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Review

Plastid intramembrane proteolysis ☆

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ABSTRACT

Progress in the field of regulated intramembrane proteolysis (RIP) in recent years has not surpassed plant biology. Nevertheless, reports on RIP in plants, and especially in chloroplasts, are still scarce. Of the four different families of intramembrane proteases, only two have been linked to chloroplasts so far, rhomboids and site-2 proteases (S2Ps). The lack of chloroplast-located rhomboid proteases was associated with reduced fertility and aberrations in flower morphology, probably due to perturbations in jasmonic acid biosynthesis, which occurs in chloroplasts. Mutations in homologues of S2P resulted in chlorophyll deficiency and impaired chloroplast development, through a yet unknown mechanism. To date, the only known substrate of RIP in chloroplasts is a PHD transcription factor, located in the envelope. Upon proteolytic cleavage by an unknown protease, the soluble N-terminal domain of this protein is released from the membrane and relocates to the nucleus, where it activates the transcription of the ABA response gene ABI4. Continuing studies on these proteases and substrates, as well as identification of the genes responsible for different chloroplast mutant phenotypes, are expected to shed more light on the roles of intramembrane proteases in chloroplast biology.

1. Introduction

Hydrolysis of a peptide bond in a transmembrane α-helix, within the hydrophobic core of a membrane, has seemed anomalous. However, this intramembrane proteolysis has been recognized in the past two decades or so as a ubiquitous process occurring in all forms of life. Such cleavage events are involved in numerous biological processes and regulate many different functions. Four different families of proteases have been demonstrated to mediate intramembrane proteolysis: rhomboid proteases, site-2 proteases, signal peptide peptidases and presenilin/γ-secretases. Together with the demonstration of their role in signaling, membrane remodeling, protein quality control, cell adhesion and communication, their fundamental role in development and physiology of eukaryotes and prokaryotes alike have been consolidated (for reviews, see [1–3].

The progress made in the field of regulated intramembrane proteolysis (RIP) in recent years has radiated into plant biology as well [4]. Although the number of reports on RIP in plants is still sparse, interesting observations have started to accumulate. The ubiquitous nature of RIP and the enzymes responsible for this process has prompted plant biologists to look for homologs of these enzymes in plants, and to incorporate the concepts associated with RIP into their hypotheses. Independently of these, analysis of specific mutants culminated in the identification of mutations in specific intramembrane proteases as responsible for certain mutant phenotypes. In yet another line of research, potential substrates for intramembrane proteolysis in plants have been identified, although the responsible proteases are still unknown. In the following, the limited available information on these subjects that is relevant to chloroplast biology is summarized and evaluated.

2. Intramembrane proteases in plastids

2.1. Rhomboid proteases

Rhomboids are widely spread intramembrane serine proteases that are found in nearly all sequenced organisms. They are involved in different biological functions such as signaling, development, apoptosis, organelle integrity, parasite invasion and more (for recent reviews, see [5,6]). The well-studied GlpG rhomboid protease of *Escherichia coli*, which can be considered as a paradigm for this family, contains six transmembrane helices, incorporating the catalytic dyad of Ser-His. These helices form a conical cavity that is open to the aqueous phase, providing the hydrophilic environment required for hydrolysis of a peptide bond within a transmembrane helix of the substrate protein (Fig. 1, [5,6].

Of the 16 genes related to rhomboid proteases found in the Arabidopsis genome, one belongs to the PARL-type (At1g18600), and three (At1g74130, At1g77860, At5g38510) are expected to be inactive due to lack of conservation in and around the active serine [6,7]. It should be noted that contradicting nomenclature already exists in the literature. Thus, we will use here the one of Lemberg and Freeman [7], along with the other published names where relevant.

The occurrence of rhomboid homologs in plants was first mentioned in 2001 [8]. Koonin et al. [9] have then identified Arabidopsis sequences

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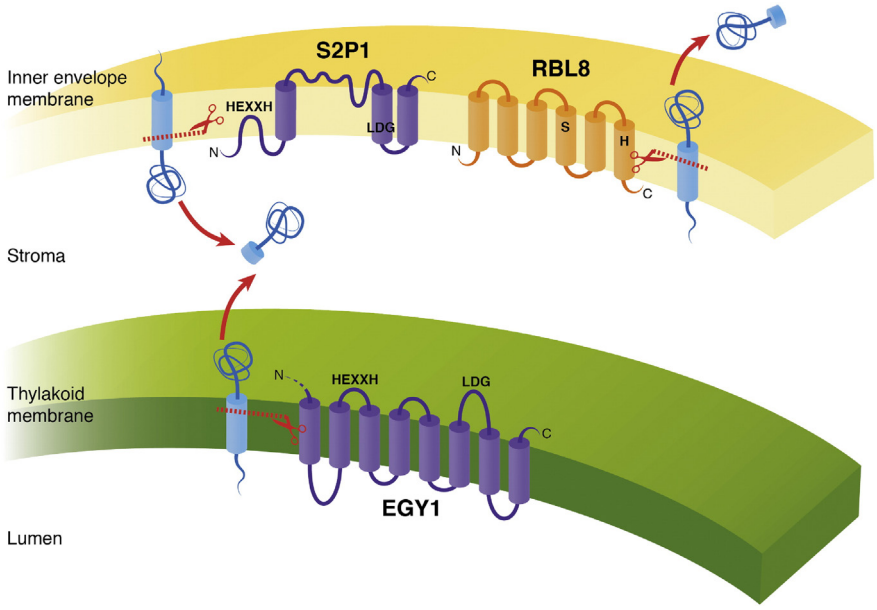

Fig. 1. Schematic model of three chloroplast intramembrane proteases. Two metalloproteases of the S2P family and one serine protease of the rhomboid family are colored in purple and orange, respectively. The HEXXH zinc-binding motif and the highly conserved LDG motif of S2Ps as well as the catalytic dyad of rhomboids (Ser and His) are indicated. As the structures of S2Ps are not yet determined, the location of transmembrane helices and the critical motifs are predicted based on hydropathy plots and the TMHMM (v. 2) program. Since the length of the N-terminus of mature EGY1 is not known, it is depicted as a broken line. Potential substrate proteins are colored in blue, and cleavage sites are indicated by red dotted lines. Predicted locations of the soluble products of proteolytic cleavages are also illustrated.

containing the conserved active serine and its surrounding residues (GA**S**GA), characteristic of rhomboid proteases. The first report on experimental work with plant rhomboids appeared shortly afterwards [10]. Based on their homology to the Drosophila Rho-1, eight sequences were identified in the Arabidopsis genome, although their overall sequence similarity was relatively low, less than 20%. Further characterization of two of these, AtRBL1 and AtRBL2 (for *Arabidopsis thaliana* rhomboid-like), revealed that their transcripts accumulated in all tissues, and transient expression assays of GFP fusions in protoplasts suggested that they located in the Golgi apparatus [10]. Testing their activity in a mammalian cell transfection system demonstrated that AtRBL2, but not AtRBL1, could cleave Drosophila substrates, suggesting that at least AtRBL2 is a *bona fide* rhomboid protease. However, since the known Drosophila substrates of Rho-1 do not have homologs in plants, it is believed that rhomboid proteases in plants have their own specific substrates.

The first report on the involvement of plant rhomboids in chloroplast biology was that of Karakasis and co-workers [11]. Reasoning that diverse rhomboid substrates have in common two features, a single transmembrane domain and a large soluble domain, they attempted to link between Tic40, a protein believed to be a component of the protein import machinery into chloroplasts, and one Arabidopsis rhomboid-like protein. Using a mitochondria-based system, they showed that Tic40 could be processed by the product of the At1g74130 gene, suggesting a role for this protein in the biogenesis of the chloroplast protein import machinery. Nevertheless, this suggestion is debatable, as the product of the aforementioned gene lacks the conserved catalytic serine and histidine residues, and thus is expected to be proteolytically inactive [6].

Two other Arabidopsis rhomboid-like proteases that were studied are AtRBL9 and AtPARL (designated AtRBL11 and AtRBL12 in the original paper) [12]). Transient expression assays of GFP fusions suggested that these proteins were targeted to chloroplasts and mitochondria, respectively [12]. However, the Arabidopsis mitochondrial rhomboid failed to complement the corresponding yeast mutant and did not recognize the yeast substrates cytochrome *c* peroxidase and a dynamin-like GTPase.

More recently, AtRBL8 was also identified in chloroplasts and was located to the envelope membrane ([13] and Table 1). Interestingly, the Arabidopsis mutant lacking this protein demonstrated reduced fertility and aberrant flower morphology [14]. Proteomic analysis of a double mutant lacking both AtRBL8 and its homolog AtRBL9 revealed that in the absence of these two rhomboid proteases the level of allene oxide synthase (AOS) was affected, although it was not determined which of these two was responsible for this effect [13]. As AOS is involved in the synthesis of the plant hormone jasmonic acid, this observation provides a link between the lack of chloroplast rhomboid proteases and the morphologic phenotype. Another interesting observation was that AtRBL9 forms homo-oligomers [13]. Although the functional significance of rhomboid oligomerization is still unknown, it is interesting to note that bacterial rhomboids were recently reported to oligomerize as well [15].

Table 1
Identified plastid intramembrane proteases and substrates for intramembrane proteolysis in *Arabidopsis thaliana*.

Protein	Gene locus	Intraplastid location	Biological function	Reference
Proteases				
Rhomboids				
AtRBL8	At1g25290	Inner envelope	Jasmonic acid, biosynthesis, flower morphology	[13,14]
AtRBL9	At5g25752	Inner envelope	Unknown	[12,13]
S2Ps				
AtS2P1	At2g32480 (AraSP)	Inner envelope and/or thylakoid	Chloroplast development	[19]
AtS2P2	At1g05140	Thylakoid		[20]
AtEGY1	At5g35220	Thylakoid	Chloroplast development, ABA signaling	[20–23]
AtEGY2	At5g05740	Thylakoid	Hypocotyl elongation	[25]
Substrates				
AtPHD	At5g35210	Envelope	Chloroplast nucleus signaling	[38]

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