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GP-2/THP gene family encodes self-binding glycosylphosphatidylinositol-anchored proteins in apical secretory compartments of pancreas and kidney

(pH- and ion-dependent self-association/protein sorting/condensation/secretory granule/vesicle/regulated apical secretion)

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ABSTRACT **A family of homologous genes is shown to encode GP-2, the major glycosylphosphatidylinositol (GPI)-linked glycoprotein of pancreatic zymogen granule membranes, and Tamm–Horsfall protein (THP), a GPI-linked glycoprotein associated with apical vesicles in kidney thick ascending limb of Henle (TALH) cells. The C-terminal regions of GP-2 (Asp^{54}–Phe^{530}) and THP (Asp^{175}–His^{644}) from rat show 53% identity, 86% similarity, and 26 conserved cysteine residues including one epidermal growth factor motif. The unique N-terminal domain of rat THP (unique-THP, Pro^{29}–Gln^{174}) shows four conserved epidermal growth factor motifs, three in tandem and one in reverse orientation. GP-2 homologues are observed in a wide variety of epithelial cells, several of which contain highly regulated secretory processes. GP-2 released from zymogen granule membranes with phosphatidylinositol phospholipase C reacts with anti-cross-reactive determinant antibody (anti-CRD), confirming the GPI nature of the pancreatic homologue. In contrast, GP-2 and THP, released endogenously from pancreas and kidney, respectively, do not react with anti-cross-reactive determinant antibody, suggesting alternative enzymatic mechanisms for their physiological release. Globular domains of GP-2 and THP, but not albumin, show pH- and ion-dependent self-association *in vitro*. The GP-2/THP family appears to represent a newly discovered class of GPI-anchored proteins, which may utilize pH- and ion-dependent self-association mechanisms for establishing membrane (micro)domains targeted to intracellular secretory compartments.**

A growing number of hydrophilic proteins have recently been shown to be linked to membranes via a glycosylphosphatidylinositol (GPI) anchor (1, 2). Demonstrating diverse activities in mammalian cells, including cell adhesion, T-cell activation, and hydrolytic activities, GPI-anchored proteins are normally found attached to the outer surface of the plasma membrane. In polarized Madin–Darby canine kidney cells in culture, endogenous GPI-anchored and phosphatidylinositol phospholipase C (PI-PLC)-releasable proteins appeared to be restricted to the apical plasma membrane (3). In some cell lines, the surface expression and release of GPI-linked proteins is modulated by serum starvation or insulin (4). However, it is not clear why these proteins are tethered to membranes by GPI–lipid anchors or what role might be served by their potential release from the membrane (1).

We have recently cloned and characterized rat (5) and dog GP-2 (6), the major glycoprotein of zymogen granule membranes (ZGMs) in the exocrine pancreas, and demonstrated that the membrane form of GP-2, targeted to apical plasma membranes via regulated exocytosis, is attached to the ectoleaflet of granule membranes by a GPI anchor. After granule assembly, we have also shown that GP-2 is released to the granule content fraction by a pH-dependent anchor-cleavage activity associated with granule membranes (6). However, the function of GP-2 in ZGMs has remained obscure and its release from granule membranes into apical ductular secretion in a high molecular weight aggregated form (7–10) is poorly understood.

Search of the GenBank data base revealed that the amino acid sequence of canine pancreatic GP-2 is similar to Tamm–Horsfall protein (THP) produced by human kidney (11, 12). Despite identification of THP as the most abundant (glyco)-protein in urine [50–100 mg excreted in normal human urine daily (13, 14)], numerous proposals regarding its putative function over the past 4 decades (15–19), and elucidation of the complete peptide sequence by gene cloning studies (15, 20), the function of THP has remained enigmatic (15). Since GP-2 and THP are present in cells demonstrating regulated protein secretion [pancreatic acinar (21)] and regulated ion transport [kidney thick ascending limb of Henle (TALH) cells (22)], respectively, we explored whether the GP2/THP sequence similarity might identify a generalized role for these molecules in regulated vesicular function at the apical plasma membrane.

MATERIALS AND METHODS

Isolation and Characterization of Rat THP cDNA. Rat THP cDNA was isolated from a rat kidney λgt10 cDNA library with a 550-base-pair (bp) *Bam*HI/*Hin*dIII fragment from plasmid UM19 (15) containing the human THP cDNA. Hybridization was conducted at 55°C in 1 M NaCl/10% dextran sulfate/1% SDS. A final wash was conducted at 55°C in 2× standard saline citrate (SSC) buffer. DNA sequences were determined in both orientations by the dideoxynucleotide chain-termination method with deoxyadenosine 5′ [α-[^{35}S]thio] triphosphate and Sequenase (23) (United States Biochemical). Overlapping clones were constructed by using pBluescript vectors (Stratagene) and nested sets of deletions prepared with exonucleases III and VII (24).§

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Abbreviations: THP, Tamm–Horsfall protein; ZG, zymogen granule; ZGM, zymogen granule membrane; GPI, glycosylphosphatidylinositol; PI-PLC, phosphatidylinositol phospholipase C; TALH, thick ascending limb of Henle; EGF, epidermal growth factor; CRD, cross-reacting determinant.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M63510).

Southern Blot Analysis. Forty micrograms of genomic DNA isolated from rat spleen was digested with 300 units of *Eco*RI or 250 units of *Bam*HI in reaction volumes of 400 μl for 20 hr. Digested DNA was electrophoresed in a 0.2% agarose gel (15 × 20 cm) at 4°C and 10 V for 24 hr and vacuum blotted to nylon membranes at 22°C for 45 min using an LKB model 2016 Vacugene apparatus. Nylon membranes were hybridized with rat GP-2 [2.0-kilobase pair (kbp) *Eco*RI/*Eco*RI fragment] or rat THP cDNA (2.0-kbp *Sac* I/*Sac* I fragment) (labeled with ^{32}P to 10^9 cpm/μg by random priming) as described (25) and washed under stringent conditions (final wash, 65°C, 0.1× SSC, 30 min).

Northern Blot Analysis. Total RNA was isolated from canine tissues by guanidinium thiocyanate (26) and poly(A)$^+$ mRNA was isolated by oligo(dT)-cellulose chromatography (27). Denatured total RNA (40 μg) and polyadenylylated RNA (5 μg) were electrophoresed in 1% agarose/40 mM Tris base/40 mM glacial acetic acid/2 mM EDTA (TAE) gels (15 × 10 cm) for 2 hr and vacuum blotted to nylon. Membranes were hybridized with dog GP-2 (2.1-kbp *Hin*dIII/*Hin*dIII fragment; Fig. 1*A*) or human THP cDNA probes (2.9-kbp *Eco*RI/*Eco*RI fragment; Fig. 1*B*) (labeled with [^{32}P]dCTP to 10^9 cpm/μg by random priming) and washed under various conditions from low (final wash, 65°C, 2× SSC, 30 min) to high [final wash, 65°C, 0.2× SSC, 30 min (three times)] stringency.

Enzymatic Release of Globular GP-2 and THP. ZGs were purified from dog pancreas on self-forming Percoll gradients and membranes were lysed and washed in 100 mM Na_2CO_3 (pH 11.2) as described (6). Globular GP-2 was released from purified ZGMs by incubation of membranes (20 μg/ml) in 50 μl of 20 mM Mes (pH 7.0) containing 80 mM KCl, 0.05 μg of PI-PLC (ICN), and trypsin inhibitors (aprotinin, 20 units/ml; FOY-305, 40 μg/ml). Membranes were sedimented by ultracentrifugation at 220,000 × *g* for 30 min. For studies that required GP-2 endogenously released from pancreatic ZGs, dog ZGs were permeabilized with saponin (45 μg/ml, below the critical micellar concentration) and incubated for 60 min at 37°C as described (6). Membrane-bound and free products were separated by sedimentation at 220,000 × *g* for 30 min. THP, endogenously released from human kidney, was purified from urine by two cycles of precipitation in the presence of 570 mM NaCl and was solubilized by dialysis against 10 mM Tris·HCl (pH 8.0) as described (13).

Immunolabeling Procedures. Western blots on Immobilon were probed, as indicated, with monospecific antisera to human THP (United States Biochemical) or monospecific rabbit antisera to dog GP-2 or cross-reacting determinant (CRD) (affinity purified; gift from Ann Gurnett, Merck Sharp & Dohme, Philadelphia). Anti-CRD antibody identifies a terminal phosphate group on the inositol ring (PLC cleavage product). Second-step reagents were either ^{125}I-labeled goat anti-rabbit IgG (ICN) or alkaline phosphatase-labeled rabbit anti-goat IgG (Bio-Rad).

pH- and Ion-Dependent Self-Association of Globular GP-2 and THP. Globular GP-2 and THP were prepared as described above and incubated under buffer and ionic conditions as indicated. Protease inhibitors (FOY-305, 100 μg/ml; phenylmethylsulfonyl fluoride, 0.1 mM) were present in all incubations. Reaction mixtures were then centrifuged at 220,000 × *g* at 20°C for 90 min (GP-2) or at 13,000 × *g* at 20°C for 30 min (THP). Supernatant and pellet fractions were adjusted to equal volumes and analyzed by SDS gel electrophoresis (10% acrylamide) and silver nitrate staining.

RESULTS AND DISCUSSION

Pancreatic GP-2 and Kidney THP Constitute Members of a Gene Family. To determine whether GP-2 and THP are encoded by separate or overlapping genes, we cloned and characterized rat THP to allow critical sequence comparisons between GP-2 and THP in a single species. Fig. 1*A* shows canine and rat GP-2 amino acid sequences aligned with rat and human THP sequences. Canine and rat GP-2 sequences show 69% identity and 92% similarity. Rat and human THP sequences show 77% identity and 95% similarity. Over the C-terminal sequences observed between Asp^{54} and Phe^{530} in the rat GP-2 sequence and Asp^{175} and His^{644} in the rat THP sequence, GP-2 and THP show 53% identity and 86% similarity. A number of peptide domains and amino acid residues are conserved between GP-2 and THP. Each sequence contains an N-terminal signal sequence required for peptide chain translocation across the rough endoplasmic reticulum membrane. In each case, this signal sequence is absent in the mature protein. Each sequence contains 20 hydrophobic residues at the C terminus, consistent with a transmembrane domain, but unassociated with a cytoplasmic tail. Immediately upstream of the transmembrane domain is a decapeptide (Fig. 1*C*) rich in amino acid residues, which may serve as potential transamidation sites for attachment of GPI anchors. The putative GPI processing site is bounded upstream by dibasic amino acids in dog GP-2 (Arg-Arg^{475}), rat GP-2 (Lys-Lys^{496}), human THP (Arg-Lys^{607}), and a homologue in rat THP (Arg-Asn^{610}). Each sequence contains eight N-linked carbohydrate attachment sites. Five sites are conserved in the dog and rat GP-2 sequences. Seven sites are conserved in the rat and human THP sequences. Two of these sites are conserved among the four sequences. Kyte–Doolittle plots show conserved hydropathy domains from Trp^{123} to Phe^{509}, as numbered in the dog sequence (data not shown). Twenty-six cysteine residues are conserved, suggesting the presence of as many as 13 disulfide bonds. Within the globular portion of the mature protein, six tryptophan, 24 negatively charged (aspartic acid, glutamic acid), and 16 positively charged (lysine, arginine) residues are conserved. A conserved epidermal growth factor (EGF) motif (31) appears in GP-2 (Cys^{162}–Cys^{201} in the dog sequence) and THP (Cys^{300}–Cys^{338} in the rat sequence). Based on constraints imposed by disulfide bond formation in EGF domains, a cluster of three or four carboxylate residues contributed variously from positions 163, 164, 169, 170, 171, 180, and 186 in the four sequences (Fig. 1; numbers refer to the dog GP-2 sequence) constitutes a potential calcium binding site similar to the calcium binding site recently demonstrated in EGF domain 1 in factor IX (32). The extent of sequence similarity and conserved domain structure suggests that GP-2 and THP are evolutionarily related genes. Analysis of genomic organization by Southern blot hybridization using rat GP-2 and THP cDNA probes (Fig. 2) indicates that GP-2 and THP are encoded by separate genes. Based on the collective sizes of restriction fragments that hybridize to the two cDNA probes, we judge that GP-2 and THP are most likely encoded by single copy genes (23–26 kbp). Together, the structural studies suggest that GP-2 and THP constitute a family of distinct but homologous genes. The high degree of structural similarity suggests further that the two genes may be functionally homologous.

The unique sequences found in human and rat THP (unique-THP; Pro^{29}–Gln^{174} in the rat sequence) contain several N-linked carbohydrate attachment sites and 22 cysteine residues arranged in four EGF motifs (31), three in tandem and one in reverse orientation (Fig. 1*B*). Based on the disulfide bond structure observed in EGF domains, unique-THP demonstrates at least two potential calcium binding sites [clusters of three or four conserved carboxylate residues (positions 61, 67, 69, and 70 and 110, 113, and 115)]. The region containing the three tandem EGF motifs shows sequence similarities (19–39%) and conserved alignments of 13–20 cysteine residues with the EGF precursor (33), coagulation and thrombolytic factors (34), cell adhesion molecules

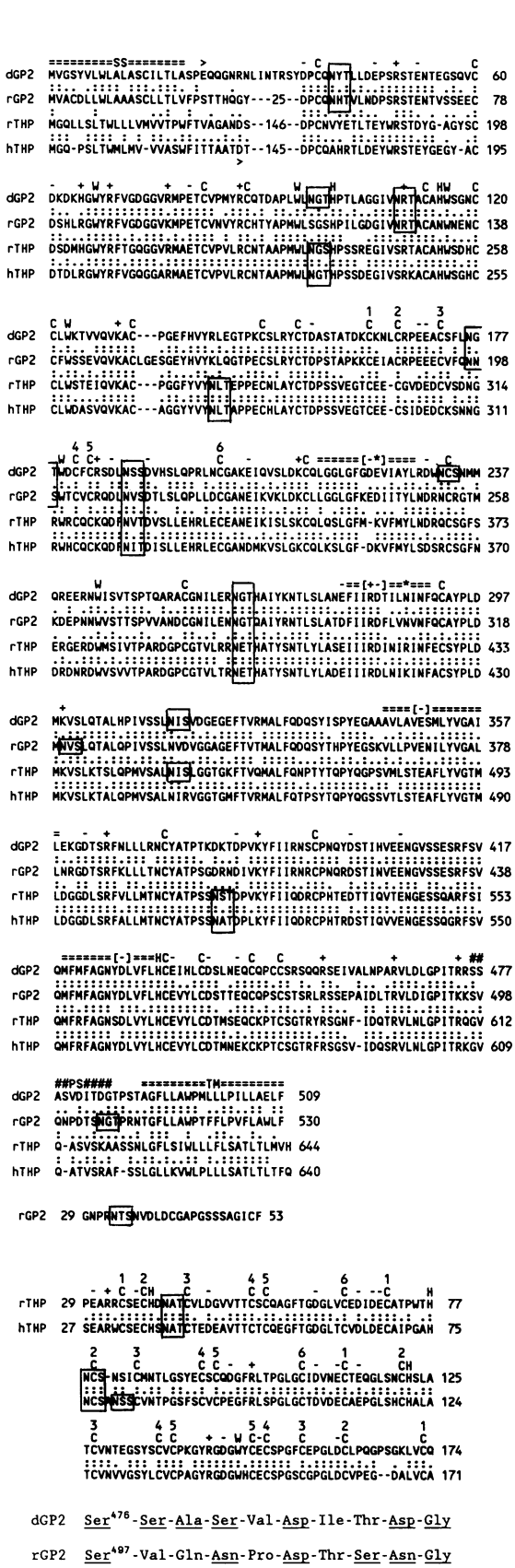

FIG. 1. Alignment of pancreatic GP-2 and kidney THP sequences. (*A*) GP-2 from dog (dGP2; ref. 6) and rat (rGP2; ref. 5) pancreas are aligned with THP from rat (rTHP; described here) and human (hTHP; refs. 15 and 20) kidney according to homology search programs of Pearson and Lipman (28). To optimize sequence alignments, 25 amino acids from rat GP-2 (Gly^{29}–Phe^{53}), 145 amino acids from hTHP (Ser^{27}–Ala^{171}), and 146 amino acids from rTHP (Pro^{29}–Gln^{174}) are shown in *B*. N-terminal signal sequences (==SS==), C-terminal hydrophobic transmembrane peptides (==TM==), putative PI-glycan processing sites (##PS##), and N-linked carbohydrate attachment sites (boxed) are indicated. Double dots indicate amino acid identity. Single dots indicate conservative amino acid changes as defined by probable replacements of one amino acid by another observed over time in evolutionarily related proteins (29). Numbers of amino acids are shown on the right. >, N-terminal sequences of mature dGP2 and hTHP. Conservation of selected amino acids is indicated above the alignment (C, cysteine; W, tryptophan; H, histidine; −, aspartic acid or glutamic acid; +, arginine or lysine). Cysteine residues comprising a conserved EGF motif (Cys^{162}–Cys^{201} in the dog GP-2 sequence) are labeled 1–6. Four hydrophobic regions (===) surrounding charged residues are indicated (residues 215–228, 279–290, 344–357, and 419–431 in the dog GP-2 sequence; *, positions of heterogeneity between charged and/or polar residues in GP-2 and THP). (*B*) Peptide sequences unique to rat GP-2 (rGP2), rat THP (rTHP), and human THP (hTHP). Similarity between the two THP sequences is analyzed as in *A*. Cysteine residues comprising three EGF motifs in tandem and one EGF motif in reverse orientation are numbered 1–6. Note that the palindromic arrangement of EGF motifs 3 and 4 suggests that a disulfide bond is formed between the two cysteines at residue 5 found in these motifs. (*C*) Putative PI-glycan processing site. Amino acid residues that serve as potential transamidation sites for attachment of the PI-glycan anchor (30) are underlined.

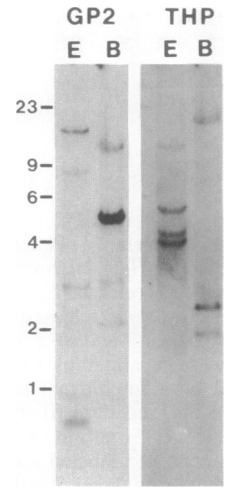

FIG. 2. Southern blot analyses of rat genomic DNA hybridized with cDNA probes encoding rat pancreatic GP-2 and rat kidney THP. E, *Eco*RI; B, *Bam*HI cleavage. Molecular size markers (λ phage DNA cleaved with *Hin*dIII) are shown on the left in kbp.

(35), integrins (36), extracellular matrix proteins (37), cell fate molecules (38), and granule membrane protein 140 (39).

GP-2 and THP Represent GPI-Anchored Proteins That Reside in Intracellular Secretory Compartments. Prior studies have suggested that GP-2 (6, 12, 40) and THP (41) are both GPI-linked membrane proteins as judged by their release from membranes by PI-PLC. Fig. 3 demonstrates that GP-2 released from ZGMs with PI-PLC contains an inositol phosphate determinant, as judged by binding of CRD antibody, which confirms the GPI nature of this molecule. In contrast, GP-2 and THP, released endogenously from pancreas and kidney, respectively, do not react with the anti-CRD antibody, suggesting that their physiological release may be due to a PI-specific phospholipase D (or protease) activity.

GP-2 and THP reside in apical secretory compartments in pancreatic acinar and kidney TALH cells, respectively. Using immunogold labeling evaluated in the electron microscope,

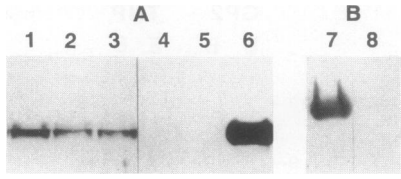

FIG. 3. Presence of CRD in GP-2 and THP released from pancreas and kidney, respectively. (*A*) Pancreatic ZGMs, lysed and washed in 100 mM Na_2CO_3 (pH 11.2), containing ≈1.0 μg of GP-2 (lanes 1 and 4). GP-2 (≈1.0 μg) released from saponin-permeabilized ZGs (lanes 2 and 5). GP-2 (≈1.0 μg) released from purified ZGMs by incubation with PI-PLC (lanes 3 and 6). Proteins contained in cell fractions and extracts were separated by SDS/PAGE in 10% acrylamide gels, transblotted to Immobilon membranes, and identified by a two-step immunolabeling procedure [first reagent, monospecific rabbit anti-dog GP-2 (lanes 1–3); affinity-purified rabbit anti-CRD (lanes 4–6); second reagent, ^{125}I-labeled goat anti-rabbit IgG]. (*B*) THP purified from human urine (lanes 7 and 8). Proteins (≈1.0 μg) separated by gel electrophoresis and analyzed on nylon membranes as described in *A*. Immunolabeling for lane 7 used monospecific goat anti-hTHP (United States Biochemical) followed by rabbit anti-goat IgG labeled with alkaline phosphatase and developed as described by the manufacturer; lane 8, affinity-purified rabbit anti-CRD followed by ^{125}I-labeled goat anti-rabbit IgG. The molecular mass of dog GP-2 is 75 kDa; that of human THP is 85 kDa.

GP-2 has been localized in pancreatic acinar cells in trans-Golgi, condensing vacuoles, ZGs (spherical particles, ≈1000 nm), and apical plasma membranes (7–10, 21). Using similar methods, THP has been localized in kidney TALH cells in trans-Golgi, apical vesicles (oblong structures, 30–150 nm), and apical plasma membranes (42, 43). The conserved molecular features of GP-2 and THP reported here and the conserved distributions of GP-2 and THP in apical secretory compartments in pancreatic acinar and kidney TALH cells, respectively, suggest that the GP-2/THP gene family targets GPI-anchored proteins to apical storage compartments destined for functional interaction at the apical plasma membrane.

Globular Domains of GP-2 and THP Show pH- and Ion-Dependent Self-Association Under *in Vitro* Conditions. Pancreatic exocrine cells show decreased pH (44) in immature granule compartments (equivalent to trans-Golgi network in other cells). Recent studies, which used the M1 protein of influenza virus as a pH-sensitive probe, indicate that trans-Golgi pH is <6.0 (45). Since decreased pH has been implicated in sorting/condensation of granule content proteins in several regulated secretory systems (46–48), we explored whether decreases in pH could be involved in membrane sorting and condensation of GP-2 and THP. Fig. 4 demonstrates, under *in vitro* conditions, that the globular protein domains of GP-2 and THP both aggregate into sedimenting complexes at pH 5.5. Sedimentation of GP-2 aggregates required >1.0 mM calcium and showed insensitivity to sodium concentration. Atomic absorption spectrometry studies have demonstrated high levels of calcium in pancreatic ZGs (36.57 nmol per mg of protein) (49). In contrast, pH-induced aggregation of THP was observed between 30 and 100 mM concentrations of sodium in the absence of calcium and required lower *g* forces for sedimentation. The differences observed in ionic requirements for pH-induced aggregation of THP may correlate with the unique 144-residue N-terminal domain demonstrated in THP. Under similar conditions little or no aggregation was observed with albumin, a protein released via a constitutive secretory pathway. These findings indicate that pH- and ion-induced self-association reactions for globular GP-2 and THP are specific homotypic-binding processes. The significance of these findings is that they provide the first clues that luminal membrane components (globular domains of GPI-anchored proteins) are capable of *in vitro* pH- and ion-induced condensation/precipitation reactions similar to those observed for soluble components targeted to regulated secretory compartments (47, 48).

Taken together, these data suggest that GPI-linked forms of GP-2 and THP may show ion-dependent and pH-induced self-association on the cisternal leaflet of trans-Golgi membranes. The capacity of the GP-2/THP family of GPI-linked proteins to cluster/sort in the plane of the membrane may be determined not only by pH and ionic composition of cisternal compartments but also by glycolipid concentrations in trans-Golgi membranes, which are known to be increased in apical secretory compartments (50). pH- and ion-induced self-association of GP-2 and THP may provide a molecular mechanism for luminal sorting of trans-Golgi membrane proteins into domains targeted to regulated secretory compartments in pancreatic acinar and kidney TALH cells, respectively. By its very nature, the process of homotypic binding (self-association/aggregation) constitutes a sorting process. Diffusion coefficients for GPI-anchored membrane proteins appear to be ≈10-fold higher than values for peptide-anchored membrane proteins (51). Thus, GPI anchors associated with GP-2 and THP may facilitate the self-association/sorting process associated with membrane assembly of apical vesicular compartments.

GP-2 Homologues Exist in Other Tissues. Because of the potential importance of GPI-linked homotypic-sorting mechanisms in highly regulated secretory systems, we explored the tissue distribution of mRNA transcripts encoding GP-2- and THP-like proteins. Fig. 5 demonstrates discrete mRNA transcripts that hybridize to GP-2 cDNA in a variety of

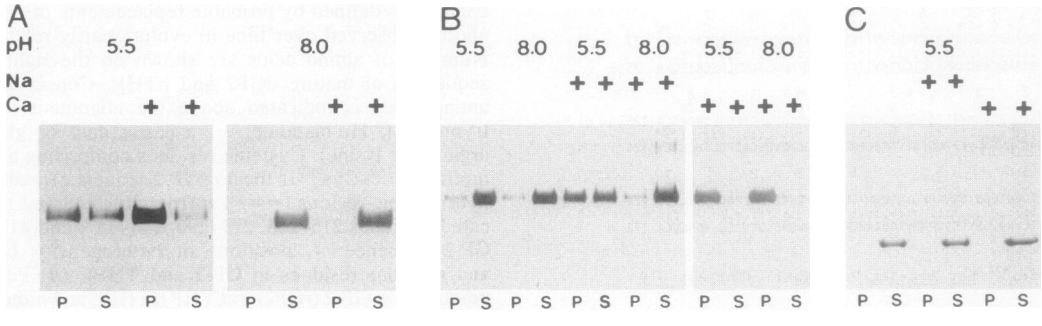

FIG. 4. pH- and ion-dependent self-association of GP-2 and THP. (*A*) GP-2 (1 μg/μl) was incubated in the presence of 100 mM Mes (pH 5.5) or 100 mM Tris·HCl (pH 8.0) in the absence (−) or presence (+) of 15 mM $CaCl_2$ at 20°C for 16 hr. Reaction mixtures (final vol, 20 μl) were then centrifuged at 220,000 × *g* at 20°C for 90 min. Supernatant (lanes S) and pellet (lanes P) fractions were adjusted to equal volumes and analyzed by SDS gel electrophoresis (10% acrylamide) and silver nitrate staining. (*B*) THP (1 μg/μl) was incubated in the presence of 100 mM Mes (pH 5.5) or 100 mM Tris·HCl (pH 8.0) in the absence or presence of 50 mM NaCl or 15 mM $CaCl_2$ at 20°C for 4–20 hr. Reaction mixtures (final vol, 20 μl) were centrifuged at 13,000 × *g* at 20°C for 30 min and pellet and supernatant fractions were analyzed as described in *A*. (*C*) Bovine serum albumin (1 μg/μl) was incubated as described in *B* but was fractionated and analyzed as described in *A*.

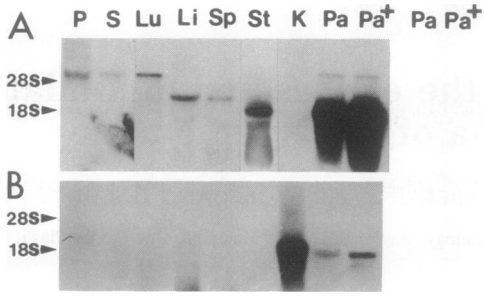

FIG. 5. Canine tissue distribution of mRNA transcripts hybridizing to dog pancreatic GP-2 and human kidney THP cDNAs. Results are shown for membranes washed under low-stringency conditions and exposed to x-ray film for 8 hr in the presence of one intensifying screen. P, parotid; S, submandibular; Lu, lung; Li, liver; Sp, spleen; St, stomach; K, kidney; Pa, pancreas; Pa^+, pancreas poly(A)$^+$ RNA. Lanes Pa and Pa^+ on the far right are a shorter exposure (1 hr). (*A*) Probed with dog pancreatic GP-2 cDNA. (*B*) Probed with human kidney THP cDNA. 28S and 18S rRNAs are indicated by arrowheads.

epithelial tissues known to contain regulated secretory processes, including parotid, submandibular gland, stomach, liver, and lung. Other segments of the intestinal tract (esophagus, jejunum, ileum, and colon) and endocrine tissues (pituitary, pineal, thyroid, parathyroid, and islet) did not react with either cDNA probe. GP-2 mRNA homologues were observed in three size classes [2.2 kb (pancreas, stomach, and duodenum), 3.6 kb (liver), and 5.2 kb (pancreas, lung, parotid, and submandibular gland)]. Reactivity of the THP probe was confined to RNA from kidney, although low levels of cross-reactivity were observed with pancreatic GP-2 mRNA. An earlier study had demonstrated that antibodies directed against THP reacted with apical granules not only in pancreatic acinar cells, but in salivary gland serous cells and jejunal epithelial cells as well (52). These studies indicate that GP-2 homologues are widely distributed among diverse epithelial tissues. Interestingly, pancreatic tissue showed two transcripts, a dominant form encoding GP-2 described here and elsewhere (6) and a 5.2-kb form, which comigrated with transcripts observed in lung, parotid, and submandibular gland. The precise characterization of the higher molecular weight transcript in pancreas as well as the three size classes of transcripts observed in other tissues awaits further investigation. Heterogeneity of this type may be due to the presence of additional members within the GP-2/THP gene family or alternative splicing mechanisms that lead to mRNA transcripts of various sizes. Sequence analysis of cDNA transcripts from these other tissues as well as genomic DNA will resolve these issues.

Collectively, our structural and functional data suggest that pancreatic GP-2 and kidney THP constitute a family of homologous genes that encode GPI-linked membrane proteins associated with apical secretory compartments. The data further suggest that GP-2 and THP engage in pH- and ion-dependent self-association/sorting events, which may play an important role in assembly of vesicular compartments targeted to apical plasma membranes via regulated exocytosis. The GP-2/THP family appears to represent a newly discovered class of GPI-anchored proteins involved in apical secretory processes in divergent epithelial cells.

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1. Low, M. G. (1989) *FASEB J.* **3**, 1600–1608.
2. Cross, G. A. M. (1990) *Annu. Rev. Cell Biol.* **6**, 1–39.
3. Lisanti, M. P., Caras, I. W., Davitz, M. A. & Rodriguez-Boulan, E. (1989) *J. Cell Biol.* **109**, 2145–2156.
4. Lisanti, M. P., Darnell, J. C., Chan, B. L., Rodriguez-Boulan, E. & Saltiel, A. R. (1989) *Biochem. Biophys. Res. Commun.* **164**, 824–832.
5. Fukuoka, S.-I. & Scheele, G. A. (1990) *Nucleic Acids Res.* **18**, 5900.
6. Fukuoka, S.-I., Freedman, S. F. & Scheele, G. A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 2898–2902.
7. Scheffer, R. C. T., Poort, C. & Slot, J. W. (1980) *Eur. J. Cell Biol.* **23**, 122–128.
8. Havinga, J. R., Strous, G. J. & Poort, C. (1984) *Eur. J. Biochem.* **144**, 177–183.
9. Rindler, M. J. & Hoops, T. C. (1990) *Eur. J. Cell Biol.* **53**, 154–163.
10. Beaudoin, A. R., St.-Jean, P. & Grondin, G. (1991) *J. Histochem. Cytochem.* **39**, 575–585.
11. Fukuoka, S.-I., Freedman, S. F. & Scheele, G. A. (1990) *J. Cell Biol.* **111**, 318a (abstr.).
12. Hoops, T. C. & Rindler, M. J. (1991) *J. Biol. Chem.* **266**, 4257–4263.
13. Tamm, I. & Horsfall, F. (1950) *Proc. Soc. Exp. Biol. Med.* **74**, 108–114.
14. Lewis, R. A., Schwartz, R. H. & Schenk, E. A. (1972) *Lab. Invest.* **26**, 728–730.
15. Hession, C., Decker, J. M., Sherblom, A. P., Kumar, S., Yue, C. C., Mattaliano, R. J., Tizard, R., Kawashima, E., Schmeissner, U., Heletky, S., Chow, E. P., Burne, C. A., Shaw, A. & Muchmore, A. V. (1987) *Science* **237**, 1479–1484.
16. Worchester, E. M., Nakagawa, Y., Wabner, C. L., Kumar, S. & Coe, F. L. (1988) *Am. J. Physiol.* **255**, F1197–F1205.
17. Dulawa, J., Thomsen, M., Rambausek, M. & Ritz, E. (1988) *Eur. J. Clin. Invest.* **18**, 87–91.
18. Bjugn, R. & Flood, P. R. (1980) *Scand. J. Urol. Nephrol.* **22**, 313–315.
19. Sikri, K. L., Foster, C. L., Bloomfield, F. J. & Marshall, R. D. (1979) *Biochem. J.* **181**, 525–532.
20. Pennica, D., Kohr, W. J., Kuang, W.-J., Glaister, D., Aggarwal, B. B., Chen, E. Y. & Goeddel, D. V. (1987) *Science* **236**, 83–88.
21. Geuze, J. J., Slot, J., van der Ley, P. A. & Scheffer, R. C. T. (1981) *J. Cell Biol.* **89**, 653–665.
22. Haas, M. (1989) *Annu. Rev. Physiol.* **51**, 443–457.
23. Tabor, S. & Richardson, C. C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4767–4771.
24. Yanisch-Perron, C., Vieira, J. & Messing, J. (1985) *Gene* **33**, 351–359.
25. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), Sect. 9.31.
26. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–160.
27. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
28. Pearson, W. R. & Lipman, D. (1985) *Proc. Natl. Acad. Sci. USA* **85**, 2444–2448.
29. Dayhoff, M., ed. (1978) *Atlas of Protein Sequence and Structure* (Natl. Biomed. Res. Found., Washington), Vol. 5, Suppl. 3.
30. Micanovic, R., Gerber, L. D., Berger, J., Kodukula, K. & Udenfriend, S. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 157–161.
31. Baron, M., Moran, D. G. & Campbell, L. D. (1991) *Trends Biochem. Sci.* **16**, 13–17.
32. Handford, P. A., Mayhew, M., Baron, M., Winship, P. R., Campbell, I. D. & Brownlee, G. G. (1991) *Nature (London)* **351**, 164–167.
33. Bell, G. I., Fong, N. M., Stempien, M. M., Wormsted, M. A., Caput, D., Ku, L., Urdea, M. S., Rall, L. B. & Sanchez-Pescador, R. (1986) *Nucleic Acids Res.* **14**, 8427–8446.
34. Cool, D. E. & McGillivray, R. T. A. (1987) *J. Biol. Chem.* **262**, 13662–13673.
35. Bevilacqua, M. P., Stengelin, S., Gimbrone, M. A. & Seed, B. (1989) *Science* **243**, 1160–1165.
36. Argraves, W. S., Suzuki, S., Arai, H., Thompson, K., Pierschbacher, M. D. & Ruoslahti, E. (1987) *J. Cell Biol.* **105**, 1183–1190.
37. Zimmerman, D. R. & Ruoslahti, E. (1989) *EMBO J.* **8**, 2975–2981.
38. Yochem, J. & Greenwald, I. (1989) *Cell* **58**, 553–563.
39. Johnston, G. I., Cook, R. G. & McEver, R. P. (1989) *Cell* **56**, 1033–1044.
40. Lebel, D. & Beattie, M. (1988) *Biochem. Biophys. Res. Commun.* **154**, 818–823.
41. Rindler, M. J., Naik, S. S., Li, N., Hoops, T. C. & Peraldi, M.-N. (1990) *J. Biol. Chem.* **265**, 20784–20789.
42. Sikri, K. L., Foster, C. L. & Marshall, R. D. (1985) *Biochem. J.* **225**, 481–486.
43. Bachmann, S., Koeppen-Hagemann, I. & Kriz, W. (1985) *Histochemistry* **83**, 531–538.
44. Orci, L. (1987) *Nature (London)* **326**, 77–79.
45. Sugrue, R. J., Bahadur, G., Zambon, M. C., Hall-Smith, M., Douglas, A. R. & Hay, A. J. (1990) *EMBO J.* **9**, 3469–3476.
46. Tartakoff, A. M. (1983) *Cell* **32**, 1026–1028.
47. Gerdes, H. H., Rosa, P., Phillips, E., Baeuerle, P. A., Frank, R., Argos, P. & Huttner, W. B. (1989) *J. Biol. Chem.* **264**, 12009–12015.
48. Yoo, S. H. & Albanesi, J. P. (1990) *J. Biol. Chem.* **265**, 14414–14421.
49. Clemente, F. & Meldolesi, J. (1975) *J. Cell Biol.* **65**, 88–102.
50. van Meer, G. & Simons, K. (1988) *J. Cell Biochem.* **36**, 51–58.
51. Noda, M., Yoon, K., Rodan, G. A. & Koppel, D. E. (1987) *J. Cell Biol.* **105**, 1671–1677.
52. Santoso, A. W. B., Scott, D. M. & Kinne, R. (1987) *Eur. J. Cell Biol.* **43**, 104–109.