Biochimica et Biophysica Acta 1797 (2010) 1071–1075

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Review

ATP-dependent proteases in biogenesis and maintenance of plant mitochondria

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ARTICLE INFO

Article history:
Received 30 October 2009
Received in revised form 22 February 2010
Accepted 22 February 2010
Available online 1 March 2010

Keywords:
ATP-dependent protease
AAA protease
FtsH
Clp
Lon
Oxidative phosphorylation
Plant mitochondria
Arabidopsis thaliana

ABSTRACT

ATP-dependent proteases from three families have been identified experimentally in *Arabidopsis* mitochondria: four FtsH proteases (AtFtsH3, AtFtsH4, AtFtsH10, and AtFtsH11), two Lon proteases (AtLon1 and AtLon4), and one Clp protease (AtClpP2 with regulatory subunit AtClpX). In this review we discuss their submitochondrial localization, expression profiles and proposed functions, with special emphasis on their impact on plant growth and development. The best characterized plant mitochondrial ATP-dependent proteases are AtLon1 and AtFtsH4. It has been proposed that AtLon1 is necessary for proper mitochondrial biogenesis during seedling establishment, whereas AtFtsH4 is involved in maintaining mitochondrial homeostasis late in rosette development under short-day photoperiod.

1. Introduction

It is now widely accepted that the mitochondrial proteome is a dynamic system whose composition changes in response to a variety of developmental stimuli. This is especially evident for multicellular organisms where the mitochondrial proteome is tailored to suit the different tasks assigned to mitochondria in particular tissues. Moreover, these tasks often vary during the lifespan of the organism. Another important cause of changes in the composition and integrity of the mitochondrial proteome is the changing environmental conditions. Environmental stresses are often connected with a risk of harmful mitochondrial protein modifications. Thus, tissue- and time-specific control of the quantity and quality of mitochondrial proteins is essential to cope with the challenges of changing developmental and environmental conditions. Recent studies indicate that in plant mitochondria, like in the yeast and animal ones, ATP-dependent proteases are the key components of such control [1–3].

2. Common features of mitochondrial ATP-dependent proteases and their types

ATP-dependent proteases have been identified in mitochondria of all types of eukaryotic cells, and, in line with the endosymbiotic origin of the mitochondria, they exhibit significant similarity to the bacterial orthologs [4–7]. A characteristic feature of these proteases is that the proteolytic domain is accompanied by an ATPase domain (also called AAA module) which is believed to act as a molecular chaperone. Typically, mitochondria contain ATP-dependent proteases of three types: Lon, Clp, and FtsH [4–7]. The two former are serine proteases, whereas FtsH (also called AAA proteases) have a catalytic site characteristic for metalloproteases. In the case of FtsH and Lon, the ATPase and proteolytic domains are formed by a single polypeptide, whereas in Clp these domains are separate proteolytic (ClpP) and chaperone-like (ClpX) subunits. The ATP-dependent proteases are typically organized into large oligomeric ring-shaped complexes composed of identical or closely related subunits. The proteolytic active sites are buried inside these complexes and substrates enter the proteolytic chamber through axial channels that are too narrow to accommodate native folded proteins [8–11]. Thus, to be degraded polypeptides have first to be unfolded by the AAA domain. Substrate recognition can be mediated by the ATPase domains or indirectly through auxiliary proteins. All substrates are degraded in an ATP-dependent manner, with the energy from ATP hydrolysis being used for unfolding and translocation of the polypeptide undergoing degradation. The substrate entering the catalytic chamber is intercepted by conservative hydrophobic loops protruding from the AAA module. Cyclic forth and back movements of the loops induced by ATP hydrolysis generate the driving force for the unfolding and pulling the polypeptide into the protease chamber. Once the polypeptide reaches the active site, it is fragmented into short 3–30 amino acid-long peptides [8–11]. Unlike other ATP-dependent proteases, bacterial FtsH has only a weak unfolding activity and is unable to degrade stably folded proteins, but instead relies on local spontaneous unfolding of substrates destined for degradation [12]. In this review we focus on the ATP-dependent proteases identified experimentally

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doi:10.1016/j.bbabio.2010.02.027

in mitochondria of *Arabidopsis*, but data on other higher plants will be discussed as well.

3. ATP-dependent proteases identified in plant mitochondria

Members of the Lon, FtsH and Clp families have been identified experimentally in plant mitochondria [7]. In *Arabidopsis*, four FtsH proteases (AtFtsH3, AtFtsH4, AtFtsH10, and AtFtsH11) [7,13,14], two Lon proteases (AtLon1 and AtLon4) [15,16], and one Clp protease (AtClpP2 with regulatory subunit ClpX) [17] have been described (Fig. 1A). In addition to mitochondria, the ATP-dependent proteases have also been identified in other higher plant organelles: FtsH and Clp in plastids while Lon in chloroplasts and peroxisomes [18]. Moreover, some of these ATP-dependent proteases show dual localizations: AtFtsH11 and AtLon4 were found in both mitochondria and chloroplasts [14,15]. Regarding their submitochondrial localization, most data concern FtsH proteases. Like in fungi and mammals, two subtypes of the FtsH proteases reside in the inner mitochondrial membrane: the m-AAA protease faces the matrix while the i-AAA protease protrudes to the intermembrane space [14,19]. Only one m-AAA protease (PsFtsH) is found in *Pisum sativum* [19], while *Arabidopsis thaliana* has two: AtFtsH3 and AtFtsH10 [7,13]. Like their yeast counterparts, the *Arabidopsis* m-AAA proteases assemble with prohibitins, integral inner mitochondrial membrane proteins, into large 2-MDa complexes [13]. However, in contrast to yeast, but similarly to mammalian ones, *Arabidopsis* m-AAA proteases can also form homo- as well as hetero-oligomeric complexes (our unpublished results). Only one i-AAA protease is present in yeast while

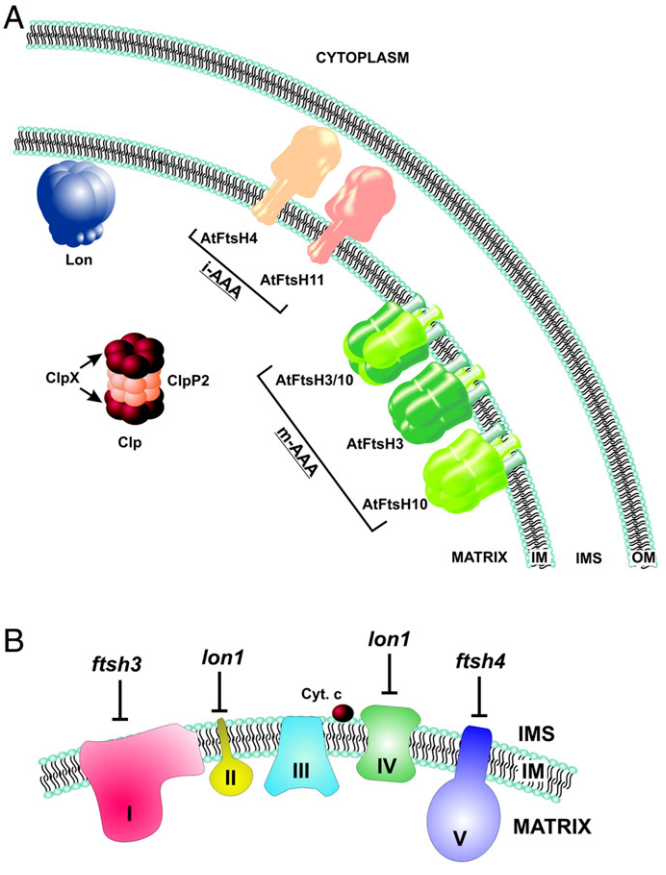

Fig. 1. Experimentally verified mitochondrial ATP-dependent proteases in *Arabidopsis thaliana*. Their intramitochondrial localizations are shown in A, and putative molecular targets inferred from mutant studies in B. Reduced activity of complexes II and IV was observed in *lon1* mutant [16], while a significant decrease in the activity and protein amount of complexes I and V in *ftsh3* and *ftsh4* mutants, respectively [45]. Abbreviations used: IM, inner membrane; IMS, intermembrane space; OM, outer membrane.

Arabidopsis mitochondria contain two: AtFtsH4 and AtFtsH11 [14]. They form two independent complexes in the inner membrane, both with apparent molecular masses of about 1.5 MDa. The submitochondrial localization of Lon proteases in *Arabidopsis* mitochondria is unknown, but it has been shown that in the common bean Lon protease is attached to the inner mitochondrial membrane [20]. It should be emphasized that in yeasts and human cells a Lon protease was detected as a soluble protein in the mitochondrial matrix [21,22]. Immunoblot analysis showed that *Arabidopsis* mitochondria contain both proteolytic and regulatory subunits of the Clp protease [17], which is in contrast to yeast mitochondria where only Clp-like proteins with a non-proteolytic function have been found [6]. Proteomic studies have revealed that in the matrix of potato mitochondria the proteolytic subunit ClpP2 forms a complex with three chaperone ClpX subunits [23]. Phylogenetic analyses indicate that the number of mitochondrial ATP-dependent proteases of a given type may vary among plant species. For example, AtFtsH4 has two orthologs in rice [14], but only one in *Populus* [24], while AtFtsH11 has one ortholog in rice [14] and two in *Populus* [24]. Moreover, it has been claimed that the orthological relationship is not clear for some of the genes encoding those proteases in *Arabidopsis* and *Populus* [24].

4. Expression of plant mitochondrial ATP-dependent proteases

In *Arabidopsis* rosettes, transcripts encoding mitochondrial ATP-dependent proteases are generally less abundant than the chloroplastic ones [25]. The Genevestigator toolbox [26] and several reports (Halperin et al. [17] for AtClpP2, Chen et al. [27] for AtFtsH11, Rigas et al. [16] for AtLon1, and Kicia et al. [2] for AtFtsH4) reveal that the *Arabidopsis* mitochondrial ATP-dependent proteases are expressed in all organs investigated. However, their expression levels differ among the analyzed organs and may change during their development. A survey of Genevestigator data [26] for roots and leaves indicates that expression of mitochondrial ATP-dependent proteases is roughly constant during their development and only minor changes, not exceeding 2–3-fold, are observed. Based on the data extracted from Genevestigator, extreme levels of expression are usually found in flowers and seeds (Table 1). It should be emphasized that the above conclusions need not be true for the dual-targeted FtsH11 protease since, basing on the transcript level, it is not possible to judge about expression of the mitochondrial protein alone. Recently, Rigas et al. [16] showed that expression of the *AtLon1* gene was high in germinating seeds, embryonic organs, including cotyledons and primary roots, and in organs with high growth rates such as developing inflorescences, while it was hardly detected in mature roots or stems of adult plants. A quantitative RT-PCR analysis of the time-course of *AtFtsH4* transcript accumulation in *Arabidopsis* plants showed that the photoperiod could influence the pattern of this gene's expression in rosette leaves [2].

Numerous studies have revealed that expression of non-mitochondrial ATP-dependent proteases is often regulated in response to environmental conditions [1,28–30]. In contrast, the steady-state transcript levels of mitochondrial ATP-dependent proteases in *Arabidopsis* are usually only minimally affected by environmental stimuli, except for AtLon4. Genevestigator [26] indicates higher-than-two-fold changes in expression level only for the *AtFtsH4*, *AtFtsH11* and *AtLon4* genes in response to heat stress. However, it should be emphasized that these conclusions are based on data obtained for certain conditions only. Different conditions can give different results. For instance, the transcript abundance of the mitochondrial members of *Arabidopsis* Lon, FtsH and Clp families was evaluated following short-term exposure of plants to high-light intensity (770 μE m^{-2} s^{-1} for 2.5 h), high temperature (42 °C for 1 h) or low temperature (4 °C for 18 h) [25]. That analysis revealed that exposure to high-light intensity had the strongest effect, but only for AtFtsH4 the expression was changed substantially (increased 11-fold). In contrast to the