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Review

The roles of intramembrane proteases in protozoan parasites ☆

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ABSTRACT

Intramembrane proteolysis is widely conserved throughout different forms of life, with three major types of proteases being known for their ability to cleave peptide bonds directly within the transmembrane domains of their substrates. Although intramembrane proteases have been extensively studied in humans and model organisms, they have only more recently been investigated in protozoan parasites, where they turn out to play important and sometimes unexpected roles. Signal peptide peptidases are involved in endoplasmic reticulum (ER) quality control and signal peptide degradation from exported proteins. Recent studies suggest that repurposing inhibitors developed for blocking presenilins may be useful for inhibiting the growth of *Plasmodium*, and possibly other protozoan parasites, by blocking signal peptide peptidases. Rhomboid proteases, originally described in the fly, are also widespread in parasites, and are especially expanded in apicomplexans. Their study in parasites has revealed novel roles that expand our understanding of how these proteases function. Within this diverse group of parasites, rhomboid proteases contribute to processing of adhesins involved in attachment, invasion, intracellular replication, phagocytosis, and immune evasion, placing them at the vertex of host–parasite interactions. This article is part of a Special Issue entitled: Intramembrane Proteases.

Contents

1. Introduction to protozoan parasites

Protozoan parasites are extremely diverse and are scattered across many of the major groups of eukaryotic lineages, while by contrast yeast and humans belong to the same major branch (*i.e.* opistokonts) [1]. The diversity of parasites likely reflects the fact that parasitism has arisen multiple times evolving independently in groups that were already phylogenetically divergent. Among the major animal and human parasites, the phylum Apicomplexa contains *Plasmodium* spp., the cause of malaria, *Cryptosporidium parvum*, a common cause of diarrheal disease, and *Toxoplasma gondii*, an opportunistic pathogen. This phylum also contains important animal pathogens such as *Theileria* spp., which causes disease in cattle, and *Babesia* spp., parasites of animals that can also cause zoonotic disease in humans. Only distantly related to the apicomplexans, members of the kinetoplastidae also cause important animal and human diseases due to infections by *Trypanosoma brucei* in

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Africa and *T. cruzi* in the New World. Members of the genus *Leishmania*, of which there are multiple species that cause important diseases in humans, are also members of the kinetoplastidae. Other important human pathogens include *Entamoeba histolytica*, a member of the amoeba group, and early branching eukaryotes such as *Trichomonas vaginalis* and *Giardia* spp.

Protozoan parasites contain a wide variety of serine, threonine, cysteine, aspartic, and metalloproteases and many of these have been implicated in important aspects of their biology including development, immune evasion, nutritional acquisition, and maturation of proteins involved in invasion and egress [2–4]. However, most of these proteases do not cleave their substrates within the membrane, and therefore will not be considered further here. Although all three families of intramembrane proteases exist in protozoan parasites, only two have been investigated experimentally: signal-peptide peptidases and rhomboid proteases [5,6]. Site-2 proteases exist in the genomes of protozoan parasites (http://eupathdb.org/eupathdb/), yet they have not been studied extensively and so will not be considered here. Signal peptide proteases exist in all protozoan parasites, but have only been examined in *Plasmodium*. Rhomboid proteases have been studied most extensively in the apicomplexans, and on a more limited basis in Entamoeba, and as such this review will focus more closely on these later examples.

2. Intramembrane proteolysis

Although only recently recognized, intramembrane proteolysis turns out to be a conserved process with an ancient ancestry that stretches across bacterial, archeal, and eukaryotic lineages [7,8]. There are three major types of intramembrane proteases: site-2 metalloproteases, aspartyl proteases consisting of the related signal-peptide peptidase and presenilin families, and rhomboid proteases that have a serine at their active site [9]. Their general functions are briefly reviewed here prior to considering what is known about them in protozoan parasites in the sections below.

Proteins destined for export in eukaryotic cells typically contain a hydrophobic signal peptide at their N-terminus that directs the protein for insertion into the lumen of the ER *via* the Sec61 complex [10]. Signal-peptide peptidases (SPP) cleave the signal peptide that remains in the ER membrane following protein export [11]. The action of SPP also generates short peptides for recognition of self *via* MHC class I HLA-E molecules, while cleavage of some substrates by SPP-like proteases can generate signals for activating transcription [11]. SPP also functions in ER quality control of MHC class I molecules in CMV infected cells [12]. SPP share common mechanistic features to presenilins such as γ-secretase, which functions in Notch signaling and in generation of amyloid β-peptide, and the bacterial prepilin IV proteases. These two classes of proteases are defined by an active site containing two conserved aspartate residues that occur within motifs consisting of the residues YD and GXGD, a feature unique to this family of aspartic proteases [11].

Site-2 proteases (S2P) are zinc metalloproteases that cleave within the TMD of their substrate after an initial cleavage, typically by a membrane-tethered site-1 protease that cleaves outside the TMD [6,9]. S2P contain a conserved HExxH motif characteristic of metalloproteases and use a H-H-D motif that coordinates a zinc ion within the active site [13]. S2P are multi-membrane spanning proteases that typically reside in the ER, or other endomembranes. S2P cleave their substrates near the inner leaflet of the membrane, releasing transcription factors that migrate to the nucleus to activate gene expression [6,9]. For example, in eukaryotes, in response to low cholesterol, sterol regulatory element binding protein (SREBP) is processed by the sequential action of site-1 protease and S2P to release a transcription factor that up-regulates sterol biosynthesis [14]. Similarly, the release of ATF transcription factors from the ER in response to the unfolded protein response requires the action of S2P [15]. In prokaryotes, S2P control a variety of responses including stress responses, lipid metabolism, toxin production, and sporulation [16].

Rhomboid proteases were originally identified in *Drosophila* based on a genetic screen for mutants that disrupted development [8]. Rhomboid 1 was shown to cleave Spitz, an EGF-like factor, within its transmembrane domain (TMD) releasing this growth promoting hormone *via* the secretory pathway to control development in neighboring cells [17]. Drosophila rhomboid 1 has 7 TMD and contains a catalytic triad that was originally proposed to contain histidine, aspartate, and an active site serine, based on mutational and inhibitor studies [17]. Rhomboid proteases are unique among intramembrane proteases in not requiring preprocessing of the substrate prior to cleaving within the TMD [8]. Rhomboid proteases are characterized by a conserved domain structure consisting of 6 TMD in most prokaryotes, 6 + 1 TMD in eukaryotes, and 1 + 6 TMD that are found in mitochondrial rhomboid proteases, as well as key catalytic residues including a conserved GxSx active site [7,8]. Although not highly conserved at the amino acid level, rhomboid proteases are phylogenetically very widespread [18]. In addition to catalytically active rhomboid proteases, many organisms contain rhomboid-like genes encoding proteins that lack key catalytic residues (so called inactive rhomboids or iRHOMs); these pseudoenzymes typically contain a Pro residue upstream of the catalytic Ser and therefore are inactive as proteases [19]. Although originally functional orphans, recent studies suggest that while iRHOMs lack enzymatic activity, they may still be biologically active in influencing the trafficking of single TMD proteins in the secretory pathway, thereby altering signaling [20].

Since their initial discovery, more precise catalytic mechanisms have been worked out based on *in vitro* cleavage assays [21–23] and structural studies on bacterial rhomboid proteases [24–27]. These studies confirmed that the active site serine is found within the membrane where it is located at the top of a short TM helix that places the serine at the base of a cavity that is open to the aqueous environment. They also revealed that the catalytic site involves a dyad of histidine that acts as a base to remove a proton from serine, which then serves as a nucleophile to attack the peptide bond. Several models have been proposed for the insertion of the substrate TMD into this pocket, where upon the helix breaking propensity of typical substrates is important in allowing access to the peptide bond [7]. Various features of rhomboid substrates have been identified, and although there are no universal rules, there are several general features. One prominent feature is the presence of small helix breaking residues in the TM segment adjacent to the site of cleavage [28]. Rhomboid proteases are fairly permissive in cleaving substrates from widely divergent sources, likely reflecting the general features of the cleavage site rather than specific residues. However, other studies have stressed the conservation of small hydrophobic residues at the cleavage site, as well as bulkier hydrophobic flanking residues, as an important sequence determinant for many, but not all rhomboid proteases [29]. These features combine to generate a meta-stable helix that is stabilized in the lipid bilayer, but which can easily be destabilized in other environments, rendering them susceptible to cleavage within the active site of rhomboid proteases [30]. Given these fairly general rules for substrate preference, rhomboid proteases are fairly permissive such that heterologous assays have been very helpful for defining their function, yet identification of native substrates that control important aspects of the biology within their respective systems remains challenging.

3. Signal-peptide peptidase in *Plasmodium*

SPP normally resides in the ER, yet surprisingly, in *P. falciparum* this protease was initially reported to be a secretory protein and to interact with Band 3 in the red blood cell, presumably after secretion to the parasite surface [31]. Antibodies raised against PfSPP blocked the invasion of red cells by *P. falciparum*, and purified PfSPP bound directly to a peptide from the 5ABC loop in Band 3 [31]. Collectively, these studies suggested that export of PfSPP to the merozoite cell surface may aid in cell binding *via* recognition of Band 3 and that this protease may act to process host