Proteomic dissection of the Arabidopsis Golgi and trans-Golgi network
Parsons, Harriet Tempé; Drakakaki, Georgia; Heazlewood, Joshua L.

Published in:
Frontiers in Plant Science

DOI:
10.3389/fpls.2012.00298

Publication date:
2013

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Proteomic dissection of the Arabidopsis Golgi and trans-Golgi network

Harriet T. Parsons1*, Georgia Drakakaki2 and Joshua L. Heazlewood3,4

1 Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark
2 Department of Plant Sciences, University of California at Davis, Davis, CA, USA
3 Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Berkeley, CA, USA
4 Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

E-mail: htpa@life.ku.dk

January 2013 | Volume 3 | Article 298 | 1

The plant Golgi apparatus and trans-Golgi network are major endomembrane trafficking hubs within the plant cell and are involved in a diverse and vital series of functions to maintain plant growth and development. Recently, a series of disparate technical approaches have been used to isolate and characterize components of these complex organelles by mass spectrometry in the model plant Arabidopsis thaliana. Collectively, these studies have increased the number of Golgi and vesicular localized proteins identified by mass spectrometry to nearly 500 proteins. We have sought to provide a brief overview of these technical approaches and bring the datasets together to examine how they can reveal insights into the secretory pathway.

Keywords: Golgi, trans-Golgi network, proteomics, LOPIT, free-flow electrophoresis, Arabidopsis, SYPER

BACKGROUND

At its simplest level, subcellular proteomics attempts to identify all proteins in a particular compartment. However, even with such a basic definition in mind, the Golgi proteome presents conceptual difficulties; functional proteins in the Golgi may also be functional elsewhere (Ondzighi et al., 2008), whilst endoplasmic reticulum (ER)-Golgi connections (Boevink et al., 1998) makes absolute divisions between the proteomes of these compartments somewhat futile. A number of proteins are known to form functional associations on the cytoplasmic face of cisternae but are part of the cytosol (Ito et al., 2011), so the very definition of the Golgi proteomes is problematic. Furthermore, in such an architecturally heterogeneous organelle, simply identifying all the proteins present in the Golgi is not that helpful unless we can classify them according to sub-Golgi location, post-Golgi compartments, cargo, resident, or dual-localized proteins. The plant Golgi poses a challenge in terms of isolation, not least because of its fragmented morphology. In mammalian cells Golgi stacks tend to be less numerous per cell with fewer, longer cisternae which are less tightly associated with the ER and could be relatively easily isolated (Morre and Mollenhauer, 1964). Excepting highly conserved pathways such as protein N-linked glycan processing, few similarities exist between plant and mammalian Golgi. Thus assuming Golgi-residency between the two systems based on homology alone is not possible. Earlier work on Golgi from rat liver was therefore of limited help other in terms of providing an isolation strategy or a comprehensive bank of marker proteins (Taylor et al., 1997).

The plant Golgi is much less structurally defined during and after cell homogenization than, for example, plastids or mitochondria. Consequently, quality control of and improvements to isolation strategies have been tricky and therefore purity limited when using sucrose density centrifugation strategies (Morre and Mollenhauer, 1964). In short, it is easy to understand why progress in Golgi proteomics has trailed behind other subcellular compartments in plants. In light of the shortcomings of sucrose density centrifugation strategies (Morre and Mollenhauer, 1964), major, large-scale proteomic characterizations in various plant systems (Tanaka et al., 2004; Asakura et al., 2006; Nikolovski et al., 2012), whilst the FFE approach identified proteins in fractions of purified Golgi, that were estimated to be enriched in medial Golgi cisternae (Parsons et al., 2012a). Immunoisolation of compartments has recently been used to great effect in separating components of the TGN, enabling comparative proteomics at the sub-Golgi level (Drakakaki et al., 2012). Characterization of Golgi-enriched fractions has been attempted in various plant systems (Tanaka et al., 2004; Asakura et al., 2006; Mast et al., 2010), major, large-scale proteomic characterizations have exclusively occurred in the model plant Arabidopsis thaliana.

AN OVERVIEW OF THE Arabidopsis Golgi-TGN PROTEOMES

Initial attempts to characterize the Arabidopsis Golgi by mass spectrometry were undertaken nearly a decade ago with the aim of distinguishing between ER- and Golgi-resident proteins (Dunkley et al., 2004). The LOPIT approach involves quantitative mass spectrometry of proteins labeled with isotope tags. A cell homogenate separated along a linear gradient is fractionated and pairwise comparisons of fractions allows abundance ratios of isotope masses to be calculated for each protein. Proteins physically located in the same compartment will have similar ratios and so cluster together...
during partial least squares discriminant analysis (Figure 1). Using LOPIT, 89 proteins were initially localized to the Golgi (Dunkley et al., 2006) but the requirement that proteins carry all four tags limited the number of proteins for which a statistically credible localization could be assigned. Recent reanalysis and analysis of existing and new datasets, incorporating values for “missing” tags assigned using partial least squares regression models and training sets based on fully tagged proteins, enabled the collective localization of 204 proteins to the Golgi/TGN (Dunkley et al., 2006; Nikolovski et al., 2012).

Although a major motivation for the development of LOPIT was the difficulty in separating the Golgi, particularly from ER contaminants, a recent study has managed to isolate Golgi vesicles with an estimated 80% purity based on protein composition. This was achieved using a combination of sucrose density centrifugation and FFE (Parsons et al., 2012a). The power of FFE for organelle isolation was demonstrated in plants several years ago when applied to the separation of mitochondria and peroxisomes, two organelles which are typically hard to separate using density centrifugation alone (Eubel et al., 2008). As separation by FFE is dependent on surface charge, the Golgi, which carries a more negative surface charge than ER vesicles and most other contaminants, is amenable to separation using this technique, which resulted in 371 proteins being localized to the Golgi (Figure 1).

A dissection of the complexity of the Golgi proteome was recently attempted using immunoisolation of specific TGN trafficking populations. Affinity purified TGN compartments from plants expressing a syntaxin from plants (SYP61)-CFP construct were enriched for the TGN by sucrose density centrifugation then exposed to anti-FP antibodies coupled to agarose beads and analyzed by mass spectrometry (Drakakaki et al., 2012). Although widely used in mammalian systems, application of this approach in plants was precedential. The technique was able to identify 145 proteins from affinity purified samples of SYP61 vesicles, providing the foundation of a TGN proteome in plants.

THE SIZE OF THE PLANT GOLGI PROTEOME

In total, 452 proteins have been characterized by mass spectrometry to the Golgi apparatus and 145 to the TGN from the model plant Arabidopsis. An ever-present question in subcellular

![Diagram of proteomic characterization](image)
A number of large gene families have been identified by both the AdaBoost, Golgi and PProwler, and positive prediction programs. The Golgi/TGN proteome is estimated to be 2239 proteins found by both FFE and LOPIT approaches (Nikolovski et al., 2012; Parsons et al., 2012a) and 145 proteins to the Golgi/TGN by fluorescent marker studies (Heazlewood et al., 2007). In total 575 unique proteins have been experimentally localized to the Golgi/TGN. Of the 22 subcellular prediction algorithms that have been applied to the Arabidopsis proteome, 14 provide a "Golgi" prediction output (Table 1).

Employing the relational capabilities of the SUBA database, it is possible to compute a size estimate of the Golgi/TGN proteome based on each algorithms performance. The overall performance of each prediction program can vary considerably with regard to the total predicted "Golgi" proteins in Arabidopsis (contrast AdaBoost, 66 Golgi and PProwler, 885 Golgi) and positive prediction rate of the experimental proteome (contrast AdaBoost <1% and PProwler >50%). However, after calculating false positive and false negative rates for each program, the final predicted Golgi proteomes are remarkably similar. Based on this analysis, the Arabidopsis Golgi/TGN proteome is estimated to be 2239 ± 465, employing the average of the predicted proteomes of these 14 subcellular prediction programs.

**USING THE PROTEOME: WHAT ARE THE ROLES OF UNCHARACTERIZED PROTEIN FAMILIES?**

A number of large gene families have been identified by both the FFE and LOPIT studies (Nikolovski et al., 2012; Parsons et al., 2012a). The quantitative mass spectrometry performed when applying LOPIT (Nikolovski et al., 2012) and spectral counts from FFE isolates (Parsons et al., 2012a), combined with localization data (Heazlewood et al., 2007), provide an important starting guide as to which members of these large families are major components and should be initially investigated in future studies.

The cyclophilin-like peptidyl-prolyl cis-trans isomerase family is consistently represented in the Golgi proteomes. These are known to catalyze conversion of cis to trans conformation of peptide bonds preceding prolyl residues in newly synthesized peptides (Chou and Gasser, 1997). In plants, they are classified associated with the thylakoid lumen where they are thought to help protein folding and assembly of photosystem complexes although their exact role is not clear (Ingelsson et al., 2009). The cyclophilins found by both FFE and LOPIT approaches (Nikolovski et al., 2012; Parsons et al., 2012a) localize either exclusively to the Golgi or are dually localized to the Golgi and plasma membrane (Dunkley et al., 2006; Benschop et al., 2007; Marmagne et al., 2007; Parsons et al., 2012a), implying a secretory-specific function, although no cyclophilins were found during immunoisolation of the TGN (Drakakaki et al., 2012).

The prenylated RAB acceptor B2 (PRA1.B2, A T2G40380) is found in both Golgi proteomes (FFE and LOPIT) but not in the TGN, implying involvement with cisternal-specific interactions and vesicle docking. Examining proteins present uniquely in the TGN, besides those involved in trafficking such as the Rab GTPases, soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs, Blatt et al., 1999; Surpin and Raikhel, 2004), transport protein particle (TRAPP) components (Barrowman et al., 2010) or present as cargo, e.g., specific cellulose synthase A (CESA) subunits (Paredes et al., 2006), one endomembrane protein/transmembrane 9 protein (EMP/TMN9) and two S-adenosyl-L-methionine-dependent methyltransferases appear to stand out. Most EMP/TMN9 proteins are found in the Golgi cisternae: 11 members from a total of 12 were identified in FFE-purified samples (Parsons et al., 2012a) and 10 during LOPIT studies. EMP/TMN9 proteins interact with COPI and COPII proteins and membrane proteins destined for post-Golgi locations but are only recently studied in plants (Gao et al., 2012).

The presence of two EMP/TMN9 proteins in both the Golgi and TGN implies trans-Golgi localization. With only one EMP/TMN9 identified uniquely in the TGN, members of the family may fulfill niche roles in trafficking depending on their location along the Golgi stack and are likely interesting subjects for future study. Apart from QUA2 (Mouille et al., 2007), a pectin methyltransferase in the S-adenosyl-L-methionine-dependent methyltransferases superfamily, no clear function has been assigned to any other members of this family of proteins in plants. The S-adenosyl-L-methionine-dependent methyltransferases which include QUA2 are prevalent in the Golgi and Golgi/TGN proteomes. A total of 20 were identified by LOPIT, 15 by FFE, and 3 in SYP9, resulting in 22 distinct proteins from this family (Drakakaki et al., 2012; Nikolovski et al., 2012; Parsons et al., 2012a). One member, AT5G64030, has been found in the plasma membrane proteome (Mitra et al., 2009; Zhang and Peck, 2011), so could conceivably function there. Assuming that all family members perform some kind of polysaccharide methylation, proteomic comparisons could be used to reveal late-acting enzymes in cell wall biosynthesis such as these examples.

Many functionally important Golgi proteins may actually be the sole members of their protein family. Of the 111 proteins not assigned to a functional protein category in the FFE proteome, 30 were also identified by LOPIT studies and many different protein families were represented. Amongst datasets such as these, dataset overlaps can provide a means to shortlist potentially important proteins about which little information is available.

Interestingly, although the proteomes comprised by the LOPIT studies and Parsons et al. (2012a) were both derived from similar starting tissues, a number of proteins are found in Parsons et al. (2012a) but not LOPIT studies and vice versa. Parsons et al. (2012a) identified more proteins overall and results included cargo proteins, unlike in LOPIT studies. Nevertheless after eliminating those annotated by Parsons et al. (2012a) as either transient or involved in protein synthesis, 81 proteins identified by LOPIT are not found in Parsons et al. (2012a) and 205 are in
Table 1 | Estimated size of the Arabidopsis Golgi/TGN proteomes utilizing data in the SUBA database and the current integrated proteome (575) employing the abilities of subcellular prediction algorithms.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>AdaBoost</td>
<td>66</td>
<td>13</td>
<td>2</td>
<td>11</td>
<td>0.85</td>
<td>10</td>
<td>100</td>
<td>2919</td>
<td>2909</td>
<td>Niu et al. (2008)</td>
</tr>
<tr>
<td>SuOxLo</td>
<td>578</td>
<td>1100</td>
<td>104</td>
<td>997</td>
<td>0.91</td>
<td>489</td>
<td>0.62</td>
<td>2704</td>
<td>2215</td>
<td>Pfeffer et al. (2006)</td>
</tr>
<tr>
<td>EpLoc</td>
<td>29</td>
<td>121</td>
<td>30</td>
<td>91</td>
<td>0.75</td>
<td>72</td>
<td>0.95</td>
<td>1378</td>
<td>3206</td>
<td>Brady and Shatkay (2008)</td>
</tr>
<tr>
<td>iPSORT</td>
<td>5688</td>
<td>1439</td>
<td>177</td>
<td>1362</td>
<td>0.88</td>
<td>700</td>
<td>0.69</td>
<td>2273</td>
<td>1573</td>
<td>Belli et al. (2002)</td>
</tr>
<tr>
<td>MultiLoc2</td>
<td>916</td>
<td>299</td>
<td>114</td>
<td>185</td>
<td>0.62</td>
<td>349</td>
<td>0.80</td>
<td>1762</td>
<td>1412</td>
<td>Blum et al. (2009)</td>
</tr>
<tr>
<td>PlanimPlac</td>
<td>1074</td>
<td>310</td>
<td>100</td>
<td>230</td>
<td>0.30</td>
<td>325</td>
<td>0.83</td>
<td>1871</td>
<td>1546</td>
<td>Chou and Shiah (2010)</td>
</tr>
<tr>
<td>PrediTar</td>
<td>5851</td>
<td>1438</td>
<td>186</td>
<td>1252</td>
<td>0.87</td>
<td>767</td>
<td>0.68</td>
<td>2340</td>
<td>1563</td>
<td>Small et al. (2004)</td>
</tr>
<tr>
<td>PredSL</td>
<td>8700</td>
<td>2012</td>
<td>259</td>
<td>1823</td>
<td>0.99</td>
<td>1092</td>
<td>0.55</td>
<td>2475</td>
<td>1333</td>
<td>Unpublished</td>
</tr>
<tr>
<td>ProProwler</td>
<td>8895</td>
<td>2121</td>
<td>236</td>
<td>1825</td>
<td>0.96</td>
<td>1240</td>
<td>0.49</td>
<td>2409</td>
<td>1169</td>
<td>Bodan and Hawkins (2009)</td>
</tr>
<tr>
<td>SLFA</td>
<td>7318</td>
<td>1733</td>
<td>142</td>
<td>1591</td>
<td>0.82</td>
<td>634</td>
<td>0.75</td>
<td>2667</td>
<td>1933</td>
<td>Tamura and Akutsu (2007)</td>
</tr>
<tr>
<td>SLPLocal</td>
<td>7406</td>
<td>1589</td>
<td>167</td>
<td>1432</td>
<td>0.90</td>
<td>773</td>
<td>0.71</td>
<td>2663</td>
<td>1890</td>
<td>Matsuda et al. (2009)</td>
</tr>
<tr>
<td>TargeP</td>
<td>6812</td>
<td>112</td>
<td>200</td>
<td>1374</td>
<td>0.87</td>
<td>825</td>
<td>0.65</td>
<td>2372</td>
<td>1547</td>
<td>Emanuelli et al. (2000)</td>
</tr>
<tr>
<td>WolfPSORT</td>
<td>112</td>
<td>45</td>
<td>11</td>
<td>34</td>
<td>0.76</td>
<td>27</td>
<td>0.98</td>
<td>1431</td>
<td>1404</td>
<td>Horan et al. (2007)</td>
</tr>
<tr>
<td>YLoc</td>
<td>1235</td>
<td>318</td>
<td>105</td>
<td>213</td>
<td>0.67</td>
<td>456</td>
<td>0.82</td>
<td>2233</td>
<td>1825</td>
<td>Sorokine et al. (2010)</td>
</tr>
</tbody>
</table>

Predicted Golgi Arabidopsis: All proteins predicted to be Golgi in Arabidopsis (TAIR10).
Expt. any location (575): Predicted Golgi and experimentally determined to be in any location by MS or FP (SUBA) or Golgi (575).
Expt. in Golgi (575): Predicted Golgi and experimentally determined to be in Golgi (575).
Expt. non-Golgi: Predicted Golgi but experimentally found to be non-Golgi [(Expt. any location) — (Expt. non-Golgi)]
FPR Golgi prediction: False positive rate for Golgi prediction [(Expt. non-Golgi) / (Expt. in Golgi)]
FNR Golgi prediction: False negative rate for Golgi prediction [1—(Expt. in Golgi) / (575)]
Estimated correct predictions: Estimation of correct predictions from total Golgi predictions in Arabidopsis (Expt. any location) — (Expt. any location) x (FPR Golgi prediction)
Estimated correct predictions: Estimation of correct predictions from total Golgi predictions in Arabidopsis (Expt. any location) — (Expt. any location) x (FPR Golgi prediction)
FPR Golgi prediction: False positive rate for Golgi prediction [(Expt. any location) — (Expt. in Golgi) / (575)]
FNR Golgi prediction: False negative rate for Golgi prediction [1—(Expt. in Golgi) / (575)]
Non-predictable expt. Golgi: The size of the unpredictable Golgi proteome [(Predicted Golgi) — (Expt. correct predictions)]
Parsons et al. (2012a) but not LOPIT. No clear pattern, e.g., protein abundance, exists between the proteins observed in either study; most probably differences arise from variations in methodologies, highlighting the value of multi-faceted approaches to proteomic characterization of the Golgi.

**WHAT IS MISSING FROM THE EXPERIMENTAL GOLGI PROTEOME?**

Specific questions concerning what has not been identified so far are obviously difficult to answer but they can be addressed in part by examining what sorts of protein have been localized by fluorescent tagging but not identified by subcellular proteomic techniques. Fluorescent localization of proteins is generally motivated by interest in a specific protein and so is more likely to represent low-abundant polypeptides. It therefore provides an initial guide to the completeness of subcellular proteomic approaches.

Notably absent from proteomic surveys, but localized to the Golgi stack by fluorescent tagging are the Golgins and GRIP domain proteins (Latijnhouwers et al., 2007). Several glycosyltransferases such as cellulose synthase-like D5 (CSDL5; Bernal et al., 2007), rhmannogalacturonan II xylosyltransferase (RGXT) 1 and 2 (Egelund et al., 2006), irregular xylem 9 (IRX9; Pena et al., 2007), reversibly glycosylated polypeptide (RGP) 1–4 (Drakakaki et al., 2006; Rautengarten et al., 2011), galacturonate acid transferase like (GATL) members from the GT8 family and a number of small GTPases are also either absent or poorly represented. Common methodological steps between these technically very different proteomes may in part explain these absences. Both the FFE and LOPIT approaches (Nikolovski et al., 2012; Parsons et al., 2012a) used cell suspension cultures whilst the immunosolubilization approach (Drakakaki et al., 2012) used 14-day-old liquid grown plantlets as the starting tissue, meaning that all proteomes were based on primary cell wall-rich tissue. This may explain the absence of CSDL5 and IRX9, which are both implicated in secondary cell wall biosynthesis and localized to the Golgi stack (Bernal et al., 2007; Lee et al., 2007). RGT1 and 2 may have been also been missed because of tissue-specific or low expression (Egelund et al., 2006). Members of the GATL clade, although localized to the Golgi stack (Kong et al., 2011), are absent from all Golgi proteomes, which could point toward some specific spatial or temporal function of these glycosyltransferases. Golgins are Golgi matrix proteins with coiled coil domains that typically locate to the cis- and trans-extremities of the Golgi stack and cisternal peripheries. They are involved in regulation of stack architecture and tethering events during trafficking (Osterrieder, 2012). Their location to cis- or trans-extremities of the Golgi stack may have precluded detection (Nikolovski et al., 2012; Parsons et al., 2012a). Peripheral golgins and those with GRIP domains which localize to the TGN, have no predicted transmembrane domain and appear to be recruited from the cytosol by interaction with small GTPases. Their absence from either the Golgi or the SVPS61 proteome (Drakakaki et al., 2012) may be due to carbonate washes used to remove cytosolic contaminants and/or centrifugation steps. Electron micrographs taken during FFE isolation procedure (Parsons et al., 2012a) show loss of vesicles from cisternal edges in with progressive centrifugation steps. Two of four data sets used in the LOPIT approach (Nikolovski et al., 2012) had been subjected to carbonate washes resulting in reduced peripheral proteins. This may explain why no RGS have been detected, as these are peripheral membrane associated proteins (Dolgado et al., 1998).

Several RAB GTPases have been localized by fluorescent protein assay to the Golgi stack (Batoko et al., 2000; Fezaru et al., 2012). LOPIT approaches have identified two RAB GTPases localized to the Golgi, five were found by FFE purification (Parsons et al., 2012a) and 19 by immunosolubilization (Drakakaki et al., 2012). RAB GTPases are involved in cargo-vesicle docking (Woodland and Moore, 2008) and are not Golgi-residents. This likely explains why fewer were present in the LOPIT Golgi proteome (Nikolovski et al., 2012). Step gradients employed prior to FFE purifications (Parsons et al., 2012a) were designed for maximal cisternal enrichment at the cost of small vesicles, so as to minimize ER contamination prior to FFE. This exemplifies the role of methodology in these technically diverse proteomes and shows how removal of contaminants may risk removal of Golgi-associated proteins.

Judging from these inconsistencies between the subcellular proteomics data and fluorescent protein localizations, it is clear that Golgi proteomics must be applied to other tissue types if the proteome is to be “completed.” This presents an even greater technical challenge as young, softer tissues are more easily homogenized to maintain Golgi stack integrity (Morre and Mollenhauer, 2009).

However, useful information may be gleaned from less pure preparations using tougher, challenging tissue types, or preparations which are less pure but contains Golgi-associated and Golgi matrix proteins, as there is now a sufficiently broad base of proteins from which to compile ever more extensive markers and training sets.

**SUB-GOLGI PROTEOMICS AND THE GOLGI IN AN ENDOMEMBRANE CONTEXT**

Comparative analyses such as those discussed above can now be formulated since a post-Golgi compartment has been characterized. The potential for distinguishing resident and cargo Golgi components can also be applied. Almost 50% of proteins identified in the TGN proteome comprise non-Golgi proteins as determined by the LOPIT approach (Drakakaki et al., 2012; Nikolovski et al., 2012). It is conceivable that with a few more post-Golgi compartments characterized, many of the endomembrane proteins currently assigned to multiple locations (Heazleswood et al., 2007) could be reassigned and more light shed on the various protein cycling routes through the secretory pathway. This could be reasonably achieved in a number of ways. For the smaller compartments such as endosomal compartments, the immunosolubilization approach (Drakakaki et al., 2012) could hold the most promise as a number of syntaxin proteins known to associate with this compartment have been identified (Sanderfoot and Raikhel, 1999). Such an approach may not be appropriate for isolating individual cisternae from the main stack as trafficked proteins destined for later cisternae and TGN may also be detected by antibodies, whilst stack architecture could prove too complex for such an approach. Several fractions containing a high proportion of known Golgi proteins were not included in the FFE proteome owing to slightly higher level of contaminants. The number of fractions in which over 25% of proteins had been localized to the Golgi by LOPIT...
studies suggest partial electrophoretic separation of cisternae may have been occurring during the isolation process (Parsons et al., 2012a,b). A collection of sub-Golgi markers have been characterized (Saint-Jore-Dupas et al., 2006), so if proteins from FFE fractions could be accurately quantified profiles of co-migrating proteins could be created to enable sub-Golgi differentiation.

**CONCLUDING REMARKS**

Although one of the most technically challenging organelles to isolate, a diversity of technologies have led to two Golgi proteomes and one proteome of TGN vesicles, resulting in nearly 500 proteins now localized to the Golgi and/or TGN by mass spectrometry. Exploring other tissue types is needed to increase the coverage of the Golgi proteome. Efforts must also be concentrated in getting the proteomes of cis-, medial-, and trans-Golgi subcompartments and specific vesicle populations. This will incur further technical challenges but will help identify more lowly expressed proteins and provide invaluable insight into plant Golgi functions.

**ACKNOWLEDGMENTS**

This work conducted by the Joint Bioenergy Institute was supported by the Office of Science, Office of Biological and Environmental Research, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. The work conducted by Drakakaki et al. (2012) was funded by a grant from the DOE (DEFG03-02ER15295) and UC Davis startup funds.

**REFERENCES**


Morre, D. J., and Mollenhauer, H. H.


January 2013 | Volume 3 | Article 298 | #7

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 November 2012; accepted 12 December 2012; published online: 05 January 2013.


This article was submitted to Frontiers in Plant Proteomics, a specialty of Frontiers in Plant Science.

Copyright © 2013 Parsons, Drakakaki and Heazlewood. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and any changes are noted.