# Proprotein Convertases and Enveloped Viruses

Subjects: Virology

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There are seven known human coronaviruses (CoV), which are enveloped positive-stranded RNA viruses belonging to the orderNidoviralesand are mostly responsible for upper respiratory tract infections. All these coronaviruses exhibit a “crown-like” structure composed of a trimeric spike (S) protein. The S-protein is a ~180–200 kDa type I transmembrane protein, with the N-terminus facing the extracellular space and anchored to the viral membrane via its transmembrane domain followed by a C-terminal short tail facing the cytosol.

Keywords: enveloped virus ; proprotein convertases ; Furin ; SKI-1/S1P ; PCSK9 ; SARS-CoV-2 ; COVID-19 ; pandemic

## 1. Introduction

Infectious diseases that threatened the life of humans since at least the Neolithic times, are believed to have started around 12,000 years ago, when roaming human hunter-gatherers became sedentary and settled into small camps and villages to domesticate animals and cultivate crops [1][2]. The domestication of animals and their proximity to humans favored the transmission of animal-human (zoonotic) diseases. The latter implicated various infectious microorganisms, such as bacteria, fungi, parasites, and viruses. Some of these pathogens caused widespread disease worldwide with “pandemic proportions” resulting in the death of a significant fraction of the human population.

## 2. Proprotein Convertases and Enveloped Viruses

### 2.1. Furin in Viral Infections and Pathogenicity

Furin is the third and best characterized member of the PCSK family of secretory convertases. It cleaves at basic amino acid motifs and recognizes a prototypical sequence R-X-(K/R)-R↓ [3]. The presence of this Furin(-like) motif in viral envelope glycoproteins constitutes a way to activate the fusion-dependant entry of several viruses such as HIV gp160, influenza hemagglutinin (**Table 2**), and some coronaviruses spike proteins [4][5].

**Table 2.** Various enveloped viruses and the sequences of surrounding their surface glycoprotein cleavage sites (designated by an arrow) by Furin(-like) proprotein convertases. The bold and underlined residues at positions P8, P6, P4, P2, P1 and P2′ emphasize the importance of these amino acids for protease recognition.

| Virus | Glycoprotein | P8 | | P6 | | P4 | | P2 | ↓ | | P2′ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **HIV** | **gp160** | | | **V** | **Q** | **R** | **E** | **K** | **R** | **A** | **V** |
| **H7N1 A/FPV/Rostock/34** | **HA** | | | **K** | **K** | **R** | **E** | **K** | **R** | **G** | **L** |
| **Avian H5N8 TKY/IRE** | **HA** | | | **R** | **K** | **R** | **K** | **K** | **R** | **G** | **L** |
| **Avian H5N1 A/HK/97** | **HA** | **R** | **E** | **R** | **R** | **R** | **K** | **K** | **R** | **G** | **L** |
| **Avian H5N1 TKY/ENG** | **HA** | **N** | **T** | **P** | **Q** | **R** | **K** | **K** | **R** | **G** | **L** |
| **Human CMV** | **gB** | | | **H** | **N** | **R** | **T** | **K** | **R** | **S** | **T** |
| **Human MPV** | **F Protein** | | | **N** | **P** | **R** | **Q** | **S** | **R** | **F** | **V** |
| **Human RSV** | **F Protein** | | | **K** | **K** | **R** | **K** | **R** | **R** | **F** | **L** |
| **Dengue Virus (DENG2)** | **PrM** | | | **H** | **R** | **R** | **E** | **K** | **R** | **S** | **V** |
| **Ebola Virus** | **gp160** | | | **G** | **R** | **R** | **T** | **R** | **R** | **E** | **A** |
| **Chikungunya (CHIKV)** | **E3E2** | | | **P** | **R** | **R** | **Q** | **R** | **R** | **S** | **I** |
| **Zika Virus** | **PrM** | | | **A** | **R** | **R** | **S** | **R** | **R** | **A** | **V** |

| Virus | Glycoprotein | P8 | P6 | | P4 | | P2 | ↓ | | P2′ |
|---|---|---|---|---|---|---|---|---|---|---|
| SARS-CoV-2 | S | | S | P | R | R | A | R | S | V |

In 1992, Furin was identified to be the cellular protease cleaving hemagglutinin (HA) of fowl plague/influenza virus (FPV; H7N1 A/FPV/Rostock/34), as well as the HIV glycoprotein gp160 into gp120 and gp41 [6][7][8]. The presence of a Furin cleavage sequence can shift the pathogenicity of certain viruses from non-virulent to virulent. For instance, Newcastle disease virus (NDV) fusion protein contains only a monobasic cleavage site, while the virulent NDV fusion protein possesses a polybasic sequence of amino acids that can be cleaved by Furin [9]. Similarly, the acquisition of a Furin cleavage site has been linked to the high pathogenicity of avian influenza [10], especially for the Hong Kong variant (H5N1 A/HK/97) [11][12] that has an insertion of RERR between the NTPQ and the Furin-site RKKR↓GL in HA (**Table 2**), resulting in a very efficient cleavage by more than one convertase, such as Furin, PC5 and PC7 [13]. Other pathogenic influenza virus do not possesses a Furin cleavage site and rely on trypsin-like proteases, e.g., HAT and TMPRSS2 [14]. The expression of these enzymes is restricted to the upper respiratory tract [15], while Furin is ubiquitously expressed, and the acquisition of a Furin(-like) site enhances the cellular tropism of influenza. However, the presence of a polybasic Furin(-like) cleavage site is not the only determining factor for cleavage by Furin, as the amino-acids adjacent to the Furin(-like) sequence are important too, as described in **Table 2**. Furthermore, the presence of oligosaccharides near the cleavage site can prevent processing [16], unless it is compensated by an increased number of basic residues as described in the non-pathogenic variant of influenza hemagglutinin A of H5N2 [17].

Aside from glycoproteins from NDV and influenza hemagglutinin, HIV glycoprotein, as well as other retrovirus glycoproteins such as Rous sarcoma virus and murine leukemia virus are cleaved by Furin. Interestingly, these glycoproteins form trimers, and some viruses assemble with un-cleaved glycoproteins that remain either inactive or can be cleaved at the plasma membrane. The Furin-specific cleavage of gp160 in HIV-1 has been described as dispensable as other proprotein convertases [18] could substitute for Furin in some tissues, and plasmin could activate the gp160 at the plasma membrane. However, these hypotheses remain to be validated in vivo in humans.

Human metapneumovirus (hMPV) is a paramyxovirus responsible for acute respiratory tract infections which can result in hospitalization of both children and adults. We showed that blocking the activity of the protease-activated receptor 1 (PAR1), a G-coupled receptor, induced by inflammation, protects against hMPV infections [19]. Furthermore, Furin activates the envelope F-protein of hMPV by cleavage at NP**R**QS**R**$_{102}$↓F**V** (**Table 2**, where bold residues emphasise the critical P1 and P4 positions) [19]. Unexpectedly, PAR1 itself potently inhibits cellular Furin activity [20]. Indeed, PAR1 exhibits in its second luminal loop a Furin(-like) motif (P**R**SFLL**R**$_{46}$-NP) with a P1 and P6 Arg and is cleaved by PC5A and PACE4 but not by Furin. The presence of an Asn$_{47}$ at the P1′ site rather made it a potent Furin-inhibitor that binds its catalytic subunit and sequesters the [PAR1-Furin] complex in the *trans*-Golgi network (TGN), thereby inactivating Furin and preventing PAR1 from reaching the cell surface [20]. The overexpression of PAR1 has been described in the brain upon neuroinflammation and in patients with neurocognitive disorders associated with HIV infection (HAND) [20]. Interestingly, while PAR1 binds and inhibits Furin, PAR2 is cleaved by Furin at the motif **R**SSKG**R**$_{36}$↓S**L**, which also exhibits a P1 and P6 Arg but a favorable Ser at P1′ and Leu at P2′ (**Table 2**) [16]. Recently, another strategy of viral host-cell defense has been identified. Guanylate-binding proteins (GBPs) are interferon stimulated genes. Upon HIV infection, the cytosolic GBP2 and 5 expressions are induced, and these proteins can bind the cytosolic tail of Furin and prevent its trafficking beyond the *cis/medial* Golgi, thus keeping it in an inactive state bound to its inhibitory prodomain. This effectively inhibits the Furin processing of gp160 into gp120 and gp41 [21][22]. Thus, while PAR1 is the first secretory endogenous natural inhibitor of Furin that blocks its enzymatic activity and prevents its exit from the TGN, the cytosolic GBP2,5 also inhibits Furin activity by blocking it in the *cis/medial* Golgi in an inactive state.

Another family of viruses requiring Furin is the *filoviridae*. Marburg and Ebola are enveloped single negative strand RNA viruses of the *filoviridae* family. These viruses are highly pathogenic in humans causing hemorrhagic fever with a very high mortality rate. The glycoprotein of Marburg and Ebola (GPs) are cleaved by Furin into GP1 containing the receptor binding domain (RBD) and membrane-bound GP2 [23]. Pathogenic viruses of the family possess a Furin(-like) cleavage site, but experiments design to establish the role of Furin in pathogenicity indicated that Furin cleavage is not required for replication in cell culture [24] and disease severity in non-human primates [25]. Recent work identified a new Furin site in Ebola GP that requires N-glycosylation to be processed [26]. Interestingly, MARCH8 a member of the Membrane-associated RING-CH-type 8 (MARCH8) that has been described to have broad antiviral activity against several viruses, can inhibit Furin by forming a complex with the GP and Furin thereby sequestering it in the Golgi, and preventing the maturation of the viral particles [27]. This is the third example of endogenous Furin-inhibitors that sequester the enzyme in a subcellular Golgi compartment where it remains inactive.

The Flaviviruses envelope also requires Furin to get activated. The first evidence was reported for the Tick Borne Encephalitis virus. Flavivirus are enveloped positive strand RNA viruses. Their surface protein is composed of a heterodimer formed by prM and E [28]. The immature prM is cleaved by Furin at GS**R**T**RR**$_{205}$↓S**V** before the viral particles are released from the cell. The cleavage allows fusion and is required for infectivity. An acidic pH is required to promote a change of conformation of the heterodimer to facilitate Furin cleavage. While, the cleavage of prM into M is inefficient especially for Dengue virus (**H**R**R**E**KR**$_{205}$↓S**V**) (**Table 2**), likely due to the presence of a Glu at P3 [29], Furin has been confirmed to be required for infectivity of Dengue virus in vitro by using LoVo cells that are Furin deficient [30]. Additionally, the role of Furin in the antibody-dependent-enhancement (ADE) of Dengue virus infection is intriguing. ADE is the mechanism by which the immunisation against Dengue could exacerbate the pathogenicity of Dengue in the context of a second infection. This has been explained by the fact that the binding of antibodies to an immature particle could enhance its infectivity. Interestingly, while Furin inhibition has been described to block anti-Envelope dependant enhancement [31], antibodies against prM (uncleaved by Furin) allow tissue entry of immature particles [32]. Recently, broadly neutralizing monoclonal antibodies were reported to protect against multiple tick-borne flaviviruses (TBFVs) [33], opening the way to the design of vaccines and antibody therapeutics against clinically relevant TBFVs.

Chikungunya virus (CHIKV) is a mosquito-transmitted α-virus that causes in humans an acute infection characterized by polyarthralgia, fever, myalgia, and headache. Since 2005 this virus has been responsible for an epidemic outbreak of unprecedented magnitude. By analogy with other α-viruses, it is thought that cellular proteases can process the viral precursor protein E3E2 to produce the receptor binding E2 protein that associates as a heterodimer with E1. Destabilization of the heterodimer by exposure to low pH allows viral fusion and infection. We demonstrated that membrane-bound Furin but also PC5B can process E3E2 from African CHIKV strains at the H**R**Q**RR**$_{642}$↓ST site, whereas a CHIKV strain of Asian origin is cleaved at R**R**Q**RR**$_{642}$↓SI (**Table 2**) by membranous and soluble Furin, PC5A, PC5B, and PACE4 but not by PC7 or SKI-1/S1P [34]. This cleavage was also observed in CHIKV-infected cells and could be blocked by Furin inhibitor decanoyl-RVKR-chloromethyl ketone. This inhibitor was compared with chloroquine for its ability to inhibit CHIKV spreading in myoblast cell cultures, a cell-type previously described as a natural target of this virus [34]. We observed an additive effect of dec-RVKR-cmk and chloroquine, supporting the concept that these two drugs act by essentially distinct mechanisms. The combinatory action of chloroquine and dec-RVKR-cmk led to almost total suppression of viral spread and yield [34].

Furin cleavage of viral glycoproteins of the *Herpesviridae* family have been reported for Herpes simplex virus 1 and 2 and Varicella Zoster (**Table 1**), contributing to the pathogenesis of the later in vivo [35][36]. Finally, while Furin cleaves many glycoproteins, its activity is required for other viral proteins such as for Hepatitis B virus (HBV) core antigen. For reviews see [29][21].

**Table 1.** Families of pathogenic viruses that depend on the basic aa-specific proprotein convertases for host cell entry. RT = reverse transcriptase. Modified from [29].

| Family | Virus | Capsid | Genome |
|---|---|---|---|
| ***Retroviridae*** | **HIV, Leukemia viruses** | **Enveloped** | **Linear ssRNA(−), RT** |
| ***Flaviridae*** | **HCV, Dengue, Zika, West Nile** | **Enveloped** | **Linear ssRNA(+)** |
| ***Togaviridae*** | **Chikungunya** | **Enveloped** | **Linear ssRNA(+)** |
| ***Coronaviridae*** | **SARS-CoV-1,2, MERS** | **Enveloped** | **Linear ssRNA(+)** |
| ***Filoviridae*** | **Ebola, Marburg** | **Enveloped** | **Linear ssRNA(−)** |
| ***Orthomyxoviridae*** | **Avian Influenza H5N1** | **Enveloped** | **Linear ssRNA(−)** |
| ***Paramixoviridae*** | **Measle, RSV, Nipah, MPV** | **Enveloped** | **Linear ssRNA(−)** |
| ***Hepadnaviridae*** | **Hepatitis B** | **Enveloped** | **Linear ssDNA (−), RT** |
| ***Herpesviridae*** | **Herpes, CMV, Varicella-Zoster** | **Enveloped** | **Linear dsDNA** |
| ***Papillomaviridae*** | **HPV** | **Naked** | **Circular dsDNA** |

HIV-1 encodes four accessory gene products—Vpr, Vif, Vpu, and Nef—which are thought to collectively manipulate host cell biology to promote viral replication, persistence, and immune escape [37]. Increasing evidence suggests that extracellular Vpr could contribute to HIV pathogenesis through its effect on bystander cells. Soluble forms of Vpr have been detected in the sera and cerebrospinal fluids of HIV-1-infected patients, and in vitro studies have implicated

extracellular Vpr as an effector of cellular responses, including G2 arrest, apoptosis, and induction of cytokines and chemokines production, presumably through its ability to transduce into multiple cell types. Thus, Vpr is a true virulence factor and is a potential and promising target in different strategies aiming to fight infected cells including latently HIV-infected cells [38]. While Furin is clearly implicated in the processing activation of multiple viral surface glycoproteins, including gp160 of HIV-1, other cell-surface associated convertases such as PC5A and PACE4 [39][40] seem to negatively regulate the activity of the HIV-1 by cleavage-inactivation of secreted Vpr at **R**Q**RR**$_{88}$↓ [41]. PC-mediated processing of extracellular Vpr results in a truncated Vpr product that was defective for the induction of cell cycle arrest and apoptosis when expressed in human cells [41]. Thus, inhibitors of PC5/PACE4 may enhance HIV-1 virulence, and Furin-specific inhibitors would restrict HIV-1 infectivity, without affecting Vpr inactivation by PC5A/PACE4.

### 2.2. PCSK9 and Viral Infections

The secreted protein PCSK9 is the 9th member of the PCSKs family that autocatalytically cleaves its prodomain in the ER allowing its exit from this compartment and its secretion from cells [42], as a protease-inactive [prodomain-PCSK9] complex [43][44]. Its high expression in the liver and the presence of its gene on chromosome 1p32 [42], led to the genetic association of gain-of-function (GOF) variants of *PCSK9* with an autosomal dominant form of hypercholesterolemia [45]. This strongly suggested that PCSK9 may play a critical role in low density lipoprotein- cholesterol (LDLc) regulation. Indeed, while GOF variants were associated with high levels LDLc [45], the reverse was true for loss-of-function (LOF) variants [46]. Mechanistically, circulating PCSK9 was shown to reduce the levels of liver LDL-receptor (LDLR) protein by sorting the PCSK9-LDLR complex to lysosomes for degradation, implicating an interaction of the PCSK9 catalytic domain with the EGF-A domain of the LDLR [43][47][48].

In a first study, we showed that PCSK9 can reduce HCV infection of HuH7 cells using the cell culture-derived HCV clone JFH1 (genotype 2a). We demonstrated that the effect was due to the PCSK9-induced decrease of the LDLR protein levels on the surface of HuH7 cells. Also, HCV upregulates the expression of LDLR in vitro and in the chronically infected liver [49], likely due to an upregulation of the LDLR promotor activity through SREBPs and a decreased expression of PCSK9 [50]. Befittingly, Alirocumab, a therapeutic human monoclonal antibody (mAb) to PCSK9 used to treat hypercholesterolemia through an increase of cell surface LDLR failed to reduce HCV entry and infectivity in hepatocyte in vitro [51].

Additionally, the role of PCSK9 during natural HCV infection is not fully understood. We showed that HCV genotype 3 (G3) infected patients with high viral load have low levels of PCSK9 and LDLc compared to HCV genotype 1 (G1) infected patients, suggesting that the particular interplay between HCV and lipid metabolism and PCSK9 might be genotype specific [52]. In contrast, in a retrospective study, PCSK9 levels were found to be elevated especially in chronic genotype 2 (G2) HCV patients with or without hepatocellular carcinoma (HCC), but the increased PCSK9 levels were not significantly associated with changes in LDLc [53]. Overall, the data reported illustrate the complexity of the relation between HCV and lipid metabolism and more work would be required to better understand the role of PCSK9 in HCV infection.

Dengue virus (DENV), a single positive-stranded RNA virus of the family Flaviviridae, is transmitted to humans by the urban-adapted *Aedes* mosquitoes. It is estimated that 400 M+ individuals are infected with 1 of the 4 types of DENV [54]. The DENV surface glycoprotein prM-E complex needs cleavage by Furin(-like) enzymes to remove the prodomain of the envelope glycoprotein prM and allow the formation of the activated M-E complex and acquisition of infectivity. However, unlike cleavage of the prM of other flaviviruses, cleavage of DENV prM is incomplete in many cell lines, likely due to the negative influence of the aspartic acid (D) at the P3 position of the processing site HR**R**D**KR**↓S**V** at the pr-M junction (**Table 2**) [55]. Therefore, it is not surprising that inhibitors of Furin(-like) enzymes, while significantly reducing viral entry, did not completely block viral infections [56] (*unpublished results*).

**Table 2.** Various enveloped viruses and the sequences of surrounding their surface glycoprotein cleavage sites (designated by an arrow) by Furin(-like) proprotein convertases. The bold and underlined residues at positions P8, P6, P4, P2, P1 and P2′ emphasize the importance of these amino acids for protease recognition.

| Virus | Glycoprotein | P8 | | P6 | | P4 | | P2 | ↓ | | P2′ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **HIV** | **gp160** | | | **V** | **Q** | **R** | **E** | **K** | **R** | **A** | **V** |
| **H7N1 A/FPV/Rostock/34** | **HA** | | | **K** | **K** | **R** | **E** | **K** | **R** | **G** | **L** |
| **Avian H5N8 TKY/IRE** | **HA** | | | **R** | **K** | **R** | **K** | **K** | **R** | **G** | **L** |
| **Avian H5N1 A/HK/97** | **HA** | **R** | **E** | **R** | **R** | **R** | **K** | **K** | **R** | **G** | **L** |
| **Avian H5N1 TKY/ENG** | **HA** | **N** | **T** | **P** | **Q** | **R** | **K** | **K** | **R** | **G** | **L** |

| Virus | Glycoprotein | P8 | P6 | | P4 | | P2 | ↓ | | P2′ |
|---|---|---|---|---|---|---|---|---|---|---|
| Human CMV | gB | | H | N | R | T | K | R | S | T |
| Human MPV | F Protein | | N | P | R | Q | S | R | F | V |
| Human RSV | F Protein | | K | K | R | K | R | R | F | L |
| Dengue Virus (DENG2) | PrM | | H | R | R | E | K | R | S | V |
| Ebola Virus | gp160 | | G | R | R | T | R | R | E | A |
| Chikungunya (CHIKV) | E3E2 | | P | R | R | Q | R | R | S | I |
| Zika Virus | PrM | | A | R | R | S | R | R | A | V |
| SARS-CoV-2 | S | | S | P | R | R | A | R | S | V |

Very recently, we showed that DENV infection reduces the antiviral response of the host hepatocytes. Thus, DENV infection induces expression of PCSK9, thereby reducing cell surface levels of LDLR and LDLc uptake resulting in enhanced *de novo* cholesterol synthesis and its enrichment in the ER. In turn, high levels of ER cholesterol suppressed the phosphorylation and activation of the ER-resident stimulator of interferon (IFN) gene (STING), leading to reduction of type I interferon (IFN) signaling through antiviral IFN-stimulated genes (ISGs) [57]. This was supported by the detection of elevated plasma PCSK9 levels in patients infected with DENV resulting in high viremia and increased severity of plasma leakage. This unexpected role of PCSK9 in dengue pathogenesis, led us to test the effect of inhibition of PCSK9 function by the mAb Alirocumab. Befittingly, this treatment resulted in higher LDLR levels and lower viremia. Our data suggested that PCSK9 inhibitors could be a suitable host-directed treatment for patients with dengue [57], possibly in combination with Furin(-like) inhibitors.

## 3. Discussion

From the above considerations, it became clear that most enveloped viruses require the processing of their surface glycoproteins for productive infectivity. Enhanced pathogenicity has been associated with the apparition of a new cleavage site in certain viruses. While clearly favoring Furin cleavage [58][59], the exact role of the acquisition of a Furin site in SARS-CoV-2 [60] and its role in the spread of the virus and/or its pathogenicity have not been adequately established. Experimental evidence is not easy to obtain. One would need to generate a virus mutant to be tested in an animal model exhibiting the multiple pathogeneses that mimic the human clinical manifestation of COVID-19. A clear advantage of SARS-CoV-2 over SARS-CoV-1 has been its ability to spread asymptomatically and with great efficiency in a large portion of the population. One can speculate that the acquisition of a Furin cleavage site has contributed to confer to SARS-CoV-2 such an advantage and to enhance the tropism of this virus, as Furin is widely expressed in most tissues, whereas TMPRSS2 implicated in SARS-CoV-1 activation [61] is more limited in its tissue expression.

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