# Innate Resistance to Flavivirus Infections and the Functions of 2´-5´ Oligoadenylate Synthetases

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**Abstract** Mouse susceptibility to experimental infections with flaviviruses is significantly influenced by a cluster of genes on chromosome 5 encoding a family of proteins with enzymatic properties, the 2´-5´ oligoadenylate synthetases (OAS). Positional cloning of the locus in question has revealed that susceptibility of laboratory inbred strains to this class of virus is associated with a nonsense mutation in the gene encoding the OAS1B isoform. Analysis of the molecular structure of the cluster in different mammalian species including human indicates that the cluster is extremely polymorphic with a highly variable number of genes and pseudogenes whose functions are not yet completely established. Although still preliminary, a few recent observations also substantiate a possible role for OAS1 in human susceptibility to viral infections (West Nile virus, SARS, etc.) and its possible involvement in some other diseases such as type 1 diabetes and multiple sclerosis. Finally, convergent observations indicate that the molecules encoded by the *2´-5´OAS* cluster might be involved in other fundamental cellular functions such as cell growth and differentiation, gene regulation, and apoptosis.

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## Introduction

The severity of the clinical manifestations of most infectious diseases is greatly influenced by environmental factors and by the physiological status and the immune competence of the infected organism. It is also strongly influenced by genetic factors controlling the virulence of the pathogen and the susceptibility of the host. For this reason, co-evolution of infectious organisms with their hosts has been often compared to the "battle of two genomes" leading in general to infections with less deleterious consequences, which is after all the best way (if not the only way?) to ensure the survival of both organisms in the long term (Lengeling et al. 2001).

Investigations made with the aim of better understanding the genetic mechanisms that operate during the initial steps of infection are of major interest because they can provide information that may, in turn, help in the development of better strategies for fighting infectious diseases. Experiments of that kind, however, are not easy to perform because many parameters interfere with the experimental protocols, often making difficult the unambiguous delineation of "resistant" and "susceptible" phenotypes in a population of experimentally infected animals. In most cases, resistance or susceptibility to a pathogen depends on the complex interactions of multiple genes that control the host response. In a few cases, however, the situation is greatly simplified by the observation of clear-cut phenotypic differences between various inbred strains of laboratory mice after experimental infection. In this review we describe the historical case of genetic resistance to flaviviruses, the experiments that led to its elucidation at the molecular level, and the consequences of these discoveries.

## The Pathogenicity of Flaviviruses

Flaviviruses are positive-sense, single-stranded, RNA viruses that are generally transmitted to warm-blooded animals through mosquito or tick bites. Many individuals exhibit flavivirus-specific antibodies, suggesting that infections by these viruses are mild or even unapparent, and revealing that some degree of adaptation has occurred between the virus and its host. In some other cases, however, flaviviruses can cause epidemic outbreaks in humans, and infected patients may exhibit a wide range of symptoms ranging from transient febrile illness to life-threatening hemorrhagic fevers (dengue and yellow fever) and meningo-encephalitis syndromes [Japanese encephalitis and West Nile (WN) fever]. The reasons why some flaviviruses cause severe clinical manifestations only in a small percentage of infected individuals are probably numerous and accordingly they have not yet been completely elucidated, but recurrent epidemiological observations and recent scientific data indicate that host-dependent genetic factors might be important.

Variations in innate flavivirus susceptibility in mice were reported for the first time in the early 1930s, and investigations performed during the following decades have fully confirmed these differences. By and large, one can consider that all laboratory

inbred strains of mice that have been tested so far, with the exception of strain PL/J, are susceptible to experimental infections while most wild mice or mice from inbred strains recently derived from wild progenitors are resistant. To mention just one example of this difference in susceptibility, we reported that a single intra-peritoneal inoculation, equivalent to 100 times the median lethal dose ($LD_{50}$) of the WN virus (strain IS-98-ST1), administered to adult mice of the classical laboratory inbred strains BALB/c or C57BL/6 was lethal for all the animals 9.5±1.5 days after inoculation, while mice from unrelated inbred strains recently derived from wild ancestors of either the *Mus musculus domesticus* (WMP/Pas), *Mus μ. musculus* (MAI/Pas, MBT/Pas, PWK/Pas), or *Mus spretus* (SEG/Pas, STF/Pas) species were totally resistant to the same treatment (Mashimo et al. 2002). During this experiment, infectious particles of WN virus could be detected in the brain of all infected mice after 5 days of infection, and the amounts of virus peaked at $10^9$ focus forming units (FFU)/g of brain tissue by day 7 (Mashimo et al. 2002). High levels of anti-WN antibody could also be detected in surviving animals, indicating that the virus replicated in resistant strains. This experiment was just one of many experiments of the same kind performed over the past 40 years with a variety of flaviviruses, using several routes of inoculation and several doses and strains of virus. All these experiments yielded similar results, confirming that the phenotype of resistance/susceptibility is not WN-specific but, on the contrary, extends to other types of flaviviruses as well.

The resistance to flaviviruses was also demonstrated to be controlled by a major locus on chromosome (Chr) 5, designated flavivirus resistance (symbol *Flv*), with basically two alleles: *Flv^r*, which is dominant and induces resistance, and *Flv^s*, which is recessive and correlates with susceptibility. Most classical laboratory inbred strains are homozygous for the *Flv^s* allele. A third allele leading to "minor resistance" (*Flv^mr*) has also been found segregating in wild mice of the *M. m. molossinus* subspecies [for historical details on the discovery and genetics of the *Flv* locus, readers may refer to Brinton and Perelygin (2003)]. Congenic "resistant" strains have been produced by back-crossing for several generations the successive resistant offspring of an initial cross between resistant wild mice (any species) with a "susceptible" laboratory inbred strain. This classical breeding strategy allowed, at the same time, refinement of the genetic localization of the *Flv* locus on mouse Chr 5 and allowed the production of unlimited populations of "resistant" and "susceptible" mice with an otherwise similar genetic constitution, a very helpful material for experimentation.

## Investigating the Molecular Basis of Susceptibility to West Nile Infection

Considering the relatively simple (monofactorial) genetic basis of WN resistance in the mouse and the "genomic" tools that became available after the genome sequencing effort in this species, we decided to embark on the positional cloning of the *Flv* locus. Readers who may be interested in reading our publication about the positional

cloning of *Flv* (Mashimo et al. 2002), must know that, since we had no evidence that the gene we were cloning was identical to *Flv* itself, we provisionally gave it another name (*Wnv* for WN virus—with two alleles *Wnv^r* and *Wnv^s*) even though we had little doubt that the two genes were presumably one-and-the-same entity. We now have molecular proof of this identity.. A first difficulty in this project arose when we found that, among the offspring of an intersubspecific backcross of the type (BALB/c×MBT/Pas)F1×BALB/c, which we expected to be a mixed population with 50% of the individuals being *Flv^r*/*Flv^s* and the other 50% being *Flv^s*/*Flv^s*, all mice heterozygous for *Flv^r* survived while not all mice with a *Flv^s*/*Flv^s* genetic constitution died as we would have expected. This is a good illustration of a major pitfall in this kind of experiment, where it is always risky or even impossible to trust in a "dead-or-alive" phenotype after an experimental infection, even if the latter is performed in the same highly standardized conditions. To bypass this difficulty and be able to achieve a high-resolution genetic mapping of the *Flv* locus, an absolutely necessary step in the positional cloning process, we derived a set of subcongenic mice by selecting, with the help of microsatellite markers, those offspring where a crossover event occurred that reduced the critical genetic interval containing the *Flv* locus. Offspring from these mice (all of the same genetic constitution) were challenged with a standardized dose of virus and finally classified as "resistant" or "susceptible." This rather tedious procedure allowed us to localize, with a very high degree of confidence, the *Flv* locus within an interval flanked by markers *D5Mit408* and *D5Mit242*, which is roughly equivalent to 300 kb of DNA (Fig. 1).
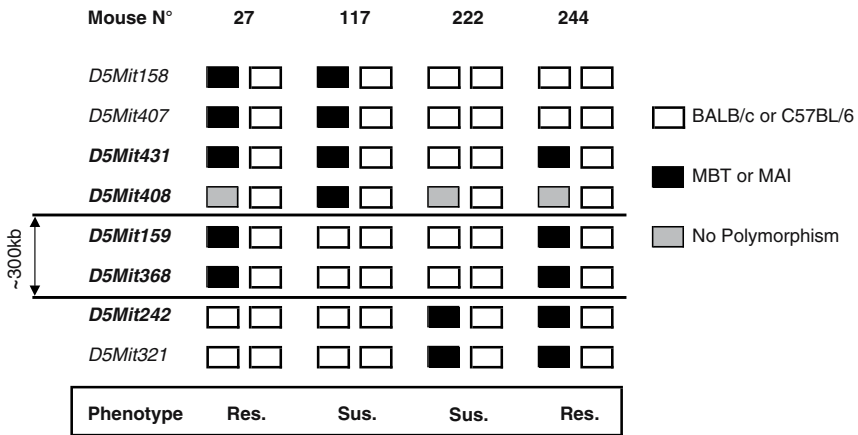


**Fig. 1** Over 350 offspring from two intersubspecific backcrosses [(BALB/c×MBT)F1×BALB/c or (C57BL/6×MAI)F1×C57BL/6] were raised and genotyped for the region of Chr 5 flanking the locus for flavivirus resistance and four mice were found with a recombinant haplotype in the critical region (Nos. 27, 117, 222, and 244). These recombinant mice were then mated with either BALB/c or C57BL/6 susceptible partners, and around 30/50 progenies of these crosses were challenged with West Nile virus and classified as "susceptible" or "resistant." This protocol, because it involves a rather large sample of animals with exactly the same genotype, is highly reliable for the purpose phenotyping based on a dead-or-alive phenotype

This genetic interval contains about 30 genes whose sequence and expression pattern are well known (Fig. 2). Among these genes, the cluster encoding the interferon inducible oligoadenylate synthetases (*Oas1, Oas2, Oas3* etc.) appeared top-ranked in the list of candidates for reasons that will be explained later. We then decided to have a more careful look at the sequence of these genes in both "resistant" and "susceptible" mice. We observed several single nucleotide polymorphisms (SNPs) among the different strains or species studied, a finding that was not surprising considering the polyphyletic origins of the laboratory inbred strains of mice (Wade et al. 2002). We found it remarkable, however, that in one of the elements of the cluster, namely the *Oas1b* gene, all susceptible mice had a T→C transition in the fourth exon of this gene, replacing an arginine residue with a premature stop codon. The perfect and absolute correlation between susceptibility to viral infection and the occurrence of a stop codon was observed independently in two laboratories (Mashimo et al. 2002; Perelygin et al. 2002) and supported the hypothesis that a truncated, and presumably inactive form of 2′-5′OAS is indeed causative of the innate susceptibility to flavivirus infection. The presence of a stop codon is also compatible with susceptibility behaving as a fully recessive trait and fits perfectly with one of the known functions of the interferon inducible enzyme 2′-5′OAS. In addition to this absolute phenotype/genotype correlation, it has also been reported that a flavivirus-resistant phenotype could be restored in a susceptible mouse strain by replacing the 3′ portion of the susceptible *Oas1b* sequence in 129/SvJ/RW4 ES cells, by homologous recombination with a 129/SvJ/RW4 DNA sequence containing four substitutions characteristic of the *Oas1b* resistance allele, in particular a reversion from TGA to CGA (Scherbik et al. 2007).

## The Molecular Organization and Evolution of the *OAS* Gene Family in Mammals

2′-5′OAS are a relatively homogeneous family of enzymatic proteins with a remote evolutionary origin, since molecules with a similar structure have been identified in a wide range of species including most mammals and birds, and even the marine sponge *Geodia cydonium* (Cayley et al. 1982; Wiens et al. 1999; Yamamoto et al. 1998). In the species where the molecular organization has been studied in detail, the genes encoding 2′-5′OAS have been found to be clustered, with variations in gene copy numbers (orthologs and paralogs) among the different species, indicating that rapid evolutionary changes occurred in these regions. In this section, we will summarize the most recent findings with reference to the corresponding publications.

The *human* cluster is the simplest with only three genes *OAS1*, *OAS2*, and *OAS3* within a 130-kb stretch of Chr 12 (12q24.13) (Hovnanian et al. 1998). These three genes share the same order of transcription and are arranged on the chromosome in the following order: centromere 5′–*OAS1*–*OAS3*–*OAS2*–3′ (Hovanessian and Justesen 2007). The size of these genes is relatively short (~12 kb for *OAS1*; ~36 kb for both *OAS2* and *OAS3*) and analysis of their sequence reveals the presence of a conserved domain of five exons (the first 346-amino acid residues of *OAS1*), designated
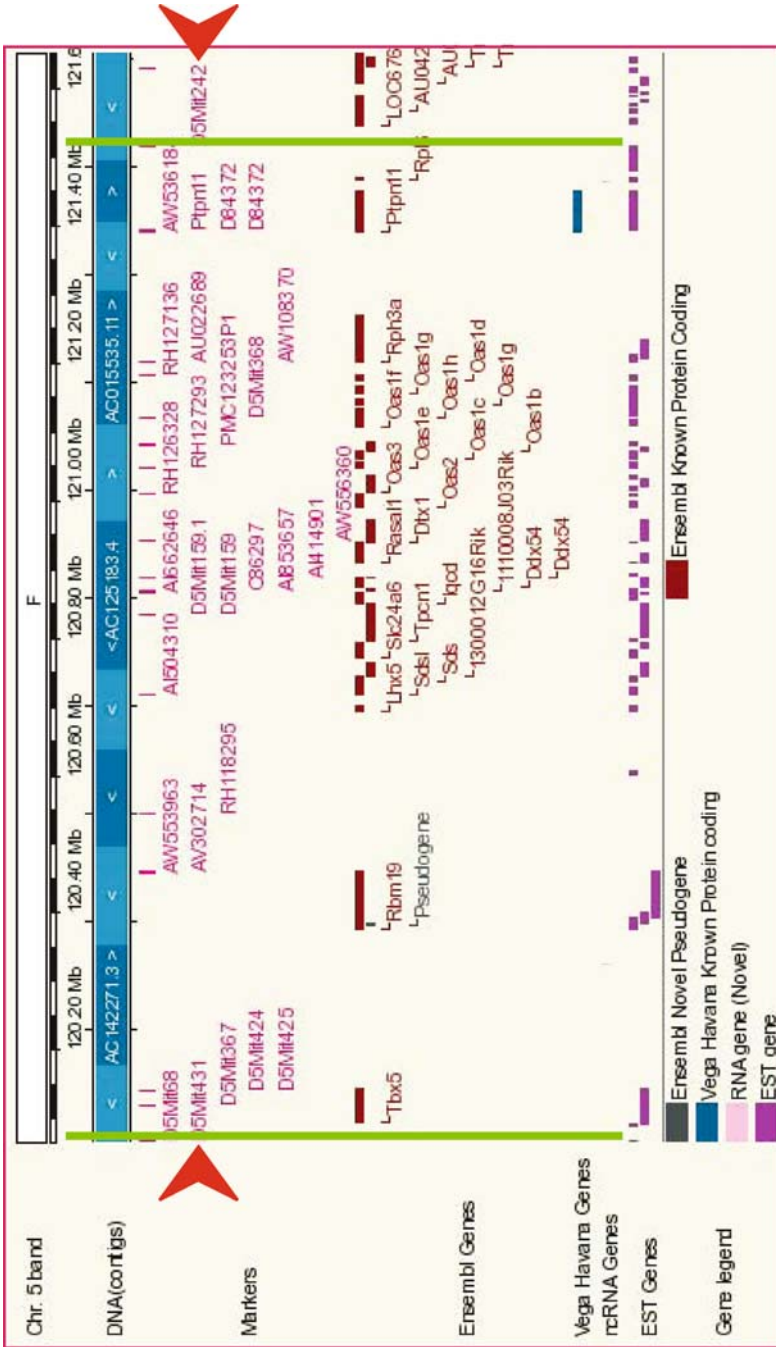
**Fig. 2** This figure, from the *Ensembl* database, represents a segment of mouse Chr 5 in the critical region between markers *D5Mit431* and *D5Mit242* and shows the approx. 30 genes that are in the region. In this sequence (released after the positional cloning of *Flv*) the genes *Oas2*, *Oas3*, and the eight genes in the *Oas1* cluster are obvious. From the knowledge we have of their function, they appear as top-ranked candidates in the determinism of susceptibility to flaviviruses

the 2′-5′OAS unit, with one copy in *OAS1*, two copies in *OAS2*, and three copies in *OAS3*. This organization and sequence homology suggest that the *OAS1* gene is probably the ancestral gene, the other two genes being derived after duplication or triplication of this ancestral gene. The promoter region of the three genes contains an interferon-stimulated response element (ISRE), which is consistent with the fact that most of the 2′-5′OAS proteins are interferon (IFN)-inducible enzymes. *OAS1* is transcribed in four isoforms of 42, 44, 46, and 48 kDa respectively, depending on alternative splicing of exons 5 and 6. These isoforms, which have identical amino-termini but different carboxyl-termini, may have different functions. Formation of a human OAS1 tetramer is essential for the catalytic activity of the protein (Torshin 2005). *OAS2* is also spliced in two isoforms of 69 and 71 kDa that share a common amino-terminus of 683 residues, with extensions of 4 and 44 amino acids, respectively. *OAS3* encodes a unique protein of 100 kDa (Hovanessian and Justesen 2007; Justesen et al. 2000; Rebouillat and Hovanessian 1999; Fig. 3).

Aside from *OAS1*, *OAS2*, and *OAS3*, another gene—identified by screening a cDNA expression library with anti-OAS3 polyclonal antibodies (Rebouillat et al. 1998) and by screening an EST library (Hartmann et al. 1998)—with a sequence similar to the one of *OAS1*, although somewhat bigger, was discovered. This gene encodes a 56-kDa protein that differs from the other OAS proteins by an approx. 160-amino acid extension at its C-terminus, which has homology to the interferon-inducible protein ISG15 and also has a tandem repeat of two ubiquitin-like domains (Hartmann et al. 1998). This protein is devoid of 2′-5′OAS activity and accordingly was named OASL for "OAS like" protein. The human *OASL* gene exhibits a high degree of homology with the chicken *OAS* gene, but the latter encodes a highly active 2′-5′OAS (Torshin 2005).
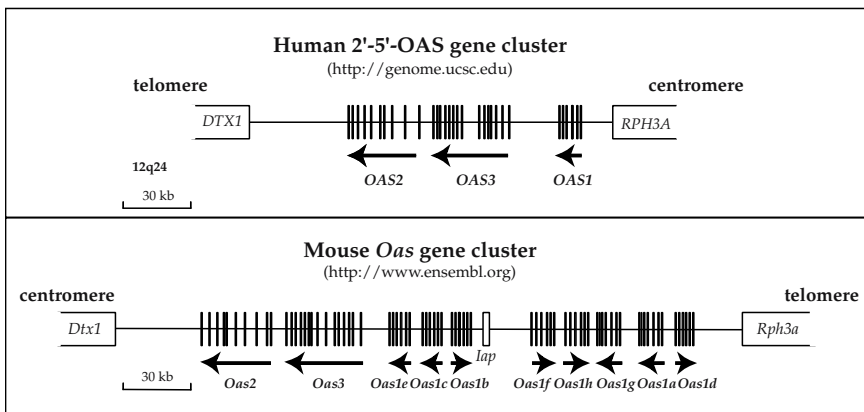


**Fig. 3a** A schematic organization of the OAS cluster in human and in mouse. Human *OAS1*, *OAS2*, and *OAS3* are paralogous copies of an ancestral gene. The mouse has at least eight orthologous copies of the human *OAS1*, some of them likely being nonfunctional pseudogenes (updated and modified from Mashimo et al. 2003)

# Human OAS transcripts

[Sarker *et al.* 1998, Rebouillat *et al.* 1999, Justesen *et al.* 2000]
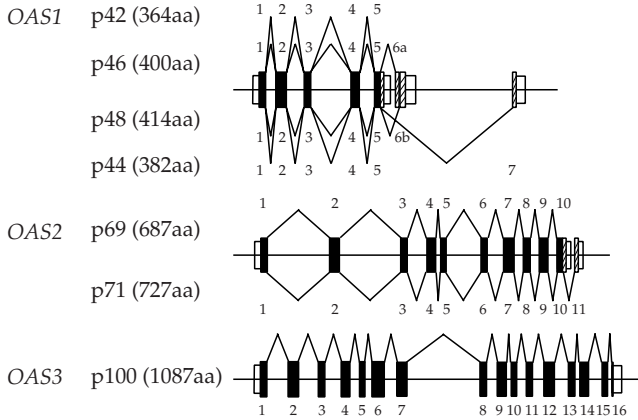


# Mouse Oas transcripts

[Mashimo *et al.* 2003]
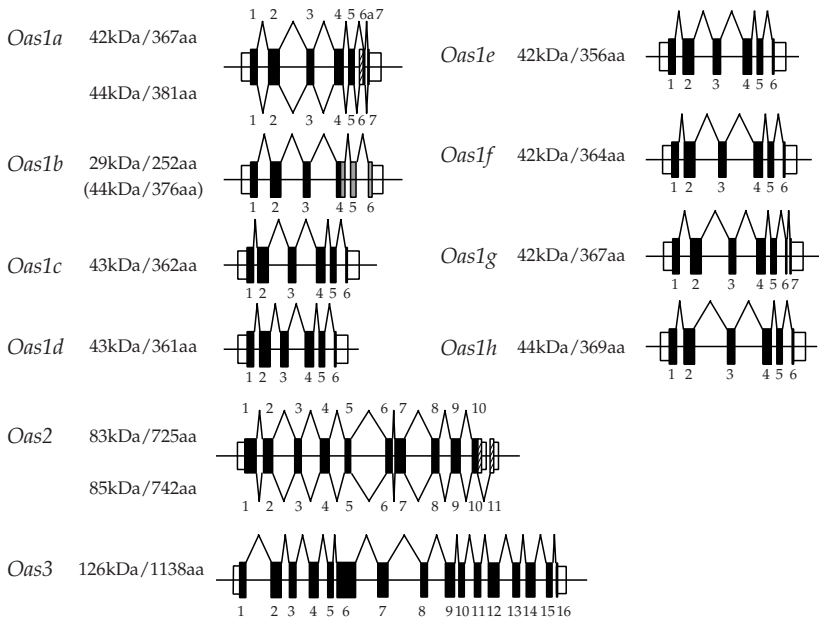


**Fig. 3 b** The transcription pattern of the different genes in the mouse *Oas* cluster. All these genes are transcribed, but with minor tissue-specific variations. Among the different genes of the mouse *Oas1* cluster, only *Oas1a* is alternatively transcribed. *Oas1a* and *Oas1g* have an extra seventh exon.

The *mouse Oas* cluster is the second most well known after human. It contains one *Oas2* and one *Oas3* gene, and ten *Oas1* genes (or pseudogenes?) designated *Oas1a* to *Oas1j* (Eskildsen et al. 2002; Kakuta et al. 2002; Mashimo et al. 2003; Perelygin et al. 2006; Figs. 2 and 3). These 12 genes are arranged in tandem within a stretch of DNA that spans approximately 230 kb of mouse Chr 5. The segment harboring the *Oas* cluster (and a few other flanking genes) is inverted when compared to the human configuration (Mashimo et al. 2003). Mouse *Oas2/Oas3* genes have a genomic structure very similar to the one of human, and a very similar pattern of transcription even if the transcription products are somewhat bigger in the mouse (725 and 742 amino acids compared to 687 and 727 for OAS2; 1,138 amino acids compared to 1,087 for OAS3). Here again, based on their sequences and orientation, one can guess that these 12 genes result from the duplication, in several steps, of an ancestral copy after divergence of the primates and rodents phyla. RT-PCR performed with a set of specific primers on RNA extracted from several adult and fetal tissues, either before or after induction with double-stranded RNA (dsRNA), indicated that nine genes of the *Oas1* family (*Oas1a* to *Oas1i*) are transcribed and some variations can be observed in the different patterns of expression. According to Mashimo and coworkers (2003) each of the nine *Oas1* genes exhibits a unique battery of transcriptional regulatory elements, suggesting that each of these units has the potential to be differentially regulated. So far only *Oas1a* has been found to be alternatively spliced yielding two transcripts each including different parts of exon 6 (Kakuta et al. 2002; Mashimo et al. 2003; Fig. 3). Two genes, homologous to the human *OASL*, have also been identified in the mouse: *Oasl1* and *Oasl2*. Analysis of their structure indicates that they are probably nonfunctional pseudogenes.

Twelve 2′-5′OAS (*Oas*) genes were identified in the *rat* genome (Chr 12) (Mashimo et al. 2003), including eight *Oas1* genes (the orthologs of mouse *Oas1a* and *Oas1e* are missing in this species, while two additional isoforms are present: *Oas1k* and *Oas1l*). Two *Oas1* pseudogenes, a single *Oas2* and a single *Oas3*, and two *Oas*-like genes, *Oasl1* and *Oasl2*, are also present. The structure and organization of the rat cluster is very similar to the one of the mouse, which is no surprise.

The structure of the 2′-5′OAS cluster has also been investigated in detail in four other mammalian species (pig, dog, cow, and horse) and in chicken (Perelygin et al. 2005, 2006). Four *OAS* genes (*OAS1X*, *OAS1Y*, *OAS1Z*, and *OAS2*) plus a single copy of *OASL1* were detected in the cow genome. The cluster in the pig genome is similar to the *OAS* cluster of cattle although *OAS1Z* is absent. Remarkably, the orthologous copy of mouse/human *OAS3* is not found in either the pig or the cow genomes. The dog and horse clusters are also similar and are more like the human cluster than either the mouse or rat clusters. Dog and horse have three copies of the

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**Fig. 3 b** (continued) Alignment of the predicted amino acid sequences for the proteins encoded by the eight *Oas1* genes indicates that *Oas1c*, *Oas1d*, *Oas1e*, *Oas1f*, and *Oas1h* are structurally similar and lack several functional domains. These observations suggest that these isoforms may actually be inactive pseudogenes rather than real isoforms. In contrast, *Oas1g* and *Oas1a* encode proteins that could be functional in the 2′,5′OAS/RNase L cascade. Note that for both **a** and **b** the scale for exons is five times larger than for introns

human/mouse *OAS3* ortholog (*OAS3C*, *OAS3M*, and *OAS3N*), two copies of *OAS2* (*OAS2N* and *OAS2C*) and a single copy of the human *OAS1* ortholog. However, while the dog has two copies of the *OASL* orthologous gene (*OASL1* and *OASL2*), the horse has only one. Two tandemly duplicated OAS-like (*OASL*) genes were identified in the dog genome but only a single *OASL* ortholog was found in both the cattle and the pig genomes. The bovine and porcine *OASL* genes contain premature stop codons and encode truncated proteins, which lack the typical C-terminal double ubiquitin-like domains. Evidence of concerted evolution of all these paralogous *2´-5´OAS* genes was obtained in rodents (*Rodentia*) and even-toed ungulates (*Artiodactyla*) (Perelygin et al. 2006).

## The Functions of the OAS Molecules

### The Antiviral Functions of OAS1 Molecules

The best known molecules among those that are encoded by the 2´-5´OAS cluster are described as interferon-induced enzymes that polymerize ATP into 2´-5´ oligomers of adenosine with the general formula pppA $(2´p5´A)_n$. These enzymes are activated by binding to double-stranded RNA, and their products, the 2´-5´ oligoadenylates, activate the latent endoribonuclease RNase L that finally degrades viral or cellular RNA molecules (Hovanessian and Justesen 2007). This is how the antiviral activity of these enzymes is generally explained. Several experiments have confirmed this crucial function in the innate, antiflavivirus mechanism of defense in the mouse (Kajaste-Rudnitski et al. 2006; Lucas et al. 2003) although some experiments suggest that RNase L activation is not a major component of the OAS1B-mediated flavivirus resistance phenotype (Scherbik et al. 2006).

Sequence alignment of human OAS1, OAS2, and OAS3 reveals the presence of highly conserved stretches of 7–14 amino acids among which the pentapeptide D-F-L-$K_{199}$-Q has been reported to represent a part of the ATP binding site, while $K_{199}$ inside this pentapeptide seems to be essential for catalytic activity (Justesen et al. 2000; Rebouillat and Hovanessian 1999). In the mouse, interferon is also an inducer of the different OAS molecules, and alignment of the predicted amino acid sequences for the proteins encoded by *Oas1c*, *Oas1d*, *Oas1e*, *Oas1f*, *Oas1h*, *Oas1i*, and *Oas1j*—although structurally very similar—lack some essential functional domains, such as the LXXXPA motif (Ghosh et al. 1997), the highly conserved aspartic acid residues in exon 2 (Sarkar et al. 1999), and the CFK motif (Ghosh et al. 1997). These observations suggest that, although these isoforms have retained their binding activity to dsRNA, they have lost their $Mg^{2+}$-dependent catalytic activity and accordingly are most probably inactive pseudogenes rather than genes encoding a protein with 2´-5´OAS activity (Sarkar et al. 1999; Shibata et al. 2001). Another possibility that should be kept in mind would be that these isoforms, encoded in the above-mentioned genes, have acquired other functions. The three

genes, *Oas1g*, *Oas1a*, and of course *Oas1b*, encode proteins that have been proved (or are likely) to be functional in the 2′-5´OAS/RNase L cascade.

The suspected role of the OAS1B isoform in the innate mechanisms of defense in the mouse, hypothesized after experimental infection of mice with flaviviruses and positional cloning of the *Flv* locus, has been confirmed by the production of a knock-in, as already mentioned. It has also been confirmed by experiments performed in vitro in which stable neuroblastoma cell clones overexpressing either the mutant or wild-type OAS1B were infected with WN virus. These experiments indicated that viral replication is less efficient in cells that produce the normal copy of OAS1B than in those expressing the mutant form of the protein (Lucas et al. 2003). The experiments have been confirmed and reinforced by other experiments performed on genetically engineered fibroblasts that could upregulate OAS1B protein expression under the control of the *Tet-Off* expression system (Kajaste-Rudnitski et al. 2006).

The role played by the OAS1B isoform of 2′-5´OAS in the innate mechanisms of defense of the mouse against flavivirus infection now seems firmly established. In human and other mammalian species, however, the role of 2′-5´OAS in viral pathogenesis is much less clear. According to Perelygin and colleagues, the flavivirus-specific activity of the mouse OAS1B isoform on flaviviral replication might be correlated with a 4-amino acid deletion in the P-loop motif that is unique to this isoform and does not appear to exist in human (Perelygin et al. 2002). This 4-amino acid deletion (12 bp) might be of special importance for the OAS1B protein to specifically interact with the ATP substrate if one considers recent data from the crystalline structure of the porcine OAS1 enzyme (Hartmann et al. 2003). Even if this hypothesis is supported by other experiments in vitro (Urosevic et al. 1999), an alternative explanation for the specific activity of OAS1B on flavivirus replication might be found in its promoter sequence, where several binding sites [for NF-κB, GAS, and interferon (IFN)-stimulated specific response element (ISRE)] exhibit a unique organization. In particular, it is noteworthy that OAS1B is the only gene where the NF-κB and ISRE binding sites are closely associated in tandem, producing a genomic structure that has previously been reported as capable of triggering gene expression upon viral induction (Cheng et al. 1998). Sequencing the promoter regions of the OAS1B isoform in remotely related mouse species did not provide evidence that some particular structural changes in this promoter might be associated with the phenotype of resistance or susceptibility after flavivirus infection. This supports the hypothesis that the stop codon found in the OAS1B coding sequence of most laboratory strains, which is the only obvious structural difference between susceptible and resistant genotypes, indeed is directly related to this phenotype. The 4-amino acid deletion in the P-loop motif has not been found in the orthologous region of the rat OAS1B isoform but does occur in rat OAS1F. The rat, like wild mice, seems to be naturally resistant when naturally infected (Eldadah et al. 1967).

Two recent observations arguing in favor of genetic control of human susceptibility to flavivirus infections have been published recently. The first, by Bonnevie-Nielsen and colleagues, reports a significant correlation between the basal activity of OAS1 and an A/G SNP at the exon 7 splice-acceptor site (AG or AA) of the OAS1 gene (Bonnevie-Nielsen et al. 2005). According to these authors, in a cohort

of 83 families each containing two parents and two children, allele G had a higher frequency in people with high enzyme activity than in those with low enzyme activity, with the activity being related to this polymorphism in a dose-dependent manner across the GG, GA, and AA genotypes. Allele G generates the p46 enzyme isoform, whereas allele A ablates the splice site and generates a dual-function antiviral/ pro-apoptotic p48 isoform and a novel p52 isoform. The discovery of this genetic polymorphism and of its influence on host susceptibility to flavivirus infections clearly underlines the likely importance of OAS1 in the innate mechanisms of defense.

The second observation was made in a survey performed on 33 individuals hospitalized with WN virus infection. The survey was designed to assess whether a structural change could be detected in the *OAS* genes of patients with a clinically severe form of the disease. Sequence comparisons between case patients and control subjects identified 23 SNPs, including a synonymous SNP in *OASL* exon 2 in which the reference allele occurred at a higher frequency in case patients ($p<.004$). According to the authors, the RNA transcripts generated from this allele may undergo increased splicing, resulting in a dominant-negative OASL isozyme similar to the nonsense/truncation mutant form of *Oas1b* in mice (Yakub et al. 2005). These two reports, although preliminary, are indicative of a possible role for the OAS1 molecules in human mechanisms of defense.

Although the role of the OAS1 molecules in innate immunity against flavivirus infections is now established, at least in the mouse, several experiments indicate that this resistance, unlike resistance to myxovirus associated with the *Mx* locus (Haller et al. 1998), does not require induction by interferon (Brinton and Perelygin 2003). This observation is totally consistent with the observation that plants transgenic for 2´-5´OAS family and for the gene encoding RNase L, were found to be resistant to experimental infections with a number of viruses such as tobacco mosaic virus, cucumber mosaic virus, and potato virus Y (Honda et al. 2003; Mitra et al. 1996; Ogawa et al. 1996).

Finally, and again concerning the *Oas1b* gene of the mouse, a likely hypothesis to account for the presence of the same stop codon in virtually all laboratory strains is that all these strains inherited the same segment of Chr 5 from a common ancestor. Such a situation is not uncommon among mouse laboratory strains and was also observed by Staeheli and colleagues when they investigated the genetic basis of susceptibility to orthomyxovirus infection (Staeheli et al. 1986). However, whether this occurred by chance only or under some sort of selective pressure is an open question. Nonetheless, it is also clear that the use of inbred strains derived from wild specimens of different species might be a rich source of information for investigating the genetic basis of resistance/susceptibility to infectious diseases.

Aside from their well-established role in the pathology generated by flavivirus infections, 2´-5´OAS molecules (and more specifically those encoded by *OAS1*) may be involved in the outcome of diseases generated by coronaviruses or hepaciviruses. Two independent surveys indicated that SNPs in the *OAS1* gene (more precisely in exons 3, 6 or in the 3´-UTR region) were associated with severe acute respiratory syndrome (SARS) susceptibility in Vietnamese or Chinese Han populations (Hamano et al. 2005; He et al. 2006). Another study suggested that a polymorphism

in the 3′-UTR of the *OAS1* gene was significantly associated with a higher frequency of self-limiting infection in patients with hepatitis C (Knapp et al. 2003). It is likely that with time, more associations between *OAS1* polymorphisms and resistance to viral infections will be discovered.

## The Other Functions of OAS Molecules

Situations where different mammalian genomes harbor orthologous genes with a variable number of copies are not uncommon and it was suggested that such variations are the result of different selective environmental pressures experienced by the ancestors of modern rodents and primates. While infectious agents in natural environments certainly play an important role in natural selection (the "battle of two genomes"), however, the number and range of pathogens is not very different among the different mammalian species. It therefore makes sense to guess that the different OAS molecules have cellular functions other than the one made obvious by the accidental discovery of differential flavivirus resistance in the mouse species. In humans, for example, *OAS1*, *OAS2*, and *OAS3* appear to be differentially induced by interferon, induced in different types of cells, and for some of them expressed even in healthy individuals, which is indicative of an eventual role under physiological conditions and not only after infections. They are also characterized by different subcellular locations. Some OAS proteins might have as-yet-unidentified catalytic activities, suggesting that they may have distinct roles in the cell. In fact, 2′-5′OAS molecules have now been demonstrated to be involved in other cellular processes such as cell growth and differentiation, gene regulation, and apoptosis (Hovanessian and Justesen 2007).

Some polymorphisms at the *OAS1* locus have been reported to be associated with a variety of human pathologies. This is the case, for example, for a SNP generating an A/G splice-site in *OAS1*-exon 7, which was found to be associated with a protective effect against type 1 diabetes (Field et al. 2005). This observation was later disputed (Smyth et al. 2006) but another polymorphism in the same gene, generating a serine/glycine substitution resulting in a functional variant, was reported as a more likely cause for the observed association with type 1 diabetes (Tessier et al. 2006). Similarly, SNPs detected in exons 3 and 7 of *OAS1* demonstrated an association with risk for multiple sclerosis in 333 patients and 424 healthy controls, suggesting that OAS1 activity is involved in the etiology of this disease (Fedetz et al. 2006).

In the mouse species, with the unlimited possibilities of genetic engineering in embryonic stem (ES) cells in vitro, a comprehensive survey of the different functions of the OAS molecules should be undertaken in the forthcoming years, for example by knocking out each and every gene of the cluster. Although of importance, making alterations in the coding sequences of these genes in order to assess their function(s) would not necessarily require that the stop codon in *Oas1b* exon 4 be "repaired" in advance. Yan and colleagues, for example, demonstrated that mutant mice lacking OAS1D (*Oas1d*$^{-/-}$) displayed reduced fertility due to defects in ovarian follicle development,

decreased efficiency of ovulation, and arrest at the one-cell stage of fertilized eggs (Yan et al. 2005). This was indeed a totally unexpected function for a protein exhibiting a very high degree of similarity with OAS1B.

As we already noted, the *2´-5´OAS* family of genes exhibits both an evolutionarily ancient origin and wide variations in the number of copies between species. Experimental data collected after experiments on mouse flavivirus susceptibility and preliminary observations made in humans suggest that the cluster in question is important for the maintenance of cellular homeostasis since evolutionary (environmental) forces contribute to its "shaping" (Godfrey et al. 2004). Since no sequences related to *2´-5´OAS* genes could be identified in either *Caenorhabditis elegans* or in *Drosophila melanogaster*, however, it seems that the OAS cluster is either not absolutely fundamental for cell physiology or that it is replaced by another structure with similar functions in other developed organisms.

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