Cell

Quantitative Proteomics Analysis of the Secretory Pathway

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SUMMARY

We report more than 1400 proteins of the secretory-pathway proteome and provide spatial information on the relative presence of each protein in the rough and smooth ER Golgi cisternae and Golgi-derived COPI vesicles. The data support a role for COPI vesicles in recycling and cisternal maturation, showing that Golgi-resident proteins are present at a higher concentration than secretory cargo. Of the 1400 proteins, 345 were identified as previously uncharacterized. Of these, 230 had their subcellular location deduced by proteomics. This study provides a comprehensive catalog of the ER and Golgi proteomes with insight into their identity and function.

INTRODUCTION

Proteomics has successfully mapped protein complements for several targets, including the nuclear pore complex in yeast (Rout et al., 2000) and clathrin-coated vesicles in rat brain and liver (Blondeau et al., 2004; Girard et al., 2005). There is evidence that these efforts have led to complete, accurate, and permanent catalogs: No additional proteins beyond those identified by Rout et al. (2000) have been identified as belonging to the nuclear pore complex, and the validity of each protein has been established. Beyond simple enumeration of the presence or absence of each individual protein in a sample, several studies (e.g., Blondeau et al., 2004; Foster et al., 2006; Kislinger et al., 2006) have also reported the relative abundance of each protein. One method gaining acceptance is to use the number of redundant peptides (obtained from tandem mass spectra) that identify a protein as a measure of the abundance of that protein. This opens up new avenues for the proteomics field. For example, the relative abundance of proteins can be clustered by similar expression profiles across tissues or organelles using standard clustering methods (Kislinger et al., 2006). Such techniques are used in microarray analysis and provide a convenient and visual way of understanding the overlap in protein composition and the associated distribution in relative abundance between compartments. This may point to common functions dictated by the compartment to which proteins cocluster.

A complete and accurate map of the secretory pathway remains elusive. For ER proteins, efforts such as HERA (Scott et al., 2004a) have compiled only 500 proteins related to folding chaperones, modifying enzymes, trafficking proteins, and signaling proteins through literature search and detailed curation. For Golgi proteins, the most comprehensive catalog contains 421 proteins characterized in Golgi fractions isolated from rat liver (Wu et al., 2004). However, the literature confirms the Golgi status of only 26% of these proteins.

This study represents a quantitative proteomic map of the rough ER, smooth ER, and Golgi apparatus isolated from rat liver homogenates. The use of highly enriched organelles and multiple biological replicates and the extension of the study to biochemical subfractions and organelle subcompartments as reported here provide experimental evidence indicating that COPI vesicles concentrate Golgi-resident proteins while largely excluding secretory cargo. In addition to uncovering 345 proteins of unknown function, these findings also open up new areas of investigation for the field.

RESULTS

Cell Map Strategy

A strategy was elaborated to map proteins to their intracellular locations using isolated subcellular organelles

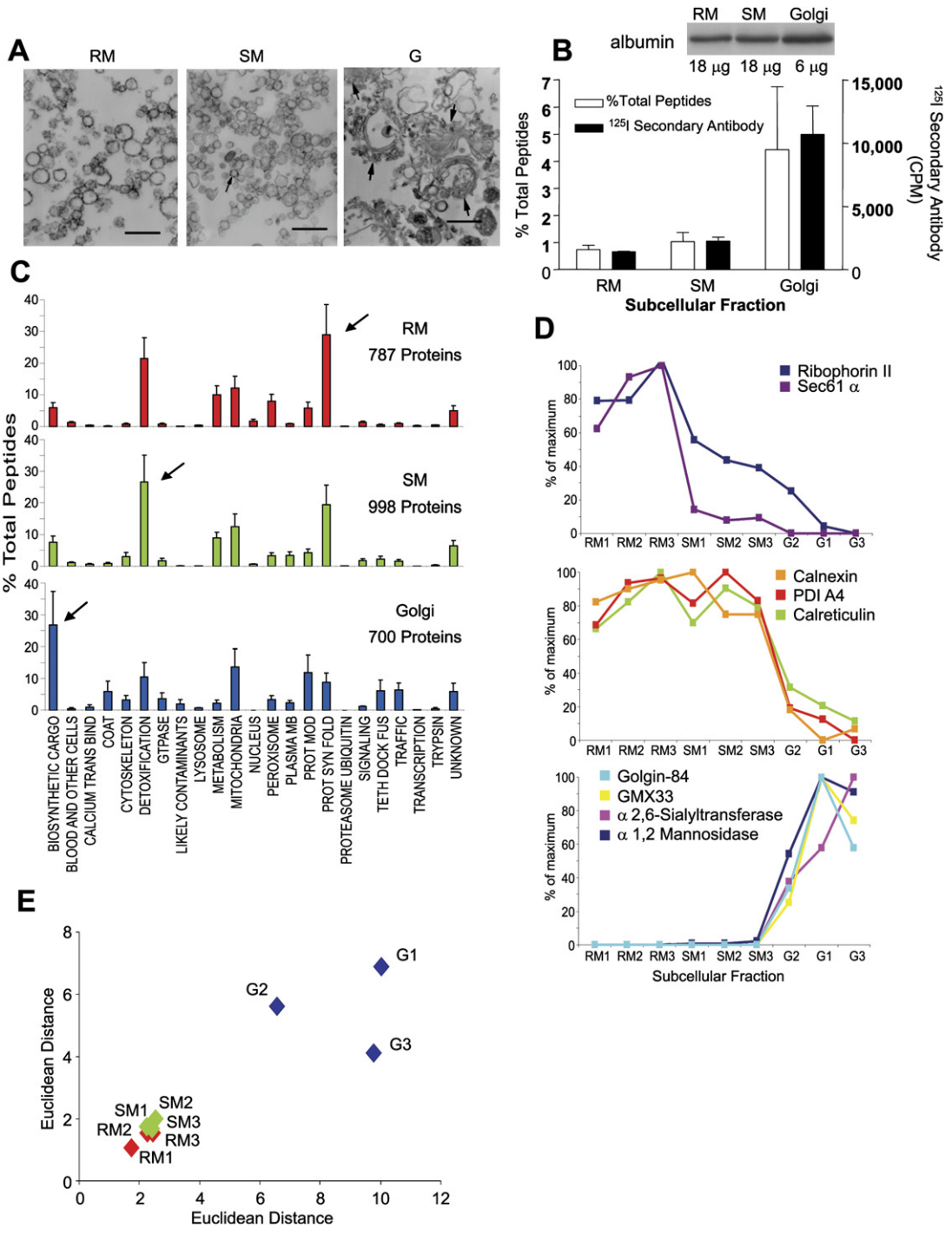

Figure 1. Proteomics of RM, SM, and Golgi Fractions Isolated from Rat Liver Homogenates

(A) EM characterization of random views of filtered preparations (Bell et al., 2001) of isolated rough microsomes (RM) with ribosome-studded microsomal profiles, smooth microsomes (SM) with a preponderance of smooth membranes but free polysomes (not shown) and occasional profiles showing ribosome-studded microsomes (arrow), and Golgi apparatus (G) with four different angles of section of Golgi profiles indicated by arrows. Scale bar = 0.5 µm.

(B) Comparison of redundant peptide counts with quantitative western blotting. The inset indicates the relative proportion of subcellular fractions within the linear response range for detecting albumin as visualized on X-ray films. The solid histograms represent the relative proportion of albumin antigenicity as deduced from γ counting (Bell et al., 2001) of the excised bands corresponding to albumin in RM, SM, and Golgi fractions. The white histograms represent peptide counts for albumin. In this and all other figures, error bars represent ±SD (n = 3) unless otherwise noted.