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A scientific journey through the 2-5A/RNase L system

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Abstract

The antiviral and antitumor actions of interferons are caused, in part, by a remarkable regulated RNA cleavage pathway known as the 2-5A/RNase L system. 2′-5′ linked oligoadenylates (2-5A) are produced from ATP by interferon-inducible synthetases. 2-5A activates pre-existing RNase L, resulting in the cleavage of RNAs within single-stranded regions. Activation of RNase L by 2-5A leads to an antiviral response, although precisely how this happens is a subject of ongoing investigations. Recently, RNase L was identified as the hereditary prostate cancer 1 gene. That finding has led to the discovery of a novel human retrovirus, XMRV. My scientific journey through the 2-5A system recounts some of the highlights of these efforts. Knowledge gained from studies on the 2-5A system could have an impact on development of therapies for important viral pathogens and cancer.

Keywords: 2-5A; RNase L; Interferon; Cancer; Virus

1. Background on the 2-5A/RNase L system

Over the past 30-years, investigations into the mechanisms of interferon (IFN) action have elucidated how antiviral innate immunity operates within and between mammalian cells. The focus of my work over this period has been, and continues to be, probing the biology and biochemistry of the 2-5A/RNase L system, and studying its roles in health and disease. My scientific journey, from basic research to clinical studies, is summarized in this monograph. The 2-5A/RNase L system is one the principal pathways by which IFNs suppress viral infections. Exposure of cells to IFNs induces expression of genes that result in an ''antiviral state''. Type I IFNs bind to the IFN-α receptor 1 (IFNAR1) and IFNAR2 polypeptide chains on cell surfaces initiating JAK-STAT signaling to the IFN stimulated genes (ISGs) [1]. Included among over a hundred different ISGs are genes encoding 2-5A synthetases (OAS) [2,3]. The OAS genes are a family of ISGs that function in the 2-5A/RNase L system (Fig. 1). In humans there are three functional OAS genes (*OAS1-3*), resulting in 8–10 OAS isoforms due to alternative mRNA splicing [4]. In mice, in addition to *OAS2* and *OAS3* there are eight separate *OAS1* genes, including *OAS1b*, the flavivirus resistance gene (Flv^r) [5–8]. When stimulated by dsRNA, the functional OAS proteins produce a series of short 5′-phosphorylated, 2′,5′-linked oligoadenylates collectively referred to as 2-5A [$p_x5'A(2'p5'A)_n$; $x = 1–3$; $n \geq 2$] from ATP [9]. The first molecular clone for an OAS was obtained by Michel Revel's lab [10]. Biochemical characterization of OAS proteins by Ara Hovanessian's lab [11–14] and Ganes Sen's lab [15–19] and a crystal structure by Rune Hartmann and Vivien Yee (in collaboration with Ganes Sen and Just Justesen) [20] have led to functional and structural insight into the OAS family of proteins. Because dsRNA is a frequent viral pathogen associated molecular pattern, 2-5A often accumulates in IFN-treated and virus-infected cells [21–23]. The principal species of 2-5A found in such cells is the trimeric form, $p_3(A2'p5')_2A$ [21]. 2-5A is a transient signaling molecule that is degraded within minutes by the combined action of 2′-phosphodiesterase and 5′-phosphatase(s) [24,25]. The only well-established function of 2-5A is activation of the latent endoribonuclease, RNase L [26]. To do so, 2-5A must have at least one 5′-phosphoryl group, the internucleotide linkages must be 2′ to 5′ and the nucleotides must by adenylyl residues for optimal

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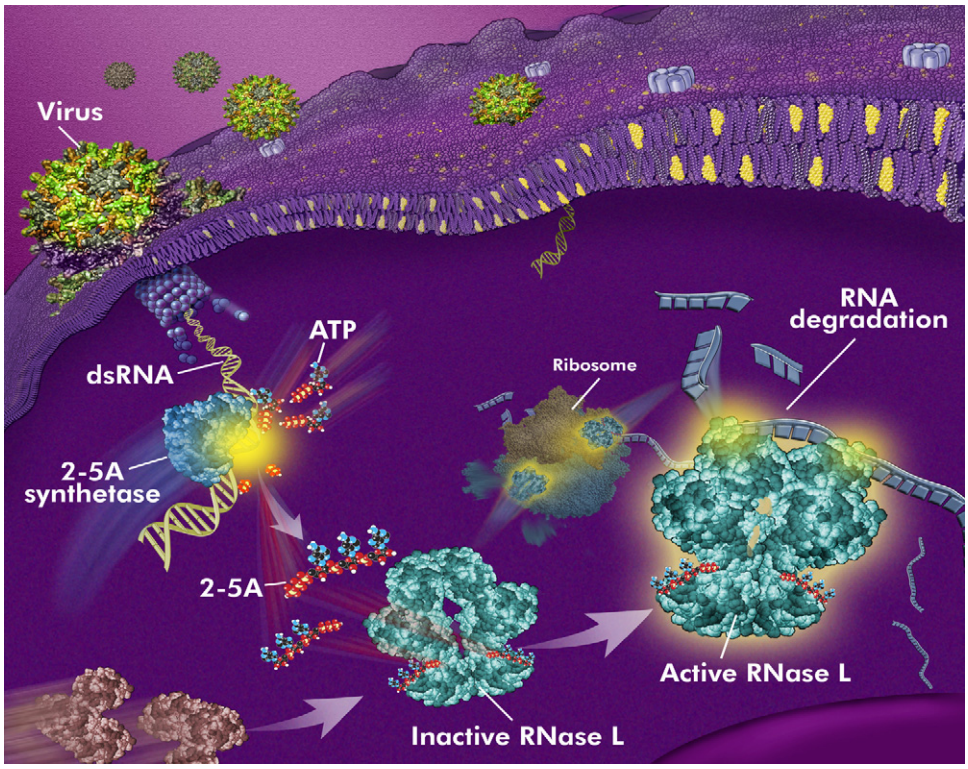

Fig. 1. The 2-5A/RNase L pathway is a classic antiviral innate immune pathway. The viral pathogen associated molecular pattern, dsRNA, activates IFN induced 2-5A synthetase (also known as oligoadenylate synthetase or OAS). This results in the synthesis of 2-5A from ATP. 2-5A binding to inactive monomeric RNase L results in formation of activated dimers of RNase L. The resulting degradation of single stranded loop regions in RNA, including rRNA in intact ribosomes, produces a potent antiviral response in the IFN treated and virus infected cell. Reprinted with the permission of The Cleveland Clinic Center for Medical Art & Photography © 2007. All Rights Reserved.

activity [27]. RNase L is activated by subnanomolar levels of 2-5A resulting in the cleavage of single-stranded regions of RNA, preferentially after UpUp and UpAp dinucleotides [28,29]. In addition, single-stranded regions of RNAs that are partially double-stranded are preferentially cleaved by RNase L [30]. Ribosomal RNAs in intact ribosomes are cleaved at specific sites providing an index of prior RNase L activity, a frequent characteristic of virus-infected cells (Fig. 1) [31].

2. Monitoring the presence and activation of RNase L in intact cells

I entered ongoing studies on the 2-5A system as a postdoctoral fellow in Ian M. Kerr's lab in late 1979. By that time, members of the Kerr lab (notably Ara Hovanessian and Bryan Williams with collaborator Michael Clemens) had firmly laid the groundwork by discovering 2-5A synthetase (OAS) activity [32], elucidating the chemical structure of 2-5A [9], and demonstrating that isolated and purified 2-5A activated the 2-5A dependent RNase [33] (now referred to as "RNase L"; the "L" stands for "latent"). Complementary work in the labs of Peter Lengyel, Michel Revel, Corrado Baglioni and Charles Samuel gave impetus to these early studies [34–37]. I came to the Kerr lab because of my interest in unusual nucleotide regulators and my fascination with Ian Kerr's remarkable discovery of 2-5A [9,38]. The discovery of 2-5A followed Ian Kerr's observation of an IFN-induced increase in the sensitivity of protein synthesis to inhibition by dsRNA [39–41]. My transition was an easy one in that I was immediately able to go to work because all of the cell-free system components were in place in Eppendorf tubes in the −70 °C freezer. The labs in the National Institute for Medical Research (NIMR) in Mill Hill, London were somewhat Spartan at that time, but it was a great place to do a post-doc because of the wonderful group of scientists that were assembled there, particularly in the area of virology. Not to mention the history, after all in the mid-1950s the NIMR is where A. Isaacs and J. Lindenmann discovered IFN [for readers who have never had the pleasure, the NIMR also appears in the film *Batman Begins* (source: Wikipedia.org)]. I made many good and longtime friends from my days at the NIMR, including George Stark, who was pursuing collaborative studies with Ian Kerr at the time [42]. Our lab group moved to the Imperial Cancer Research Fund (now Cancer Research UK) labs in Lincoln's Inn Fields in the summer of 1980.

My first task was to develop methods for detecting and characterizing the RNase and to collaborate in the development of quantitative and highly sensitive methods for conveniently measuring 2-5A from intact cells. It was not