"Nonclassical" Secretion of Annexin A2 to the Lumenal Side of the Enterocyte Brush Border Membrane†

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ABSTRACT: Annexin A2 is a member of the annexin family of Ca^{2+}-dependent lipid binding proteins and believed to be engaged in membrane transport processes in a number of cell types. In small intestinal enterocytes, we localized annexin A2 to the brush border region, where it was found mainly on the lumenal side of the microvilli, showing an apical secretion by a "nonclassical" mechanism. In addition, annexin A2 was associated with surface-connected, deep apical tubules in the apical terminal web region and with an underlying pleiomorphic, tubulo-vesicular compartment (subapical compartment/multivesicular bodies). By subcellular fractionation, the 36 kDa full-length form of annexin A2 was approximately equally distributed between the Mg^{2+}-precipitated fraction (containing intracellular and basolateral membranes) and the microvillar membrane fraction. In addition, a 33 kDa molecular form of annexin A2 was seen in the latter fraction that could be generated from the full-length annexin A2 by digestion with trypsin. Taken together, the results suggest that annexin A2 acts in exocytic apical membrane trafficking and is proteolytically cleaved *in situ* by pancreatic proteinases once it has become externalized to the lumenal side of the brush border membrane. On the basis of its well-known membrane fusogenic properties, we propose a model for the nonclassical membrane translocation of annexin A2.

The annexin multigene family numbers ~100 members from all eukaryotic kingdoms except fungi (*1*). Structurally the annexins are defined by a conserved core domain consisting of four homologous repeats responsible for Ca^{2+}-dependent binding to anionic phospholipids, the cytoskeleton, and extracellular matrix proteins (*2*), and the three-dimensional structure of several members of the family has now been determined (*3*). Collectively, annexins are thought to play a role in a host of biological functions, including membrane trafficking and organization, ion channels, and extracellular activities, and they have been implicated in a number of diseases, for instance, heart disease and cancer (*4*, *5*). Thus, annexins A1 and A2 were first discovered as targets for tyrosine phosphorylation by the EGF receptor and the transforming gene product of the Rous sarcoma virus, respectively, implicating these annexins in signaling pathways involved in cancer (*5*).

Several annexins, including annexins A1, A2, A4, A6, and A13, are expressed in the small intestine enterocyte (*6*−*10*). During its short life span of only 3−5 days, this highly specialized cell must generate and maintain a dense lumenal brush border, enabling it to fulfill the digestive and absorptive functions that are vitally important for the whole organism (*11*). This task is accomplished by a massive biosynthesis and constitutive exocytic transport of digestive enzymes to the developing brush border. In general, apical membrane trafficking in epithelial cells involves formation of glycosphingolipid- and cholesterol-rich microdomains, commonly known as lipid rafts, that act as sorting platforms for proteins destined for the lumenal cell surface (*12*−*14*). Two members of the annexin family, annexin A2 (*15*−*18*) and annexin A13b (*19*, *20*), have been shown to be associated with lipid rafts. Whereas the latter annexin is epithelial cell specific (*8*), annexin A2 has a more widespread tissue distribution and has been implicated in both regulated secretion and endocytosis (*4*, *5*, *21*−*25*). It forms a heterotetramer with S100A10, a member of the S100 family of proteins, and is a substrate for the *src* tyrosine kinase as well as for protein kinase C. Both S100A10 binding and phosphorylation are able to modulate the properties of annexin A2, but the mechanistic aspects of the function of this annexin are not fully understood (*4*, *5*).

We recently observed that enterocyte annexin A2 is associated with lipid rafts of both intracellular and microvillar origin (*26*), suggesting its involvement in apical membrane trafficking. In the work presented here, we studied this annexin in more detail. The results indicate that the lumenal surface of the brush border is a main destination for annexin A2 in the enterocyte. In addition, a model for its "nonclassical" membrane translocation is presented.

MATERIALS AND METHODS

Materials. A mouse monoclonal antibody to bovine annexin A2 was from Transduction Laboratories (Lexington,

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KY), and a goat antibody to human annexin A2 was from Santa Cruz Biotechnology (Santa Cruz, CA). A rabbit antibody to pig intestinal aminopeptidase N was previously described (*27*). Protein A-gold was obtained from J. W. Slot and G. Posthuma (Utrecht University School of Medicine, Utrecht, The Netherlands), and rabbit anti-goat IgG-gold[1] was from Amersham Pharmacia Biotech (Hørsholm, Denmark).

Pig small intestine was kindly provided by L. Klarskov and M. Olesen from the Department of Experimental Medicine, The Panum Institute, University of Copenhagen.

Tissue Fractionation of Intestinal Mucosa. Mucosal segments were excised from freshly obtained pig small intestine, taken ~2 m from the pylorus, as previously described (*28*). In all subsequent tissue fractionation experiments, leupeptin (10 μg/mL) and aprotinin (10 μg/mL) were present. Subcellular fractionation of intestinal mucosa into Mg^{2+}-precipitated (intracellular and basolateral) membranes and outside-out microvillar membranes was performed by the method of Booth and Kenny (*29*). Briefly, mucosal scrapings were homogenized in 2 mM Tris-HCl and 50 mM mannitol (pH 7.1) using a manually operated Potter-Elvehjem homogenizer. The homogenate was cleared by centrifugation at 500*g* for 10 min, and $MgCl_2$ was added to a final concentration of 10 mM. After incubation for 15 min on ice, the preparation was centrifuged at 1500*g* for 10 min to pellet aggregated intracellular and basolateral membranes. Finally, the supernatant was centrifuged at 48000*g* for 1 h to obtain a pellet of outside-out microvillar membrane vesicles and a supernatant of soluble proteins.

Electron Microscopy. Pieces of mucosal segments of pig small intestine were fixed overnight by immersion in 0.1% glutaraldehyde and 2% formaldehyde in 0.1 M cacodylate buffer (pH 7.2). After fixation, the specimens were washed in the above buffer, infiltrated with sucrose, mounted on stubs, and frozen in liquid nitrogen. Ultracryosections were labeled with goat anti-human annexin A2 followed by protein A-gold.

For surface staining with ruthenium red, mucosal segments were fixed in 3% glutaraldehyde and 2% formaldehyde in 0.1 M cacodylate buffer (pH 7.2) for 20 h in the presence of 0.2% ruthenium red followed by postfixation in 1% osmium tetroxide and 0.3% ruthenium red for 2 h. After dehydration, the mucosal segments were embedded in Epon. Postembedding immunogold labeling of ultrathin Epon sections of ruthenium red-treated specimens was performed with goat anti-human annexin A2 and rabbit anti-goat IgG-gold.

For pre-embedding immunogold labeling, microvillar membrane vesicles were fixed in 0.1% glutaraldehyde and 2% formaldehyde in 0.1 M sodium phosphate (pH 7.2) for 10 min. The labeling was performed in Eppendorf tubes using goat anti-annexin A2 or rabbit anti-aminopeptidase N, essentially as described previously (*30*). The vesicles were then postfixed in 1% osmium tetroxide in 0.1 M sodium phosphate (pH 7.2) for 1 h at 4 °C, dehydrated in graded concentrations of acetone, and finally embedded in Epon directly in the Eppendorf tubes.

[1] Abbreviations: IgA, immunoglobulin A; IgG, immunoglobulin G; SAC, subapical compartment; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; Snare, Snap receptor.

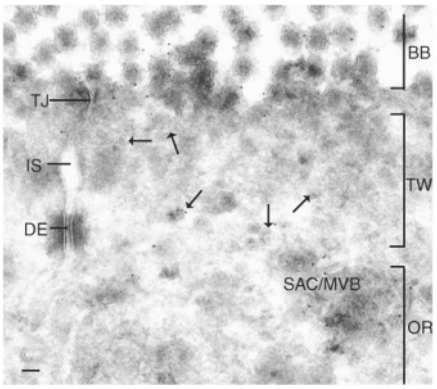

FIGURE 1: Survey electron micrograph of the apical region of an enterocyte. The ultracryosection has been labeled with immunogold (10 nm gold) to detect annexin A2. Gold labeling is seen in the brush border (BB), associated with apical tubular and vesicular profiles (arrows) of the terminal web region (TW), and with the subapical compartment/multivesicular bodies (SAC/MVB) of the organelle region (OR). TJ is the tight junction, IS the intercellular space, and DE the desmosome. The bar is 200 nm.

In all labeling experiments, controls were performed by omission of the primary antibody. Finally, the sections were inspected in a Philips 100 CM or Zeiss EM 900 electron microscope. Photographs were obtained using positives that were subsequently digitized. Alternatively, digital electron micrographs were obtained directly, using a Mega View II camera system.

SDS−PAGE and Immunoblotting. SDS−PAGE in 10% gels was performed according to the method of Laemmli (*31*). After electrotransfer onto Immobilon membranes, immunoblotting was performed using a chemiluminescence (ecl) reagent from NEN (Boston, MA) according to the protocol supplied by the manufacturer. Annexin A2 was detected using either a mouse monoclonal or goat polyclonal antibody. Image analysis was carried out using a LAS-1000plus luminescent image analyzer (Fujifilm, Tokyo, Japan) and a software program supplied by the manufacturer.

RESULTS

Immunogold Localization of Annexin A2 in the Enterocyte Apex. By immunogold electron microscopy, we observed annexin A2 mainly in the apical region of the enterocyte (the 5−10 μm zone between the nucleus and the lumen), as previously reported by others (*9*). In this apical region, ~40% of the gold particles were associated with microvilli while the remaining labeling was found on membranous structures in the underlying cytoplasm (the terminal web and the organelle region in Figure 1). The microvillar labeling was seen predominantly on the lumenal side of the membrane (Figure 2A). Thus, a quantification of 412 gold particles localized on appropriate sections (i.e., where the microvilli were well separated and the microvillar membrane was cut at a perpendicular angle and a precise localization of the indiviual gold particles therefore could be determined) revealed that 72% of the particles were on the exterior side of the membrane, 15% were over the membrane, and 13% were on the interior side of the membrane. This indicates that a major part of microvillar annexin A2 (in the range of 72−87%) must reside on the lumenal side of the brush border membrane. For comparison, Figure 2B shows a similar labeling for aminopeptidase N, a digestive enzyme located

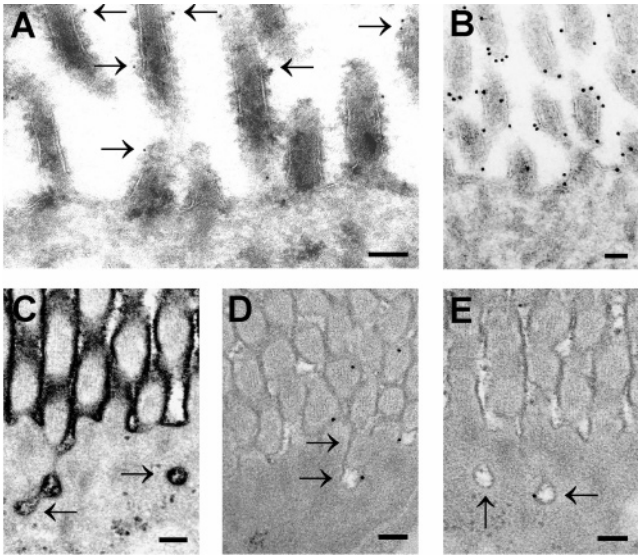

FIGURE 2: Annexin A2 associated with microvilli and deep apical tubules. (A) An ultracryosection, showing immunogold labeling for annexin A2 predominantly localized to the outside of the microvilli (arrows). (B) A similar immunogold labeling for aminopeptidase N. Notice that most, but not all, gold particles for this lumenal marker localize on the lumenal side of the microvilli. Panels C−E are ultrathin Epon sections from ruthenium red-treated mucosal segments. In panel C, deep apical tubules are seen outlined by distinct ruthenium red staining (arrows). In panels D and E, sections of ruthenium red-stained specimens have further been labeled with immunogold (10 nm gold) for annexin A2. The ruthenium red staining (now weaker due to washout) outlines the gold-labeled, deep apical tubules (arrows). Bars are 200 nm (A and C−E) and 100 nm (B).

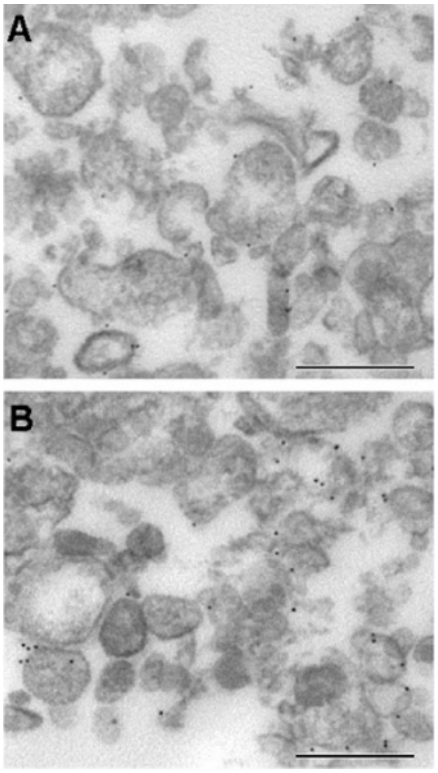

FIGURE 3: Immunogold labeling of microvillar membrane vesicles. Epon sections showing pre-embedding immunogold labeling of outside-out microvillar membrane vesicles for annexin A2 (A) and aminopeptidase N (B). For both proteins, labeling of vesicular membranes is seen. Bars are 500 nm.

at the extracellular side of the membrane. Here, gold particles were also found predominantly, but not exclusively, on the lumenal side of the membrane.

To ascertain the extracellular localization of annexin A2, Figure 3A shows an annexin A2 immunogold labeling of microvillar membrane vesicles performed by a pre-embedding protocol. Microvilli form regular closed, outside-out vesicles upon tissue homogenization (*29*), leaving only the extracellular surface accessible for labeling. Since the vesicles were conspicuously labeled, this experiment independently corroborates the extracellular localization of annexin A2 shown in Figure 2A. For comparison, Figure 3B shows a similar labeling for aminopeptidase N.

In the underlying apical cytoplasm, annexin A2 labeling was associated with tubulo-vesicular membranes localized in the terminal web region (Figure 1, TW, and Figure 2D,E), which contains a complex meshwork of filaments interdigitating between the actin rootlets of the microvillar cytoskeleton (*32*). The high density of filaments functions as a barrier, preventing larger organelles such as mitochondria, endoplasmic reticulum, and endosomal compartments from direct access to the apical surface. Figure 2C shows an Epon section of intestinal mucosa after treatment with the nonpermeable surface marker ruthenium red. In addition to staining the entire microvillar surface, ruthenium red also labeled deep apical tubules (*26*) penetrating into the terminal web region. Some vesicular profiles were also labeled by the surface marker, indicating a connection with the cell surface (Figure 2C). The tubules and vesicular profiles (most likely cross-sectioned tubules) had a diameter of 50−100 nm, and penetrated up to 1 μm into the cytoplasm. Annexin A2 was seen at the membrane of both the deep apical tubules and the vesicle-like structures (Figure 2D,E).

Below the terminal web region in the organelle region (Figure 1), annexin A2 labeling was seen in tubulo-vesicular and endosomal structures also known as the subapical compartment (SAC) (*33*).

Subcellular Distribution and Proteolytic Processing of Enterocyte Annexin A2. When small intestine mucosa was subcellularly fractionated by the divalent cation precipitation method into Mg^{2+}-precipitated (intracellular and basolateral) membranes, outside-out microvillar membrane vesicles, and soluble protein, approximately equal amounts of the 36 kDa polypeptide of annexin A2 were found in the two membrane fractions, whereas it was essentially absent from the soluble pool of proteins (Figure 4). This distribution agrees well with the immunogold labeling shown in Figure 1. When one considers that the microvillar fraction only constitutes 5−10% of the protein of a total mucosal homogenate, it indicates that the apical cell surface is a major destination for annexin A2 trafficking in the enterocyte. For comparison, Figure 4 also shows that the transient, high-mannose glycosylated 140 kDa band of the resident microvillar enzyme aminopeptidase N was only seen in the Mg^{2+}-precipitated fraction, whereas the mature, complex 160 kDa glycosylated enzyme was highly enriched in the microvillar fraction, indicating that a major cross contamination between the two membrane fractions did not occur. In addition, a fragment (120 kDa) of the mature form of aminopeptidase N was present in the microvillar fraction, indicative of proteolytic cleavage *in situ* by pancreatic proteinases (*34*).

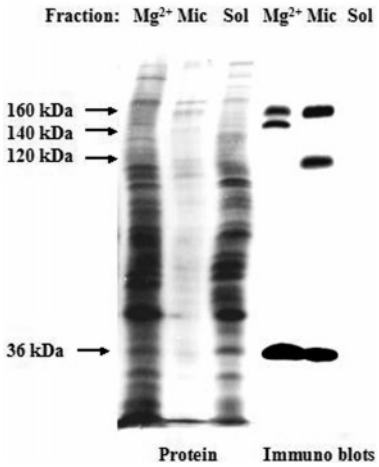

FIGURE 4: Subcellular distribution of enterocyte annexin A2. Mucosal scrapings were homogenized and fractionated into Mg^{2+}-precipitated membranes (Mg^{2+}), microvillar membrane vesicles (Mic), and soluble proteins (Sol) as described in Materials and Methods. Proportional amounts of the three fractions were analyzed by SDS−PAGE followed by immunoblotting with a monoclonal antibody to annexin A2 and a polyclonal antibody to aminopeptidase N. After immunoblotting had been carried out, total protein was visualized by staining with Coomassie brilliant blue. Molecular mass values of annexin A2 (36 kDa) and aminopeptidase N (160, 140, and 120 kDa) are indicated.

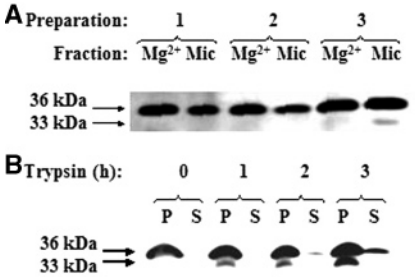

FIGURE 5: A 33 kDa molecular form of annexin A2. (A) Mucosal scrapings from three different pigs were homogenized and fractionated into Mg^{2+}-precipitated (Mg^{2+}) and microvillar (Mic) membranes as described in Materials and Methods. Proportional amounts of the two membrane fractions were analyzed by SDS−PAGE followed by immunoblotting with a monoclonal antibody to annexin A2. Notice the presence of a 33 kDa band in the microvillar fraction in preparation 3. (B) Mg^{2+}-precipitated membranes, resuspended in 25 mM HEPES-HCl and 150 mM NaCl (pH 7.1), were incubated at 37 °C in the presence of 10 μg/mL trypsin for the indicated periods of time. After incubation, the samples were rapidly cooled on ice and centrifuged at 20000*g* for 20 min. The pellets (P) and supernatants (S) were collected and analyzed by SDS−PAGE followed by immunoblotting with a monoclonal antibody to annexin A2.

In intestinal preparations from some but not all animals, an additional molecular form of 33 kDa annexin A2 was detected in the microvillar fraction by immunoblotting, using both a monoclonal (Figure 5A) and a polyclonal (Figure 7) antibody, suggestive of a partial proteolysis. Since not only aminopeptidase N (*34*) but also several other microvillar proteins, including sucrase isomaltase (*35*) and maltase glucoamylase (*36*), are known to be partially proteolytically cleaved *in situ* by pancreatic proteinases, annexin A2 might undergo a similar processing at the lumenal surface of the brush border membrane. Consequently, the animal to animal variation with regard to the presence of the 33 kDa form seen in Figure 5A most likely can be attributed to differences in lumenal contents and activity of pancreatic enzymes at the time of slaughter. Figure 5B shows that the 36 kDa molecular form of annexin A2 was converted to the 33 kDa form *in vitro* by incubation with trypsin. Since the total amount of annexin A2 was not markedly reduced during the incubation, the result indicates the existence of a trypsin-sensitive bond in pig annexin A2. Interestingly, the cleavage of full-length annexin A2 was not accompanied by a release

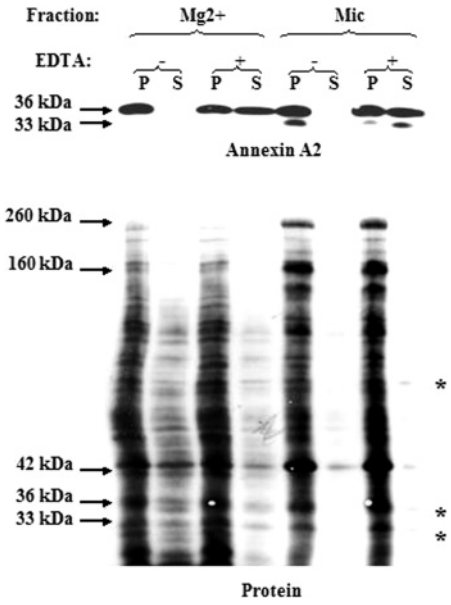

FIGURE 6: Membrane association of annexin A2 is Ca^{2+}-dependent. Mg^{2+}-precipitated (Mg^{2+}) and microvillar (Mic) membranes, resuspended in 25 mM HEPES-HCl and 150 mM NaCl (pH 7.1), were incubated at room temperature for 10 min in the absence or presence of 1 mM EDTA. After incubation, the samples were centrifuged at 20000*g* for 20 min, and the pellets (P) and supernatants (S) were collected and subjected to SDS−PAGE. After electrophoresis and electrotransfer onto Immobilon, annexin A2 was visualized by immunoblotting with a monoclonal antibody and total protein of the same gel tracks by staining with Coomassie brilliant blue. Asterisks denote proteins, including annexin A2, specifically released from the microvillar fraction by EDTA. Molecular mass values are indicated.

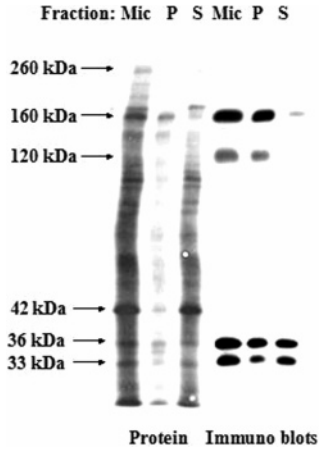

FIGURE 7: Release of microvillar annexin A2 by alkaline treatment. Microvillar membranes (Mic) were resuspended in 5 mM Na_2CO_3 (pH 11.0) and incubated on ice for 30 min. After centrifugation at 48000*g* for 1 h, the pellet (P) and supernatant (S) were collected. Proportional amounts of the fractions were analyzed by SDS−PAGE, followed by immunoblotting with polyclonal antibodies to annexin A2 or aminopeptidase N. Total protein was visualized by staining with Coomassie brilliant blue. Molecular mass values are indicated.

of the 33 kDa form from the membrane. Taken together, the data strongly indicate that annexin A2, like other lumenal brush border proteins directly exposed to the proteolytic enzymes of pancreatic origin during the digestive process, becomes partially cleaved *in situ*.

Figure 6 shows that annexin A2 was released from both Mg^{2+}-precipitated and microvillar fractions by a brief treatment with EDTA, indicating the requirement for Ca^{2+} in membrane binding. For both membrane fractions, approximately half of the total amount of the 36 kDa form of annexin A2 was released by 1 mM EDTA, whereas the 33 kDa form of microvillar annexin A2 was almost completely released. Increasing the concentration of EDTA to 5 mM did not cause any further dissociation of annexin A2 from either membrane fraction (data not shown). This result indicates that a major part of annexin A2 resides on the cytoplasmic side of intracellular vesicles of the Mg^{2+}-precipitated fraction. In addition, since the microvillar fraction essentially consists of closed, outside-out vesicles (*29*), the significant release of annexin A2 from this fraction confirms the lumenal localization of microvillar annexin A2 observed by immunogold electron microscopy. Finally, the result shows that annexin A2 associates with the lumenal side of the membrane in a Ca^{2+}-dependent manner similar to its binding at the cytosolic face of the membrane.

Figure 7 shows the release from microvillar membranes of both the 36 and 33 kDa forms of annexin A2 by alkaline extraction (pH 11.0), a method that opens membrane vesicles and releases peripheral proteins on both sides of the membrane but leaves integral membrane proteins, including aminopeptidase N, in the pellet after centrifugation (*37*). Unlike the EDTA treatment shown in Figure 6, alkaline extraction therefore released most of the vesicular content of actin (42 kDa). Nevertheless, some annexin A2 remained in the pellet, indicating a strong membrane association.

DISCUSSION

Although annexin A2 is known to be a membrane-associated protein in the brush border region of enterocytes (*9*), its presence in the deep apical tubules (*26*) at the apical cell surface and lumenal localization along the microvilli to our knowledge have not been described previously. An additional finding was that lumenal annexin A2 appears to undergo proteolytic cleavage to a 33 kDa form, presumably by the action of trypsin and possibly other pancreatic proteinases *in situ*, in a manner similar to that of a number of digestive brush border ectoenzymes that are likewise directly exposed to the harsh environment of the gut. The cleavage of annexin A2 probably occurs near the N-terminus of the molecule that consists of an α-helix that is joined by an exposed flexible link to the remainder of the molecule (*5*). The finding that the cleaved 33 kDa form of annexin A2 was present predominantly in the microvillar fraction and not distributed to intracellular membranes implies that it most likely represents a major end point in the membrane trafficking of the molecule. Hypothetically, this could be explained by the proteolytic loss of the N-terminal helix that contains the binding site for S100A10, enabling it to form the functional heterotetramer that is capable of bridging opposite membrane surfaces (*5*). However, it is equally likely that annexin A2, once externalized at the microvillar surface, loses its ability to mediate membrane trafficking, whether cleaved or not, simply by virtue of its membrane translocation.

The fact that a major amount of enterocyte annexin A2 is found in the microvillar fraction implies that annexin A2 in this cell type mainly seems to act in apical exocytosis. This role is in agreement with its localization in the deep apical tubules and the SAC below the terminal web region. In addition, it fits with the constitutive apical exocytosis being a major trafficking pathway during the short life span of the enterocyte. Together with the likewise constitutive transcytosis of dimeric IgA from the basolateral cell surface to secretion from the brush border, this demands an apical membrane trafficking system with a "high-throughput" capacity. Annexin A2's well-known membrane fusogenic properties and actin binding capability (*5*) would seem particularly useful for the protein acting as a facilitator in this process. Endocytosis of nutrients from the brush border surface, on the other hand, is generally a rare phenomenon under normal fasting conditions after "closure" when the enterocyte ceases to take up macromolecules from the gut lumen (*38*).

The lumenal localization of microvillar annexin A2 implies that it is a protein secreted apically by a nonclassical mechanism (i.e., not involving a signal peptide for membrane translocation). The mechanism underlying nonclassical secretion of proteins is as yet poorly understood, but in light of the membrane fusogenic properties of heterotetrameric annexin A2, we propose a mechanism whereby this might occur (Figure 8). First, annexin A2 acts to mediate the docking of transport vesicles and carriers at the apical surface, most likely at a site like the easily accessible deep apical tubules between the rootlets of adjacent microvilli. During the preparation of the subsequent membrane fusion process, the two bilayers next have to come in close apposition with one another. This process where water molecules are forced out of the closing gap between the two bilayers requires energy, but we theorize that annexin A2's Ca^{2+}-dependent membrane affinity is sufficiently strong for it to remain at the membrane interface. Second, when the two monolayers, now in direct opposition to one another, form a "stalk" and undergo hemifusion, annexin A2 is forced into the hydrophobic interior of the stalk. We hypothesize that the energy requirement for this step is provided by the tight association of *v*- and *t*-snares believed to be general components of the membrane docking and fusion machinery (*39*). Third, as the fusion process proceeds to completion, annexin A2 is rapidly expelled from the interior to the surface of the membrane. The expulsion is most likely a spontaneous, stochastic process that may occur to either the cytoplasmic or extracellular surface of the membrane. When the latter is the case, annexin A2 is translocated in the process, whereas annexin A2 not translocated across the membrane may engage in a new round of vesicle trafficking. This model is consistent with the majority of microvillar annexin A2 residing on the lumenal side of the membrane and predicts that the nonclassical membrane translocation occurs as an integrated part of the general membrane fusion process. The proposed mechanism may also operate for other types of nonclassically secreted proteins, provided they have a sufficiently high affinity for the membranes that undergo fusion to avoid expulsion in the process. One such protein might be galectin-4, which like annexin A2 is secreted nonclassically and subsequently attached to the brush border membrane (*30*, *40*).

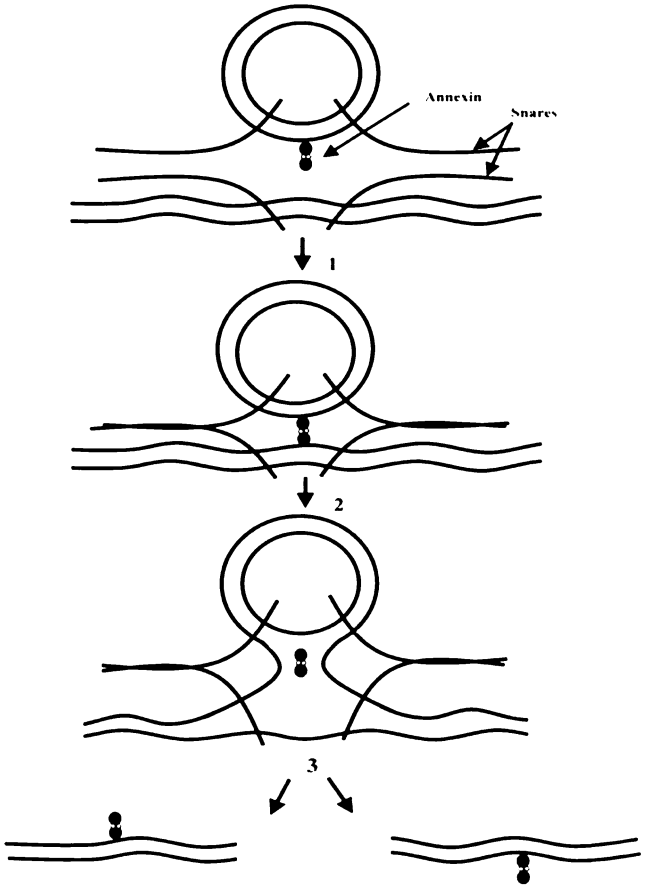

FIGURE 8: Model for apical nonclassical secretion of annexin A2. (1) A transport vesicle, carrying an annexin A2−S100A10 heterotetramer at its cytosolic surface, docks at the apical cell surface. The membrane fusogenic properties of annexin A2 facilitate the docking of the vesicle, allowing *v* and *t* snares to interact and initiate the membrane fusion process. (2) During formation of the "stalk" in the hemifusion of the two opposite lipid monolayers, annexin A2 is forced into the hydrophobic interior of the stalk. The energy requirement for this step is provided by the general vesicle fusion machinery involving *v* and *t* snares. (3) As the fusion process proceeds, annexin A2 is rapidly extruded from the hydrophobic phase of the membrane. Extrusion may take place equally well to either side of the cell membrane; thus, a fraction of annexin A2 becomes secreted in the overall process of vesicle fusion.

In addition to its role in apical membrane trafficking, it is tempting to speculate that annexin A2 may also serve a function at the lumenal side of the microvillar membrane. Annexin A2 has been found on the surface of endothelial cells where it has been proposed to serve as a platform for the binding of plasminogen and tissue plasminogen activator to promote fibrinolysis (*5*, *41*). If annexin A2 acts as a receptor in the gut lumen, physiological ligands for it have yet to be defined. One possibility could be during fat digestion, where annexin A2 might act as a receptor for phospholipid-containing mixed micelles, enabling these to remain in the unstirred layer in the proximity of the brush border during their digestion by pancreatic lipases.

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