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GolgiP: prediction of Golgi-resident proteins in plants

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ABSTRACT

Summary: We present a novel Golgi-prediction server, *GolgiP*, for computational prediction of both membrane- and non-membrane-associated Golgi-resident proteins in plants. We have employed a support vector machine-based classification method for the prediction of such Golgi proteins, based on three types of information, dipeptide composition, transmembrane domain(s) (TMDs) and functional domain(s) of a protein, where the functional domain information is generated through searching against the Conserved Domains Database, and the TMD information includes the number of TMDs, the length of TMD and the number of TMDs at the N-terminus of a protein. Using GolgiP, we have made genome-scale predictions of Golgi-resident proteins in 18 plant genomes, and have made the preliminary analysis of the predicted data.

Availability: The GolgiP web service is publically available at http://csbl1.bmb.uga.edu/GolgiP/

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

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1 INTRODUCTION

The Golgi apparatus is an essential cellular organelle found in most, if not all, eukaryotic cells, serving as an intermediate station of the secretory pathway that transports proteins out of a cell. Besides, Golgi is also a major site for protein post-translational modifications (e.g. glycosylation; Nilsson *et al*., 2009) and synthesis of various polysaccharides. The plant cell walls are mainly comprised of lignins, glycosylated proteins and polysaccharides, most of which are synthesized in Golgi (Lerouxel *et al*., 2006).

Identification of the Golgi-resident proteins represents a very challenging and a highly important problem for the understanding of the biological processes taking place in Golgi. Although there are 1183 mouse and human Golgi-resident proteins identified (Sprenger *et al*., 2007), only a little over 400 plant Golgi proteins have been experimentally identified. A key challenging issue is that plant Golgi proteins do not seem to have obvious targeting signals as proteins targeted at other cellular compartments, such as nucleus or extra-cellular space. Most of the existing computational tools for subcellular localization predictions are designed for the general subcellular localization prediction, and their predictions for Golgi-resident proteins are less than adequate (Sprenger *et al*., 2006). Only one program has been specifically designed for the prediction of Golgi-localized proteins but it focuses only on transmembrane Golgi proteins (Yuan and Teasdale, 2002). The issue is that only 25% of Golgi proteins of *Arabidopsis thaliana* are estimated to contain transmembrane regions (Schwacke *et al*., 2003), indicating the inadequacy of the current programs. Based on this consideration, we have designed a support vector machine (SVM)-based classifier, called GolgiP, to predict both Golgi-localized transmembrane proteins and non-transmembrane proteins. GolgiP currently provides multiple models for predicting plant Golgi proteins, based on the specific needs of a user.

2 METHODS AND DATASET

We have collected a large dataset comprising of 402 known Golgi proteins and 5703 known non-Golgi proteins of *A.thaliana* (91.2%), *Oryza sativa* (8.2%) and other plants (0.7%), from the SUBA (Heazlewood *et al*., 2007) and the UniProt (Apweiler *et al*., 2004) databases, as well as manually curated from the published literature. The non-Golgi proteins are proteins that have subcellular localization annotations, but not in Golgi according to the above databases. The redundant sequences in our dataset are removed by CD-hit using 95% sequence identity as the cut-off (Li and Godzik, 2006). Four-fifth of the data was used to train the classifier and the remaining one-fifth was used to test the trained classifier, where the dataset was randomly partitioned into training and test datasets.

To train an SVM-based classifier for Golgi proteins, we have examined three different sets of features, all computed from protein sequences. The first set of features is related to the dipeptide composition (DiAA). For each protein in our training set, we calculated the composition of dipeptides. The second set of features is related to transmembrane domains (TMDs). We used TMHMM (Krogh *et al*., 2001) and Phobius (Kall *et al*., 2004) to predict the number of TMDs, the average length of TMDs, the number of TMDs within the N-terminal region consisting of 70 amino acids, the length of the first TMD within the N-terminal region, and the orientation of the N-terminal (i.e. in the cytosol side or in the Golgi lumen side). The third set of features is related to functional domains (FunD). We searched proteins in our datasets against the Conserved Domains Database (CDD) using RPS-BLAST (Marchler-Bauer *et al*., 2009) with an *e*-value cutoff <0.01. We did this because the Golgi apparatus is where proteins get post-translational modifications such as glycosylation (Nilsson *et al*., 2009), and where the syntheses of most polysaccharides take place (Nilsson *et al*., 2009). In addition, Komatsu *et al*. (2007) found that the distributions of functional categories of proteins are different in different membranes such as plasma membrane, vascular membrane and Golgi membrane, respectively. Hence, it is expected that enzymes for the Golgi-related activities should be located in Golgi. The CDDs found for the Golgi proteins are then collected as the third set of features.

We applied the LIBSVM package (Fan *et al*., 2005) to train the classifier. We used the Radial Basis Function kernel, and tuned the cost (c) and gamma (γ) parameters to optimize the classification performance on the training dataset.

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Table 1. Evaluation of Golgi protein prediction tools

Tools	Sensitivity (%)	Specificity (%)	Accuracy (%)	MCC
Yuan	71.64	23.18	26.37	−0.03
WoLF PSROT	15.92	92.69	87.63	0.08
GolgiP-TMD	61.73	67.75	67.75	0.15
PSORT	43.53	83.10	80.49	0.17
GolgiP-DiAA	71.64	80.76	80.16	0.31
GolgiP-Comprehensive	72.84	98.42	96.73	0.73
GolgiP-FunD	57.50	100.00	97.21	0.75

The performances are sorted by MCC.

3 RESULTS AND DISCUSSION

We used the aforementioned three sets of sequence features, and trained three SVM classification models. Besides, we combined all three sets of features to train a comprehensive model. The training performances are shown in Supplementary Material.

We have compared the models with the other Golgi protein prediction tools, including PSORT (Nakai and Horton, 1999), WoLF PSORT (Horton *et al.*, 2007) and Yuan's Golgi predictor (Yuan and Teasdale, 2002) by using the testing dataset.

As shown in Table 1, Yuan's Golgi predictor has the good sensitivity but the lowest specificity and the lowest accuracy. PSORT and WoLF PSORT are two general subcellular localization prediction tools, and have moderate level of classification performance, which may not be adequate to serve as a plant Golgi protein predictor based on our analysis. Our program, GolgiP, exhibits the better overall performances with a higher accuracy and Matthews correlation coefficient (MCC).

We have applied GolgiP with the functional domain model to predict Golgi proteins on 18 selected fully sequenced plant genomes using the same cutoff. The reason we chose the functional domain model is that the model performs the best specificity, and therefore tends to avoid false positive results in this genome-wide prediction. The numbers and percentages of the predicted Golgi proteins in these organisms are shown in Table 2. Across algae, moss, monocot and dicot plants, the average percentages of predicted Golgi proteins is 7.25% among all the encoded proteins by these genomes. The stability in the percentage of the predicted Golgi proteins across different genomes indirectly suggests the reliability of our predictions. The trend of distribution of Golgi proteins from lower to higher plant species shows the similar percentage of Golgi proteins. This may suggest that the functionality of the Golgi apparatus has evolved and matured fairly early in the plant evolution.

In conclusion, we developed a Golgi protein prediction tool, GolgiP, and demonstrated its superior performance in predicting plant Golgi proteins over the existing prediction servers. In addition, our predictions across multiple plant genomes give an estimation of the percentage of plant Golgi proteins across different plant organisms, which is in general agreement with the previous estimations.

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Table 2. Application of the GolgiP program on 18 plant genomes

Clade	Species	# predicted Golgi proteins/# Total proteins	Percentage
Red algae	*Cyanidioschyzon merolae 10D*	430/5014	8.58
Green algae	*Micromonas pusilla CCMP1545*	716/10475	6.84
Green algae	*Micromonas strain RCC299*	833/9815	8.49
Green algae	*Ostreococcus lucimarinus*	706/7651	9.23
Green algae	*Ostreococcus tauri*	656/7725	8.49
Green algae	*Chlamydomonas reinhardtii*	982/14598	6.73
Green algae	*Volvox carteri f. nagariensis*	1025/15544	6.59
Moss	*Physcomitrella patens ssp patens*	2344/35938	6.52
Spike moss	*Selaginella moellendorffii*	2912/34697	8.39
Monocot	*Oryza sativa*	4240/67393	6.29
Monocot	*Brachypodium distachyon*	2446/32255	7.58
Monocot	*Sorghum bicolor*	2197/35899	6.12
Monocot	*Zea mays*	4748/75387	6.30
Dicot	*Vitis vinifera*	2008/30434	6.60
Dicot	*Arabidopsis thaliana*	2727/33410	8.16
Dicot	*Medicago truncatula*	1856/30028	6.18
Dicot	*Glycine max*	5262/75778	6.94

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Conflict of Interest: none declared.

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