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Diverse functions of RNase L and implications in pathology

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Abstract

The endoribonuclease L (RNase L) is the effector of the 2–5A system, a major enzymatic pathway involved in the molecular mechanism of interferons (IFN). RNase L is a very unusual nuclease with a complex mechanism of regulation. It is a latent enzyme, expressed in nearly every mammalian cell type. Its activation requires its binding to a small oligonucleotide, 2–5A. 2–5A is a series of unique 5′-triphosphorylated oligoadenylates with 2′–5′ phosphodiester bonds. By regulating viral and cellular RNA expression, RNase L plays an important role in the antiviral and antiproliferative activities of IFN and contributes to innate immunity and cell metabolism. The 2–5A/RNase L pathway is implicated in mediating apoptosis in response to viral infections and to several types of external stimuli. Several recent studies have suggested that RNase L could have a role in cancer biology and evidence of a tumor suppressor function of RNase L has emerged from studies on the genetics of hereditary prostate cancer.

Keywords

Interferon; RNase L; RNA expression; apoptosis; prostate; virus; cancer

I) Introduction

The endoribonuclease L (RNase L) is the effector of the 2–5A system, a major enzymatic pathway regulated by interferons (IFN) [1] (Figure 1). The 2–5A system is one of the two antiviral pathways induced by IFN and activated by double stranded RNA (dsRNA), the other is mediated by the dsRNA dependent protein kinase (PKR) [2]. RNase L is a very unusual nuclease. It is a latent enzyme, expressed in nearly every mammalian cell type. Its activation requires its binding to a small oligonucleotide, 2–5A (Figure 1). 2–5A itself is very unusual, consisting of a series of 5′-triphosphorylated oligoadenylates with 2′–5′ phosphodiester bonds in contrast to the 3′–5′ linkages found in RNA and DNA. The initial and essential observation was made by Ian Kerr's group in 1974 reporting an IFN-induced increase in the sensitivity of protein synthesis to inhibition by dsRNA [3]. Peter Lengyel's group observed increased nuclease activity in extracts of interferon treated cells incubated with dsRNA [4,5]. The identification by Ian Kerr's group of the activators of this nuclease, 2–5A [6] and of the enzyme responsible for their synthesis, the 2–5A-synthetase [7–9], led to the discovery of the 2–5A pathway. Clemens and Williams directly demonstrated a nuclease, now recognized as RNase

L, that was activated by 2–5A [10]. In brief, IFN induces the transcription of several 2–5A synthetase genes whose protein products are in turn activated by dsRNA and produce 5′-triphosphorylated, 2′,5′-oligoadenylates (2–5A) from ATP [11]. 2–5A bind specifically to RNase L, which is activated to cleave single stranded RNAs at UpN (mainly UpU and UpA) sequences in single-stranded regions of RNA [12,13]. The only known function of 2–5A is to bind and activate RNase L. 2–5A is very unstable in cells and in serum. It is dephosphorylated by general phosphatases at its 5′ end, leaving the so-called core oligoadenylate that is unable to efficiently activate RNase L [14]. 2–5A can also be degraded from the 2′,3′-termini by a 2′-phosphodiesterase [15].

The chemical and/or enzymatic synthesis of several analogues of 2–5A with increased metabolic stability has allowed the determination of the regions of the oligomer which interact with RNase L and that are absolutely necessary for the activation of RNase L [16]. Only oligomers with more than two adenylates are able to activate RNase L. The 5′-monophosphate, 2′, 5′-phosphodiester bonds and the 3′-hydroxyl groups of the second adenosine (from the 5′ terminus) are critical for its biological activity. The 5′ terminal adenine base of 2–5A is vital for binding with RNase L, the adenine of the 2′ terminal adenosine is absolutely critical for activation of the nuclease function of the enzyme but not for its binding. The cloning of RNase L in 1993, allowed the subsequent elucidation of its remarkable properties [1] (Figure 2). The recent crystal structure of the 2–5A binding domain of RNase L in a complex with 2–5A provides a detailed view of these interactions at the atomic level [17].

II) RNase L structure

RNase L was first described as a 185 kDa complex by gel filtration experiments [18] then as a 78–80 kDa protein in gel denaturing conditions [19,20]. These two forms seem to coexist in cells, their ratio depending on the cell type and experimental procedures use to detect RNase L [21–23]. The two forms of RNase L bind 2–5A and have 2–5A-dependent nuclease activity. Another small protein (40–46 kDa) which binds 2–5A and cleaves poly(U) has been observed in mouse spleen and liver and in EAT (Ehrlich ascites tumor) cells [19,21,24]. This low molecular weight protein seems to be due to a proteolytic cleavage of RNase L [25,26].

Cloning of RNase L demonstrates that human RNase L is a 741-amino-acid protein with a molecular mass of 83,539 kDa [1]. RNase L is widely expressed in different cell types of mammals. Recently the expression of an alternative spliced RNase L variant, lacking the third exon, was shown in peripheral blood leukocytes [27]. RNase L consists of three domains: an N-terminal ankyrin repeat domain, a protein kinase homology domain and a C-terminal ribonuclease domain (Figure 2). The N-terminal domain could be considered as the regulatory domain of RNase L. It is composed of eight complete and one partial ankyrin motifs (R1–R9). Two walker A motifs (ATP or GTP fixation) are located within R7 and R8 [28]. Ankyrin motifs function in mediating many different protein-protein interactions [29]. Surprisingly, the ankyrin motifs in RNase L interact with an oligonucleotide, 2–5A. Several studies with N-terminal truncations of RNase L in ankyrin motifs or mutation in the two walker A motifs have shown that R1, 7, 8, 9 are essential for 2–5A binding [1,30,31]. The crystal structure of the N-terminal ankyrin repeat domain of RNase L complexed with 2–5A shows that the bound 2–5A directly interacts with R2–R4 [17]. That study indicates that R2–R4 constitute the 2–5A binding pocket and that R7–R9 may be necessary for structural integrity of RNase L rather than directly binding 2–5A. To determine more precisely the role of the amino acids residues surrounding the 2–5A binding site, several mutants were constructed based on the crystal structure [32].

Binding with 2–5A induces a conformational change in the ankyrin domain of RNase L believed to unmask the C-terminal ribonuclease domain allowing homodimerization of RNase L and activation of its nuclease activity [30,33–35]. The dimerization and activation of RNase

L requires a molar ratio of 1:1 between RNase L and 2–5A [33,36,37]. The C-terminal half of RNase L is homologous with the kinase/endoribonuclease, IRE1p, which functions in the unfolded protein response (UPR) in *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and vertebrates including *Homo sapiens* [34,38,39]. RNase L and IRE1 have homology in their nuclease domains but also in their kinase or kinase-like domains. However, whereas the kinase function of IRE1p is well established, RNase L has not been shown to have kinase activity. However, mutation of the lysine 392 in the protein kinase II domain leads to a greatly reduced activity of RNase L which was correlated to a defect in the ability of RNase L to dimerize [40]. Several amino acids in C-terminal domain of RNase L are required for catalysis, including R667 and H672 [34]. In addition, Tyr712 and Phe716 are important for both binding and cleavage of RNA [41]. As mentioned above, RNase L is an endoribonuclease with minimal sequence specificity. Its first RNA targets to be identified were viral mRNA and rRNA [42–45]. But more recently, several cellular mRNAs regulated by RNase L were identified. This is an indication that RNase L, and the 2–5A pathway, could have a wide biological role in cell physiology.

III) Biological activities of RNase L

1) Antiviral activity

The type I IFN response is the host's frontline defence against viral infections and occurs prior to the onset of the adaptive immune response. IFN activity is critical to limit virus propagation before the development of a full immune response. Type I IFN regulates transcription of a number of genes which inhibit or block viral replication through diverse mechanisms. Since the 1980's, many studies have established that the 2–5A/RNase L pathway plays a central role in the antiviral activity of IFN. During viral infections, many viruses produce dsRNA structures that can activate 2–5A-synthetases. The presence of 2–5A has been demonstrated in cells infected with encephalomyocarditis (EMC) virus [46], vaccinia virus [47] or reovirus [48]. The role played by RNase L in IFN-induced antiviral activity has clearly been demonstrated by transfection of 2–5A or stabilized 2–5A analogues in intact cells [49–54]. The cloning of 2–5A-synthetase [55,56] and RNase L [1] allowed the definitive demonstration of the antiviral activity of RNase L. The constitutive expression of the 40 kDa 2–5A-synthetase confers resistance to EMCV and mengovirus [57,58] and over expression of the 69 kDa form of 2–5A-synthetase protein in human cells inhibits the replication of EMCV [59]. Similarly, the antiviral activity of IFN against EMCV was inhibited by the expression of a dominant negative mutant of RNase L [28] or treatment with a 2–5A antagonist [49,53,54]. *In vivo* evidence for the antiviral role of the 2–5A system was provided by studies with RNase $L^{-/-}$ mice, which have enhanced susceptibility to infections with EMCV, Coxsackievirus B4,West Nile virus and herpes simplex virus 1 [60–63]. In addition, it has been shown that RNase L is activated in West Nile virus-infected cells and plays a role in the cellular antiviral response to flaviviruses [64]. The RNase L pathway has also been implicated in the response to IFN-beta during acute ocular herpes simplex virus type 1 infection (HSV-1) of mouse trigeminal-ganglia (TG) cells [65].

a) Mechanism of action—A localized model of activation of RNase L could lead to preferential degradation of viral vs. cellular RNA. Viral replicative intermediates or viral mRNA possessing double-stranded structures can activate 2–5A-synthetase resulting in localized production of 2–5A and activation of RNase L [66–68]. 2–5A synthetase may bind to replicative intermediates of RNA viruses and be activated by these RNA structures [69]. Moreover, complexes between EMCV dsRNA and 2–5A-synthetase that produce 2–5A from ATP have been isolated from virus-infected cells [70]. Several viral RNAs can activate 2–5A-synthetase including adenoviral VAI RNA [71], TAR RNA of human immunodeficiency virus type 1 (HIV-1) [72], REX-RE RNA of Human T-cell Leukemia virus type I (HTLV-1) [73],

and EBER-1 RNA of Epstein-Barr virus [74]. Cleavage of viral mRNA by RNase L in intact cells has been demonstrated during treatment of vesicular stomatitis virus-infected cells with 2–5A [75], HIV-1 and EMCV infection [44,45]. Interestingly, limited activation of RNase L depends on the levels of 2–5A. Low levels of 2–5A are sufficient to observe a biological response: a reduction in EMCV RNA. In presence of high concentrations of 2–5A an extensive rRNA cleavage is observed, indicating of a widespread degradation of host cell RNA [44].

b) Inhibition of RNase L activity by viruses—Several viruses have developed different strategies to counteract the antiviral activity of 2–5A/RNase L pathway. In some virus infected cells, despite the presence of viral dsRNA structures able to activate 2–5A-synthetase and the presence of high concentrations of 2–5A, no RNase L activity could be detected. During herpes simplex virus type 1 and 2 infections, natural 2–5A analogs are synthesized in parallel with authentic 2–5A. These 2–5A derivatives are weak activator of RNase L and able to inhibit RNase L activation by authentic 2–5A [76]. Such 2–5A related compounds, unable to activate RNase L, are also observed in simian virus 40 (SV40) infected cells [77]. Hepatitis C virus (HCV) may escape RNase L activity through selection of sequence variants [78,79]. As expected, RNase L cleaves HCV mRNA predominantly after UA and UU dinucleotides in single-stranded (loop) regions leading to fragments of 200 to 500 bases in length. HCV mRNAs from relatively IFN-resistant genotypes (HCV 1a and 1b) have fewer UA and UU dinucleotides than HCV mRNAs from more IFN-sensitive genotypes (HCV 2a, 2b, 3a and 3b). During IFN therapy, HCV 1b mRNA accumulates silent mutations preferentially at UA and UU dinucleotides, possibly to escape RNase L activity. Perhaps as a consequence, patients infected with HCV 1b viruses are cured less frequently than patients infected with HCV genotype 2 or 3 [78]. To synthesize 2–5A, 2–5A-synthetase needs to be activated by dsRNA. Some viral proteins which possess the capacity of binding dsRNA directly compete with 2–5A synthetase. In fact, during vaccinia virus infection, 2–5A-synthetase is inhibited and 2–5A is degraded [80]. Inhibition of 2–5A synthetase during vaccinia virus infection is mediated by the E3L, viral encoded dsRNA binding proteins [81–83]. During influenza A virus infection, the viral protein NS1A sequesters dsRNA and inhibits 2–5A-synthetase activation [84]. Some viruses recruit a host protein to inhibit RNase L activity. This is the case for EMCV and HIV-1 which lead to increase expression of the Ribonuclease L Inhibitor (RLI) [45,85,86]. RLI is an ATP binding cassette (ABC) protein now classified as ABCE1 protein. RLI can form a heterodimer with RNase L and inhibit the binding of 2–5A by RNase L in a noncompetitive manner [85]. During HIV-1 infection, RLI also interacts with the nucleocapsid domain of Gag and play a role in HIV-1 capsid assembly [87].

2) Regulation of mRNA translation

RLI is not the only cellular protein which can form heterodimer with RNase L. One of us (C.B.) has previously identified an RNA binding protein (RNABP) which associates with RNase L. This complex has been observed in different cell extracts by co-immunoprecipitation, gel filtration and pull-down assays in a 2–5A dependent and single-stranded RNA independent manner [23,88]. Recently RNABP was identified (by C.B. and collaborators) as the translation termination release factor eRF3/GSPT1 [89]. After activation by 2–5A, RNase L can interact with eRF3. This association can help to localize RNase L to its mRNA target, but it is also a way to modulate eRF3 activity. Importantly the regulation of eRF3 activity depends of the 2–5A oligomer size activating RNase L. Binding of $2–5A_3$ or $2–5A_4$ induces a conformational change in RNase L that promotes its interaction with eRF3. In one conformational change, the eRF3-RNase L interaction brings RNase L into close association with the mRNA, where it can act as an endoribonuclease. But binding with $2–5A_3$ can induce another conformational change leading to an RNase L-eRF3 complex that can modulate translation termination and promote ribosomal readthrough of a termination codon. Moreover, RNase L regulates the +1 frameshifting of the antizyme 1 mRNA in IFN treated cells. This was the first report implicating

a nuclease, RNase L, in the translation regulation of a cellular mRNA independently of its nuclease activity.

3) Regulation of cellular mRNA stability

2–5A-synthetase levels vary with growth and hormonal status in a variety of cell types and tissues [90,91]. These results suggested that 2–5A levels, and consequently RNase L activity, may play a role in metabolism of uninfected cells and in the antiproliferative effects of IFN. It has been known for more than 30 years that IFN-treated cells show enhanced sensitivity to inhibition of protein synthesis by dsRNA [3], this is also true for cells treated by 2–5A [92], mediated by RNase L that degrades mRNA and rRNA [69,93–95]. 2–5A-synthetases are activated by double stranded region of cellular RNA which leads to 2–5A synthesis [96,97]. But the identification of specific mRNAs regulated by RNase L was difficult to detect in intact cells until the advent of DNA microarray technology [98]. Activation of RNase L by exogenous 2–5A leads to general degradation (70%) of cellular RNA [99]. Studies of subcellular localization of RNase L showed that RNase L could be associated with polysomes [24], mitochondria [100], cytoskeleton [101] and nuclei [102], suggesting that RNase L could regulates the stability of different cellular RNA species in different cellular compartments. Conditional expression of RNase L showed that RNase L regulates MyoD mRNA stability and muscle cell differentiation [103]. RNase L in mitochondria regulates mitochondrial mRNA stability and possibly contributing to the antiproliferative activity of IFNs [100]. Interestingly, RNase L regulates the stability of PKR mRNA and of two interferon-stimulated genes: ISG43 and ISG15 [104,105] suggesting one function of RNase L is to limit the IFN response.

4) Apoptosis

The 2–5A/RNase L pathway is implicated in mediating apoptosis in response to viral infections and to several types of external stimuli [60,106–111]. How might RNase L activation lead to apoptosis? The degradation of 28S and 18S rRNA by RNase L in intact ribosomes has been long known as a hallmark of IFN and viral infections [42,43]. Cleavage of 28S rRNA by RNase L maps to the L1 protuberance implicated in formation of the exit or E site of the ribosome, possibly interfering with release of deacylated tRNA [112]. By comparison, the fungal ribonuclease, α-sarcin, and the RNA modifying enzyme, ricin A chain, and uv light each lead to ribotoxic stress responses involving damage to the 3′-end of the large ribosomal RNA. These treatments activate the stress-activate protein kinases, c-jun NH2-terminal kinases (JNKs) [113,114]. JNK activation in response to uv irradiation has been linked to apoptosis through Bax [115]. Furthermore, JNK activation by the topoisomerase I inhibitor, camptothecin, or by the protein synthesis inhibitor, anisomycin, sensitizes prostate cancer cells to anti-fas mediated apoptosis [116]. Interestingly, phosphorylation of JNK in response to dsRNA is deficient in RNase L-null cells [112]. Furthermore, it has been shown using *JNK1*$^{-/-}$*JNK2*$^{-/-}$ mouse embryonic fibroblast cells that the JNKs are essential for apoptosis mediated by RNase L [117]. Apoptosis initiated by RNase L requires caspase 3 activity and is characterized by the appearance of cytosolic cytochrome c indicating involvement of mitochondria [110]. In fact, IFNα treatment induces down regulation of mitochondrial mRNAs, which leads to a decrease in cellular ATP levels and suppression of mitochondrial functions and in particular a decrease in mitochondrial protein levels [118,119]. The consequence of such a degradation of mitochondrial mRNA and inhibition of protein synthesis is a loss of mitochondrial membrane potential which results in osmotic swelling and cytochrome c release [120]. Yanase and co-workers have shown that during IFNα induced apoptosis there is a release of cytochrome c from mitochondria, a decline in mitochondrial membrane potential and caspase 3 activation [121]. The different partners of the 2–5A pathway are localized in mitochondria: RNase L, RLI and p69 form of 2–5A-synthetase [100,122,123]. This pathway is induced by IFNα treatment and RNase L participates in mitochondrial mRNA degradation. Mitochondrial mRNA remained stable during IFNα treatment in cells where RNase L activity was decreased

by transfection of RNase L antisense or RLI sense cDNAs [100]. A similar stabilization of mitochondrial mRNA is observed in *RNASEL* $^{-/-}$ mouse embryonic fibroblast cells [124]. If mitochondrial mRNAs are stabilized by inhibiting RNase L activity, no cytochrome c release or caspase 3 activation are observed during IFNα treatment (CB, unpublished observation).

Ultimately, sustained activation of RNase L triggers a mitochondrial pathway of apoptosis that eliminates virus-infected cells [106,111]. Both the antiviral and tumor suppressor activities of RNase L *in vivo* could be due to its pro-apoptotic activity, limiting viral spread or tumor growth. In fact, Aimin Zhou and co-workers demonstrated recently that RNase L can potently inhibit fibrosarcoma growth *in vivo* [125].

IV) Implications in pathology

1) Role of RNase L in the biology of prostate cancer

Because genetic lesions in RNase L impair apoptotic responses, such mutations could possibly contribute to malignancy [60,107]. Recently, evidence of a tumor suppressor function of RNase L has emerged from studies on the genetics of hereditary prostate cancer. Prostate cancer has a complex etiology and is influenced by genetics, but also by androgens, diet, and other environmental factors [126]. Sporadic prostate cancer displays an age-related increase in prevalence, while familial prostate cancer often displays earlier-onset disease. Hereditary prostate cancer (HPC) stricken families, with three or more first degree relatives per family, account for 43% of early onset cases (<55 years old) and 9% of all cases [127]. HPC genetics is complex with many genes proposed as susceptibility factors in this syndrome. Interestingly, the prototype of these genes, *HPC1*, maps to the RNase L gene, *RNASEL* [128,129]. Several germline mutations or variants in *HPC1/RNASEL* have been observed among hereditary prostate cancer cases (reviewed in ref. [130]). For instance, a common (35% allelic frequency) missense variant of RNase L, in which a G to A transition at nucleotide position 1385 (G1385A) results in a glutamine instead of arginine at amino acid position 462 (R462Q). A controlled sib-pair study implicated the RNase L “Q” variant in up to 13% of unselected prostate cancer cases [129]. One and two copies of the mutated gene increased the risk of prostate cancer by about 150% and 200%, respectively. The RNase L “Q” variant at residue 462 in the kinase-like region had a 3-fold decrease in catalytic activity compared to the wild-type enzyme, due to an impairment in enzyme dimerization [129,131]. However, while several case-controlled genetic and epidemiologic studies support the involvement of *RNASEL* (and notably the R462Q variant) in prostate cancer etiology [128,129,132,133], others do not [134–136], suggesting that either population differences or environmental factors, such as viral infection, may modulate the impact of *RNASEL* on prostatic carcinogenesis. Therefore, there is a possibility that the linkage of RNase L alterations to HPC might reflect enhanced susceptibility to a viral agent. To test this hypothesis, RNA derived from wild-type and RNase L variant (R462Q) prostate tumors was examined for evidence of viral sequences, by hybridization to a DNA microarray composed of the most conserved sequences of all known human, animal, plant and bacterial viruses [137,138]. Because the array contains highly conserved sequences within viral nucleic acids, it can detect viruses not explicitly represented. These studies identified the novel retrovirus, xenotropic murine leukemia related virus (XMRV) in 8 (40%) of 20 R462Q homozygous prostate cancer tissues, and in 1 (1.5%) of 66 tissues that harbored at least one copy of the wild-type allele. Three complete genomes were sequenced, sharing >98% nucleotide and >99% protein sequence identity. The virus encodes four major proteins, gag, pro, pol, and env. XMRV is more closely related to the xenotropic and polytropic than to the ecotropic murine retroviruses. Complete viral genome sequences were obtained from three strains and partial sequences were obtained for another six XMRV strains. XMRV is a canonical gammaretrovirus, with *gag*, *pro-pol* and *env* genes, and is not closely related to any endogenous human retroviral (HERV) elements. In addition, XMRV sequences are not present

in any human genome sequences that have been reported to date [139]. Recently, the group of one of us (R.H.S.) constructed a complete infectious clone for XMRV strain VP62 [140]. In the same study, XMRV provirus integration sites were mapped in DNA isolated from human prostate tumor tissue. These findings represent the first detection of xenotropic MuLV-like agents in humans, and reveal a strong association between infection with the virus and defects in RNase L activity. In addition, these studies provide evidence that RNase L functions as an antiviral enzyme in humans.

2) Pancreatic cancer and colorectal cancer

As prostate cancer occurs in some familial pancreatic families, Bartsch and co-workers evaluated the role of two variants of *RNASEL* gene: E265X and R462Q in the etiology of pancreatic cancer [141]. That study showed that the *RNASEL* R462Q variant might be associated with an increased risk for sporadic pancreas cancer and with more aggressive tumors in familial pancreatic cancer. Additional, large scale efforts will be important to validate these results.

Also, the R462Q variant of *RNase L* correlated with earlier age of onset of hereditary non-polyposis colorectal cancer [142].

3) Chronique fatigue syndrome (CFS)

Chronic fatigue syndrome (CFS) is an illness characterized by long-lasting fatigue accompanied by non specific symptoms [143,144]. Several reports indicated the up-regulation of components of the 2–5A/RNase L pathway in extracts of peripheral blood mononuclear cells (PBMCs) from CFS patients [145,146] as well as the accumulation of a low molecular weight 2–5A-binding protein of 37 kDa [147]. It has been proposed that this polypeptide could be a biochemical marker for CFS [148,149]. However, levels of the 37 kDa polypeptide appeared to vary with time within individual patients [150]. The polypeptide is an apparent degradation product of the native RNase L due to an increased proteolytic activity in CFS PBMC extracts. *In vitro*, an equivalent degradation of RNase L could be observed when recombinant RNase L was incubated with human leucocyte elastase [151]. In CFS patients, the majority of 2–5A oligomers produced are 2–5A dimers which fail to bind or activate RNase L [33]. 2–5A trimer and tetramer binding appeared to stabilize RNase L in PBMC cell extracts from CFS patients. These observations suggest that in CFS there is increased proteolytic activity in PBMC causing accumulation of the 37 kDa polypeptide [152].

V) Conc lusion

RNase L is an unusual and fascinating enzyme unique among know nucleases in its complex mechanism of action. Considerable progress has been made since the discovery of the 2–5A pathway in 1978 and its cloning in 1993. Nevertheless, much work remains to be done in understanding how RNase L contributes to innate immunity and cell metabolism. We anticipate many additional findings over the coming years of the roles of RNase L in human pathology, in particular cancer and viral diseases.

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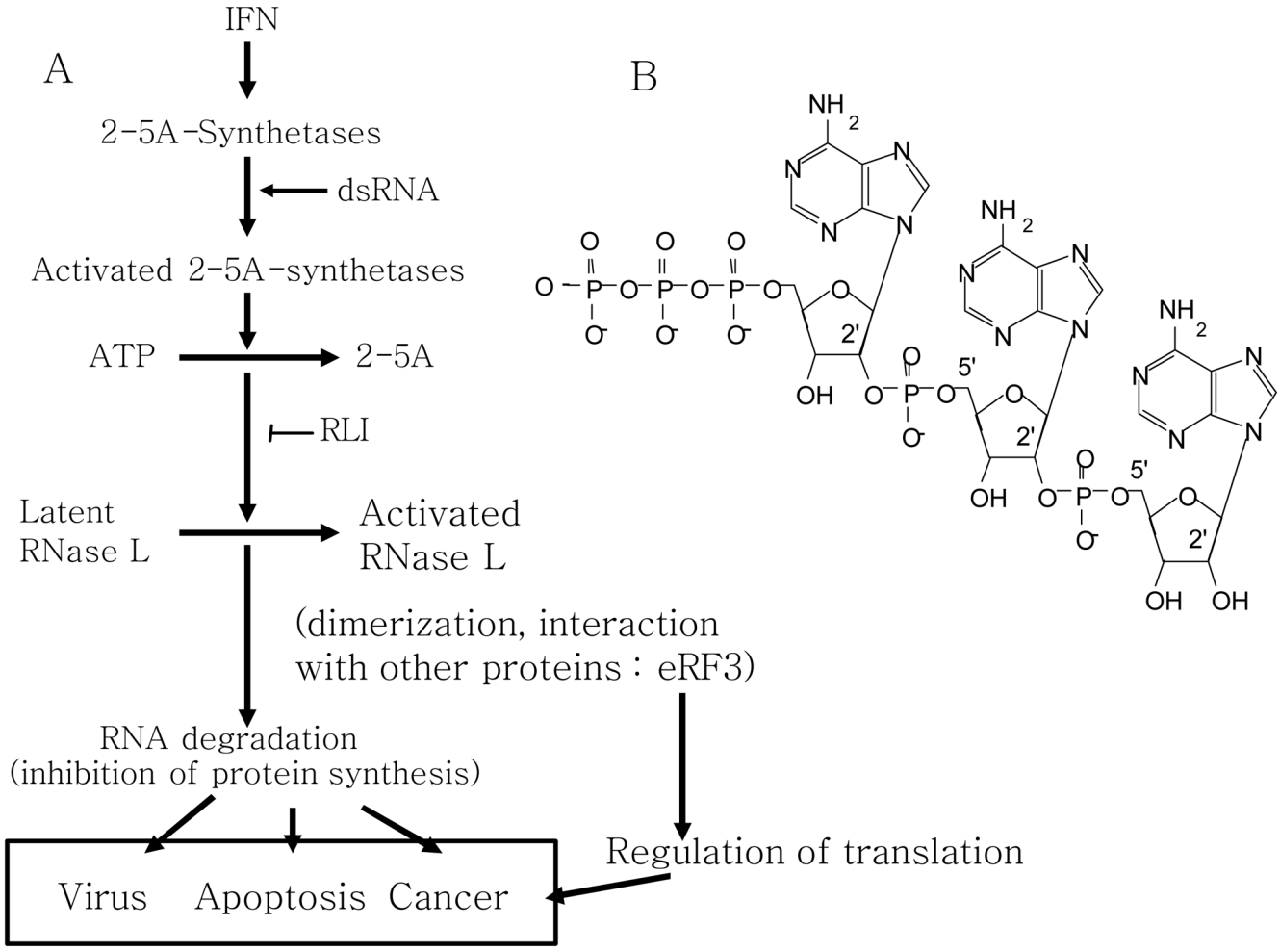

Figure 1.
A) The 2–5A/RNase L pathway
B) Structure of the 2–5A4: 2′–5′ oligoadenylates tetramer.

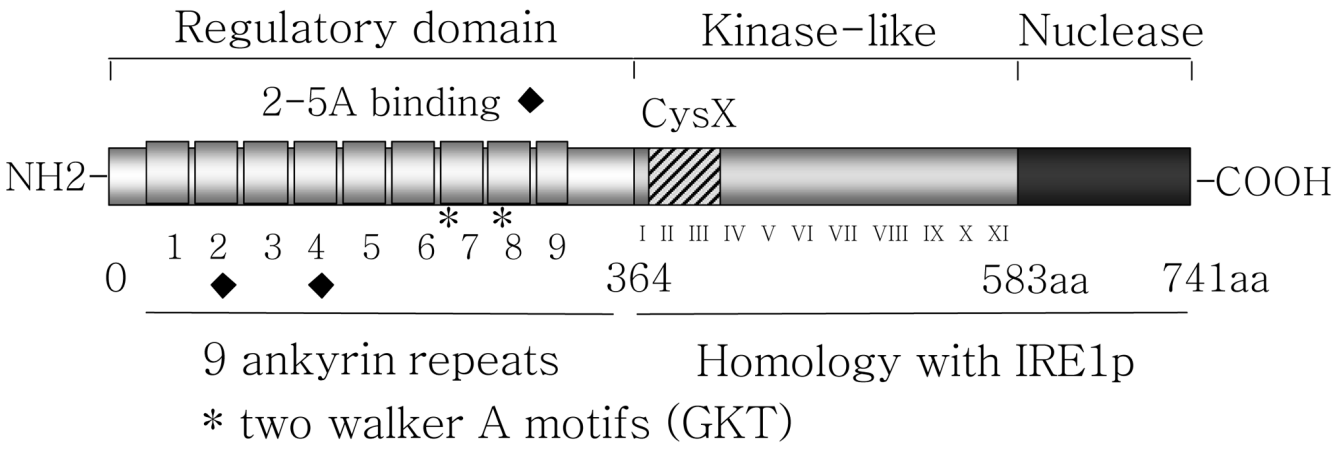

Figure 2.
Structure of the RNase L.