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Review

The mitochondrial rhomboid protease: Its rise from obscurity to the pinnacle disease-relevant genes☆

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ABSTRACT

The Rhomboid proteases belong to a highly conserved family of proteins that are present in all branches of life. In *Drosophila*, the secretory pathway-localized rhomboid proteases are crucial for epidermal growth factor (EGF) signaling. The identification of a mitochondrial-localized rhomboid protease shed light on other functions of rhomboid proteases including the maintenance of mitochondrial morphology and the regulation of apoptosis. More recent work has revealed other functions of the mitochondrial rhomboid protease in mitochondrial and cellular biology, failure of which have been implicated in human diseases. In this review, we will summarize the current knowledge and disease relevance of the mitochondrial-localized rhomboid protease. This article is part of a Special Issue entitled: Intramembrane Proteases.

Contents

Abbreviations: TMD, transmembrane domain; IMS, intermembrane space; PD, Parkinson's disease; T2D, type 2 diabetes

☆ This article is part of a Special Issue entitled: Intramembrane Proteases.

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1. Introduction

Proteolysis within the membrane bilayer is conceptually challenging, but several groups of proteases have evolved elegant and sophisticated mechanisms to conduct such a feat. In this review, we will discuss in detail the discovery and ongoing characterization of mitochondrial-localized rhomboid proteases. This family has emerged as a critical mediator of mitochondrial biology and is also deeply rooted in human disease etiologies.

1.1. Intramembrane proteolysis

Intramembrane proteolysis is a key regulatory mechanism conserved throughout evolution [1–4]. To date, intramembrane proteases can be classified into three major classes: (i) the site-2 metalloproteases (S2P), (ii) the signal peptide peptidase (SPP) and presenilin (PS) aspartyl proteases and (iii) the Rhomboid family of serine proteases. One of the major challenges of intramembrane proteolysis is the requirement for water in the hydrophobic lipid bilayer to allow for the hydrolysis of a peptide bond. Until recently, it was inconceivable that proteolysis can occur within the membrane. Intense research in the field has elucidated the structure, function and regulatory mechanisms of these intramembrane proteases. The S2P, SPP and PS proteases are highlighted in other reviews of this special issue. We will focus our review on the Rhomboid family of serine proteases, and more specifically, the mitochondrial sub-family of Rhomboids.

1.2. Identification of the Rhomboid superfamily of enzymes

Rhomboids are the newest class of intramembrane proteases and are a relatively new superfamily of proteins. The first rhomboid was identified in a genetic screen performed in *Drosophila*, where fly embryos of the mutant had a mis-shapened rhombus-like head skeleton. This mutant phenotype led to the naming of the gene as "rhomboid" [5]. It was later discovered that Rhomboid was also required for the establishment of the dorsal–ventral axis during oogenesis. It was proposed that the spatial localization of Rhomboid was important in selectively activating the epidermal growth factor (EGF) [6]. The identification of six other rhomboids in *Drosophila* and the finding that Rhomboid-1 activates the EGF-like protein, Spitz, by cleaving it in its transmembrane domain (TMD) defined a new family of intramembrane proteases [7–9]. It is now known that rhomboid proteases are indispensible regulators of EGF signaling in *Drosophila*. The Golgi-localized rhomboid proteases, Rhomboid-1, -2, -3 and -4 can activate EGF signaling *in vivo* by cleaving the EGF-like proteins Spitz, Keren and Gurken [10]. However, Rhomboids are also present in organisms that lack EGF signaling, and hence, must have additional conserved functions [11].

1.3. Identification of the mitochondrial rhomboid protease

The identification of a mitochondrial-localized rhomboid protease and its substrates shed light on another function of Rhomboids that is distinct from EGF signaling. The mitochondrial rhomboid proteases regulate mitochondrial morphology and function [12–17]. First identified in yeast as a protein required for the cleavage and maturation of cytochrome c peroxidase, Ccp1, the role of the mitochondrial rhomboid proteases in mitochondrial biology was not highlighted until the identification of Mgm1 as the other substrate of the yeast mitochondrial rhomboid protease, Rbd1/Pcp1 [12–14,18]. The mitochondrial rhomboid proteases are now known to be crucial regulators of mitochondrial dynamics and function. Impaired function of the mammalian mitochondrial rhomboid protease is associated with impaired mitochondrial function and quality control that are proposed to contribute to type 2 diabetes and Parkinson's disease (discussed below).

1.4. Structure and catalysis of mitochondrial-localized rhomboid proteases

The specificity of rhomboid proteases for their substrates is better characterized for the non-mitochondrial rhomboid proteases than it is for the mitochondrial rhomboid proteases, and is discussed in great detail in other reviews of this special issue. A recent study examining substrate specificity of the mitochondrial rhomboid proteases indicates that the yeast and human mitochondrial rhomboid proteases are not selective in the sequence that they cleave. Although hydrophobicity is required, the sequence can be highly variable. Replacing the entire rhomboid cleavage region (RCR) of Mgm1 with two different hydrophobic sequences not physiologically cleaved by Rbd1 did not alter the efficiency of Rbd1-dependent cleavage of Mgm1. However, this cleavage was dependent on a 13 amino acid stretch of negatively charged residues C-terminal to the RCR. Mutating these residues resulted in impaired Mgm1 cleavage by both the yeast and mammalian mitochondrial rhomboid proteases, demonstrating possible conservation of substrate recognition [21].

Although the Rhomboid family was discovered to be proteases only a decade ago, intense research has enabled us to learn more about their localization and biological functions *in vivo*. It is now clear that the Rhomboid superfamily includes the secretory pathway-localized rhomboid proteases, the inactive rhomboids (iRhoms), the mitochondrial-localized rhomboid proteases and more recently, the Derlin proteins [11,22]. In this review, we will focus on the mitochondrial-localized rhomboid proteases, summarizing the current findings on their substrates, regulatory mechanisms and disease-relevance.

2. The mitochondrial rhomboid proteases

2.1. The yeast mitochondrial rhomboid protease, Rbd1

The existence of a mitochondrial-localized rhomboid protease was first discovered in yeast in 2002. Rbd1/Pcp1 was found to be required for the cleavage and maturation of cytochrome c peroxidase, Ccp1 [18]. Shortly after, in 2003, three independent studies identified another substrate of Rbd1, the dynamin-like GTPase, Mgm1 [12–14]. The defect in the proteolytic processing of Mgm1 and Ccp1 in the Δ*rbd1* strain could by rescued by a plasmid-borne copy of WT Rbd1 but not a catalytically inactive mutant. More importantly, this defect could also be rescued by human PARL (presenilins-associated rhomboid-like), the human homolog of Rbd1 that also localized to mitochondria, although its function was still unknown at the time. This was the first indication that the localization and function of the mitochondrial rhomboid proteases are conserved from yeast to mammals [12]. Despite these important findings in yeast, Ccp1 and Mgm1 remain the only known substrates of the yeast mitochondrial rhomboid protease (Table 1). Although Ccp1 was the first identified substrate of Rbd1, Mgm1 has since become its key substrate in yeast and most studies have focused on understanding the regulation of Mgm1 processing and its biological function in mitochondrial membrane dynamics.

Table 1
List of mitochondrial rhomboids and their known substrates.

Species	Rhomboid	Substrate	Function	Refs.
Saccharomyces cerevisiae	Rbd1/Pcp1	Ccp1		[12,18]
		Mgm1	Mitochondrial fusion	[12–14]
Drosophila melanogaster	Rhomboid-7	Opa1-like	Mitochondrial fusion, apoptosis	[16]
		Pink1	Mitophagy	[28]
		Omi	Apoptosis	[28]
Mammals	PARL	PINK1	Mitophagy	[43,53–55]
		OMI	Apoptosis	[96]
		PGAM5	Apoptosis	[17]