The zymogen granule (ZG) is the specialized organelle in pancreatic acinar cells for digestive enzyme storage and regulated secretion and has been a model for studying secretory granule functions. In an initial effort to comprehensively understand the functions of this organelle, we conducted a proteomic study to identify proteins from highly purified ZG membranes. By combining two-dimensional gel electrophoresis and two-dimensional LC with tandem mass spectrometry, 101 proteins were identified from purified ZG membranes including 28 known ZG proteins and 73 previously unknown proteins, including SNAP29, Rab27B, Rab11A, Rab6, Rap1, and myosin Vc. Moreover, several hypothetical proteins were identified that represent potential novel proteins. The ZG localization of nine of these proteins was further confirmed by immunocytochemistry. To distinguish intrinsic membrane proteins from soluble and peripheral membrane proteins, a quantitative proteomic strategy was used to measure the enrichment of intrinsic membrane proteins through the purification process. The iTRAQ™ ratios correlated well with known or Transmembrane Hidden Markov Model-predicted soluble or membrane proteins. By combining subcellular fractionation with high resolution separation and comprehensive identification of proteins, we have begun to elucidate zymogen granule functions through proteomic and subsequent functional analysis of its membrane components. 

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Pancreatic acinar cells are the functional units of digestive enzyme synthesis, storage, and secretion and have been a classical model for studying regulated exocytosis (1, 2). In acinar cells digestive enzymes are stored in a specialized organelle, the zymogen granule (ZG). Stimulation of acinar cells by secretagogues triggers fusion of ZG membranes with the apical membrane and the subsequent release of digestive enzymes. Early studies using SDS-PAGE indicated a relatively simple protein structure for the ZG membrane. Several more recent studies using 2D GE led to the identification of two additional major ZG membrane components, GP3 (3) and membrane dipeptidase (4). In another study, 14 spots were identified as small GTPases by [35S]GTPγS overlay on a 2D gel of ZG membrane proteins, but the identities of the spots remained unknown (5). In addition, a number of low abundance proteins, including Rab3D and several SNARE proteins, have been identified on ZG membranes by immunoblotting and immunocytochemistry (2). Despite its importance, a comprehensive proteomic analysis of the membrane protein components of ZGs has not been achieved.

By combining 2D GE and 2D LC with tandem mass spectrometry, we report the identification of 101 proteins on ZG membranes, many of which had not been localized previously on ZGs including multiple small GTP-binding proteins, SNARE proteins, and molecular motor proteins. In addition, to distinguish intrinsic membrane proteins from peripheral and soluble proteins, a quantitative proteomic strategy was used to measure the relative abundances of ZG proteins during membrane purification. The intrinsic membrane proteins characterized based on iTRAQ™ (6) ratios correlated well with known or TMHMM-predicted membrane proteins.

EXPERIMENTAL PROCEDURES

Isolation of ZGs and Purification of ZG Membranes—A previously validated Percoll gradient procedure was utilized (7). Briefly, rat pancreata were homogenized in ice-cold buffer containing 0.25 M sucrose, 25 mM MES, 2 mM EGTA (pH 6.0). Homogenates were centrifuged at low speed to generate a crude particulate enriched in ZGs. The particulate was resuspended in homogenization buffer and mixed with an equal volume of Percoll. The mixture was centrifuged at 60,000 × g for 20 min. The dense white ZG band was collected and washed with homogenization buffer. To purify ZG membrane, the isolated ZGs were lysed with nigericin and centrifuged at 100,000 × g for 1 h. The membrane pellet was washed with 250 mM KBr and then 0.1 M Na2CO3 (pH 11.0) each for 1 h.

Immunocytochemistry and Confocal Microscopy—Sample preparation and methods for immunostaining of isolated rat acini and ZGs were performed as described previously (8, 9). Dilutions of primary rabbit antibodies were as follows: Rab27B and Rab11A, 1:500–1:1000; Rab6, myosin Vc, and VAMP 2, 1:50–1:100; SNAP29, 1:800–1:1200; Rap1, 1:200–1:400. Secondary antibody was a 1:200 dilution of Cy3-conjugated donkey anti-rabbit IgG (Jackson Immunoresearch Laboratory, West Creek, VA).
Laboratories, Inc.), which also contained 0.17 μM Oregon Green-conjugated phalloidin to counterstain actin. 4',6-Diamidino-2-phenylindole (DAPI) was added to mounting medium to counterstain nuclei. Immunostained samples were viewed with Zeiss LSM 510 and Olympus FluoView 500 confocal microscopes, and digitized images were processed using Photoshop 6.0 software.

RESULTS

The ZG membrane purification and iTRAQ tagging are outlined in Fig. 1, left. The high purity of isolated ZGs using Percoll gradient was confirmed in a previous study by electron microscopy and enrichment of the ZG enzyme marker amylase (7). In the current study, a similar degree of amylase enrichment was achieved, and the ZGs were highly homogeneous as indicated by Nomarski images and positive staining for the known ZG membrane marker Rab3D (Fig. 1, right). To characterize the ZG membrane proteome, 2D gel maps were generated for Na2CO3-washed ZG membrane. In a representative gel, about 130 spots were visualized among which 61 were identified by tandem mass spectrometry (Supplemental Fig. 1). These 61 spots represented 29 unique proteins (Table I). To uncover additional ZG membrane proteins, the same samples were also run on one-dimensional gels as well as broad range and basic 2D gels (data not shown). Sixteen additional proteins were identified from these gels, the majority of which were basic proteins. Altogether a total of 45 unique proteins were identified by the gel-based proteomic analysis (supplemental table).

To distinguish intrinsic ZG membrane proteins from other identified proteins, a quantitative proteomic strategy was implemented using iTRAQ reagents and 2D LC separation (Supplemental Fig. 2). The peak areas of reporter ions, 114, 116, and 117 iTRAQ™ reagents, respectively, were measured. Because of their enrichment through wash steps, it is expected that intrinsic ZG membrane proteins will have 117:114 ratios greater than one, whereas soluble or peripheral membrane proteins will have ratios less than one. A representative MS/MS spectrum of an iTRAQ-labeled peptide from the intrinsic membrane glycoprotein GP2 is shown in Supplemental Fig. 3. The iTRAQ ratios of all proteins in Table I with at least two independent measurements are summarized in Table II. These proteins are classified in two groups with 117:114 ratios in group 1 less than one (not membrane proteins) and in group 2 greater than one (membrane proteins). In another column, TM domains, the presence or absence of transmembrane structures in these proteins is summarized based on literature or predictions using TMHMM (10). It was found that the two columns, 117:114 ratios and TM domains, correlated very well for the characterization of intrinsic membrane proteins. The only exception in group 1, syncollin, has been reported to be washed off ZG membranes when incubated with 0.1 M Na2CO3 (11). In group 2, 18 proteins had either transmembrane domains or...
| Protein name                                      | NCBI accession no. | Molecular weight | pl  | 2D GE<sup>a</sup> | 2D GE<sup>b</sup> | 1D GE | 2D LC |
|--------------------------------------------------|--------------------|------------------|-----|-------------------|-------------------|-------|-------|
| Proton pumps and ion channels                    |                    |                  |     |                   |                   |       |       |
| ATP synthase α chain                             | 114523             | 63,493           | 9.22|x                  |                   |       |       |
| ATP synthase β chain                             | 54792127           | 59,786           | 5.14| 22, 23            |                   |       |       |
| Vacuolar-type H<sup>+</sup>-ATPase 115-kDa subunit, a1 isoform | 13928826 | 102,385.04 | 6.04 | x | | |
| Voltage-dependent anion channel 1                | 4873487            | 36,013           | 8.31|x | | | |
| Voltage-dependent anion channel 2                | 13786202           | 35,690           | 7.44|x | | | |
| Enzymes                                          |                    |                  |     |                   |                   |       |       |
| Acid sphingomyelinase-like phosphodiesterase 3A | 53850608           | 49,701           | 5.97| 19, 20            |                   |       |       |
| Dipeptidase 1                                    | 16758372           | 48,023           | 5.68| 7, 8, 25–30       |                   |       |       |
| γ-Glutamyl transpeptidase (heavy chain)          | 16758696           | 41,515           | 7.66|x | | | |
| γ-Glutamyl transpeptidase (light chain)          | 16758696           | 20,059           | 6.47| 57, 58            |                   |       |       |
| Phosphodiesterase 3                              | 9506849            | 101,041          | 5.93| 12–14             |                   |       |       |
| Protein-disulfide isomerase precursor            | 129731             | 64,731           | 4.82| 21                |                   |       |       |
| Content proteins                                 |                    |                  |     |                   |                   |       |       |
| α-Amylase                                        | 62644218           | 51,020           | 8.42| x                 |                   |       |       |
| Anionic trypsin precursor                        | 67548              | 28,363           | 4.69|x | | | |
| Caldecrin precursor (chymotrypsin c)             | 1705913            | 30,919           | 5.64| 43, 45, 51        |                   |       |       |
| Carboxypeptidase A1 precursor                    | 8393183            | 50,282           | 5.38|x | | | |
| Carboxypeptidase A2 precursor                    | 61556903           | 50,269           | 5.17|x | | | |
| Chymotrypsin B precursor                         | 6978717            | 25,934           | 4.90| 48                |                   |       |       |
| Colipase                                         | 203503             | 13,597           | 8.04|x | | | |
| Elastase 2 precursor                             | 6978803            | 27,274           | 8.81|x | | | |
| Elastase 3B precursor                            | 62648990           | 30,806           | 5.47| 49, 50            |                   |       |       |
| Pancreatic lipase                                | 1865644            | 54,494           | 6.6 | | | | |
| Sterol esterase                                  | 1083805            | 72,537           | 5.37| 16, 17, 39        |                   |       |       |
| Small GTP-binding proteins                       |                    |                  |     |                   |                   |       |       |
| Rab1                                             | 56605816           | 25,670           | 5.95|x | | | |
| Rab3D                                            | 18034781           | 26,332           | 4.75| 59                |                   |       |       |
| Rab6                                             | 62654200           | 23,590           | 5.42|x | | | |
| Rab8A                                            | 49522647           | 26,686           | 9.15|x | | | |
| Rab11A                                           | 2463536            | 24,509           | 5.85| 54, 56            |                   |       |       |
| Rab14                                            | 420272             | 24,078           | 5.85| 55                |                   |       |       |
| Rab27B                                           | 16758202           | 27,382           | 5.38| 52, 53            |                   |       |       |
| Rac1                                             | 54607147           | 24,326           | 8.77|x | | | |
| Rap1                                             | 52138628           | 21,201           | 5.37|x | | | |
| Vesicular trafficking                            |                    |                  |     |                   |                   |       |       |
| 21-kDa transmembrane trafficking protein         | 3915137            | 25,994           | 6.03|x | | | |
| Cysteine string protein                          | 1095322            | 24,892           | 4.93|x | | | |
| Dynactin 2                                       | 50926127           | 44,148           | 5.14| 24                |                   |       |       |
| Exocyst complex component Sec8                   | 24418659           | 120,821          | 6.3 | | | | |
| Myosin Vc                                        | 62653910           | 228,341          | 8.17|x | | | |
| SNAP29                                           | 7769720            | 29,000           | 5.4 | 42                | | | |
| VAMP 2                                           | 51704188           | 12,691           | 7.84| 60                | | | |
| VAMP 8                                           | 13929182           | 12,512           | 8.93|x | | | |
| Matrix and glycoproteins                         |                    |                  |     |                   |                   |       |       |
| Clusterin                                        | 46048420           | 56,070           | 5.53| 18                |                   |       |       |
| GP2                                              | 121538             | 62,355           | 4.9 | 1–4               |                   |       |       |
| GP3                                              | 17105374           | 58,695           | 6.03| 32–37             |                   |       |       |
| Lysosomal associated membrane protein 2          | 40254785           | 48,941           | 8.02| 9, 10             |                   |       |       |
| Pancreatic lipase-related protein 1              | 14091772           | 57,122           | 5.79|x | | | |
| Proteoglycan 2                                   | 13928848           | 25,570           | 5.78|x | | | |
| Syncollin                                        | 20806121           | 17,780           | 8.61| x                 |                   |       |       |
| ZG16                                             | 19705541           | 17,316           | 9.79|x | | | |
| Others                                           |                    |                  |     |                   |                   |       |       |
| Glucose-regulated protein, 58 kDa                | 38382858           | 57,044           | 5.88| 31                | | | |
| Integral membrane-associated protein 1           | 5916203            | 72,874           | 6.07| 5, 6              | | | |

**Table I**

*Functional categories of ZG membrane proteins identified from both gel-based and LC-based separations*

NCBI, National Center for Biotechnology Information; 1D, one-dimensional.
The high 117:114 ratio of ubiquitin may suggest that some ZG membrane proteins were preferentially ubiquitinated. However, the specific proteins that are ubiquitinated have not been identified. The very large 117:114 ratio observed for myosin Vc indicates that it is very strongly bound to the ZG. The mechanism of this strong attachment is unknown and requires further investigation. In contrast to 117:114 ratios, the 116:114 ratios were less informative in distinguishing membrane from soluble proteins, reflecting the fact that the KBr wash did not remove proteins from crude ZG membranes very efficiently.

In addition to the quantitative results, the 2D LC experiments also led to the identification of more proteins than from 2D gels. In these experiments, a total of 2,498 peptides were fragmented for MS/MS analysis, and 630 peptides were assigned to 84 non-redundant proteins. By combining the LC- and gel-based approaches, a total of 101 proteins was identified from purified ZG membranes among which 17 proteins were uniquely identified by the gel-based approach, 56 were uniquely identified by the LC-based approach, and 28 were identified by both approaches. The complete list of identified proteins is summarized in the supplemental table. After ex-identification by both approaches. The complete list of identified proteins was included that verified our approach. In addition, a large number of proteins previously unknown on ZG membranes were also identified including several hypothetical proteins such as Similar to c20orf178.

The new discoveries come primarily from the small GTP-binding proteins and vesicular trafficking categories. These include eight small G proteins, Rab1, Rab6, Rab8A, Rab14, Rab11A, Rab27B, Rac1, and Rap1; one SNARE protein, SNAP29; and the molecular motor protein myosin Vc. Six of these proteins were further confirmed by immunocytochemistry. In isolated acini, Rab27B was localized throughout the granule region as described previously (9). SNAP29 and myosin Vc shared this distribution (Fig. 2, left). Although Rap1 was present in most of the ZG area, staining appeared more intensive in the deeper ZG regions, likely including Golgi elements. In contrast, Rab6 was sharply confined to the deepest region of the apical ZGs, and the reticular nature of the staining suggested localization primarily to Golgi. Although Rab11A was present to varying degrees throughout the ZG region, intense staining was generally seen immediately proximal to the luminal area. The presence of Rap1, Rab11A, Rab6, VAMP 2, and Rab27B on ZGs was confirmed by immunolocalization at the purified ZG level. As shown in Fig. 2, right, essentially all ZGs were stained with Rab27B or Rap1 antibodies, whereas only a moderate number showed strong staining for Rab11A, and just a few granules were clearly stained for Rab6.

**DISCUSSION**

Here we report the first comprehensive proteomic study of ZG membrane proteins with the identification of 101 proteins. As a verification of our approach, 28 previously reported ZG membrane proteins were identified in this study including the low abundance SNARE proteins VAMP 2 and VAMP 8 (12) but not Syntaxin 3, another SNARE protein localized on ZG membrane previously by immunocytochemistry (13). Seventy-three new proteins were identified on ZG membrane for the first time, nine of which were confirmed by immunocytochemistry at both the isolated acini and ZG levels. Despite the high

**TABLE I—continued**

| Protein name                      | NCBI accession no. | Molecular weight | pl  | 2D GE\(^a\) | 2D GE\(^b\) | 1D GE | 2D LC |
|-----------------------------------|--------------------|------------------|-----|-------------|-------------|-------|-------|
| Prohibitin                        | 13937353           | 29,820           | 5.57| 44, 46, 47   | x           | x     |       |
| Protein transport protein SEC61 β subunit | 27714473          | 10,749           | 11.57|             |             | x     |       |
| Signal sequence receptor, δ       | 8394364            | 20,016           | 5.5  | 61          |             | x     |       |
| Signal sequence receptor, α       | 57114346           | 37,186           | 4.35 |             |             | x     |       |
| Similar to osmotic stress protein | 34856875           | 165,550          | 6.29 |             |             | x     |       |
| Similar to stomatin-like protein 2 | 34867290           | 26,294           | 5.63 | 38          |             | x     |       |
| Ubiquitin                         | 1050930            | 12,675           | 5.43 |             |             | x     |       |
| Unknown                           |                    |                  |     |             |             |       |       |
| Similar to c20orf178              | 28077049           | 24,941           | 4.76 | 40, 41      | x           |       |       |
| Similar to hypothetical protein MGC10854 | 34872667          | 87,656           | 6.49 | 11          |             |       |       |
| Similar to RIKEN cDNA D030041N15  | 34868591           | 74,901           | 7.01 | 15          |             |       |       |
| Similar to RIKEN cDNA D130054N24 | 62653608           | 76,796           | 6.54 |             |             | x     |       |

\(^a\) Proteins identified by 2D GE using IPG strips, pH 4–7; corresponding spot numbers in Supplemental Fig. 1 are listed.

\(^b\) Proteins identified by 2D GE using IPG strips pH 3–10 or 7–11 nonlinear are labeled with “x” (gel images are not shown).
Pancreatic Zymogen Granule Membrane Proteins

The number of transmembrane (TM) domains is listed in the column. The transmembrane domains are either predicted by TMHMM or known from the database or literature. The known transmembrane domains or posttranslational modifications are included in parentheses.

| Protein name | NCBI accession no. | No. of spectra | Ratios (mean ± S.D.) | TM domains* |
|--------------|-------------------|----------------|----------------------|-------------|
| ATP synthase β chain | 54792127 | 6 | 0.75 ± 0.19 0.24 ± 0.09 | 0 |
| GP3 | 17105374 | 21 | 0.88 ± 0.29 0.43 ± 0.15 | 0 |
| Collipase | 203503 | 11 | 1.11 ± 0.08 0.44 ± 0.16 | 0 |
| Pancreatic lipase | 1865644 | 21 | 0.96 ± 0.05 0.44 ± 0.05 | 0 |
| Sterol esterase | 1083805 | 17 | 0.86 ± 0.14 0.47 ± 0.21 | 0 |
| Anionic trypsin precursor | 67548 | 4 | 0.6 ± 0.28 0.49 ± 0.16 | 0 |
| Syncollin | 20806129 | 9 | 0.77 ± 0.10 0.52 ± 0.10 | 1 |
| α-Amylase | 62644218 | 26 | 0.72 ± 0.12 0.60 ± 0.17 | 0 |
| ATP synthase α chain | 114523 | 8 | 1.03 ± 0.10 0.69 ± 0.20 | 0 |
| Carboxypeptidase A1 precursor | 8393183 | 8 | 1.12 ± 0.12 0.70 ± 0.30 | 0 |
| Carboxypeptidase A2 precursor | 6156903 | 3 | 1.15 ± 0.21 0.70 ± 0.25 | 0 |
| ZG16 | 19705541 | 6 | 1.09 ± 0.20 0.70 ± 0.15 | 1 |
| Elastase 3B precursor | 62649890 | 6 | 1.52 ± 0.11 0.73 ± 0.22 | 0 |
| Calcecin precursor (chymotrypsin c) | 1705913 | 2 | 1.20 ± 0.10 0.79 ± 0.31 | 0 |
| Protein-disulfide isomerase precursor | 129731 | 2 | 1.10 ± 0.19 0.85 ± 0.16 | 0 |
| Pancreatic lipase-related protein 1 | 14091772 | 29 | 0.98 ± 0.12 0.87 ± 0.22 | 0 |
| Clusterin | 46048420 | 3 | 1.10 ± 0.17 0.93 ± 0.10 | 0 |
| Rab27B | 16758202 | 7 | 0.99 ± 0.10 1.35 ± 0.13 | 1 (prenyl) |
| Rac1 | 54607147 | 3 | 1.05 ± 0.11 1.40 ± 0.15 | 1 (prenyl) |
| 21-kDa transmembrane trafficking protein | 3913137 | 2 | 1.23 ± 0.30 1.40 ± 0.19 | 1 |
| Similar to osmotic stress protein | 3485875 | 6 | 1.10 ± 0.14 1.48 ± 0.47 | 2 |
| Ubiquitin | 1050930 | 5 | 1.31 ± 0.18 1.54 ± 0.32 | 0 |
| Vacuolar-type H+-ATPase 115-kDa subunit, a1 isofrom | 13928826 | 7 | 1.13 ± 0.23 1.74 ± 0.14 | 6 |
| Lyosomal associated membrane protein 2 | 40254785 | 2 | 1.25 ± 0.23 1.85 ± 0.31 | 1 (GPI) |
| γ-Glutamyl transpeptidase | 16758696 | 19 | 1.27 ± 0.19 2.02 ± 0.33 | 1 |
| Rab8A | 49522647 | 6 | 1.24 ± 0.15 2.12 ± 0.76 | 1 (prenyl) |
| Rab6 | 62654200 | 6 | 1.19 ± 0.18 2.35 ± 0.73 | 1 (prenyl) |
| Rab1 | 5665816 | 12 | 1.34 ± 0.26 2.37 ± 0.75 | 0 (prenyl) |
| Signal sequence receptor, α | 57114346 | 3 | 0.98 ± 0.25 2.68 ± 1.20 | 1 |
| RAB3D | 18034781 | 8 | 1.48 ± 0.06 2.94 ± 0.10 | 1 (prenyl) |
| GP2 | 121538 | 28 | 1.64 ± 0.67 3.06 ± 1.11 | 1 (GPI) |
| Protein transport protein SEC61 β subunit | 27714473 | 4 | 1.27 ± 0.11 3.13 ± 0.60 | 1 |
| Integral membrane-associated protein 1 (Itmap 1) | 5916203 | 11 | 0.94 ± 0.20 3.24 ± 0.35 | 2 |
| Dipetidase | 16758372 | 7 | 1.23 ± 0.17 3.30 ± 1.10 | 1 |
| Voltage-dependent anion channel 2 | 13786202 | 2 | 1.48 ± 0.21 3.48 ± 0.48 | 1 (β-sheet) |
| Voltage-dependent anion channel 1 | 48734887 | 5 | 1.50 ± 0.21 3.58 ± 0.62 | 1 (β-sheet) |
| Myosin Vc | 62659310 | 7 | 1.10 ± 0.10 3.67 ± 0.71 | 0 |

*The number of transmembrane (TM) domains is listed in the column. The transmembrane domains are either predicted by TMHMM or known from the database or literature. The known transmembrane domains or posttranslational modifications are included in parentheses.*

The newly identified ZG membrane proteins provide new directions for studying the molecular mechanisms of ZG trafficking and regulated exocytosis. According to the current model for regulated exocytosis, secretory vesicles are regulated at multiple steps during vesicular transport from the Golgi network to the plasma membrane. It is generally believed that two families of proteins govern this process with the SNARE proteins mediating plasma membrane docking and probably also fusion of secretory vesicles with plasma membrane and at least one specific Rab protein regulating each vesicular transport step (14). Although it is believed that ZGs share the above common mechanisms with other secretory vesicles, most of the players in this model have been missing for ZGs including most of the Rab proteins and the complete set of SNARE complexes.

Rab3D has been the only candidate for regulating ZG exocytosis (15), although recently stimulated amylase release was reported to be normal in Rab3D-deficient mice (16), implying the presence of additional Rab(s) to regulate ZG exocytosis. Here Rab27B, the closest homologue of Rab3, was identified on ZG membranes for the first time and was further demonstrated to play a positive role in regulating acinar exocytosis in a separate study (9). It is of interest that Rab27A was found in a complex with myosin Va in melanosomes and thus tethered melanosomes to the apical actin cytoskeleton (17). The fact that myosin Vc was also identified...
on ZG membranes in this study leads us to hypothesize that myosin Vc and Rab27B may form a complex and thus tether ZGs at the apical actin web. While Rab27B is very likely responsible for ZG tethering, Rab6 may play a role in budding from Golgi or in regulating ZG transport along microtubules; Rab11A, based on work in other cell types, is possibly in-

Fig. 2. Immunolocalization of novel proteins identified from ZG membranes. ZG localization of a subset of the novel proteins was demonstrated by immunocytochemistry and confocal microscopy using corresponding antibodies (red). The subluminal actin is stained with Oregon Green-conjugated phalloidin, and nuclei are stained with 4’,6-diamidino-2-phenylindole (DAPI) (blue). Left, immunostaining of isolated acini for Rab27B, SNAP29, myosin Vc, Rap1, Rab6, and Rab11A. Right, immunostaining of purified isolated ZGs for Rab27B, VAMP 2, Rap1, Rab6, and Rab11A. ZG fluorescence images are paired with the corresponding Nomarski images.
involved in ZG membrane recycling after exocytosis. The fact that Rab6 and Rab11A localized to only a fraction of ZGs may indicate that different Rabs target to ZGs at different stages in the secretory pathway. The roles of Rab8 and Rab14 are currently unknown, although it was recently reported that Rab8 was partially associated with mature melanosomes and regulated actin-dependent movement of melanosomes (18). In addition to the small G proteins, a novel SNARE protein, SNAP29, was identified on ZGs. SNAP29 has been considered to be a ubiquitous cytoplasmic SNARE protein involved in general membrane trafficking steps (19), and its role in ZG exocytosis needs to be further investigated.

In summary, the catalog of protein components comprising the ZG membrane described in this study evokes a range of new hypotheses regarding mechanisms of ZG function and will aid future efforts to identify higher order architectural components of the ZG, including protein complexes, leading the field to a better understanding of ZG architecture and functions.

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