Secretion of Milk Constituents

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Introduction

The major constituents of milk are either directly synthesized and secreted from the mammary epithelial cells into the alveolar lumen or transported across the epithelial barrier from other sources. At least five pathways are recognized (**Figure 1**): (1) lipid droplets bud from the cell apex and are secreted into milk with a membrane derived from intracellular sources and the cell surface (Pathway 1); (2) mammary epithelial cell-specific proteins such as the caseins, α-lactalbumin, and β-lactoglobulin, the disaccharide lactose, and at least some of the minerals and water are secreted by exocytosis (Pathway 2); (3) some proteins such as serum albumin and immunoglobulins, peptide hormones, and other constituents that originate from cells other than milk-secreting cells are conveyed across the mammary epithelium by transcytosis (Pathway 3); (4) a fraction of the minerals, small molecules, and water is transported across the basal/lateral and apical membranes by membrane-bound transporters (Pathway 4); and (5) constituents equilibrate between cells by a paracellular route during times when the epithelial tight junctions are permeable (Pathway 5). The last pathway may be important in some species during established lactation, and in production animals like cows during the earliest (colostral) and perhaps very late stages of lactation. In cows with healthy mammary glands the paracellular pathway appears to play a minor role during an established lactation because the junctional complexes are tight and effectively seal off the epithelium to extracellular small molecules.

This article will focus on the first four major pathways for milk secretion in ruminants and laboratory rodents. As far as is known, secretory mechanisms involved in milk production are the same or generally similar across species, judged primarily by morphological observations and analysis of milk composition. However, milk secretion has been studied in detail in a very few species; hence, the general conclusions discussed in this article should be considered with this caveat in mind.

Milk Lipid Secretion (Pathway 1)

Most of the lipid in cow's milk is present in globules that range in diameter from less than 0.5 to more than 8 μm. Each globule consists of a triacylglycerol-rich core surrounded by a membrane known as the milk lipid or milk fat globule membrane (MFGM) (*see* **Milk Lipids**: Milk Fat Globule Membrane). Within the mammary epithelial cells, milk lipid globules originate from triacylglycerols synthesized in the rough endoplasmic reticulum (ER) and are then assembled into microlipid droplets, each of which comprises a triacylglycerol-rich core surrounded by a surface coat composed of proteins and polar lipids. The details of how such droplets are initially formed in any species are unclear and the suggested mechanisms are contradictory and controversial.

The current paradigm for the genesis of lipid droplets in many cells, including the mammary epithelium, is that nascent triacylglycerols associate between the two leaflets within the hydrophobic core of the ER membrane. In the mammary gland, accretion of neutral lipid between the two phospholipid monolayers is presumed to lead to the formation of small droplets of triacylglycerols ($\leq$0.5 μm in diameter) that are released into the cytoplasm coated with the cytoplasmic-facing leaflet of the ER membrane. Thus, the droplets are assumed to acquire a monolayer of phospholipid and some proteins directly from the ER. Evidence for this mechanism comes from electron micrographs showing specialized areas of the membrane with osmiophilic material (presumptive nascent lipid droplets) accumulated between the bilayer halves. In addition, ER-associated phospholipids and resident ER proteins have been detected in lipid droplet fractions from isolated lactating mouse mammary tissue by lipid and proteomic analysis. That ER proteins are associated with secreted milk lipid droplets has been recently confirmed by proteomic analysis of the MFGM (*see* **Milk Lipids**: Milk Fat Globule Membrane). However, specialized budding sites within the ER membrane have not been routinely identified, and especially not at the frequency expected in the many cell types in which lipid is extensively synthesized and turned over.

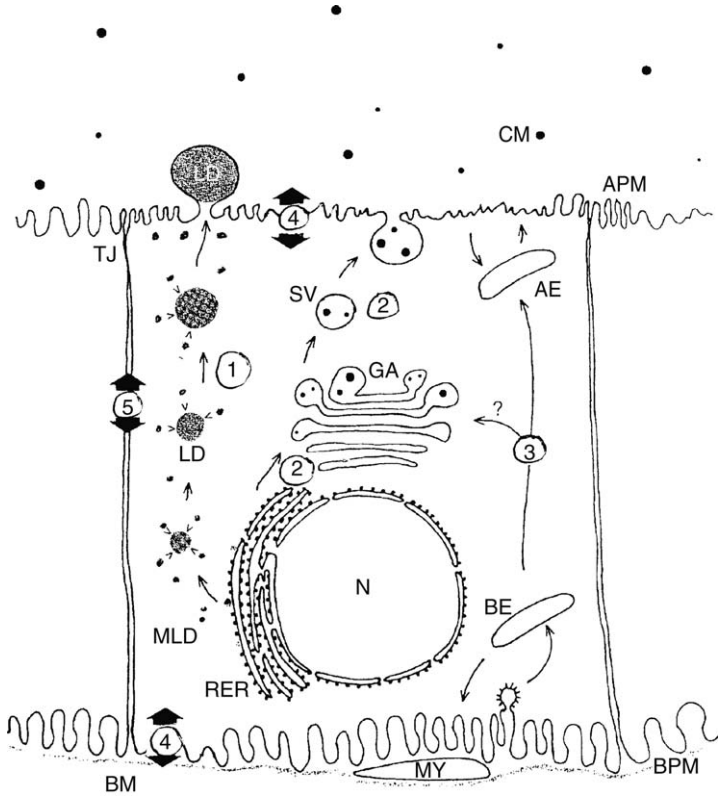

Figure 1 Diagram showing the major pathways for the secretion of milk constituents, discussed in the text. Pathway 1: Lipid secretion. Lipid droplets originate from the rough endoplasmic reticulum (RER) as small droplets called microlipid droplets (MLDs). Droplets can grow in volume by fusion with each other to form larger droplets (LDs), especially at the apical surface, and are secreted by envelopment in apical plasma membrane (APM), giving rise to milk lipid droplets. Pathway 2: Protein secretion. Proteins, lactose, water, and some ions are synthesized and processed through the classical secretory pathway, involving the RER, Golgi apparatus (GA), and secretory vesicles (SV). Pathway 3: Transcytosis. Molecules may transit the cell in either direction in vesicles formed at either the APM or basal/lateral plasma membranes (BPM). Basal (BE) and apical (AE) endosomes serve as sorting stations. Pathway 4: Ion transport. Ions and water are transported into and across the cell by specific transporters and channels. Pathway 5: Paracellular transport. Constituents may pass between the cells by a paracellular route if the tight junctions (TJ) are permeable. Pathway not shown: Secretion of membrane-bounded 'exosomes', which are presumed to be formed either by direct blebbing from the apical surface or by exocytosis of vesicles from multivesicular bodies. BM, basement membrane; CM, casein micelle; MY, myoepithelial cell. Reproduced with modifications by permission from Mather IH and Keenan TW (1998) Origin and secretion of milk lipids. *Journal of Mammary Gland Biology and Neoplasia* 3: 259–273.

An alternative possibility is that lipid droplets initially form in the cytoplasm close to the ER membrane surface. In one recent freeze-fracture study from Robenek's laboratory, presumptive nascent lipid droplets in macrophages were identified in proximity to the ER membrane, which formed egg-cup-shaped folds around the droplets. The lipid droplet-associated protein, adipophilin (ADPH), was localized by immunolabeling of the fractured surfaces on both the lipid droplet surface and the cytoplasmic leaflet of the ER adjacent to the droplets. These specialized regions of the ER were postulated to be the sites of lipid droplet assembly, and ADPH was suggested to play a role in facilitating the transport of triacylglycerols and fatty acids between the ER and the forming droplets. ADPH may also promote lipid accretion by sterically blocking lipase-mediated turnover. Whether the many other proteins and enzymes required for the assembly of nascent lipid droplets are also concentrated in such specialized areas of the ER is unknown, but this could be evaluated by similar immunolabeling techniques using lactating mammary tissue.

The size range of the lipid globules in milk argues that lipid droplets can grow substantially in volume after their formation. Globules with diameters >1 μm account for 90% or more of the total volume of lipid in cow's milk even though 80% or more of the droplets are <1 μm in diameter. Given this distribution, one can infer that many droplets grow little in volume, if at all, after their initial formation, but that the larger droplets must grow appreciably before secretion. Whether droplet growth occurs randomly or is a controlled process is unknown. Potential mechanisms include droplet expansion by the self-synthesis of triacylglycerols through the activity of droplet-associated lipogenic enzymes, or by the incorporation of

triacylglycerols shuttled from sites of synthesis in the ER to the droplets by lipid carrier proteins. However, the only mechanism for which there is morphological and biochemical evidence is the direct fusion of microlipid droplets with each other. Fusion appears to be restricted to small droplets; these can fuse with each other and with larger droplets, but large droplets over ~2 μm in diameter do not appear capable of fusing with each other. From morphometric studies, it is apparent that lipid droplets can grow appreciably in volume just before and even during the secretion process as the droplet is being coated with the apical plasma membrane. That fusion may be a regulated process is suggested by observations that calcium promotes fusion and that small-molecular-weight GTP-binding proteins are present on droplet surfaces.

Lipid droplets traverse the cell from their sites of origin, primarily in basal and medial/lateral regions, to the cell apex, from whence they are secreted. Judging from electron micrographs of fixed tissue, droplets may associate with other organelles besides the ER during transit, including mitochondria, the Golgi complex, and secretory vesicles. Whether these associations are fortuitous, or are functionally important to droplet assembly, is unclear. In this context, proteomic analysis of the MFGM from several species has identified protein constituents from several intracellular sources in the secreted membrane, indicating that such associations may well modify the composition of the MFGM (*see* **Milk Lipids**: Milk Fat Globule Membrane; also see below).

Transit of the lipid droplets is almost certainly guided by cytoskeletal elements, but exactly how they function is not clear. The potential involvement of microtubules is suggested by live-cell imaging studies of the trafficking of lipid droplets in other cells that show the association and movement of lipid droplets along microtubule tracks. Microtubules are abundant in milk-secreting cells, and proteomic analysis has identified microtubule motors and microtubule-associated proteins in fractions of cytoplasmic lipid droplets from lactating mouse mammary gland. Drugs that disrupt microtubules or interfere with microtubule assembly suppressed milk lipid secretion when infused into the mammary gland via the teat canal. In glands treated with such drugs lipid synthesis was not inhibited and the cytosolic lipid droplets were larger than those in contralateral untreated control glands. Unfortunately, the secretion of milk serum was also inhibited, which precluded any definitive conclusions. The potential role of microtubules in the trafficking of lipid droplets in mammary epithelial cells deserves further study especially using live-cell imaging approaches to follow the movement in real time of droplet-associated proteins tagged with green fluorescent protein analogues. Actin-containing microfilaments and keratin intermediate filaments are also abundant in milk-secreting cells and keratin filaments show a strong propensity to bind lipids. In adipocytes, intermediate filaments form a cage around the lipid droplets. However, the potential role of such elements in lipid droplet assembly and trafficking in mammary cells has not been investigated.

Upon arrival at the cell apex, lipid droplets are discharged into the alveolar lumen by budding from the apical surface, such that the droplets are entirely coated with membrane (**Figures 1** and **2**(**a**)). During this process, a 10–20 nm thick electron-dense layer becomes visible in electron micrographs between the lipid droplet surface and the plasma membrane bilayer. This coat is presumed to consist of the cytoplasmic tails of integral plasma membrane proteins and peripheral proteins derived from multiple sources, including the lipid droplet surface, the cytoplasmic face of the apical membrane, and the cytoplasm. In static electron micrographs, droplets of all size classes, from very small to large, appear to be secreted by the same mechanism. In some cases, cytoplasm and even whole organelles may become trapped between the plasma membrane and the droplet, resulting in the formation of a structure known as a cytoplasmic crescent (*see* **Milk Lipids**: Milk Fat Globule Membrane). Crescent formation appears to be much less common in cows than it is in some other species.

In an alternative mechanism, Wooding has proposed that lipid droplets are surrounded by secretory vesicles in the cytoplasm. Progressive fusion of neighboring vesicles on the surface of the droplets is postulated to generate vacuoles, in which the lipid droplets are enveloped in secretory vesicle membrane. The contents of such vacuoles, which would include lipid droplets and skim milk components, are then presumed to be released by exocytosis from the apical surface. A combination of both mechanisms is also possible, with contributions to the MFGM coming from both the apical surface and the secretory vesicle membrane as the droplets engage with both membranes at the cell surface.

The bulk of available morphological and biochemical evidence favors the direct plasma membrane mechanism because some of the major integral proteins of the MFGM, such as butyrophilin and the mucin, MUC-1, are concentrated in the apical plasma membrane but not in secretory vesicle membranes. However, the possibility that both mechanisms may operate, perhaps with one or the other being more prevalent at different stages of lactation, cannot be excluded.

Little is known about the actual processes involved in recognition between lipid droplets and the plasma membrane, and what forces are required to expel the droplets from the cell. Several molecular mechanisms have been proposed based on the phenotypic analysis of knockout mouse strains, immunohistochemistry of selected MFGM proteins, and protein–protein interaction assays. Most models are based on the assumption that interaction between the lipid droplet and membrane occurs between

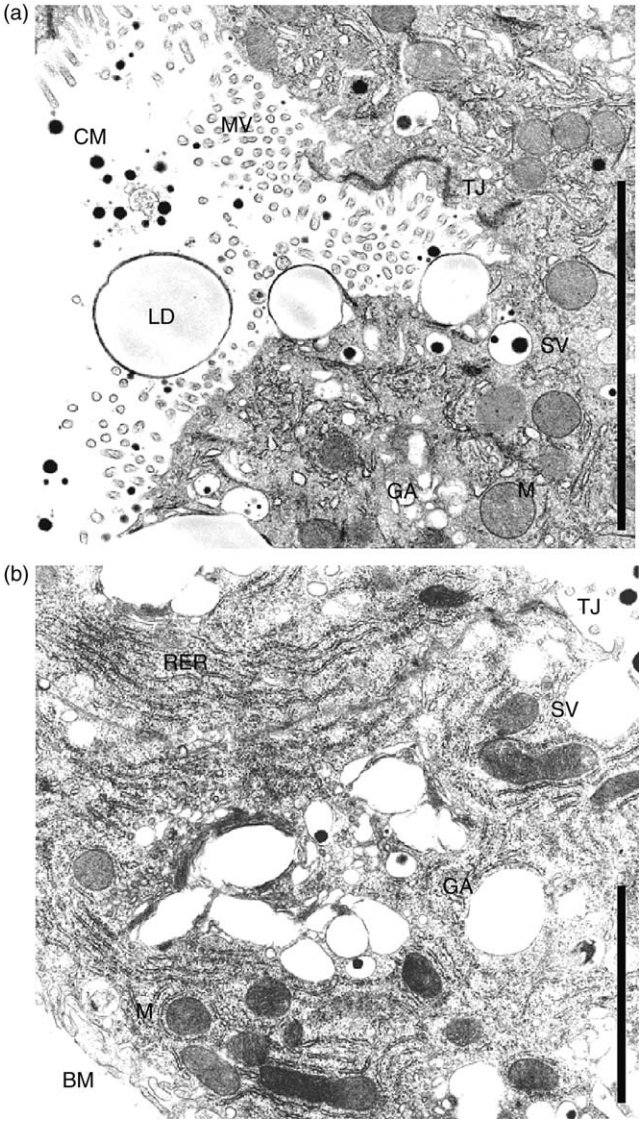

Figure 2 Electron micrographs of milk-secreting cells. (a) Lipid droplet secretion from the apical surface. One lipid droplet has been secreted and two are budding from the apical plasma membrane. (b) Entire cell showing centrally located Golgi apparatus with the associated swollen secretory vesicles and casein micelles. A secretory vesicle at the apical surface has fused with the apical plasma membrane. BM, basement membrane; CM, casein micelle; GA, Golgi apparatus; LD, lipid droplet; M, mitochondrion; MV, microvilli; RER, rough endoplasmic reticulum; SV, secretory vesicle; TJ, tight junction. Scale = 5 μm. Part (**a**) is reproduced with modifications by permission of the National Academy of Sciences, USA, from Ogg SL, Weldon AK, Dobbie L, and Mather IH (2004) Expression of butyrophilin (Btn1a1) in lactating mammary gland is essential for the regulated secretion of milk-lipid droplets. *Proceedings of the National Academy of Sciences of the United States of America* 101: 10084–10089.

proteins and protein domains exposed on the inner face of the apical plasma membrane, those on the droplet surface, and proteins from the cytoplasm. These protein complexes are presumed to give rise to the electron-dense layer sandwiched between the lipid droplet surface and the outer membrane bilayer visible in electron micrographs of osmium-fixed tissue. Proteins that contribute to this coat complex include butyrophilin 1A1 (BTN), xanthine dehydrogenase/oxidase (XDH/XO), and ADPH, which are all major constituents of the MFGM in many species. Knockout of the BTN (*Btn*) and XDH/XO (*Xdh*) genes severely disrupts the secretion of lipid droplets in $Btn^{-/-}$ and $Xdh^{+/-}$ mice, and large lipid droplets accumulate in the epithelial cell cytoplasm. In *in vitro* assays, XDH/XO binds to the B30.2 domain of BTN in the cytoplasmic tail, and in one model it is postulated to aggregate BTN in the plane of the lipid bilayer to form a multimeric complex with other proteins including ADPH on the lipid droplet surface. Thus BTN is seen to function as a transmembrane scaffold linking the membrane bilayer to the droplet surface. In distinct contrast, Robenek has proposed that formation of the MFGM is

mediated through homophilic interactions between BTN on the lipid droplet and BTN in the outer membrane. However, this latter model requires the direct association of BTN, which is an integral transmembrane protein, with the lipid droplet in an unconventional topology. In a third model, ADPH on the lipid droplet surface is proposed to directly bind to the lipid bilayer via a putative hydrophobic cleft in the C-terminus. There is some evidence that lipid globule secretion may be controlled by protein kinases or phosphatases, by calcium, and by GTP-binding proteins, although nothing is known about how such regulatory molecules may function. Resolution of all the above issues would be aided by analysis of lipid secretion in cultured mammary cells *in vitro*. Unfortunately, no established mammary cell lines that secrete membrane-coated lipid droplets in a regulated manner are available.

Following secretion, the MFGM undergoes structural rearrangements, and some membrane vesiculates and is lost to the skim milk phase (*see* **Milk Lipids**: Milk Fat Globule Membrane).

Exocytosis (Pathway 2)

Unlike the mechanism of lipid secretion, which is unique to mammary cells, milk protein transport and secretion follow the universal secretory pathway. Milk proteins synthesized in the rough ER are processed through the Golgi complex, packaged into secretory vesicles, and secreted from the apical surface by exocytosis (**Figure 1**).

Major secretory proteins of cow's milk comprise the caseins (α_{s1}-, α_{s2}-, β-, and κ-), α-lactalbumin, and β-lactoglobulin (*see* **Milk Proteins**: Casein Nomenclature, Structure, and Association). All of these proteins are synthesized with N-terminal signal peptides, which target the respective mRNAs to the ER for translocation of the nascent peptides across the ER membrane. Within the ER lumen, the proteins are co- and posttranslationally modified and the signal peptides are proteolytically removed. The secretory proteins are then transported to the *cis* face of the Golgi apparatus in vesicles, which, by analogy with other better-characterized secretory systems, are most likely coated with COPII proteins. These coat proteins serve to recruit protein cargo to specialized exit sites in the ER and form a cage on the cytoplasmic side of the membrane, thus inducing curvature. Completed vesicles are released, which then transit to the *cis* face of the Golgi apparatus via an intermediate sorting station comprising tubular–vesicular elements, which is commonly referred to as the intermediate compartment. Whether such vesicles and tubules fuse together to form new Golgi cisternae or shuttle to preexisting Golgi membranes is currently the subject of intense debate. This point is yet to be studied in mammary epithelial cells.

Transport of proteins through the Golgi stack to the *trans* side is accompanied by further processing, although the exact sites for such reactions in mammary cells are unknown. At some point, the caseins are phosphorylated, and the *O*-linked glycan chains associated with κ-casein terminally sialylated. The caseins begin to assemble into micelles as they transit the Golgi apparatus and as they are packaged into secretory vesicles (*see* **Milk Proteins**: Caseins, Micellar Structure). Calcium, which is needed for casein micelle formation, is actively transported across Golgi membranes by Ca^{2+}-stimulated ATPases. However, in a recent paradigm shift, the bulk of the calcium in milk appears to be transported directly across the apical surface by a plasma membrane type Ca^{2+} ATPase (see section 'Secretion of Minerals and Water (Pathway 4)'). This implies that casein micelle formation may well continue in the alveolar lumen following secretion.

Following processing, the caseins and other secretory proteins are packaged into secretory vesicles. Whether such vesicles selectively contain just caseins or whey proteins, or both, has not been firmly established, although in one study rat caseins and α-lactalbumin were identified in the same secretory vesicles. Compared with secretory granules in other cell types, mature vesicles in mammary cells are swollen and distended, and as such they constitute a morphological hallmark of milk-secreting cells during lactation (**Figures 2**(**a**) and **2**(**b**)). Extensive swelling of the vesicles is presumed to be due to the presence of lactose, which is synthesized in the Golgi apparatus and draws water and ions into the vesicles by osmosis.

After formation, the secretory vesicles transit to the apical pole, possibly guided by microtubules. However, as is the case with the transport of lipid droplets, the experimental evidence for this is equivocal. Secretion of vesicle contents is accomplished by exocytosis, during which the vesicles fuse with the apical plasma membrane and the contents empty into the alveolar lumen. By analogy with secretion in more widely characterized secretory systems, the targeting and fusion of the vesicle membrane will be mediated by SNAREs, which form four-helix protein bundles (SNAREpins) that drive fusion of the apposing lipid bilayers. The identity of the SNAREs responsible in bovine mammary cells is uncertain. The mammalian plasma membrane/vesicle SNAREs, SNAP 23, syntaxin 3, and VAMP 2, have all been identified in bovine MFGM by proteomics, thus plausibly suggesting that they may function in secretory vesicle–plasma membrane fusion before being incorporated into the MFGM by default.

Exocytosis may occur by either simple or compound mechanisms. In simple exocytosis, single vesicles

individually fuse with the plasma membrane. In compound exocytosis, vesicles in the cytoplasm interconnect in chains that fuse with each other. Thus, the contents of a number of vesicles can be released from the cell in a single vesicle–plasma membrane fusion event. Although both mechanisms have been observed in mammary cells by electron microscopy, simple exocytosis appears to be the most prevalent mechanism (**Figures 1** and **2**(**b**)).

Secretion from most cells can be classified into one of two main types: constitutive or regulated. Constitutive secretion is typically a continuous 'housekeeping' process, which is common to most cells. In regulated secretion, secretory protein is stored in membrane-bounded vesicles as highly condensed aggregates, which are secreted only when an external signal stimulates rapid exocytosis of the vesicle contents in a secretory burst. Milk protein secretion defies such a straightforward classification. On the one hand, it appears to be continuous, apparently needing no regulatory signal and thus could be considered constitutive. On the other hand, mature casein micelles are morphologically like regulated protein aggregates, even though they are surrounded by milk serum in swollen atypical vesicles. By the use of isolated primary acinar preparations, Burgoyne and colleagues have shown that casein secretion may occur at basal levels by an apparently constitutive mechanism. However, addition of calcium to these preparations stimulated secretion in an apparently regulated manner. This suggests that milk protein secretion may occur by both constitutive and acute calcium-regulated mechanisms, but, as yet, no external regulator of cytosolic calcium levels has been identified.

For many years, it was assumed that the secretory vesicle membrane added to the plasma membrane during exocytotic fusion serves to replenish that portion of the plasma membrane expended in fat globule envelopment. Although this may occur, it is obvious from the size and surface area of secretory vesicles and the volume of secreted skim milk that more vesicle membrane is added to the surface than is needed for the formation of the MFGM. Thus, a large fraction of the added membrane is most likely retrieved by endocytosis and is possibly recycled for further use, as occurs in many other cell types. Nothing is currently known about such possibilities in mammary epithelial cells. Membrane may also vesiculate at the surface and bleb off into the lumen, and contribute to the skim milk membrane fraction. In this context, vesicles derived from mammary epithelial cells and containing marker proteins such as BTN, XDH/XO, and immune molecules have been isolated and characterized from human breast milk. Such 'exosomes' are assumed to play immunomodulatory roles in protecting the gland from infections.

Transcytosis (Pathway 3)

Proteins found in milk that originate from cells other than milk-secreting cells most probably cross the mammary epithelium by transcytosis. Such proteins may include the immunoglobulins, transferrin, serum albumin, and prolactin. Similarly one can infer that at least some of the other peptide hormones, in addition to prolactin, found in milk cross the mammary epithelium by similar means. The only other possible route is between the cells by paracellular transport (Pathway 5), which, as indicated in the section, Introduction, is unlikely to occur in an established lactation because the epithelial tight junctions are sealed, at least in cows and mice.

Transcytosis begins by the uptake of extracellular material by endocytosis. Uptake into membrane-bounded vesicles can occur by either a clathrin-dependent or a clathrin-independent mechanism. Either mechanism may be receptor-mediated, or involve nonspecific uptake of extracellular fluid, the latter of which is known as fluid-phase endocytosis or pinocytosis. If the transport is in a basal to apical direction, the material is first delivered into a basally located sorting endosome (**Figure 1**) and is then re-sorted for delivery in a second vesicle through the cytoplasm to an apical endosomal sorting station. A final sorting step ensures delivery to the apical surface and secretion of the vesicle contents to the other side of the cell. During transit through the cell some constituents may be delivered to intracellular organelles for processing. For example, in rabbit and mouse mammary cells, transferrin and prolactin are taken up into coated vesicles, transported to basal endosomes, and then delivered either to multivesicular bodies or to the Golgi complex, wherein they enter the apically directed secretory pathway.

In the mammary gland, transcytosis is critically important for the transport of immunoglobulins, especially during the formation of colostrum, but also at lower levels throughout lactation. In cows, IgG_1 is selectively taken up from the serum via the interstitial fluid into vesicles by a specific receptor, which is most likely to be the neonatal Fc receptor (FcRn), a receptor that is widely expressed in epithelia that transport IgGs. Although the exact intracellular route remains unclear, it is assumed that the internalized IgG_1 is transported without modification to the apical side of the epithelium for secretion into colostrum or milk. IgG_2 is presumed to be taken up nonselectively into either the same IgG_1-containing vesicle, or a different class of vesicle by fluid-phase endocytosis and similarly transported. Thus IgG_1 is selectively concentrated four- to fivefold over levels in the serum, compared to IgG_2, whose concentration is more than threefold higher in the serum. In contrast, IgA is synthesized in plasma cells underlying the mammary epithelium, secreted as a dimer in association with

J chain, and taken up by the secretory cells into clathrin-coated vesicles by binding to the polyimmunoglobulin receptor. At some point during transcytosis, the exoplasmic portion of the polyimmunoglobulin receptor is cleaved and subsequently secreted as 'secretory component' bound to the IgA dimer, thus giving rise to secretory IgA (sIgA) in colostrum and milk.

Cells can transport material by transcytosis in either direction (apical to basal, or basal to apical). Depending upon cell type and physiological need, the amount of material transported in either direction may be substantially different. In mammary cells, it is assumed that most material (e.g., IgG, sIgA, transferrin, serum albumin) is transported in a basal to apical direction. However, there is fragmentary experimental evidence for the uptake of material from the milk and transport to the basal side of the cell. The potential physiological importance and magnitude of this pathway is unclear. Unfortunately, we know little else about the transcytosis pathway as it operates specifically in the mammary gland.

Secretion of Minerals and Water (Pathway 4)

Water is the most abundant constituent of cow's milk and, as indicated above, is most probably drawn into secretory vesicles in the Golgi complex by osmosis following the synthesis of lactose. In many cells, water transport is facilitated by the aquaporins, a family of integral membrane proteins that form pores in membrane bilayers. Aquaporin 3 has been histochemically localized to the basal and lateral plasma membranes of secretory and ductal epithelial cells in the rat mammary glands, but apparently no such proteins were detected in Golgi membranes, secretory vesicles, or the apical surface. Therefore, aquaporin 3 most probably functions in the uptake of water from the interstitial fluid across basal membranes. The secretion of water across apical membranes is presumed to be driven by ion gradients (see below).

There is an extensive history of investigation of transport mechanisms for univalent and divalent ions into milk, which is beyond the scope of this review. Briefly, the K^+/Na^+ ratio in secretory cells is maintained by a classical Na^+/K^+ ATPase on basal/lateral membranes that maintains an excess of K^+ over Na^+ in the secretory cell cytoplasm. This same K^+/Na^+ ratio is maintained in milk, albeit at lower total concentrations, by transport of the two ions across the apical membrane. A Na^+–K^+–Cl^- cotransporter on basal/lateral and possibly apical membranes serves as an inward-facing Cl^- pump and ensures that the concentration of Cl^- in the cytoplasm is higher than in milk. Thus milk is electrically positive relative to the cell cytoplasm, and it is this electrical gradient across the apical plasma membrane that is presumed to be the driving force for the secretion of water into milk. Osmotic balance between the cell cytoplasm and milk is modulated by the level of lactose, such that for any decrease in the amount of milk lactose there is a compensatory rise in the amount of K^+ and Na^+ in milk, on a day-to-day basis.

Ca^{2+} is the most abundant divalent cation in many milks, with total concentrations of 31 and 7.5 mmol l^{-1} in bovine and human milk, respectively. It was long thought that the major pathway for the transport of Ca^{2+} into milk was via a Golgi-located Ca^{2+} ATPase, which provided more than enough Ca^{2+} for the formation of casein micelles in Golgi-derived vesicles. However, recent work by VanHouten and Wysolmerski has shown that the principal transporter of Ca^{2+} in the mouse mammary gland is a spliced variant of the plasma membrane Ca^{2+} ATPase isoform 2 (PMCA2bw), which is targeted to the apical plasma membrane. This transporter is estimated to account for about 70% of the Ca^{2+} secreted into mouse milk, whereas the two Golgi-located transporters, called secretory pathway Ca^{2+} ATPases 1 and 2 (SPCA1 and 2), account for the remaining 30%.

Conclusions and Perspective

Today there is a good understanding of the general pathways by which lipids, milk-specific proteins, lactose, and some of the ions and water of milk are secreted, and we have a general idea of how non-milk-specific proteins can transverse the mammary epithelium to enter milk. What is missing is specific information about the mechanisms and molecular events involved in any of these processes in mammary epithelial cells. The revised paradigm for Ca^{2+} secretion discussed in the last section neatly highlights the potential surprises that may be in store.

Perhaps because the mechanism of lipid secretion is unique to the mammary gland, this has been studied in some detail, and we may be on the verge of understanding the molecular and regulatory processes involved. On the other hand, possible mechanisms for the exocytotic secretion of skim milk proteins and the molecules involved have been formulated by analogy with better-characterized model secretory systems (e.g., yeast and the neuronal synapse). In addition, much of what we know about mammary gland secretion is based on the examination of static electron micrographs and from some dated biochemical studies. There is no reason, *a priori*, to suppose that secretory mechanisms will be any different in mammary epithelial cells than those in other cells. However, several activities are unique to mammary cells, notably the synthesis of lactose, the assembly of casein micelles, and the secretion of membrane-coated lipid droplets followed by the continual loss of apical membrane that this process entails. Furthermore, intracellular and apical membrane

may be lost in the form of 'exosomes' and contribute to the skim milk membrane fraction. The synthesis of lactose stimulates water transport into Golgi vesicles, which alters the morphology of the entire apparatus (**Figure 2**(**b**)). How this might affect transport of material through the Golgi stack is unknown. Mammary cells are ripe for study using contemporary live-cell imaging approaches and by the application of other modern cellular and molecular biological approaches. It remains a pity that we have so little specific information about the secretion of a fluid that is so critical for the survival of mammalian life.

See also: **Lactation**: Galactopoiesis, Effects of Hormones and Growth Factors; Galactopoiesis, Effect of Treatment with Bovine Somatotropin; Lactogenesis. **Mammary Gland, Milk Biosynthesis and Secretion**: Milk Fat; Milk Protein; Lactose. **Milk Lipids**: Milk Fat Globule Membrane. **Milk Proteins**: Casein, Micellar Structure; Casein Nomenclature, Structure, and Association; Immunoglobulins; α-Lactalbumin; β-Lactoglobulin.

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