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Chapter 501

Rubella Virus Non-Structural Protease

Databanks

MEROPS name: rubella virus peptidase
MEROPS classification: clan unassigned, family C27, peptidase C27.001
Species distribution: known only from Rubella virus
Reference sequence from: Rubella virus (UniProt: Q99IE5)

Name and History

Rubella virus, a positive-strand RNA virus belonging to the Rubivirus genus of the Togaviridae family (reviewed in [1]), contains two open reading frames within its genome of roughly 10 000 nucleotides: a 5′-proximal ORF encoding non-structural proteins involved in RNA replication (NSP-ORF) and a 3′-proximal ORF encoding the virion structural proteins, a capsid protein and two envelope glycoproteins (SP-ORF) (Figure 501.1A). Whilst the proteins encoded by the SP-ORF are cleaved co-translationally by cellular signal peptidase (Chapter 778), the product of the NSP-ORF is processed by an embedded papain-family cysteine protease. The name rubella virus non-structural protease is applied to this enzyme as it is a virally encoded endopeptidase functioning in the processing of the non-structural proteins of the virus.

Activity and Specificity

The product of the rubella virus NSP-ORF is 2116 amino acids in length (roughly 240 kDa but termed P200) (Figure 501.1A). The protease domain resides roughly between residues 1000 and 1301 of the NSP-ORF. The catalytic dyad consists of Cys1152 and His1273. The protease catalyzes a single cleavage between Gly1301 and Gly1302 (28 residues from the catalytic His) producing two products: an N-terminal 150 kDa protein (termed P150) and a C-terminal 90 kDa protein (P90); the protease is thus the C-terminal domain of P150. The protease cleaves both in *cis* and in *trans* [2,3]. Whilst no other cleavages within P200 have been detected, it is unknown if the protease cleaves any cellular proteins.

Requirements for Divalent Cations and Calmodulin

The rubella virus non-structural protease contains binding sites for both Zn^{2+} and Ca^{2+} ions. Zn binding is coordinated by four Cys residues within a C-X-X-C-(X48)-C-X-C linear context [4] (Figure 501.1A,B). Ca binding is coordinated by a 12 amino acid linear ‘EF hand’ motif [5] (Figure 501.1A,B). Binding of both ions plays an essential structural role in protease function. Zn binding serves to shield hydrophobic residues from the solvent while Ca binding enhances the thermal stability. In fact, a mutation which abrogates Ca binding renders the protease temperature sensitive at 39 *versus* 35°C. Recently, it was found that the rubella virus non-structural protease binds Ca-charged calmodulin [6] (Figure 501.1A,B). Binding is to an alpha helix adjacent to the Cys1152 catalytic residue and serves to stabilize the helix.

Biological Aspects

In the replication cycle of a positive-strand RNA virus, the genomic positive stand is first copied into a negative strand genomic equivalent that serves as the template for the synthesis of more positive strands. Positive strand RNAs are needed in stoichiometric amounts to serve as mRNAs and to be packaged into progeny virus particles in contrast to negative strands that only serve as re-usable templates. Therefore, positive-strand RNA viruses exhibit asymmetrical production of these species with positive-strands predominating over negative-strands. In the case of rubella virus, asymmetrical production appears to be mediated by the non-structural protease in the following fashion [7]: Whereas the P200 precursor catalyzes synthesis of the negative strand following its translation from the genomic RNA, the P150/P90 complex that is produced subsequent to cleavage only synthesizes positive strands. Thus, the proteolysis both alters the catalytic activity of the replicase complex, and removes the negative-strand synthase ensuring asymmetric production of positive strands. This scheme was first worked out with the alphaviruses, the other Togavirus genus [8,9].

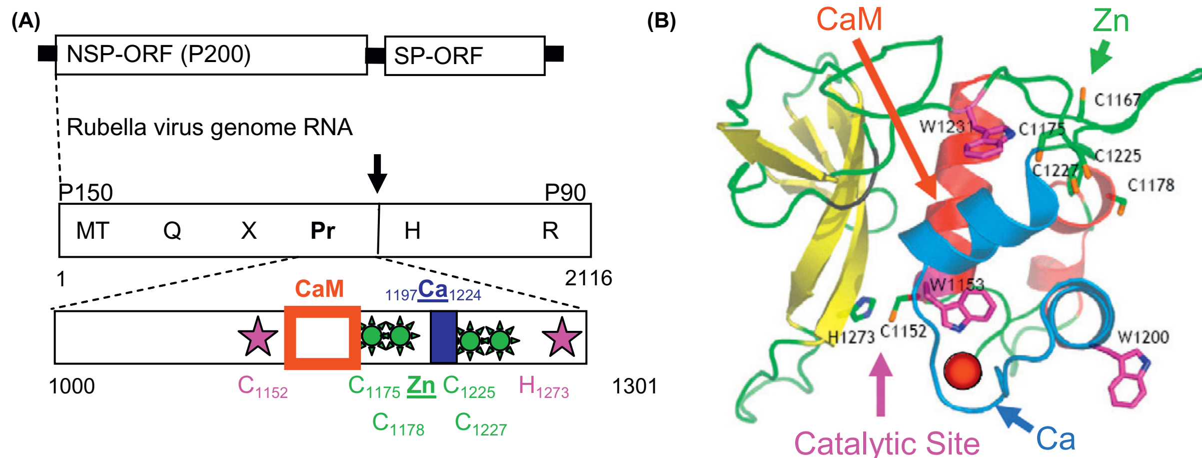

FIGURE 501.1 (A) Contextual diagram of the rubella virus non-structural protease. At the top is a representation of the rubella virus genomic RNA with open reading frames as boxes and untranslated regions as lines. Below this is a blow-up of the P200 translational product of the NSP-ORF with the boundaries of the two ultimate products, P150 and P90, and functional domains within these products indicated (MT = methyl transferase, Q = domain with undefined function, X = poly ADP ribose binding domain, Pr = non-structural protease, H = helicase, R = RNA-dependent-RNA polymerase). The cleavage site within P200 of the non-structural protease (between residues 1301 and 1302) is indicated by an arrow. Below the P200 diagram is a blow-up of the protease domain, indicating catalytic residues, Zn binding residues, the Ca-binding EF hand domain, and the calmodulin-binding domain; (B) Model of the rubella virus non-structural protease catalytic site with associated metal and calmodulin binding domains. Panel (B) reprinted from Zhou *et al.* [5], Copyright © 2007, American Society for Microbiology.

The rubella virus non-structural protease is related to a number of other viral papain-like proteases, including proteases expressed by the alphaviruses, the arteriviruses, the coronaviruses and foot-and-mouth disease virus of animals, and the potyviruses and hypovirulence-associated viruses of plants and fungi (reviewed in Chen *et al.* [10]). The existence of these proteases in these diverse viruses (which belong to different families as well as infecting animals and plants) and their relatedness to cellular papain-like proteases indicates both that these viral proteases were 'captured' from cells and that this capture event occurred several times independently during evolution of these viruses. Considering the apparent independent acquisition of these proteases, it is not surprising that the function of the proteolytic cleavage mediated by these proteases differs among these diverse viruses [11].

Further Reading

For a review, see Ten Dam *et al.* [12].

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