Characterizing an “Inactive” Organellar Rhomboid Protein in Arabidopsis

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Introduction

Rhomboid serine proteases are frequently observed to play regulatory roles in different cellular processes. Such cellular processes are not limited to a particular species or closely-related species, but come from a range of organisms. The regulatory roles of rhomboid proteases are often based on the cleavage of specific substrate targets located in membranes. This cleavage phenomenon have been discovered in prokaryotic and eukaryotic cells and are referred to collectively as Regulated Intramembrane Proteolysis (RIP) (Brown et al. 2000; Urban 2006; 2009; Freeman 2008; Wolfe 2009).

A sub-group of catalytically inactive proteins related to rhomboid proteases was recently identified through enhanced genomic analysis (Lemberg and Freeman 2007). Despite being catalytically inactive, these rhomboid-like proteins have been discovered to be equally active in development related roles. The human rhomboid homolog p100Rho/RHBDF1/IRhom1 interacts with TGF-alpha-like ligands (Nagakawa et al. 2005), is needed for epithelial cancer cell survival (Yan et al. 2008), and participates in GPCR-mediated transactivation of EGFR signals in head and squamous cancer cells (Zou et al. 2009). More recently, Drosophila Rhomboid-5/I-Rhom was found to regulate intercellular signaling by preventing the cleavage of rhomboid substrates through the ER quality control mechanism (Zettl et al. 2011).

The latest entrants to the RIP phenomenon are the plant rhomboid serine proteases and related proteins. Although a number of natural rhomboid protease substrates (and their corresponding processes) have been revealed from insects to mammals, the situation in plants remains relatively unknown. The lack of knowledge regarding plant rhomboid proteases exists even though Arabidopsis and rice are predicted to date to encode numerous rhomboid protease encoding genes.

At1g74130 is an Arabidopsis rhomboid protein which has been classified as a mixed inactive homologue through genomic analysis investigations (Lemberg and Freeman, 2007). The purpose of this study is to uncover potential developmental roles associated with At1g74130. This particular rhomboid protein was selected as it is one of the few known catalytically inactive organellar rhomboid proteins known. Such studies may provide great insight to the understanding of various human related developmental diseases.

Methods

Plants were propagated at 21°C with a 16:8 h light/dark photoperiod (fluorescent and incandescent lighting). Chloroplasts were prepared from soil-grown plants as described (Aronson and Jarvis, 2002; Fitzpatrick and Keegstra, 2001). Chlorophyll determinations were performed as reported by Porra et al. (1989).

Transmission electron microscopy was carried out using frequently described methods of chemical fixation, mostly adapted from Karnovsky (1965). The embedding protocol used was provided by the manufacturer of the kit. The transmission electron microscope used for our study was a Hitachi H-7000.

Results

• Arabidopsis development and growth is dependent upon the cellular roles of At1g74130 (see figure 1)

• Plastid development is altered with the lack of At1g74130. Chloroplasts appear underdeveloped with large starch granules present (see figure 2)

Discussion

In this study, we were interested in gaining insight into the roles of organellar rhomboid proteases. The overall rhomboid protein level in mature chloroplasts are low relative to most of the other plastid proteins (see figure 3). The low levels observed may be reflecting the types of roles rhomboid proteins potentially play in the various tissues and during plant development and growth. Since many of the nonplant rhomboid proteins have been assigned regulatory roles in a variety of cellular processes (Brown et al. 2000; Urban 2006; 2009; Freeman 2008; Wolfe 2009), it is likely that the situation is similar in plant systems.

In summary, when taken together, the data reported here suggest that inactive organellar rhomboid proteins, in particular At1g74130, are likely involved with plant growth and development. In the case of At1g74130, involvement appears to be focused on the early phases of organelle biogenesis and development, which in turn may affect later stages of growth, such as that documented here with leaves.

Literature Cited


Plastid development is altered with the lack of At1g74130. Chloroplasts appear underdeveloped with large starch granules present (see figure 2)