

2A Protease from Human Rhinovirus 2

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Abstract

Human rhinoviruses (HRVs), a major cause of the common cold, usually produce mild illness, but in asthmatics they can trigger serious exacerbations. Severe HRV infections in young children increase their odds of becoming asthmatic. HRVs belong to the Picornavirus family and like their close relative, the poliovirus, their short, single-stranded, positive-sense RNA genomes code for one polyprotein that is cleaved by the virus's two proteases into several viral proteins. The St. Dominic SMART Team has modeled the 2A protease (2A) of HRV2 using 3D-printing technology. 2A is a homodimer with an active site and structurally essential zinc ion in each chain [1]. 2A promotes viral replication in host cells by shutting down both protein synthesis and nuclear-cytoplasmic import and signaling. By cleaving eIF4G (eukaryotic initiation factor 4G), 2A prevents localization of host mRNAs to ribosomes, which are then free to synthesize the viral polyprotein using the viral RNA's IRES (internal ribosome entry site). When 2A cleaves specific nucleoporins (nups) in the nuclear pore complex (NPC), proteolytic damage to Nup62, Nup153, and Nup98 inhibits immune signaling and many other cell functions. The HRV-A, HRV-B, and HRV-C species differ in their abilities to cleave both NPC nups and eIF4G. HRV-A and HRV-C 2A proteases are more efficient at cleaving Nup62 and eIF4G than HRV-B 2A proteases. Since HRV-A and HRV-C are also significantly better at triggering asthma exacerbations than HRV-B [2], it has been proposed that the effectiveness of a virus strain's 2A protease can predict the likelihood of that strain to cause asthma exacerbations [3].



Human Rhinovirus and Its 2A Protease

Human rhinoviruses (HRVs) are a major cause of the common cold, but in asthmatics they can trigger serious life-threatening exacerbations. HRVs belong to the Picornavirus family and like their close relative, the poliovirus, their short, single-stranded, positive-sense RNA genomes code for one polyprotein that is cleaved by the virus' two proteases into several viral proteins. 2A protease (2A) also promotes viral replication in host cells by shutting down both protein synthesis and nuclear-cytoplasmic import and signaling. Of the three rhinovirus species (HRVA, HRVB, HRVC), recent research [3] indicates that species A and C have 2A proteases more efficient at cleaving eIF4G and Nup62 and they are also associated with life-threatening asthma exacerbations in children [2]. It has been proposed that 2A protease may be the virulence factor that determines whether a virus causes a severe asthmatic reaction.

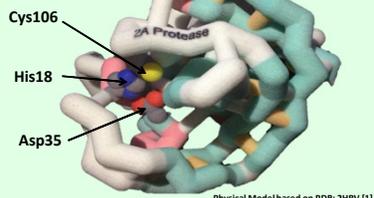
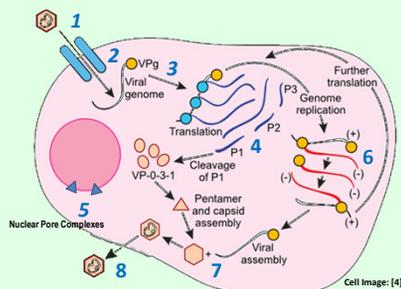


Image 1: Human Rhinovirus Infection of a Cell

Image 2: Chain B of 2A Protease

Image 1: The virus binds to an ICAM-1 receptor to get into the cell (1) and releases its single-stranded positive-sense RNA (ss+RNA) genome into the cytoplasm (2). Ribosomes bind to the viral RNA (3) and make the viral polyprotein which is cleaved into the viral proteins by 2A protease and 3C protease as it is being translated (4). 2A protease quickly shuts down host cell protein synthesis by cleaving eukaryotic initiation factor 4G (eIF4G) on host capped mRNAs (shown in Image 3). 2A protease damages the Nuclear Pore Complex (5) by cleaving several of the proteins called nucleoporins (Nups) making up the pore, which inhibits many cell functions including immune signaling. Rhinovirus cannot use host cell polymerases to replicate its RNA. Therefore HRV codes for its own replicase to make copies of its genome (6). The virus self-assembles (7), lyses the cell, and spreads to other cells. (8)

Image 2: 2A protease is a homodimer, meaning it has two identical subunits, each with its own active site. (Chain B is shown here.) The active site contains a catalytic triad made up of Cysteine 106, Aspartic Acid 35, and Histidine 18. These amino acid sidechains cut the target protein by adding a water to a peptide bond.

How the 2A Protease Makes a Cell Sick

Cleavage of Nucleoporins (Nups) by HRV 2A Proteases

Western Blot Assessing the Ability of Different HRV 2A Proteases to Cleave Nups

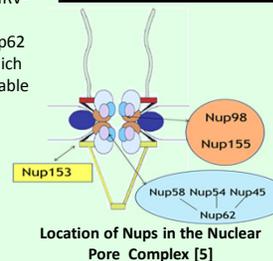
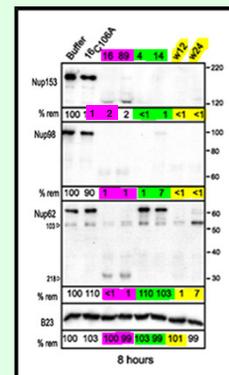
Watters and Palmenberg [3]

Nup153 and Nup98:

After 8 hours, 2As from all three species, (HRV-A, HRV-B, and HRV-C) cleaved all but 1 to 7% of Nup153 and Nup98.

Nup62: After 8 hours, the HRV-A and HRV-C 2A proteases cleaved all but 1-7% of Nup62 while 2As from HRV-B left almost all of Nup62 uncleaved.

Summary: The 2A proteases of HRV-A and HRV-C viruses were remarkably better at cleaving Nup62 than the HRV-B 2A proteases, which did not cleave Nup62 to a measurable extent in 8 hours.



Location of Nups in the Nuclear Pore Complex [5]

Cleavage of Eukaryotic Initiation Factor 4G (eIF4G) by HRV 2A Proteases

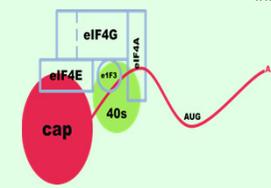
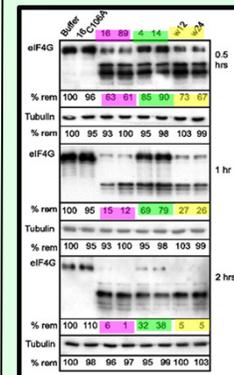
Western Blot Assessing the Ability of Different HRV 2A Proteases to cleave eIF4G

Watters and Palmenberg [3]

eIF4G:

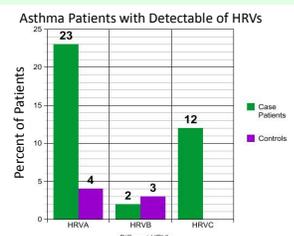
After 1 hour, the two HRV-A 2a proteases (16 and 89) cleaved all but 12 to 15% of eIF4G. The two HRV-Cs (w12 and w24) cleaved all but 26 to 27%, while the HRV-Bs (4 and 14) left 69 to 79% of eIF4G uncleaved.

Summary: HRV-A and HRV-C cleave 94 to 99% of eIF4G in 2hrs. In a cell, translation of mRNAs would be quickly inhibited at this rate.



Location of eIF4G on Host mRNAs [6]

2A Protease and Asthma



In this study, by Khetsuriani *et al* [2], the case patients were children experiencing serious asthma exacerbations. The controls were children with stable asthma. 37% of case patients with detectable HRV infections had HRV-A or HRV-C while only 5% had HRV-B. No controls had HRV-C infections and 7% had either HRV-A or HRV-B infections.

Conclusions

Human rhinoviruses have long been associated clinically with exacerbations of asthma. In the studies discussed in this poster, the HRV-A and HRV-C species were linked to both severe asthma exacerbations [2] and were also shown to have highly effective 2A proteases at cleaving Nup 62 in the Nuclear Pore Complex and eIF4G on host mRNAs [3]. It has been proposed that the effectiveness of a virus strain's 2A protease can predict the likelihood of that strain to cause asthma.

References

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- [2] Khetsuriani, M. Lu, X. Teague, W.F.G., Kazerooni, N., Anderson, L.I. and Erdman, D.D. (2008). Novel Human Rhinoviruses and Exacerbation of Asthma in Children. *Emerging Infectious Diseases*. 14:1793-1796.
- [3] Watters, K. and Palmenberg, A.C. (2011). Differential Processing of Nuclear Pore Complex Proteins by Rhinovirus 2A Proteases from Different Species and Serotypes. *Journal of Virology*. 85:10874-10883.
- [4] www.microbiologytext.com Figure 19-3 Replication of Rhinovirus.
- [5] Image of Nuclear Pore Complex Fig. 1 Lim, R.Y.H., Fahrenkrog, B. (2006). The nuclear pore complex up close. *Current Opinion in Cell Biology*. 18:342-347.
- [6] Image of capped mRNA. *Microbiology and Immunology On-line textbook*. University of South Carolina School of Medicine.
- [7] Image of Rhinovirus 14, color coded by protein, as solved by X-ray crystallography. R. M. Bock Laboratories University of Wisconsin-Madison.