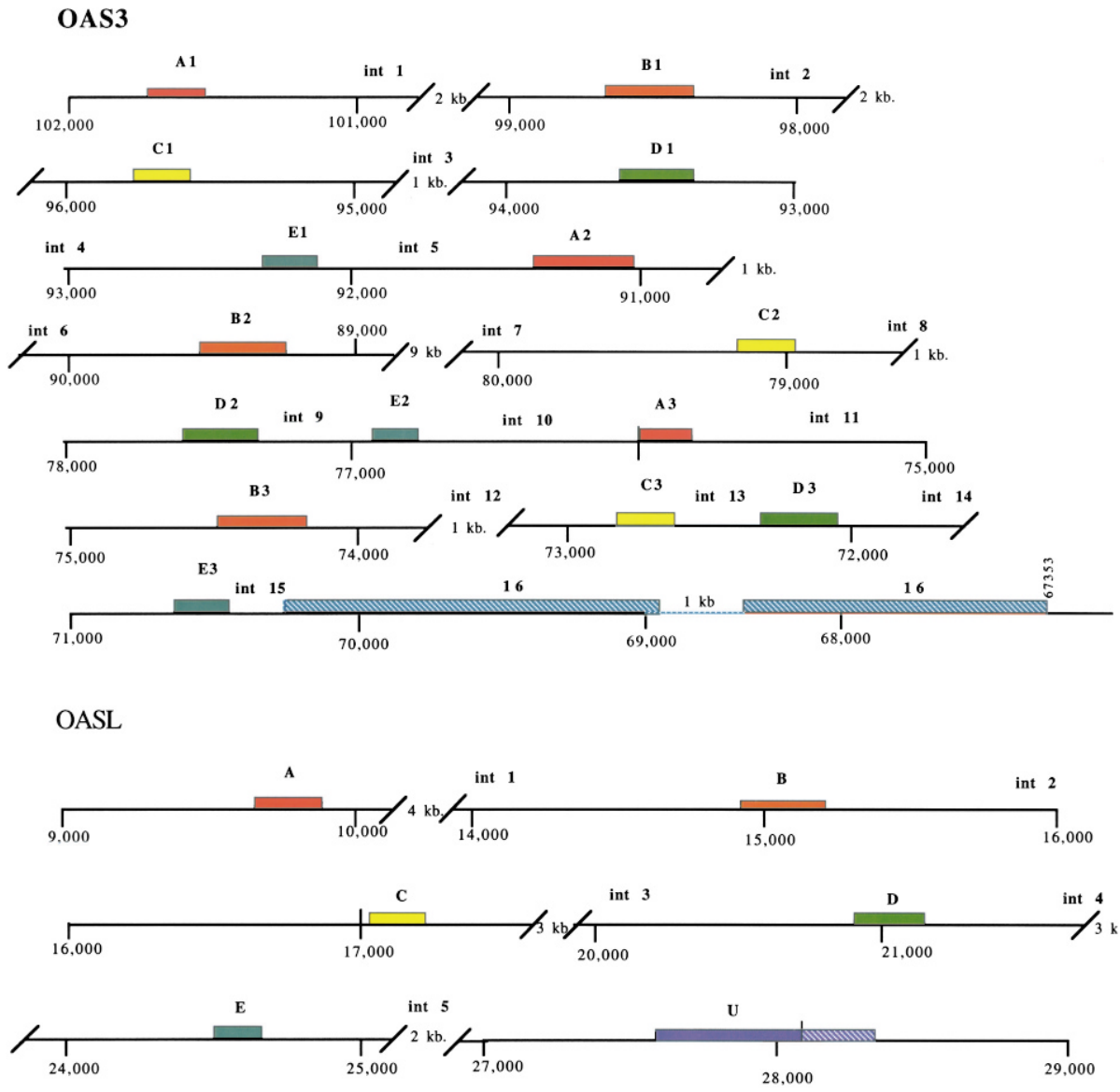

Figure 8. Exon/intron structure of OAS3 and OASL genes. Exons are marked by coloured bars, and the non-translated parts of the C-terminal exons are cross-hatched. The numbers refer to positions on the PAC clone RP1-71H24 for OAS3 and the BAC clone bk92N15 for OASL.

Independently, we have identified the OAS3 gene by searching the genomic PAC clone, RP1-71H24, for open reading frames encoding more than 50 amino acids having amino acid sequences homologous to the conserved amino acid motifs of exons A–E of OAS1. This search revealed the presence of three groups of five exons highly homologous to exons A–E of OAS1, which we call exons A1–E1, exons A2–E2 and exons A3–E3 (figs 4, 8). The OAS3 gene is located between the OAS1 and OAS2 genes in the region 102–70 kb on the PAC clone, RP1-71H24 (fig. 9). The direction of transcription is identical to that of the other two OAS genes. All of the predicted exons contain consensus splice donor and acceptor sites and again, the reading frames across the exon/intron barriers are identical to those observed for the OAS1 and OAS2 exons (fig. 7). The amino acid sequences of all three exon groups show homology to the A–E exons of OAS1 (fig. 4, table 3), and are especially pronounced for the exon group A3–E3.

Exon A2 is about twice as large (115 amino acids) as the other exons in the A group (fig. 4, table 3). Analysis of the sequences in the PAC clone, RP1-71H24, shows that the consensus splice site at the conventional position in exon A2 has been changed, and a new consensus splice site defines the 5′ end of the enlarged exon (fig. 7). This extension of exon A2 codes for the amino acids described as a linker region by Rebouillat et al. [69].
The p100 sequence of Rebouillat et al. [69] has a 3′-terminal exon 16 of about 3000 nt, only coding for the 3 C-terminal amino acids of the p100 sequence (fig. 5). Exon 16 was found to be located at position 70–67 kb of the PAC clone, RP1-71H24 (fig. 8).

OASL

Searching the EST clone library of Human Genome Sciences (Rockville, Md.) for possible clones with homology to the OAS genes, we identified a clone (HG # 1173451) derived from Jurkat cell cDNA with N-terminal sequences homologous to exons A–E of OAS1 [17]. In this EST clone, the start codon for protein synthesis is missing, but the remaining 5′ sequence of the mRNA was retrieved from HeLa cell RNA using the rapid amplification of cDNA ends, and was found to comprise 6 nt upstream of the initiation codon. The entire EST clone (accession no. AJ225089) encodes a protein of 514 amino acids (p59) with a predicted molecular weight of 59.3 kDa. The first 349 amino acids are highly homologous to those of OAS1 proteins (fig. 4), and the 165-amino-acid C-terminal domain (fig. 5) was found to be homologous to ubiquitin [17]. During their efforts to clone the p100 OAS cDNA, Rebouillat et al. [18] isolated two Daudi cell cDNA clones with OAS homology. The clones were sequenced and found to code for two OASL proteins, p56 and p30 (accession no. AF063611 and AF063612). The p56 sequence is similar to that of p59, except for differences in two early regions of 10 and 20 amino acids and a further five single-amino-acid differences. The p30 OASL seems to be a splice variant with exon D omitted. This results in a change in reading frame in exon E and a subsequent early termination of the protein chain. Hovnanian et al. [24] found that the OASL gene was localized to chromosome 12q24.2.
The cDNA sequence of the p59 OASL was identical to sequences in a 51,943-bp region of the human genomic BAC clone, bK92N15 (contig 03615, accession no. Z93097), localized to chromosome 12q24.2. This allowed us to identify the OASL gene, which consists of six coding exons (fig. 8) in the 9,000–29,000 nt region of bK92N15. The five 5′ exons show homology to the A–E exons of the OAS1 protein (fig. 4, table 3), and the

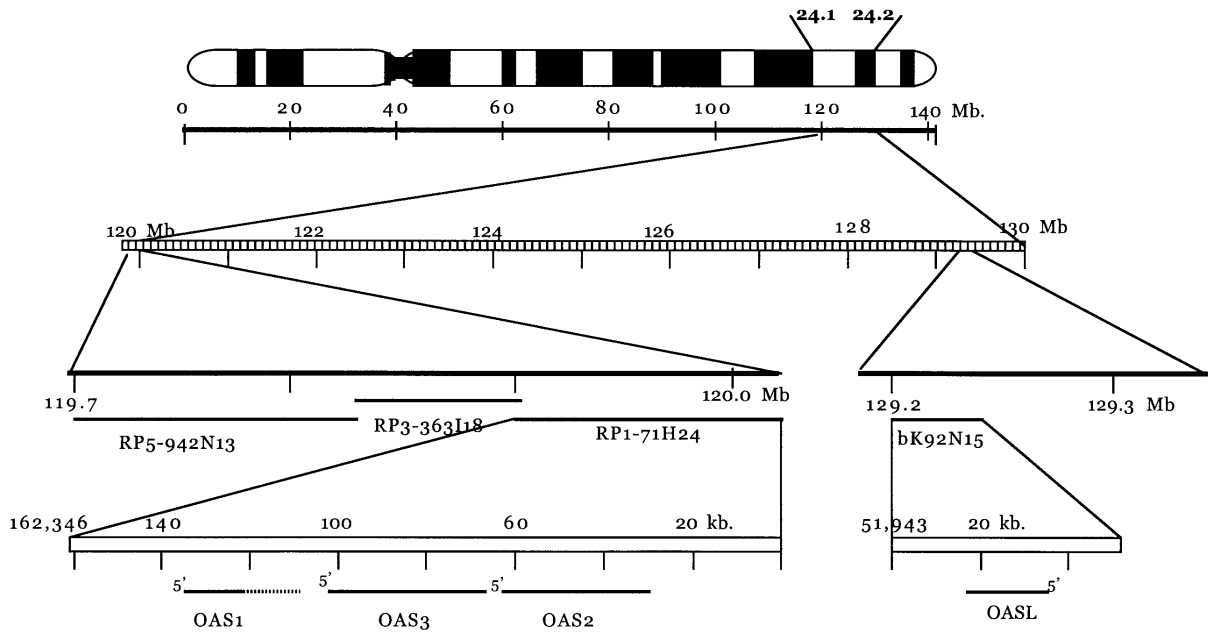

Figure 9. Localization of the OAS family genes on human chromosome 12. The scale of the three top lines is in Mb as indicated in the NCBI database (December 1999). For the lower lines representing the PAC clone RP1-71H24 and the BAC clone bk92N15, the scale is in kb.

766-nt C-terminal exon U (fig. 5) encodes two repeats of ubiquitin-like sequences. Transcription of the OASL gene occurs in the centromer to telomer direction (fig. 9).

In contrast to the p59 OASL clone, the cDNA clones of p56 and p30 harbour identical 5′ sequences of 346 nucleotides upstream of the ATG initiation codon [18]. Of this sequence, nucleotides −172 to −346 can be found in the genomic bK92N15 sequence, whereas the initial 171 nucleotides are absent from it. A BLAST search revealed identity of the first 171 nucleotides of the p56/p30 clones to a DNA sequence within a 100,000-bp PAC clone on chromosome 8p21.3–22 (accession no. AB020865). This suggests that the human lymphoblastoid Daudi cell line used for preparing the cDNA has a translocation between q-terminal sequences of chromosome 12 and p-terminal sequences of chromosome 8. This might explain the different transcriptional start sites observed in Daudi cells [18] and the HeLa cell line used by us [17].

Recently, Tiefenthaler et al. [19] cloned an analogous OASL mRNA from murine spleen dendritic cells, as a 3665-bp cDNA, M1204 (accession no. AF068835) encoding a protein with an amino acid sequence strongly homologous to that of the human p59 OASL. Like the human OASL, this protein has a ubiquitin-like C-terminal end.

Promoter regions

Analysis of the promotor regions of several IFN-inducible proteins has revealed the presence of different regulatory DNA elements that are binding sites for transcriptional regulators. The IFN-γ activation sequence (GAS, 5′-TTN5AA-3′) binds a dimer of the phosphorylated STAT1-α protein (fig. 1) and the IFN regulatory factor element (IRF-E, 5′-TCNNTTT-3′) binds a family of IFN regulatory factors (IRFs) that can either stimulate or repress transcription [70, 71]. Among the IRFs, the IFN-stimulated response element (ISRE, 5′-AGTTTCNNTTTCNY-3′) binds IFN-stimulated gene factor-3 (ISGF-3), forming a complex of transcription factors with the phosphorylated STAT1 and STAT2 proteins (fig. 1) [27, 70, 72].

When the OAS1 genomic clone was isolated, the promoter region was studied and two regions, A and B, about 150 and 100 nt upstream of the start codon for OAS synthesis, were characterized [73, 74]. The B site, which carries an ISRE site, was found responsible for IFN-β induction, while the A site, carrying an IRF-E and GAS site, seems to act as a constitutive enhancer. Later, the promoter region of the OAS2 gene was isolated and studied [68, 75]. The region upstream of the start codon contains several regulatory elements such as two ISRE sites, two IRF-E sites and sites for NFκB and interleukin-6 (IL-6). The latter is thought to account for the induction by IL-6 [68, 76].

Screening of the PAC clone RP1-71H24 sequences upstream of the start codon for the OAS3 protein shows the presence of two ISRE sites, three GAS sites and an NFκB site.

Chromosomal localizations and exon structure

It has been suggested that all human OAS genes are localized to chromosome 12q24.2 [24, 25]. However, according to database information, the PAC clone RP1-71H24 which harbours the OAS1, OAS2 and OAS3 genes is located in the region 12q24.1, and the BAC clone bK92N15, harbouring the OASL gene, is located at 12q24.2.

The genomic databases at The National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) and the Sanger Centre (www.sanger.ac.uk) indicate a total size of human chromosome 12 of 147 Mb (as of December 1999). This corresponds to a location of the PAC clone RP1-71H24 at about 120 Mb, and the BAC clone bK92N15 at about 129 Mb, counting from the p-telomeric end of chromosome 12 (fig. 9).

By studying the exon structure of the OAS genes, we observed strong homologies between the amino acid sequences of exons A–E of p42 and exons A–E of all other OAS-type proteins (fig. 4), suggesting a very similar gene structure. The reading frames across the exon/intron boundaries are also maintained for all the OAS exons (fig. 7). The conservation of the A–E exon structure as a single copy in OASL, as two repeats in OAS2 and three repeats in OAS3 supports the assumption of duplications of an ancestral OAS gene as the origin for these three genes. In contrast to the homologies of the OAS exons, the introns show no similarities and vary greatly in size between the OAS genes (fig. 7). These intron size variations must indicate that the suggested gene duplications establishing the OAS2, OAS3 and OASL genes occurred rather early during evolution. However, as discussed below, the lack of information about OAS gene structure in organisms other than the human means that this hypothesis cannot be tested for the time being.

Table 3 summarizes the homology data, giving the number of amino acids in each exon and their percentage identity compared to the OAS1 exons. In all cases, the clusters of exons C and D show the highest degree of amino acid identity. That the last group of exons of OAS2 (A2–E2) and OAS3 (A3–E3) shows the highest degree of amino acid identity to that of OAS1 implies that the enzymatic activity of the polymeric OAS proteins is associated with the C-terminal amino acid sequences. This view is in agreement with a recent study

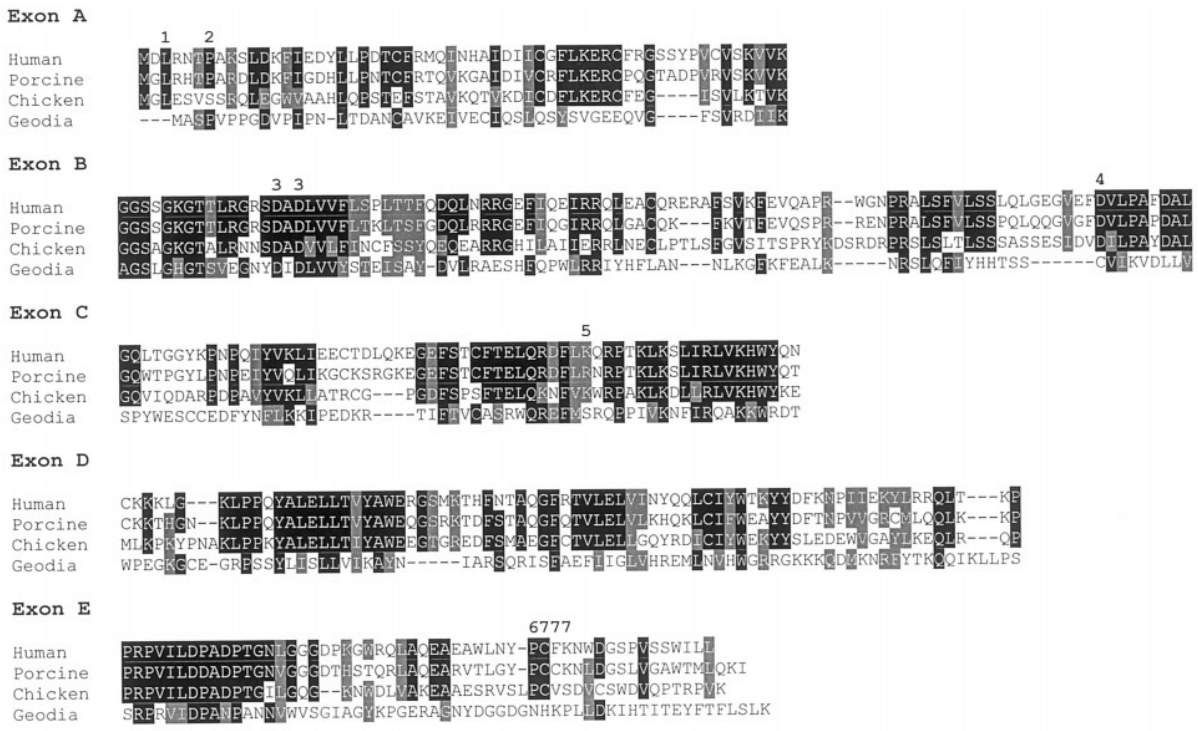

Figure 10. Alignment of the amino acid sequences of the human OAS1 gene with those of non-human OAS proteins. The amino acid sequences of the porcine (*Sus*), chicken (*Gallus*) and marine sponge (*Geodia*) proteins are aligned with the sequences of the A–E exons of the human OAS1 gene. Black boxes indicate positions at which the residues are identical, and grey boxes show residues that are similar.

showing that p69 has only one active site located to the C-terminal repeat [55].

The non-human OASs

From several mammalian species as well as from chicken, cDNA clones corresponding to the small species of OAS have been cloned and sequenced (mouse, rat, pig, cow, chicken). At the amino acid level, all these cDNA clones show sequence homology to that of the human OAS1 exons (fig. 10), when the sequences of the human OAS1 exons A–E are aligned with the corresponding amino acid sequences of non-human OAS proteins. The concordance between the sequences suggests that the exon structure of the human genes is conserved in other organisms as well. However, no sequence data of genomic clones are available in the databases for any of these species to verify such an assumption. In the case of the mouse, some genomic clones exist containing part of or the entire gene of the small OAS protein [73, 77].

The murine OAS

Besides the human OAS, the mouse enzyme has been the most thoroughly studied and small (40 kDa), medium (75 kDa) and large (105 kDa) OAS proteins and their mRNAs have been observed in the mouse [12, 13].

By cross-hybridization to human OAS1 cDNA, Ichii et al. [66] isolated a cDNA from IFN-treated mouse L cells (accession no. X04958) and obtained a sequence encoding a 367-amino-acid protein, with strong homology to human OAS1. Using the human OAS1 cDNA (E18) as a probe, Coccia et al. [67] isolated a full-length cDNA clone L3 (accession no. M33863), which was found to be sequence identical to the Ichii clone. By transfection of murine NIH-3T3 cells with a vector harbouring the L3 cDNA under transcriptional control of the LTR of Moloney murine leukaemia virus, the cells were found to express OAS activity rendering them resistant to infection by EMC virus. From JLSV9R cells, Rutherford et al. [77] isolated another cDNA clone L1 (accession no. X55982), which is incomplete and only contains the sequences corresponding to exon B and part of exon C. The sequence of L1 is ho-

mologous, but only 82% identical to that of clone L3, suggesting that it might represent another OAS gene. Two cDNA clones (9-2, 3-9) were isolated from Ehrlich ascites tumour cells and sequenced by Ghosh et al. [56]. Although published as murine clones, these sequences are identical to those of the human OAS1 cDNA clones and are probably derived from a human mRNA contaminant of the murine RNA preparation used for the cloning.

Cohen et al. [73] isolated a murine genomic clone harbouring a complete gene of one of the murine OAS proteins (ME-12), and two genomic clones, ME-8 and ME-5, which seem to be overlaps of a different OAS gene. Thus, mice likely have at least two OAS genes. This was confirmed by Rutherford et al. [77] who isolated a 41-kDa cosmid clone of mouse genomic DNA which, by restriction enzyme mapping and hybridization to the L3 cDNA clone, was found to harbour the OAS genes present in both ME-12 and ME-8/ME-5. Both the M-12 and the M-8 clones were found to contain two N-terminal exons within the OAS genes, but no sequence data were released [73].

Other mammalian OAS cDNA clones

Two cDNA clones of OAS from rat have been isolated (accession no. Z18877) [78]; and Shimizu et al., accession no. AF068268). Although similar, the two clones show many differences in their nucleotide sequences and might be transcripts of two different genes. A partial OAS cDNA clone from woodchuck has also been isolated and sequenced (accession no. AF082498).

Two porcine cDNA clones have been sequenced. A complete 1192-bp cDNA sequence from a porcine small intestine cDNA library codes for 349 amino acids corresponding to exons A–E (fig. 10) and a terminal 3′ exon (Hartmann, accession no AJ225090). An incomplete cDNA clone of 992 bp (Ozawa et al., accession no. D84331) is identical to the former clone, but sequences corresponding to exon E and the 3′-terminal exon are missing.

All these mammalian OAS sequences show strong homologies to the human OAS1 protein (fig. 10).

Non-mammalian cDNA clones

Two types of complete chicken OAS cDNAs (accession nos. AB002585 and AB002586) were cloned and sequenced by Yamamoto et al. [15]. The A-type cDNA codes for 508 amino acids whereas type B codes for 476 amino acids. The parts of the coding regions corresponding to exons A–E are identical and homologous to those of the human OAS1 protein (fig. 10). The differences between the two sequences are found in the 3′ region, which might correspond to the terminal exon, where a 100-nt sequence in clone A has been replaced by 3 nt in clone B. PCR amplifications using chicken erythrocyte DNA indicate that the two cDNA clones represent alleles of an OAS gene. Furthermore, the PCR analysis revealed the presence of a small intron with the 5′ end corresponding to the terminus of human exon E.

The marine sponge *G. cydonium* possesses a high level of OAS activity [79] and by radioimmunoassay and high-performance liquid chromatography, OASs were demonstrated to be present in the sponge [80]. A potential OAS cDNA clone was recently isolated from *G. cydonium* [16]; it encodes a protein of 327 amino acids with some but limited homology to human OAS1 (fig. 10). However, this cDNA clone has not yet been expressed by transfection, to test if the protein has OAS activity.

Evolution of the OAS family

As discussed above, the human OAS family has four members. Homologues of the small OAS protein have been characterized in a number of mammals, in chicken and as far back in evolution as the marine sponge *G. cydonium* (phylum Porifera). The presence of an OAS1 gene in *G. cydonium* suggests that OAS is widely distributed among the metazoans, although a data search has not unequivocally revealed such genes in the genome of *Caenorhabditis elegans*. Indications of the presence of the larger OAS proteins and OASL protein have been obtained for some species. Thus, the OAS family has likely evolved by multiple duplications of an ancestral OAS gene, probably similar to the OAS1 gene.

The organization of the OAS gene family will probably be similar throughout the mammalian class, even though we cannot determine whether one or more genes have been lost in some species through secondary deletions. Mouse also appears to contain four members of the OAS family—OAS1 and OASL have already been cloned [66, 67, 81], and a 100- to 110-kDa protein with OAS activity has been described [13, 35]. Finally, by searching the mouse EST database for sequences homologous to the small OAS protein, we found four groups of EST clones, of which two represent the already known OAS1 and OASL genes, and the other two are probably equivalents of OAS2 and OAS3 [R. Hartmann, unpublished data].

Acknowledgements. This work was supported by the Danish Natural Science Research Council and the Danish Cancer Society.

Note added in proof:
While this review was in press a paper was published discussing the molecular evolution of the OASgene family: Kumar S., Mitnik C., Valente G., Floyd-Smith G. (2000) Mol. Biol. Evol. **17:** 738–50.

1 Chebath J., Benech P., Revel M. and Vigneron M. (1987) Constitutive expression of (2′-5′) oligo A synthetase confers resistance to picornavirus infection. Nature **330:** 587–588
2 Mitra A., Higgins D. W., Langenberg W. G., Nie H., Sen-Gupta D. N. and Silverman R. H. (1996) A mammalian 2-5A system functions as an antiviral pathway in transgenic plants. Proc. Natl. Acad. Sci. USA **93:** 6780–6785
3 Ogawa T., Hori T. and Ishida I. (1996) Virus-induced cell death in plants expressing the mammalian 2′,5′ oligoadenylate system. Nat. Biotechnol. **14:** 1566–1569
4 Dong B. and Silverman R. H. (1997) A bipartite model of 2-5A-dependent RNase L. J. Biol. Chem. **272:** 22236–22242
5 Clemens M. J. and Williams B. R. (1978) Inhibition of cell-free protein synthesis by pppA2′p5′A2′p5′A: a novel oligonucleotide synthesized by interferon-treated L cell extracts. Cell **13:** 565–572
6 Baglioni C., Minks M. A. and Maroney P. A. (1978) Interferon action may be mediated by activation of a nuclease by pppA2′p5′A2′p5′A. Nature **273:** 684–687
7 Zhou A., Hassel B. A. and Silverman R. H. (1993) Expression cloning of 2-5A-dependent RNAase: a uniquely regulated mediator of interferon action. Cell **72:** 753–765
8 Zhou A., Paranjape J., Brown T. L., Nie H., Naik S., Dhong B. et al. (1997) Interferon action and apoptosis are defective in mice devoid of 2′,5′-oligoadenylate-dependent RNase L. EMBO J. **16:** 6355–6363
9 Diaz Guerra M., Rivas C. and Esteban M. (1997) Activation of the IFN-inducible enzyme RNase L causes apoptosis of animal cells. Virology **236:** 354–363
10 Castelli J. C., Hassel B. A., Maran A., Paranjape J., Hewitt J. A., Li X. L. et al. (1998) The role of 2′-5′ oligoadenylate-activated ribonuclease L in apoptosis. Cell Death Differ. **5:** 313–320
11 Chebath J., Benech P., Hovanessian A., Galabru J. and Revel M. (1987) Four different forms of interferon-induced 2′,5′-oligo(A) synthetase identified by immunoblotting in human cells. J. Biol. Chem. **262:** 3852–3857
12 Hovanessian A. G., Laurent A. G., Chebath J., Galabru J., Robert N. and Svab J. (1987) Identification of 69-kd and 100-kd forms of 2-5A synthetase in interferon-treated human cells by specific monoclonal antibodies. EMBO J. **6:** 1273–1280
13 St Laurent G., Yoshie O., Floyd-Smith G., Samanta H., Sehgal P. B. and Lengyel P. (1983) Interferon action: two (2′-5′)(A)n synthetases specified by distinct mRNAs in Ehrlich ascites tumor cells treated with interferon. Cell **33:** 95–102
14 Kelve M., Truve E., Aaspollu A., Kuusksalu A., Dapper J., Perovic S. et al. (1994) Rapid reduction of mRNA coding for 2′-5′-oligoadenylate synthetase in rat pheochromocytoma PC12 cells during apoptosis. Cell. Mol. Biol. **40:** 165–173
15 Yamamoto A., Iwata A., Koh Y., Kawai S., Murayama S., Hamada K. et al. (1998) Two types of chicken 2′,5′-oligoadenylate synthetase mRNA derived from alleles at a single locus. Biochim. Biophys. Acta **1395:** 181–191
16 Wiens M., Kuusksalu A., Kelve M. and Muller W. E. (1999) Origin of the interferon-inducible (2′-5′)oligoadenylate synthetases: cloning of the (2′-5′)oligoadenylate synthetase from the marine sponge *Geodia cydonium*. FEBS Lett. **462:** 12–18
17 Hartmann R., Olsen H. S., Widder S., Jorgensen R. and Justesen J. (1998) p59OASL, a 2′-5′ oligoadenylate synthetase like protein: a novel human gene related to the 2′-5′ oligoadenylate synthetase family. Nucleic Acids Res. **26:** 4121–4128
18 Rebouillat D., Marié I. and Hovanessian A. G. (1998) Molecular cloning and characterization of two related and interferon-induced 56-kDa and 30-kDa proteins highly similar to 2′-5′ oligoadenylate synthetase. Eur. J. Biochem. **257:** 319–330
19 Tiefenthaler M., Marksteiner R., Neyer S., Koch F., Hofer S., Schuler G. et al. (1999) M1204, a novel 2′,5′ oligoadenylate synthetase with a ubiquitin-like extension, is induced during maturation of murine dendritic cells. J. Immunol. **163:** 760–765
20 Benech P., Mory Y., Revel M. and Chebath J. (1985) Structure of two forms of the interferon-induced (2′-5′) oligo A synthetase of human cells based on cDNAs and gene sequences. EMBO J. **4:** 2249–2256
21 Marié I. and Hovanessian A. G. (1992) The 69-kDa 2-5A synthetase is composed of two homologous and adjacent functional domains. J. Biol. Chem. **267:** 9933–9939
22 Rebouillat D. and Hovanessian A. G. (1999) The human 2′,5′-oligoadenylate synthetase family: interferon-induced proteins with unique enzymatic properties. J. Interferon Cytokine Res. **19:** 295–308
23 Saunders M. E., Gewert D. R., Tugwell M. E., McMahon M. and Williams B. R. (1985) Human 2-5A synthetase: characterization of a novel cDNA and corresponding gene structure. EMBO J. **4:** 1761–1768
24 Hovnanian A., Rebouillat D., Levy E. R., Mattei M. G. and Hovanessian A. G. (1999) The human 2′,5′-oligoadenylate synthetase-like gene (OASL) encoding the interferon-induced 56-kDa protein maps to chromosome 12q24.2 in the proximity of the 2′,5′-OAS locus. Genomics **56:** 362–363
25 Hovnanian A., Rebouillat D., Mattei M. G., Levy E. R., Marié I., Monaco A. P. et al. (1998) The human 2′,5′-oligoadenylate synthetase locus is composed of three distinct genes clustered on chromosome 12q24.2 encoding the 100-, 69-, and 40-kDa forms. Genomics **52:** 267–277
26 Sarkar S. N. and Sen G. C. (1998) Production, purification, and characterization of recombinant 2′, 5′-oligoadenylate synthetases. Methods Enzymol. **15:** 233–242
27 Stark G. R., Kerr I. M., Williams B. R., Silverman R. H. and Schreiber R. D. (1998) How cells respond to interferons. Annu. Rev. Biochem. **67:** 227–264
28 Roberts W. K., Hovanessian A., Brown R. E., Clemens M. J. and Kerr I. M. (1976) Interferon-mediated protein kinase and low-molecular-weight inhibitor of protein synthesis. Nature **264:** 477–480
29 Kerr I. M., Brown R. E. and Hovanessian A. G. (1977) Nature of inhibitor of cell-free protein synthesis formed in response to interferon and double-stranded RNA. Nature **268:** 540–542
30 Hovanessian A. G., Brown R. E. and Kerr I. M. (1977) Synthesis of low molecular weight inhibitor of protein synthesis with enzyme from interferon-treated cells. Nature **268:** 537–540
31 Kerr I. M. and Brown R. E. (1978) pppA2′p5′A2′p5′A: an inhibitor of protein synthesis synthesized with an enzyme fraction from interferon-treated cells. Proc. Natl. Acad. Sci. USA **75:** 256–260
32 Zilberstein A., Kimchi A., Schmidt A. and Revel M. (1978) Isolation of two interferon-induced translational inhibitors: a protein kinase and an oligo-isoadenylate synthetase. Proc. Natl. Acad. Sci. USA **75:** 4734–4738
33 Samuel C. E. (1979) Mechanism of interferon action: phosphorylation of protein synthesis initiation factor eIF-2 in interferon-treated human cells by a ribosome-associated kinase processing site specificity similar to hemin-regulated rabbit reticulocyte kinase. Proc. Natl. Acad. Sci. USA **76:** 600–604
34 Dougherty J. P., Samanta H., Farrell P. J. and Lengyel P. (1980) Interferon, double-stranded RNA, and RNA degradation: isolation of homogeneous pppA(2′p5′A)n-1 synthetase from Ehrlich ascites tumor cells. J. Biol. Chem. **255:** 3813–3816
35 Yang K., Samanta H., Dougherty J., Jayaram B., Broeze R. and Lengyel P. (1981) Interferons, double-stranded RNA, and RNA degradation: isolation and characterization of homogeneous human (2′-5′)(A)n synthetase. J. Biol. Chem. **256:** 9324–9328

36 Rovnak J. and Ranu R. S. (1987) Purification of 2′,5′-oligoadenylate synthetase from rabbit reticulocytes. J. Interferon Res. **7:** 231–241

37 Revel M., Wallach D., Merlin G., Schattner A., Schmidt A., Wolf D. et al. (1981) Interferon-induced enzymes: microassays and their applications – purification and assay of (2′-5′)-oligoadenylate synthetase and assay of 2′-phosphodiesterase. Methods Enzymol. **79:** 149–161

38 Marié I., Svab J., Robert N., Galabru J. and Hovanessian A. G. (1990) Differential expression and distinct structure of 69- and 100-kDa forms of 2-5A synthetase in human cells treated with interferon. J. Biol. Chem. **265:** 18601–18607

39 Besse S., Rebouillat D., Marié I., Puvion-Dutilleul F. and Hovanessian A. G. (1998) Ultrastructural localization of interferon-inducible double-stranded RNA-activated enzymes in human cells. Exp. Cell Res. **239:** 379–392

40 Zhang Y., Zhang G., Mollat P., Carles C., Riva M., Frobert Y. et al. (1993) Purification, characterization, and cellular localization of the 100-kDa human placental GTPase-activating protein. J. Biol. Chem. **268:** 18875–18881

41 Johnston M. I. and Torrence P. F. (1984) The role of interferon-induced proteins, double-stranded RNA and 2′,5′-oligoadenylate in the interferon-mediated inhibition of viral translation. In: Interferon 3: Mechanisms of Production and Action, pp. 189–298, Friedman R. M. (ed), Elsevier, Amsterdam

42 Justesen J., Ferbus D. and Thang M. N. (1980) Elongation mechanism and substrate specificity of 2′,5′-oligoadenylate synthetase. Proc. Natl. Acad. Sci. USA **77:** 4618–4622

43 Justesen J., Ferbus D. and Thang M. N. (1980) 2′5′ oligoadenylate synthetase, an interferon induced enzyme: direct assay methods for the products, 2′5′ oligoadenylates and 2′5′ co-oligonucleotides. Nucleic Acids Res. **8:** 3073–3085

44 Justesen J. and Kjeldgaard N. O. (1992) Spectrophotometric pyrophosphate assay of 2′,5′-oligoadenylate synthetase. Anal. Biochem. **207:** 90–93

45 Ferbus D., Justesen J., Besancon F. and Thang M. N. (1981) The 2′5′ oligoadenylate synthetase has a multifunctional 2′5′ nucleotidyl-transferase activity. Biochem. Biophys. Res. Commun. **100:** 847–856

46 Rice A. P., Kerr S. M., Roberts W. K., Brown R. E. and Kerr I. M. (1985) Novel 2′,5′-oligoadenylates synthesized in interferon-treated, vaccinia virus-infected cells. J. Virol. **56:** 1041–1044

47 Turpaev K., Hartmann R., Kisselev L. and Justesen J. (1997) Ap3A and Ap4A are primers for oligoadenylate synthesis catalyzed by interferon-inducible 2-5A synthetase. FEBS Lett. **408:** 177–181

48 Turpaev K., Hartmann R. and Justesen J. (1999) 2′-Adenylated derivatives of Ap3A activate RNase L. FEBS Lett. **457:** 9–12

49 Gribaudo G., Lembo D., Cavallo G., Landolfo S. and Lengyel P. (1991) Interferon action: binding of viral RNA to the 40-kilodalton 2′-5′-oligoadenylate synthetase in interferon-treated HeLa cells infected with encephalomyocarditis virus. J. Virol. **65:** 1748–1757

50 Minks M. A., West D. K., Benvin S., Greene J. J., Ts'o P. O. and Baglioni C. (1980) Activation of 2′,5′-oligo(A) polymerase and protein kinase of interferon-treated HeLa cells by 2′-O-methylated poly (inosinic acid) · poly(cytidylic acid): correlations with interferon-inducing activity. J. Biol. Chem. **255:** 6403–6407

51 Hartmann R., Nørby P. L., Martensen P. M., Jørgensen P., James M. C., Jacobsen C. et al. (1998) Activation of 2-5 oligoadenylate synthetase by single-stranded and double-stranded RNA aptamers. J. Biol. Chem. **273:** 3236–3246

52 Minks M. A., West D. K., Benvin S. and Baglioni C. (1979) Structural requirements of double-stranded RNA for the activation of 2′,5′-oligo(A) polymerase and protein kinase of interferon-treated HeLa cells. J. Biol. Chem. **254:** 10180–10183

53 Desai S. Y. and Sen G. C. (1997) Effects of varying lengths of double-stranded RNA on binding and activation of 2′-5′-oligoadenylate synthetase. J. Interferon Cytokine Res. **17:** 531–536

54 Kon N. and Suhadolnik R. J. (1996) Identification of the ATP binding domain of recombinant human 40-kDa 2′,5′-oligoadenylate synthetase by photoaffinity labeling with 8-azido-[alpha-32P]ATP. J. Biol. Chem. **271:** 19983–19990

55 Sarkar S. N., Ghosh A., Wang H. W., Sung S. S. and Sen G. C. (1999) The nature of the catalytic domain of 2′-5′-oligoadenylate synthetases. J. Biol. Chem. **274:** 25535–25542

56 Ghosh S. K., Kusari J., Bandyopadhyay S. K., Samanta H., Kumar R. and Sen G. C. (1991) Cloning, sequencing, and expression of two murine 2′-5′-oligoadenylate synthetases: structure-function relationships. J. Biol. Chem. **266:** 15293–15299

57 Ghosh A., Sarkar S. N., Guo W., Bandyopadhyay S. and Sen G. C. (1997) Enzymatic activity of 2′-5′-oligoadenylate synthetase is impaired by specific mutations that affect oligomerization of the protein. J. Biol. Chem. **272:** 33220–33226

58 Ghosh A., Desai S. Y., Sarkar S. N., Ramaraj P., Ghosh S. K., Bandyopadhyay S. et al. (1997) Effects of mutating specific residues present near the amino terminus of 2′-5′-oligoadenylate synthetase. J. Biol. Chem. **272:** 15452–15458

59 Bandyopadhyay S., Ghosh A., Sarkar S. N. and Sen G. C. (1998) Production and purification of recombinant 2′-5′ oligoadenylate synthetase and its mutants using the baculovirus system. Biochemistry **37:** 3824–3830

60 Bisbal C., Martinand C., Silhol M., Lebleu B. and Salehzada T. (1995) Cloning and characterization of a RNAse L inhibitor: a new component of the interferon-regulated 2-5A pathway. J. Biol. Chem. **270:** 13308–13317

61 Merlin G., Chebath J., Benech P., Metz R. and Revel M. (1983) Molecular cloning and sequence of partial cDNA for interferon-induced (2′-5′)oligo(A) synthetase mRNA from human cells. Proc. Natl. Acad. Sci. USA **80:** 4904–4908

62 Benech P., Merlin G., Revel M. and Chebath J. (1985) 3′ end structure of the human (2′-5′) oligo A synthetase gene: prediction of two distinct proteins with cell type-specific expression. Nucleic Acids Res. **13:** 1267–1281

63 Wathelet M., Moutschen S., Cravador A., DeWit L., Defilippi P., Huez G. et al. (1986) Full-length sequence and expression of the 42 kDa 2-5A synthetase induced by human interferon. FEBS Lett. **196:** 113–120

64 Wathelet M. G., Szpirer J., Nols C. B., Clauss I. M., De Wit L., Islam M. Q. et al. (1988) Cloning and chromosomal location of human genes inducible by type I interferon. Somat. Cell. Mol. Genet. **14:** 415–426

65 Benech P., Vigneron M., Peretz D., Revel M. and Chebath J. (1987) Interferon-responsive regulatory elements in the promoter of the human 2′,5′-oligo(A) synthetase gene. Mol. Cell. Biol. **7:** 4498–4504

66 Ichii Y., Fukunaga R., Shiojiri S. and Sokawa Y. (1986) Mouse 2-5A synthetase cDNA: nucleotide sequence and comparison to human 2-5A synthetase. Nucleic Acids Res. **14:** 10117

67 Coccia E. M., Romeo G., Nissim A., Marziali G., Albertini R., Affabris E. et al. (1990) A full-length murine 2-5A synthetase cDNA transfected in NIH-3T3 cells impairs EMCV but not VSV replication. Virology **179:** 228–233

68 Wang Q. and Floyd-Smith G. (1997) The p69/71 2-5A synthetase promoter contains multiple regulatory elements required for interferon-alpha-induced expression. DNA Cell Biol. **16:** 1385–1394

69 Rebouillat D., Hovnanian A., Marié I. and Hovanessian A. G. (1999) The 100-kDa 2′,5′-oligoadenylate synthetase catalyzing preferentially the synthesis of dimeric pppA2′p5′A molecules is composed of three homologous domains. J. Biol. Chem. **274:** 1557–1565

70 Darnell J. E. Jr, Kerr I. M. and Stark G. R. (1994) Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science **264:** 1415–1421

71 Mamane Y., Heylbroeck C., Genin P., Algarte M., Servant M. J., LePage C. et al. (1999) Interferon regulatory factors: the next generation. Gene **237:** 1–14

72 Kalvakolanu D. V. and Borden E. C. (1996) An overview of the interferon system: signal transduction and mechanisms of action. Cancer Invest. **14:** 25–53
73 Cohen B., Peretz D., Vaiman D., Benech P. and Chebath J. (1988) Enhancer-like interferon responsive sequences of the human and murine (2′-5′) oligoadenylate synthetase gene promoters. EMBO J. **7:** 1411–1419
74 Rutherford M. N., Hannigan G. E. and Williams B. R. (1988) Interferon-induced binding of nuclear factors to promoter elements of the 2-5A synthetase gene. EMBO J. **7:** 751–759
75 Yu F., Wang Q. and Floyd-Smith G. (1999) Transcriptional induction of p69 2′-5′-oligoadenylate synthetase by interferon-alpha is stimulated by 12-O-tetradecanoyl phorbol-13-acetate through IRF/ISRE binding motifs. Gene **237:** 177–184
76 Yu F. and Floyd-Smith G. (1999) Protein synthesis-dependent and -independent induction of p69 2′-5′-oligoadenylate synthetase by interferon-alpha. Cytokine **11:** 744–750
77 Rutherford M. N., Kumar A., Nissim A., Chebath J. and Williams B. R. (1991) The murine 2-5A synthetase locus: three distinct transcripts from two linked genes. Nucleic Acids Res. **19:** 1917–1924
78 Truve E., Aaspollu A., Honkanen J., Puska R., Mehto M., Hassi A. et al. (1993) Transgenic potato plants expressing mammalian 2′-5′ oligoadenylate synthetase are protected from potato virus X infection under field conditions. Biotechnology **11:** 1048–1052
79 Kuusksalu A., Pihlak A., Muller W. E. and Kelve M. (1995) The (2′-5′)oligoadenylate synthetase is present in the lowest multicellular organisms, the marine sponges: demonstration of the existence and identification of its reaction products. Eur. J. Biochem. **232:** 351–357
80 Kuusksalu A., Subbi J., Pehk T., Reintamm T., Muller W. E. and Kelve M. (1998) Identification of the reaction products of (2′-5′)oligoadenylate synthetase in the marine sponge. Eur. J. Biochem. **257:** 420–426
81 Tiefenthaler M., Marksteiner R., Neyer S., Koch F., Hofer S., Schuler G. et al. (1999) M1204, a novel 2′,5′ oligoadenylate synthetase with a ubiquitin-like extension, is induced during maturation of murine dendritic cells. J. Immunol. **163:** 760–765