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Review

Biology of rhomboid proteases in infectious diseases

Sunil Kumar Dogga*, Dominique Soldati-Favre

Department of Microbiology and Molecular Medicine, University of Geneva, CMU, 1 Rue Michel-Servet, CH-1211 Geneva 4, Switzerland

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ABSTRACT

Rhomboids are a well-conserved class of intramembrane serine proteases found in all kingdoms of life, sharing a conserved core structure of at least six transmembrane (TM) domains that contain the catalytic serine-histidine dyad. The rhomboid proteases, which cleave membrane embedded substrates within their TM domains, are emerging as an important group of enzymes controlling a myriad of biological processes. These enzymes are found in a wide variety of pathogens manifesting important roles in their pathological processes. Accordingly, they have received considerable attention as potential targets for pharmacological intervention over the past few years. This review provides a general update on rhomboid proteases and their roles in pathogenesis of human infectious agents.

Contents

1. Introduction

Intramembrane proteases (IMPs) cleave proteins within the plane of a membrane and their substrates usually contain a single pass transmembrane domain harboring a scissile peptide bond, which is cleaved in the TM or juxtamembrane region.

Rhomboid proteases are a well-conserved class of polytopic integral membrane serine proteases found in all kingdoms of life. They are the most widespread and well characterized IMPs, impacting on a large variety of key processes in different taxa ranging from cell–cell signaling and regulation of apoptosis in metazoans [1–3], quorum sensing in bacteria [4], mitochondrial membrane fusion in yeast [5] and as invasion factors in apicomplexans [6]. Rhomboid proteases activate membrane-bound substrates by cleaving within their transmembrane (TM) domains and releasing soluble domains from the membrane anchor, which, in turn, leads to downstream events that influence diverse biological processes.

Rhomboid proteases share a conserved core structure of six TM domains that contains the catalytic serine-histidine diad, with highly variable amino termini and additional TM domains at either

* Corresponding author.
E-mail address: Sunil.Dogga@unige.ch (S.K. Dogga).

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N- or C-termini of the core six [7–11]. A typical rhomboid protease in prokaryotes has a six membrane spanning topology with an N-terminal cytoplasmic domain. Some rhomboid proteases have an additional TM helix at the C-terminus, while the mitochondrial PARL type rhomboid has an extra TM helix at the N-terminus. A subgroup of rhomboid-like proteins, called inactive rhomboids (iRhoms) lack the essential catalytic residues and are devoid of enzymatic activity [11].

The activity of rhomboid proteases on substrates needs to be regulated for a proper physiological role, which can be achieved at the transcriptional level, where the protease is expressed as and when needed. It can also be controlled by physically segregating the substrate and protease until they are needed to interact, as manifested in *Drosophila* Rho1 and apicomplexan rhomboid proteases. The *Drosophila* Rho1 substrate, Spitz, is chaperoned by Star to the Golgi apparatus, where it encounters rhomboid and gets cleaved. Members of Apicomplexa including *Toxoplasma gondii* and *Plasmodium* spp., sequester their adhesins in apical secretory organelles called micronemes. During invasion, these are released onto the parasite plasma membrane (PM), where they encounter rhomboid proteases and get cleaved.

Besides these, the soluble domains of rhomboid proteases are believed to exert a more direct regulatory role, providing rapid responses to tight cell signaling. For example, in a study analyzing rhomboid proteases containing a calcium-binding EF-hand domains appended to their cytosolic N-termini, it was shown that calcium significantly stimulates proteolytic activity of Rhomboid-4 in *Drosophila* cells [12]. Deletion of the EF-hand leads to a dysregulation of rhomboid activity and consequently to premature activation of proteolysis.

2. Rhomboid proteases and pathogenesis

Ever since their discovery, rhomboid proteases have been identified in other species, playing key roles in many human pathogens. This necessitates a deeper understanding of their mode of activity and potential substrates, in the quest for novel therapeutic interventions.

2.1. *AarA in* Providencia stuartii

Providencia stuartii is a gram-negative bacterium belonging to the Enterobacteriaceae family and is responsible for urinary tract infections in humans. A common cause of nosocomial infections leading to severities such as fatal bacteremia, diarrhea and peritonitis, the strain is resistant to many disinfectants and antimicrobial drugs.

The rhomboid protease AarA of *P. stuartii* has a 6 + 1 TM topology, with the active site close to the periplasmic side of the membrane. The aarA gene was first identified during a search for a regulatory loci controlling aac(2′)-la expression, a chromosomal 2-*N*-acetyltransferase important for peptidoglycan and aminoglycoside acetylation. The expression of aac(2′)-la is known to be regulated by quorum sensing through an unknown extracellular factor (named acetyltransferase-repressing factor or AR-factor). The deduced AarA protein was found to be highly hydrophobic, with several TM domains and displaying no significant homology to proteins in the databases [13]. In a screen to identify *Proteus mirabilis* genes that can complement the loss of AarA in *P. stuartii*, *tatA* was found to be a multicopy suppressor that restored extracellular signal production as well as complementing all other phenotypes of the AarA mutant [4]. These breakthroughs led to uncovering the close interaction of AarA with the Tat system in playing an important role for quorum sensing in *P. stuartii*.

The twin arginine translocase (Tat) system transports folded proteins across the prokaryotic cytoplasmic membrane and its machinery consists of three essential membrane proteins – TatA, TatB, and TatC. TatA from *P. stuartii* is homologous to *E. coli* TatA, and has a single TM helix, followed by an amphipathic helix, unstructured C-terminal tat and a periplasmic N-terminus [14–16]. *P. stuartii* TatA is synthesized as an inactive pre-protein with an N-terminal extension of eight amino acids. This short N-terminal extension is atypical of TatA proteins and is proteolytically removed by AarA both *in vivo* and *in vitro*, which is required to activate *P. stuartii* TatA. The unprocessed form of the protein is shown to be defective in the formation of large homooligomeric TatA complexes and in interacting with TatC for complex formation [17], leading to a defective Tat export system and failure in producing an extracellular quorum-sensing signal (Fig. 1). The AarA mutant shows Tat-dependent phenotypes previously observed in *E. coli* tat mutant, such as cell chaining, detergent sensitivity, and inability to grow anaerobically on glycerol TMAO plates. However, the exact mechanism by which AarA regulates signal production is still unknown and identification of the signaling molecule should help in expanding the picture of this rhomboid's role in *P. stuartii*.

2.2. *Rhomboid proteases in mycobacteria*

Tuberculosis is one of the major human health concerns in the world with *Mycobacterium tuberculosis* causing mortality in the range of millions every year. Despite the availability of a live attenuated vaccine and a battery of antibiotics, the difficult treatment regimen, inferior quality drugs, and emergence of multidrug resistance pose major threats. Factors such as hiding intracellularly, preventing phagosome maturation, slow growth, dormancy, and complex cell envelope are hurdles in the fights against this pathogen. It is vital to understand the interplays between *M. tuberculosis* and its human host to understand how these bacteria circumvent host defense mechanisms and emerge as successful pathogens.

Most mycobacterial species possess two phylogenetically distinct active rhomboid proteases containing the putative catalytic signatures with the residues Ser and His. The majority of the mycobacterial rhomboid proteases exhibit a phenylalanine in the active catalytic site instead of the homologous tyrosine residue known to stabilize the active site of GlpG and many other rhomboid proteases [18]. *M. tuberculosis* Rv0110 (rhomboid protease 1) and the other mycobacterial orthologs cluster with the eukaryotic rhomboid proteases, sharing a 6 + 1 TM domain topology [18]. In contrast Rv1337 (rhomboid protease 2) is a prototypical prokaryotic rhomboid with 6 TM domains like AarA from *P. stuartii*. Rv0110 orthologs have seven transmembrane helices (TMHs) with the catalytic GxSx & H residues localized in TMH4 and TMH6. The orthologs of Rv1337 contain either six (pathogenic) or five TMHs (non-pathogenic), with the GxSx and H residues localized, either in TMH4-TMH6 or in TMH3-TMH5 respectively (Fig. 2). Many Rv0110 mycobacterial orthologs possess extra eukaryotic protein features and have topologies similar to that of *D. melanogaster* Rho1. In contrast, the Rv1337 orthologs maintain a fairly constant number and type of motifs, either fungal cellulose binding domain or bacterial putative redox-active protein domains [18].

The mycobacterium rhomboid proteases were characterized by functional complementation of the null-rhomboid mutant of *P. stuartii* and through gene deletion experiments in the model organism *Mycobacterium smegmatis* [19]. Expectedly, the genes encoding orthologs of Rv1337 fully restore rhomboid activity in *P. stuartii* lacking AarA but not the Rv0110 orthologs. Moreover, individual deletion of the mycobacterial orthologs in *M. smegmatis* (ΔDMSME_4904 or ΔDMSME_5036) impairs biofilm formation, even more so in the ΔMSMEG_4904 mutant (orthologous