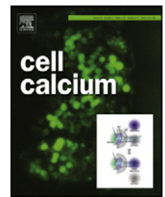
Cell Calcium 52 (2012) 397–404

Contents lists available at SciVerse ScienceDirect

Cell Calcium

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



In vivo analysis of the calcium signature in the plant Golgi apparatus reveals unique dynamics

Viviana R. Ordenes [a,b], Ignacio Moreno [c,f,1], Daniel Maturana [c,1], Lorena Norambuena [d], Anthony J. Trewavas [e], Ariel Orellana [f,*]

[a] Calcium Signalling Research Unit, Metallomics-Chile Corporation, La Serena, Chile
[b] Brotar Ltd., La Serena, Chile
[c] Laboratorio de Fisiología Celular, Facultad de Ciencias, Universidad de Chile, Santiago, Chile
[d] Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile
[e] Institute of Molecular Plant Sciences, University of Edinburgh, Edinburgh, UK
[f] FONDAP Center for Genome Regulation, Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas, Universidad Andres Bello, Santiago, Chile

ARTICLE INFO

Article history:
Received 18 February 2012
Received in revised form 12 June 2012
Accepted 23 June 2012
Available online 23 July 2012

Keywords:
Plant
Golgi apparatus
Calcium
Auxin
Aequorin

ABSTRACT

The Golgi apparatus is thought to play a role in calcium homeostasis in plant cells. However, the calcium dynamics in this organelle is unknown in plants. To monitor the $[Ca^{2+}]_{Golgi}$ *in vivo*, we obtained and analyzed *Arabidopsis thaliana* plants that express aequorin in the Golgi. Our results show that free $[Ca^{2+}]$ levels in the Golgi are higher than in the cytosol (0.70 μM *vs.* 0.05 μM, respectively). Stimuli such as cold shock, mechanical stimulation and hyperosmotic stress, led to a transient increase in cytosolic calcium; however, no instant change in the $[Ca^{2+}]_{Golgi}$ concentration was detected. Nevertheless, a delayed increase in the $[Ca^{2+}]_{Golgi}$ up to 2–3 μM was observed. Cyclopiazonic acid and thapsigargin inhibited the stimuli-induced $[Ca^{2+}]_{Golgi}$ increase, suggesting that $[Ca^{2+}]_{Golgi}$ levels are dependent upon the activity of Ca^{2+}-ATPases. Treatment of these plants with the synthetic auxin analog, 2,4-dichlorophenoxy acetic acid (2,4-D), produced a slow decrease of free calcium in the organelle. Our results indicate that the plant Golgi apparatus is not involved in the generation of cytosolic calcium transients and exhibits its own dynamics modulated in part by the activity of Ca^{2+} pumps and hormones.

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1. Introduction

The plant Golgi apparatus is the organelle where the biosynthesis of cell wall polysaccharides such as pectin and hemicelluloses take place. This organelle also plays a role in the synthesis and modification of glycoproteins and glycoconjugates [1–3]. Unlike mammalian cells, in which Golgi stacks are practically immobile and condensed in a limited perinuclear region, plant cells contain a large number of smaller motile versions of this organelle [1–3]. Thus, the plant Golgi apparatus possesses some features that are different to animal cells or model systems such as yeast.

One of the functions of the plant Golgi apparatus that have been less studied is the ionic homeostasis. In particular, the role this organelle plays in the cellular dynamics of calcium in plant cells is not known. Calcium is an important signaling molecule in plants [4–6] and it is involved in plant growth, development and responses to biotic and abiotic stresses among others [6]. Calcium homeostasis in plant cells is maintained by calcium influx and efflux systems [7]. The influx system controls calcium flux into the cytosol *via* calcium channels that reside in the plasma membrane and endomembranes [8]. Calcium efflux is controlled by calcium-transporting ATPases (Ca^{2+} pumps) and calcium transporters that actively transport calcium back to the extracellular space and/or organelles to maintain a low calcium level in the cytoplasm [9–11]. In animal cells, the Golgi apparatus takes up Ca^{2+} using both the sarco(endo)plasmic-reticulum Ca^{2+}-ATPases (SERCA) and secretory-pathway Ca^{2+}-ATPases (SPCA) [12], while in yeast Pmr1 is an ATPase involved in the uptake of Ca^{2+} and Mn^{2+} into the Golgi apparatus [13]. These findings suggest that the Golgi contributes to the regulation of calcium signaling in animal and yeast cells. Recent reports of Ca^{2+}-ATPases located in the plant Golgi apparatus [14,15], suggest that this organelle may play a role in regulating the cellular calcium, but to date, there is almost no information regarding the role of the Golgi apparatus in the calcium homeostasis in plant cells. To investigate this process and to monitor the changes in $[Ca^{2+}]_{Golgi}$ *in vivo*, we targeted the photoprotein aequorin into this organelle in *Arabidopsis*. Our results indicate that

* Corresponding author at: Centro de Biotecnología Vegetal, Facultad de Ciencias Biológicas, Universidad Andrés Bello, Av. República 217, Santiago, Chile.
Tel.: +56 2 6618628.
E-mail address: aorellana@unab.cl (A. Orellana).
[1] These authors contributed equally to this work.

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http://dx.doi.org/10.1016/j.ceca.2012.06.008

the plant Golgi apparatus has a resting [Ca^{2+}] higher than the cytosol. In addition, our results suggest that the Golgi does not release calcium when abiotic stimuli are applied. We instead observed a delayed increase in $[Ca^{2+}]_{Golgi}$ followed by a return to the resting levels after several minutes. This delayed increase in the $[Ca^{2+}]_{Golgi}$ was blocked both by cyclopiazonic acid (CPA) and thapsigargin (TG), both calcium pump inhibitors, suggesting that the increase in $[Ca^{2+}]_{Golgi}$ levels is dependent on the activity of these types of calcium transporters. On the other hand, 2,4-dichlorophenoxy acetic acid (2,4-D) treatment produced a slow decrease in the $[Ca^{2+}]_{Golgi}$ concentration but a slight increase in the $[Ca^{2+}]_{cyt}$, consistent with previous observations [16]. These results suggest that the Golgi apparatus in plants has a calcium dynamics that is different from the one observed in this organelle in animal cells.

2. Results

2.1. At-FUT1tmd-AEQ localizes at the Golgi apparatus in transgenic Arabidopsis plants

To target the Ca^{2+}-dependent photoprotein aequorin specifically to the Golgi apparatus in *Arabidopsis*, we used the first 100 N-terminal amino acids of At-FUT1 (*Arabidopsis thaliana* xyloglucan fucosyltransferase 1), a type II membrane-anchored protein; which is located in the plant Golgi apparatus [17,18]. Once we confirmed that N-terminal of At-FUT1tmd has the information to target a protein to the Golgi apparatus (supplemental material Fig. 1), we fused it to aequorin (AEQ) and Arabidopsis transgenic plants expressing AtFUT1tmd-AEQ were obtained. The subcellular localization of the chimeric protein was analyzed by immunofluorescence in root hair cells from the transgenic plants using an anti-aequorin monoclonal antibody (Fig. 1C). The results revealed a punctuate pattern of fluorescence throughout the cell for At-FUT1tmd-AEQ. To confirm that this fluorescent pattern corresponded to Golgi stacks, the cells were double labeled using an antibody against RGP (reversible-glycosylated protein), a Golgi-localized protein [19] (Fig. 1D). The immunofluorescence patterns for At-FUT1tmd-AEQ and RGP showed an extensive overlap (Fig. 1E) suggesting that AtFUTtmd-AEQ was indeed successfully targeted to the Golgi apparatus in *Arabidopsis*.

At-FUT1tmd-AEQ should be located at the Golgi membrane; therefore, to determine that aequorin was facing the lumen of the Golgi the membrane topology of At-FUT1tmd-AEQ was evaluated using a proteolysis assay of right-side-in Golgi vesicles [15,20] obtained from the entire transgenic plants. Western blot analyses showed that control Golgi vesicles contain a 33 kD protein, corresponding to the predicted size of At-FUT1tmd-AEQ (Fig. 1B, lane 1). When intact Golgi vesicles were incubated with trypsin, the size of this band did not change (Fig. 1B, lane 2). However, when the incubation with trypsin was done on Golgi vesicles whose membranes were permeabilized with detergent (TX-100), a shift in the electrophoretic mobility was observed by immunoblotting (Fig. 1B,

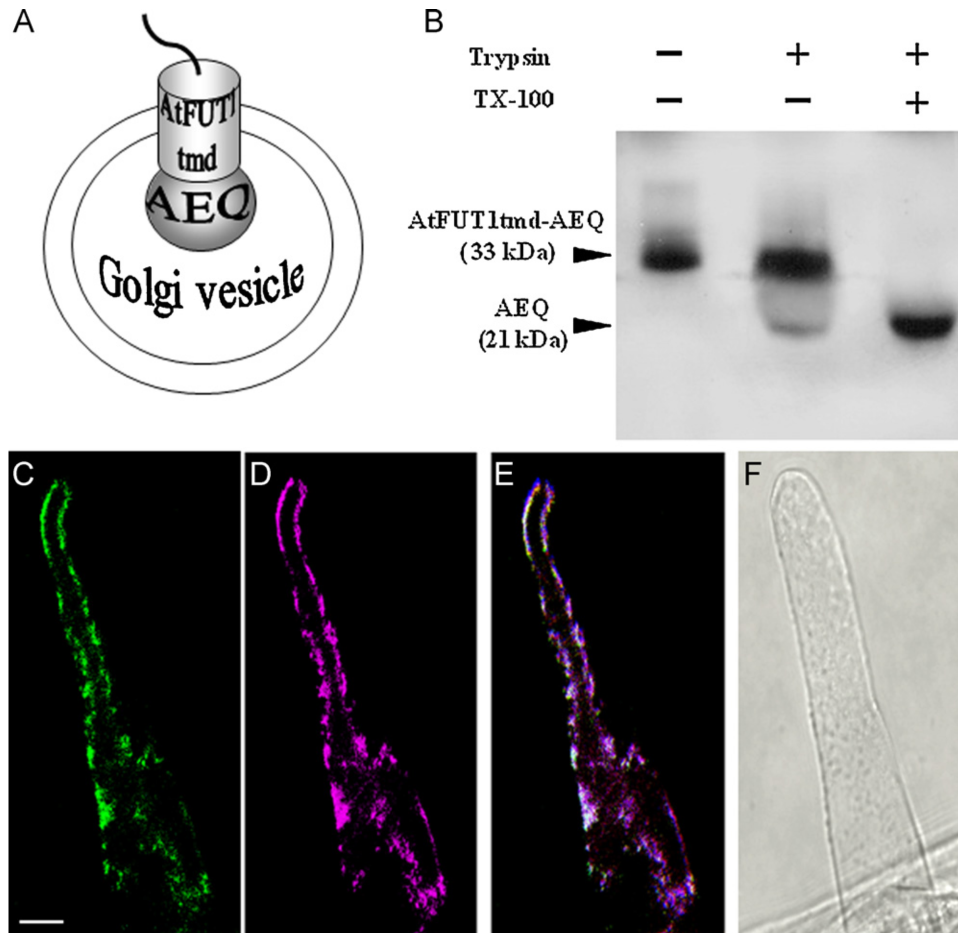

Fig. 1. At-FUT1tmd-AEQ is located within the Golgi apparatus and faces the lumen. (A) Schematic depiction of the expected membrane topology of At-FUT1tmd-AEQ. (B) Protease protection assay performed on Golgi vesicles isolated from transgenic plants expressing At-FUT1tmd-AEQ. Control (lane 1); treatment with trypsin alone (lane 2); treatment with trypsin plus TX-100 (lane 3). (C and D) The subcellular distribution of At-FUT1tmd-AEQ in root hairs was analyzed by immunofluorescence and compared with a Golgi marker. (C) Distribution of the chimera analyzed using an AEQ antibody. (D) Distribution of the Golgi assessed using an RGP antibody. (E) A merge of the images obtained in (C) and (D). (F) Contrast phase image of the root hair analyzed by immunofluorescence. Bar, 20 μm.

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