Inhibition of the OAS/RNase L pathway by viruses

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CrossMark

The OAS/RNase L system was one of the first characterized interferon effector pathways. It relies on the synthesis, by oligoadenylate synthetases (OAS), of short oligonucleotides that act as second messengers to activate the latent cellular RNase L. Viruses have developed diverse strategies to escape its antiviral effects. This underscores the importance of the OAS/RNase L pathway in antiviral defenses. Viral proteins such as the NS1 protein of Influenza virus A act upstream of the pathway while other viral proteins such as Theiler's virus L* protein act downstream. The diversity of escape strategies used by viruses likely stems from their relative susceptibility to OAS/RNase L and other antiviral pathways, which may depend on their host and cellular tropism.

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Introduction

The OAS/RNase L system, discovered in the 1970s, was one of the first characterized interferon (IFN) effector pathways. The pioneering work of P. Lengyel's and I. Kerr's groups revealed a cellular RNase whose activity depended on IFN and correlated with the synthesis of $2'$–$5'$ oligoadenylates (2–5A) in infected cells. This led to the model presented in Figure 1, where IFN induces the expression of oligoadenylate synthetases (OAS) that synthesize 2–5A in response to viral infection. 2–5A act as second messengers to trigger dimerization and activation of latent RNase L. Activated RNase L cleaves viral and cellular single-stranded RNA (ssRNA) and thereby limits virus replication and triggers the apoptosis of infected cells.

Since the discovery of this pathway, the antiviral role of RNase L has been widely documented *in vitro* and *in vivo*, notably via RNase L-knockout mice [1]. Viruses have developed various strategies to escape the OAS/RNase

L pathway, underlining its importance in the antiviral defense. Numerous reviews have covered the cellular and antiviral activities of RNase L [2,3]. This review focuses on the many strategies whereby viruses escape this defense pathway and discusses their implication for the biology of RNase L.

OAS/RNase L pathway

The OAS family consists of homologous enzymes encoded by interferon-stimulated genes (ISGs). The three OAS (OAS1, OAS2 and OAS3) differ in their number of OAS domains, oligomerization level and type of synthesized 2–5A. In addition to OAS, human and mouse genomes encode 'OAS-like' (OASL) proteins. The unique human OASL is catalytically inactive. In mice, Oasl1 is inactive while Oasl2 can synthesize 2–5A (reviewed in [4,5]). Binding of dsRNA triggers the catalytic activity of OAS, which convert ATP into 2–5A. 2–5A are short oligoadenylates linked by $2',5'$-phosphodiester bonds, whose general formula is $[pxA(2'p5'A)n; x = 1–3; n \geq 2]$ [6,7]. The only known function of 2–5A is RNase L activation [8].

RNase L is a 741 amino acid latent endoribonuclease ubiquitously expressed in mammalian tissues [8]. It is described as a cytosolic enzyme, but is also detected in other subcellular compartments such as mitochondria where it regulates mitochondrial mRNA abundance [3,9]. RNase L comprises three domains: (i) a N-terminal ankyrin domain composed of 9 ankyrin repeats (R1–R9) involved in 2–5A recognition; (ii) a central catalytically inactive pseudokinase domain which contributes to RNase L dimerization; and (iii) a C-terminal enzymatic domain that cleaves target RNA. Recent structural studies show that 2–5A bind R2 and R4 of one RNase L protomer, and R9 and the pseudokinase N-terminal lobe of the other, thereby triggering RNase L dimerization [10••,11,12••]. RNase L cleaves viral and cellular ssRNA with little specificity (UN^N sequence), leaving a 5'-OH and 3'-monophosphate [10••].

2–5A are degraded within minutes of their synthesis, by $2'$-phosphodiesterases and phosphatases. This allows a tight regulation of RNase L activity, which mirrors OAS activity. RNase L activity can also be restrained by association with a cellular factor known as inhibitor/ATP-binding cassette, sub-family E member 1 (RLI/ABCE).

Mechanisms of antiviral activity

RNase L restricts viral propagation through both direct and indirect mechanisms that include:

Figure 1
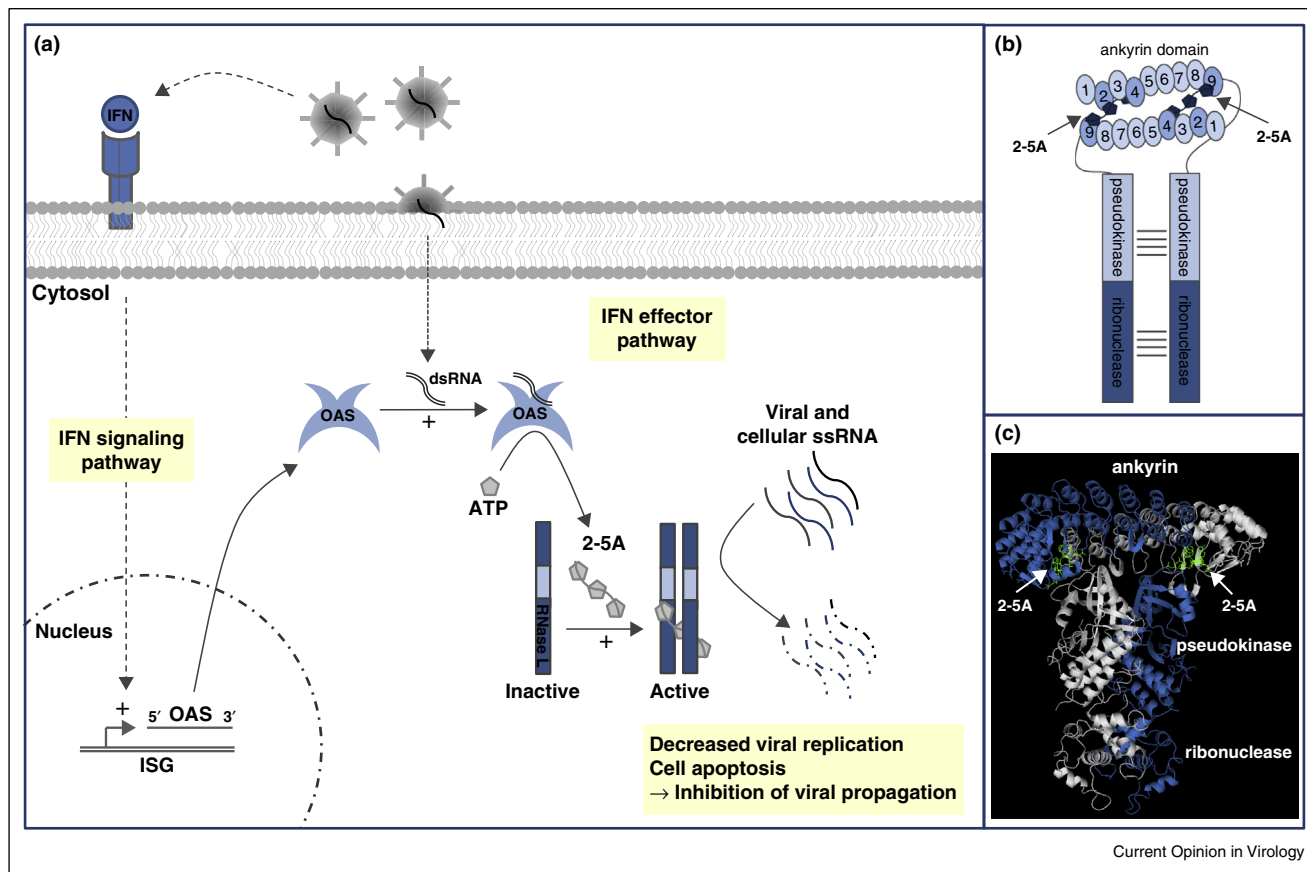
Activation of the OAS/RNase L pathway. **(a)** IFN secreted upon viral infection activates the transcription of hundreds of genes including oligoadenylate synthetase (OAS) genes. dsRNA resulting from viral replication switches on OAS, which convert ATP into 2′–5′ oligoadenylates (2–5A). 2–5A then bind to RNase L and trigger its dimerization and activation. RNase L degrades single-stranded viral and cellular RNA, decreasing viral replication and inducing apoptosis. **(b,c)** Schematic (b) and crystallographic structure (PDB ref 4OAV) (c) of active dimeric human RNase L, showing 2–5A bound to two RNase L protomers. 2–5A bind ankyrin repeats 2 and 4 of the first protomer and ankyrin repeat 9 of the other protomer (as well as the N-terminal lobe of the pseudokinase domain).

(i) *Viral genome degradation*: This is reported for EMCV [13] and is predicted for all ssRNA viruses.
(ii) *Viral mRNA degradation*: This potentially affects both DNA and RNA viruses. It has been suggested that activation of OAS and consequent 2–5A production preferentially occur at sites of dsRNA production (i.e. close to RNA virus replication complexes), which may impart some specificity toward viral mRNA [13,14].
(iii) *Cellular mRNA and rRNA degradation*: rRNA damage should limit translation, including that of viral mRNA. Sustained degradation of cellular RNA, including mitochondrial RNA, leads to apoptosis, which reduces viral propagation.
(iv) *Amplification of IFN signaling*: The release, by RNase L, of short RNA fragments into the cytoplasm can activate cytoplasmic helicases that, in turn, activate type I IFN synthesis, creating a positive feed-back in antiviral defense [15].

Antiviral activity

A major step forward in the analysis of RNase L was the development of RNase L-KO mice [1], which contributed to uncovering the role of RNase L *in vivo*, against encephalomyocarditis virus, Coxsackie virus B4 and West Nile virus [16–18].

Activity of the OAS/RNase L pathway was also demonstrated *in vitro*, against many viruses, in particular RNA viruses (Table 1). However, some RNA viruses, such as influenza A virus, Theiler's virus and murine hepatitis virus are hardly affected, because they express antagonist proteins (see following section and Figure 2).

Inhibition of OAS/RNase L system by viruses

Many viruses counteract the antiviral activity of OAS/RNase L (Figure 2). Some viruses act upstream of the pathway by masking dsRNA or by acting on OAS

Table 1

Effect of the OAS/RNase L pathway on viral infection

Virus	Effect of the OAS/RNase L pathway on viral infection in vitro (cell culture) or in vivo[a]
RNA viruses	
Picornaviridae	Induction of 2–5A production by dsRNA in replication complexes
Encephalomyocarditis virus	Effect of dominant negative RNase L or overexpression of OAS1
	In vivo: increased infection and mortality of RNase L-KO mice
Coxsackievirus B4	*In vivo*: increased infection and mortality of RNase L-KO mice
Theiler's virus[b]	*In vitro*: increased replication in RNase L-KO macrophages
Poliovirus	Minor effect of RNase L overexpression or dominant negative RNase L
Flaviviridae	Induction of 2–5A production by dsRNA in replication complexes and by 5′ and 3′UTR structures
Hepatitis C virus	Degradation of viral genome by RNase L
West Nile virus	Degradation of viral genome by RNase L
	Dominant negative RNase L renders cells more permissive
	In vivo: enhanced susceptibility of RNase L-KO mice (footpad inoculation)
Togaviridae	
Sindbis virus	*In vitro*: increased replication in RNase L-KO fibroblasts
	Minor effect *in vivo* (TD[c] mice versus IFNAR-KO mice)
Coronaviridae	
Murine hepatitis virus[b]	*In vitro and in vivo*: increased replication and mortality of the ns2 mutant in RNase L-KO macrophages and mice
Ortho/Para-myxoviridae	
Syncytial respiratory virus	Minor effect of OAS inhibition or RLI expression
	In vitro and *in vivo*: inhibition of infection by a 2–5A/oligonucleotide complex
Influenza A virus[b]	Increased replication of the NS1 mutant in RNase L-KO or RNase L-KD fibroblasts
Reoviridae	
Reovirus	Minor or deleterious effects in RNase L-KO fibroblasts
DNA viruses	
Poxviridae	
Vaccinia virus	*In vivo*: minor effect (TD[c] versus IFNAR mice)
Herpesviridae	
Herpes simplex virus 1	*In vitro: effects of the McKrae strain*
	In vivo: contradictory effects, depending on the viral strain and the inoculation route
Herpes simplex virus 2	Deleterious proinflammatory effect of RNase L
Polyomaviridae	
Simian virus 40	No cleavage is observed *in vitro*
Retrovirus and HBV	
Retroviridae	HIV: TAR sequence can activate the OAS but is inhibited by Tat
Human immunodeficiency virus	*In vitro:* RNase L overexpression inhibits HIV replication
	RNase L inhibition or RLI overexpression activates HIV replication
Hepadnaviridae	
Hepatitis B virus	Identical HBV replication in RNase L-KO HBV transgenic mice

[a] Detailed references can be found in the review [42], from which this table was adapted.
[b] In absence of the inhibitory viral protein.
[c] TD, *triply deficient* mice: RNase L, PKR and Mx.

enzymes. Others act downstream, through 2–5A degradation or RNase L inhibition.

dsRNA sequestration by a viral protein
Some viruses sequester dsRNA and thereby prevent OAS activation. Examples of proteins with this action include Influenza A virus NS1 [19], vaccinia virus (VV) E3L [20] and the σ3 outer capsid protein of reoviruses [21], which remarkably both plays a structural role in the capsid and counteracts antiviral responses.

The human immunodeficiency virus (HIV) Tat protein binds to *tar*, a dsRNA structure in the HIV mRNA, to prevent OAS activation by *tar* [22].

Expression of viral mRNA decapping enzymes
In cells infected by DNA viruses, dsRNA can arise by convergent transcription from opposite DNA strands. With vaccinia virus, up to 15% of polyA RNA synthesized late in replication is predicted to form duplexes. However, VV encodes two decapping enzymes, D9 and D10, that degrade methylated mRNA cap structures and render them susceptible to cellular 5′ exonuclease Xrn1. Accordingly, infection with a D9 and D10 catalytic mutant virus triggers a drastic increase in dsRNA-mediated activation of OAS and PKR, as does the depletion of Xrn1 [23••,24••].

2–5A degradation by a viral phosphodiesterase
A recently discovered mechanism to prevent RNase L activation is the expression of 2′,5′-phosphodiesterases

Figure 2
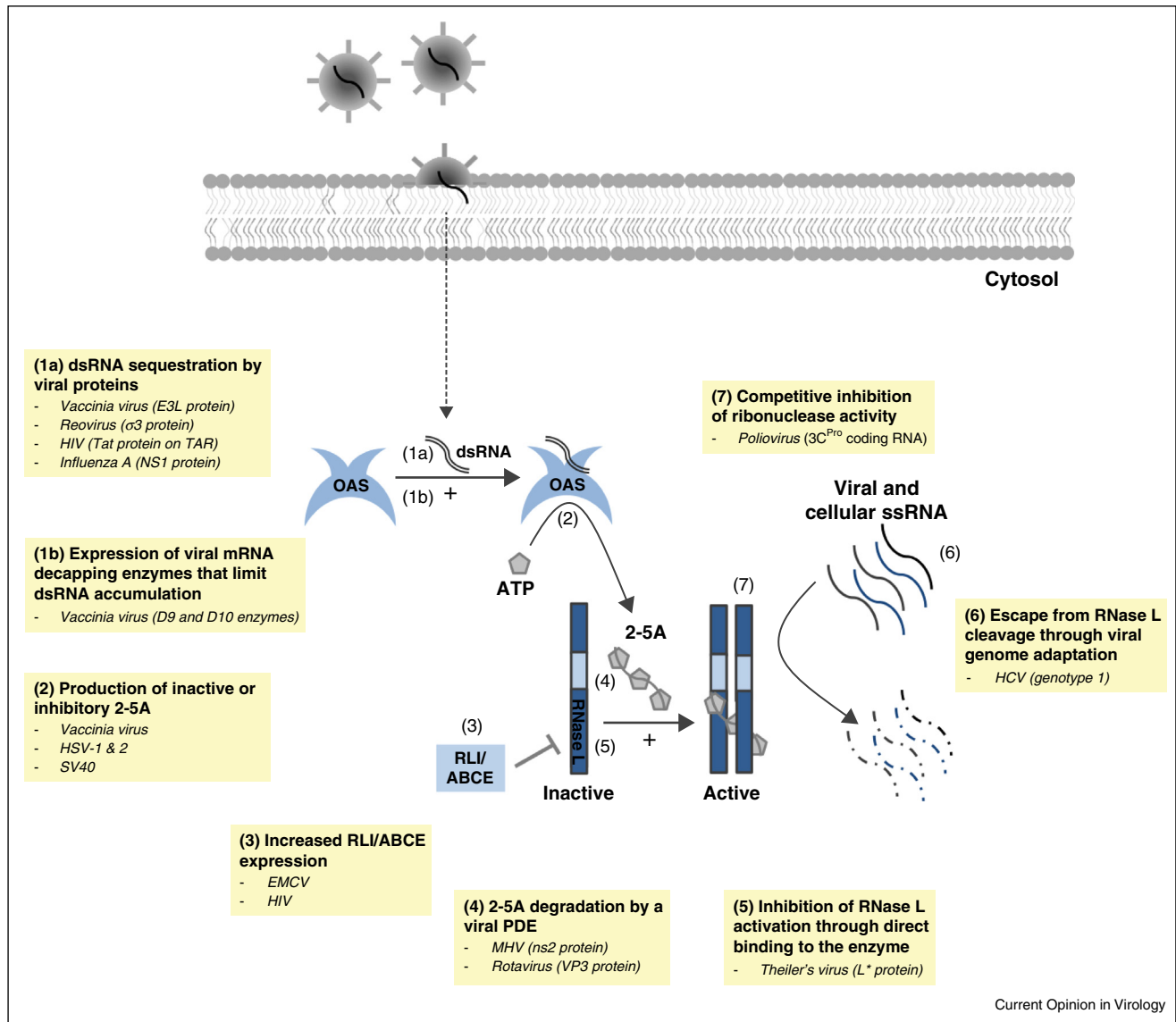
Strategies developed by viruses to escape the OAS/RNase L pathway.

(PDE) that degrade 2–5A into ATP and AMP. PDE activity was first shown for the ns2 protein of mouse hepatitis virus (MHV), a coronavirus. A catalytically inactive ns2-H126R mutant virus was strongly attenuated for liver infection of wild type but not RNase L-KO mice [25].

The C-terminal domain of rotavirus protein VP3 was identified, by sequence data mining, as another potential $2'$,$5'$-PDE. In recombinant MHV viruses, rotavirus VP3 can substitute for ns2 and rescue replication in bone marrow-derived macrophages and in mouse liver. Sequence alignments suggest that a similar PDE occurs

in all group A and likely group B and C rotavirus strains [26,27].

Production of inactive or inhibitory 2–5A

It was proposed that some DNA viruses hijack OAS to promote the synthesis of inactive or inhibitory 2–5A. For instance, upon herpes simplex virus (HSV-1 and HSV-2) infection, 2–5A synthesis is induced but 2–5A accumulation does not contribute to ribosomal RNA degradation [28]. Likewise, in simian virus 40 (SV40) and vaccinia virus infection of IFN-primed cells, 2–5A concentration can reach 2–5 μM without inducing a clear RNase L activation [29,30]. These 2–5A molecules, which remain

to be analyzed, could include phosphorylated and unphosphorylated 2–5A as well as related compounds inactive on RNase L.

Increased RLI/ABCE expression

EMCV infection induces RLI/ABCE [31], which correlates with RNase L inhibition. Accordingly, RLI/ABCE overexpression partially suppresses the action of IFN against EMCV [32]. Similar observations were made for HIV-1 [33]. Interpretation of these data is, however, complicated by the contribution of RLI/ABCE to HIV-1 capsid assembly.

Inhibition of RNase L activation through direct binding to the enzyme

Theiler's murine encephalomyelitis virus (TMEV) encodes an L* accessory protein which enhances macrophage infection *in vitro* and is required to persist in the mouse central nervous system [34]. To date, L* is the only viral protein shown to inhibit RNase L through a direct protein–protein interaction. This activity of L* is species-specific [35].

Competitive inhibition of ribonuclease activity

Poliovirus antagonizes RNase L through a highly structured hairpin in its genomic RNA. This hairpin in the 3C protein coding region acts as a cleavage-resistant substrate of RNase L. This renders poliovirus RNA resistant to RNase L cleavage despite hundreds of UU and UA dinucleotides [36].

Escape from RNase L cleavage through genome adaptation

Hepatitis C virus (HCV) genotype 1 has evolved to decrease the number of cleavage sites recognized by RNase L. As a result, HCV1 is more resistant to IFN than HCV2 or HCV3. Viral strains resistant to RNase L have fewer UU and UA dinucleotides (the main RNase L targets). Moreover, silent mutations in these cleavage sites accumulate during IFN therapy [37].

Discussion

Amplification and bottleneck in the RNase L pathway

It is surprising that the OAS/RNase L pathway starts with a two-step amplification, which activates a single enzyme (Figure 3). Indeed, IFN strongly induces the expression of several OAS, which, upon viral dsRNA binding, synthesize large amounts of 2–5A. Then, 2–5A activate a single target, RNase L, which is present in low amounts in the cell and therefore limits the cellular response. One may wonder why evolution did not select a more direct way to activate RNase L.

One reason may be that factors upstream of the cascade have additional functions. This is indeed the case for some OAS, which have RNase L-independent antiviral activity [38*,39]. For example, upon dsRNA binding, the

catalytically inactive human OASL can activate RIG-I. Even more surprising is the intensity of 2–5A production since these compounds have a unique known function, the activation of RNase L [8]. First, it is possible that 2–5A have another function. Second, the use of 2–5A as second messengers may allow tight regulation of RNase L, as 2–5A are quickly degraded by phosphodiesterases. Third, some analogy exists between the OAS/RNase L pathway and the cGAS-STING pathway, which leads to IFN expression. Both involve the synthesis of non-canonical nucleotides acting as second messengers: 2–5A for OAS/RNase L and cyclic AMP-GMP for the cGAS/STING pathway. Cyclic AMP–GMP can be transferred between cells, in a gap junction-dependent manner, to activate IFN synthesis in neighboring cells [40**]. 2–5A could similarly signal to neighboring cells to prime the RNase L-mediated positive feed-back into the IFN response.
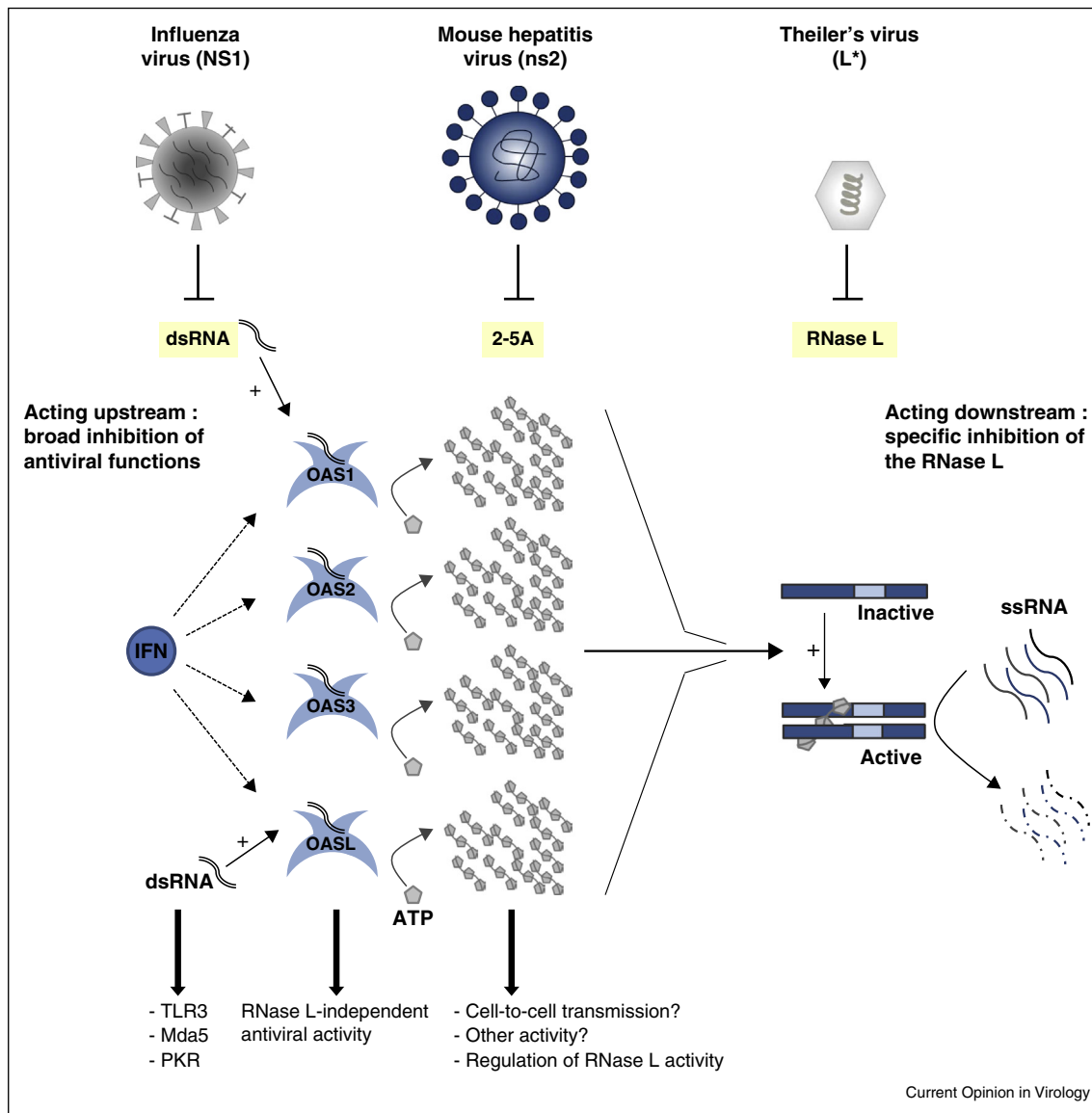
Acting upstream or downstream of the RNase L pathway?

Almost every step of the OAS/RNase L pathway is targeted by a viral protein. Inhibiting the downstream effector enzyme (RNase L), as does TMEV L* [35], would seem to be the most efficient mechanism. Indeed, RNase L is much less abundant than OAS that are induced by IFN or than 2–5A that are synthesized by activated OAS. Nonetheless, some viruses target molecules upstream of the pathway, such as dsRNA or 2–5A, despite their abundance. Targeting dsRNA may less potently counteract RNase L activity, but also inhibits other antiviral pathways such as PKR, Mda5 and TLR3. Targeting 2–5A, as coronaviruses and rotaviruses do, is a more difficult-to-understand strategy, unless 2–5A have an alternative function that would also be inhibited by these viruses (Figure 3).

Is there a host/tissue specificity for RNase L activity?

It is noteworthy that RNA viruses, like TMEV or MHV, devote a protein to inhibiting RNase L, despite their limited coding capacity [25,35]. This indicates that the OAS/RNase L pathway exerts strong selective pressure. The fact that both viruses are murine may suggest that the OAS/RNase L pathway is particularly active in this species. However, human enteric (HEC4408 strain) and respiratory (OC43 strain) coronaviruses produce a protein, homologous to MHV ns2, likely sharing phosphodiesterase activity. Moreover, human rotaviruses encode a PDE [27].

Another trait common to MHV and TMEV is macrophage tropism. In macrophages, OAS and, to a lesser extent, RNase L basal expression is higher than in other cell types [35,41*]. Incidentally, the L* protein was first described as a protein that facilitates the infection of macrophages. Similar observations were made that ns2 promotes MHV replication specifically in macrophages [41*]. The PDE activity of the rotavirus VP3 is not required to infect enterocytes of small intestinal villi

Figure 3

Inhibition of upstream or downstream steps of the OAS/RNase L pathway. Influenza virus NS1, mouse hepatitis virus ns2 and Theiler's virus L* proteins are examples that act on different steps of the pathway. NS1 is a broad spectrum inhibitor that acts upstream, by sequestering dsRNA. It therefore concomitantly inhibits other antiviral pathways that depend on dsRNA such as PKR, Mda5 and TLR3. ns2 degrades 2–5A and thus acts downstream of OAS. It is unclear whether 2–5A have functions other than RNase L activation. L* directly interacts with RNase L, which ensures a specific effect.

but might contribute to infecting a subpopulation of plasmacytoid dendritic cells thought to play an important role in virus dissemination [27].

In conclusion, viruses have developed various strategies to escape the OAS/RNase L pathway, underlining its physiological importance. The multiplicity of evasion strategies may stem from the diversity of viral replication cycles and from the variety of antiviral defenses exerted by different cell types and organisms. Some viruses rather act upstream, on triggers of the OAS/RNase L pathway, thereby antagonizing other antiviral pathways that depend on the same triggers. Others act downstream for more selective RNase L inhibition. This latter option may reflect a tropism for macrophages, in which the OAS/RNase L system is particularly active [35,41•].

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