A NEW LOOK AT TRANSUDATION:
THE APOCRINE CONNECTION

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Abstract

Transcellular trafficking in which various molecules are transported across the interior of a cell, is commonly classified as transcytosis. However, historically this term has been used synonymously with transudation. In both cases transcellular trafficking starts with the internalization of proteins or other compounds on the basal or basolateral side of a cell and continues by their transport across the interior to the apical pole (or vice versa) where they are subsequently released. This allows a cell to release products which are synthesized elsewhere. Here, we discuss the common features of both transcytosis and transudation, and that which differentiates them. It appears that transcytosis and transudation are identical in terms of vesicular import and endosomal sorting of cargo, but completely differ in the re-secretion process. Specialized epithelial cells re-release substantial quantities of the endocytosed material, and often also a great variety. Some recent studies indicate that this is achieved by non-canonical apocrine secretion rather than by the regular vesicular mechanism of exocytosis, and takes place only on the apical pole. This massive re-release of endocytosed proteins, and potentially other compounds via the apocrine mechanism should be considered as transudation, distinct from transcytosis.
Abbreviations:

AAT = alpha 1-antitrypsin
BAR = Bin/Amphiphysin/Rvs
BSA = bovine serum albumin
CF = cationized ferritin
CI-MPR = cation-independent mannose 6-phosphate receptor
ECP = eosinophil cationic protein
EE = early endosome
EGF = epidermal growth factor
EGFR = EGF receptor
ER = endoplasmic reticulum
Fc = fragment crystallizable
FcRn = neonatal Fc receptor
GFP = green fluorescent protein
HRP = horseradish peroxidase
Ig = immunoglobulin
MEF = middle ear fluid
MVB = multivesicular bodies
NF = native ferritin
PI(3)P = phosphoinositide phosphate
plgA = polymeric immunoglobulin A
plgR = polymeric immunoglobulin receptor
PX = phox homology
Rab = Ras-related proteins in brain
Rac = Ras-related C3 botulinum toxin substrate
Ras = Rat sarcoma
RFP = red fluorescent protein
RIP-1 = Rab6-interacting protein-1
SNX = sorting nexins
TGF-β1 = transforming growth factor-beta1
TGN = trans-Golgi network
TNF-α = tumor necrosis factor α
vATPase = vacuolar ATPase proton pump
VPS = vacuolar protein sorting
YFP = yellow fluorescent protein
1. Introduction

In contrast to the widely-known and well-defined process of exocytosis, apocrine secretion can be characterized as a non-vesicular transport and non-canonical secretory pathway that entails the loss of part of the cytoplasm. Exocytosis (also known as merocrine secretion), is an intensely studied mechanism of eukaryotic cells with more than two dozen identified factors and their corresponding genes (Jahn, 2004; Rutter and Tsuboi, 2004; Sudhof, 2004; Chieregatti and Meldolesi, 2005; Barclay et al., 2005; Snyder et al., 2006; Westerink, 2006; Leitzell, 2007; Deak et al., 2008; Beck et al., 2009; Südhof and Rothman, 2009; Anantharam et al., 2010; He and Guo, 2010; Morgan et al., 2013; Murray and Stow, 2014; Kokotos and Cousin, 2015; Cousin, 2017; Milosevic, 2018; Anantharam and Kreutzberger, 2019; Maj et al., 2019; Zuber and Lučić, 2019). Exocytosis is the process relaying on specific membrane contact, priming and fusion events required for the selective release of compartmentalized compounds such as signaling molecules (neurotransmitters or hormones). The exocytotic secretory pathway involves the formation of vesicles in the trans-Golgi in its initial phase, then targeted translocation of these vesicles to sites on the plasma membrane, nucleation, zippering, budding, the preparation of the docked vesicles for full fusion competence (priming), and the subsequent triggered fusion of these membranes; this results in their coalescence and the release of vesicular contents to the extracellular space. In addition to exocytosis which occurs by the targeted fusion of secretory vesicles with the plasma membrane, two additional types of non-canonical secretion exist: apocrine and holocrine. During theses secretions entire pieces of the cell are released and homotypic membrane fusion is not required. Apocrine secretion involves apical protrusions and generates cytoplasmic fragments inside a secretory lumen. As we discovered, this process is accompanied by the release of large fragments of cellular structures and entire organelles that include mitochondria, Golgi, and portions of the endoplasmic reticulum (ER), among others (Farkaš et al. 2014), during its most intense phase. Proteomic
analyses revealed that the secretion is composed of hundreds to thousands of microsomal, mitochondrial, ribosomal, membranous, cytoskeletal, and even nuclear as well as nucleolar proteins. Strikingly, although many nuclear proteins are released, the nuclear DNA itself remains intact (Farkaš et al. 2014). In spite of this complexity, it appears that several protein components of apocrine secretion are identical, regardless of the location of the apocrine gland (Farkaš 2015, 2016).

From a historical point of view, the first published study on an apocrine secretory organ was performed by Harder (1694) who described a special lachrymal gland in rodents. Some 140 years later, the human sweat gland, currently the most intensely studied apocrine organ, was discovered by Purkyně (1833a,b) and described in detail by his pupil Wendt (1833, 1834). Almost simultaneously, their findings were confirmed and extended by Breschet and Roussel de Vouzeme (1834), and by Gurlt (1835). The axillary armpit glands, which contain the highest known concentration of apocrine sweat glands in the human skin, were first recognized by Horner (1846). Ranvier (1879) was the first author to distinguish „holocrine“ secretion in the sebaceous gland from „eccrine/merocrine“ secretion in the sweat glands. But it was not until 1922 when Schiefferdecker, based on Ranvier’s observations, suggested that the secretory gland cells could be classified functionally according to how they secreted their contents, to an eccrine/merocrine, apocrine or holocrine mechanism. This made a breakthrough contribution as it established a clear functional definition of three substantially different categories of secretion based on the mechanism underlying the externalization of cellular materials.

Since that time, apocrine secretion has been confirmed and studied by many authors (Holmgren, 1922; Herzenberg, 1927; Mathis, 1927; Moriyama, 1927; Richter, 1932; Richter and Schmidt, 1934; Kato, 1936; Kuno, 1938; Wolf, 1940; Minamitani, 1941a,b,c). It has also been described in other tissues including mammary (Loeb, 1932; Dabelow, 1957; Bargmann and Knoop, 1959; Bunting, 1984) and ceruminous glands (Kawabata and Kurosumi, 1976;
Kurosumi and Kawabata, 1977; Testa-Riva and Puxeddu, 1980). Interestingly, when we discovered apocrine secretion in *Drosophila* (Farkaš *et al.* 2014), a popular and widely used genetic model organism, it allowed a seminal reappraisal of our understanding of apocrine secretion. The insights gained from molecular genetic analyses of their salivary glands has provided a glimmer of hope for elucidating the mechanistic aspects of this fundamental process (Farkaš, 2015). In the course of these and following studies we uncovered that the developmental period of the salivary glands shortly prior to apocrine secretion is dominated by high intense vacuolation due to abundant clathrin-mediated endosomal trafficking. This trafficking is affiliated with the re-secretion of numerous endocytosed proteins that had been produced by distant tissues (Farkaš *et al.*, 2015, 2018). Resecretory activity in the literature is recurrently associated with transcytosis; however, until now the releasing phase of transcytosis has been almost uniformly linked to exocytotic secretion (Hermo and Mello, 1987; Kobayashi *et al.*, 2002; Vergés *et al.*, 2004, 2007; Vergés, 2016). This prompted us to reinvestigate the relationship between (endosomal) uptake of extracellular cargo and its re-release by two substantially different mechanisms: exocytosis or apocrine secretion.

As shown in Figure 1, in this context, transcytosis is most frequently used term to describe the process where vesicle-dependent endocytosis on one pole of the cell is employed to uptake a cargo, and vesicle-dependent exocytosis on the other pole of the cell is used to release it (Rodman *et al.*, 1990; Frank *et al.*, 2009; de Lange, 2012; Preston *et al.*, 2014; Ayloo and Gu 2019). By other words, transcytosis is a type of transcellular passage in which various droplets, molecules, or particles are transported across the interior of a polarized cell (Apodaca *et al.*, 1991; Antohe *et al.*, 1997; Simionescu *et al.*, 2002; Lodish *et al.*, 2013; Alberts *et al.*, 2015; Villaseñor *et al.*, 2017). Cytopempsis appears to be a specific form of transcytosis of a substance into a cell (and through the cytoplasm in a micropinocytotic vesicle) followed by its release to the cell exterior without utilization by the cell (Staubesand, 1965; Ma *et al.*, 1974;
Weiss, 1979; Thorn et al., 1983; Platz, 2006). As described in detail in this review, transcytosis is a complex endosomal process with numerous players and routes, and can serve both unidirectional and bidirectional transport with or without modification of a cargo. A significantly less abundant term used in association with transcellular traffic is transudation, the process of oozing or seeping when a substance is released along with some fluid. Transudation is usually connected to the release of sweat or the process of vascular exuding, the escape of liquids from blood vessels through pores or breaks in the cell membranes, and is caused by disturbances of hydrostatic or colloid osmotic pressure (Auerswald et al., 1952; Schorn et al., 1975; Predescu et al., 2007; Sun et al., 2009). In broader terms, transudation describes the passage of a fluid or solute through a membrane by hydrostatic or osmotic pressure. In medicine, it is often, but not exclusively, used to identify vascular leakage of vessels fluid that can cause cirrhosis-, nephrotic syndrome- or malnutrition-associated hypoalbuminism along with some other pathologies (Venkatarami and Govindappa, 1985; Dubey et al., 2001; Vlachogiannakos et al., 2013; Bañares and Bernardi, 2019; Di Pascoli et al., 2019). In the present study we discuss several cases where transudation has been observed and is involved in the release of various serum components, colonic mucosa, ascitic fluid, pulmonary fluids, saliva, tears, ascitic fluid, cervicovaginal and oviductal secretions, etc., which share numerous common protein entities many of which are re-released but not produced by the tissue. A common feature of these tissue fluids is that they are released by apocrine secretion. Taking into consideration our recent clarification and re-definition of apocrine secretion as a non-canonical and non-vesicular transport and secretory mechanism (Farkaš, 2015), we herein summarize the collective and prevailing attributes of transcytosis and transudation which may share only the uptake mechanism (endocytosis). Furthermore, we establish crucial differences on the discharge side, where emission of the cargo is mediated, via exocytosis vs. apocrine machinery, respectively.
2. Transcytosis

2.1 Entrance phase - endocytosis and early sorting

Transcytosis has been observed in connection with numerous transport and externalization processes of the cell. Several detailed studies of transcytosis-linked processes described in the literature are associated with engulfment or endocytosis and baso-apical recycling of the vacuolar ATPase proton pump (vATPase) required for luminal acidification of endosomes, characteristic for many transporting epithelia (Brown and Sabolić, 1993; Brown and Breton, 2000). Although under various circumstances transport from the basal and basolateral pole to the apical pole or vice versa does not fulfill the features of complete transcytosis, it does represent the bipolar transcellular transport of membrane constituents (Schneider et al., 1979; de Chastellier et al., 1987); therefore we still can learn important information from these studies. In experiments with artificial cargo such as native ferritin (NF) or horseradish peroxidase (HRP) to trace fluid-phase endocytosis, or with cationized ferritin (CF) to examine adsorptive endocytosis, it is possible to follow clathrin-mediated endocytosis when tracers first enter an apical tubulovesicular endosomal system, then larger apical endosomal vesicles and later multivesicular bodies (MVB).

It is widely accepted that the intracellular network of endosomes is a highly organized cluster of membrane-derived compartments that act as sorting sites for both, endosomal and biosynthetic cargo. The subsequent fate of the engulfed cargo depends upon its interaction with a variety of molecularly distinct processing complexes, which tightly regulate the close relationship between the sorting of their particular load and the native process of membrane re-carving, essential for the proper formation of transport carriers (McGough and Cullen, 2011; Shen et al., 2018). One such complex, retromer, among others primarily mediates retrograde transport from endosomes to the trans-Golgi network (TGN) (Burd and Cullen, 2014; Gallon
and Cullen, 2015; Abubakar et al., 2017; Kvainickas et al., 2017; Simonetti et al., 2017).

Nonetheless, the bipolar transport of membrane components, such as vATPase, must be able to escape these degradative routes. Even though this membrane component is not necessarily produced by other distal tissues (and therefore not has undergone adsorptive endocytosis), it is still able to enter the same efficient intracellular transport and delivery mechanism as foreign cargo, which is either identical or similar to the transcytotic route.

Kumagai et al. (2011) described the binding of IgG to the neonatal Fc receptor (FcRn) followed by internalization of this complex, and suggested apical receptor-mediated transcytosis via the TGN. Subsequently, the FcRn located inside the apical plasma membrane binds maternal IgG, and becomes internalized by endocytosis into the absorptive cells. The FcRn plays a key role in providing the fetus or newborn with humoral immunity before its immune system becomes fully developed as it transports maternal IgG across various epithelial barriers. The authors concluded that in newborns, FcRn transfers IgG from milk to blood by apical-to-basolateral transcytosis across intestinal epithelial cells. The work of He et al. (2008) showed that the efficient unidirectional transport of IgG within the intestinal epithelial cells is facilitated by a pH difference between the apical (pH 6.0-6.5) and basolateral (pH 7.4) sides, since FcRn binds IgG at pH 6.0-6.5 but not at pH 7 or more; the basolateral pH 7.4 facilitates release of the cargo. The maternal IgG is absorbed by FcRn-expressing cells in the proximal portion of the small intestine (the duodenum and jejunum) when milk passes through the neonatal digestion; the remaining proteins are absorbed or degraded by FcRn-negative cells in the distal portion of the small intestine (ileum). In this context, a more complex view in which Fc moves throughout networks of entangled tubular and irregular vesicles before it reaches the basolateral surface was investigated by a combination of electron tomography and non-perturbing endocytic labeling (He et al., 2008). Distinct markers for early, late and recycling endosomes, each labeling vesicles in different and overlapping morphological classes, have
helped these authors to reveal the spatial complexity in endolysosomal trafficking. These observations elucidated important features of transcytosis, including transport involving multivesicular bodies, inner vesicles/tubules and exocytosis through clathrin-coated pits. They also indicate that MVBs, most likely a specific subclass of them, are involved in processive transcytosis and act in a distinct non-lysosomal (non-degradative) pathway, possibly requiring a specific sorting mechanism.

2.2 Post-entrance phase - the central role of retromer

The endosomal system is composed of an interconnected network of diverse membrane-bound structures and subcellular compartments whose primary function is to receive, dissociate, and sort cargo that originates either at the plasma membrane or from biosynthetic pathways (McGough and Cullen, 2011; Burd and Cullen, 2014; Gallon and Cullen, 2015). Several years ago, there was a large amount of interest in a system associated with transcytosis after endosomal uptake and trafficking of polymeric immunoglobulin receptor (pIgR) and the role of a retromer complex in this process (Kobayashi et al., 2002; Vergés et al., 2004, 2007).

Retromer consist of two major components: (1) a heterotrimer encoded by the vacuolar protein sorting (Vps) genes Vps26, Vps29, and Vps35, the role of which is to select cargo, and (2) a dimer of phosphoinositide-binding sorting nexins (SNX), whose function is to deform the membrane (Bonifacino and Hurley, 2008; Vergés, 2016). In other words, retromer is a heteropentameric complex that associates with the cytosolic face of vesicles and mediates the retrograde transport of transmembrane cargo from endosomes to the TGN. It comprises a sorting nexin dimer composed of a combination of SNX1, SNX2, SNX5, or SNX6, and a cargo-recognition trimer containing Vps26, Vps29 and Vps35. The SNX subunits contribute by Bin/Amphipysin/Rvs (BAR) and phosphoinositide-binding phox homology (PX) domains that
allow binding to the PI3P-enriched highly curved membranes of endosomal vesicles, whereas Vps26, Vps29 and Vps35 subunits functionally contribute by phosphoesterase, arrestin and α-solenoid folds, respectively (Bujny et al., 2007; Bonifacino and Hurley, 2008; van Weering et al., 2010; Vergés, 2008, 2016).

Following endocytosis, pIgR is trafficked from the basal to the apical membrane. This trafficking pathway may be attenuated by the suppression of Vps35, a key constituent of retromer (McGough et al., 2014). Similarly, cells expressing some pIgR mutations defective in trafficking can have their transcytosis phenotype rescued by the overexpression of wild-type Vps35. In a study using endosomal fractions prepared from liver, pIgR antibody co-immunoprecipitated Vps26, Vps29 and Vps35, showing the close interactions and overall biochemical relationship between the trimer components of retromer and pIgR (Vergés et al., 2004). Although only negligible amounts of sorting nexin SNX1 and SNX2 were pulled down in these experiments, it still remains possible that sorting nexins are also required for this process, as the rescue by Vps35 is PI3P sensitive (Vergés et al., 2007). In connection with the above mentioned MVBS capable of avoiding the degradative route, these findings suggest that retromer complex may play a central or crucial role in escaping pIgR, and potentially other cargoes, from the lysosomal pathway and directing it for transcytosis.

2.3 Game of multiple traffick and its fine tuning

Retromer is known as a critical regulator of multiple export and sorting pathways from endosomes. Other cargo proteins that rely on retromer for trafficking also exist, including Drosophila crumbs, a protein essential for maintaining apico-basolateral polarity (Pocha et al., 2011; Zhou et al., 2011), serpentine, a protein required for tracheal development in Drosophila (Dong et al., 2013) and auxin efflux carriers in plants (Kleine-Vehn et al., 2008). Originally,
Vps35 was identified in a screen for genes that function in endocytosis in the *D. melanogaster* Schneider 2 cultured cell line (Korolchuk *et al.*, 2007), and further characterized as a protein required for the endocytosis of mBSA, the scavenger receptor Croquemort, EGF receptor and various other proteins, but not for synaptic vesicle endocytosis. These and other authors speculate that the endocytosis defect is a result of the regulation of Rac by Vps35, by an undefined mechanism (Korolchuk *et al.*, 2007; Dong *et al.*, 2013). The proposed theory is based upon a genetic interaction between Rac and Vps35, as well as the increased levels of F-actin present in the Vps35 mutant background. As such, it is logical to assume that the list of proteins that are known to be carried by retromer will grow significantly. However, the specific mechanism that distinguishes whether a particular cargo should avoid the endo-lysosomal pathway and instead undergo transcytosis remains to be elucidated.

When considering pIgR, one working hypothesis is that the retromer could function by directly promoting pIgR transcytosis by rescuing the receptor-ligand complex from the degradative pathway (Vergés *et al.*, 2004, 2007). To test this hypothesis, the region of the pIgR cytoplasmic domain that interacts with Vps35 was mapped. Interaction between the cytoplasmic domain of pIgR and Vps35 is necessary for retromer to promote transcytosis. However, such an interaction is not necessary to avoid cargo degradation, as very little pIgA internalized by pIgR-725t is degraded, with or without altering Vps35 levels (Luton *et al.*, 1998), suggesting that retromer does not function primarily by rescuing pIgR from degradation. At present, transcytosis and degradation are considered to be alternative pathways, and thus anything that directly affects one is likely to indirectly affect the other (Breitfeld *et al.*, 1990; Vergés *et al.*, 2004, 2007; Vergés, 2008, 2016). Because of this tight connection, it is difficult to completely exclude a function for retromer in avoidance of, or retrieval from, the degradative pathway. However, a plausible mechanistic explanation may involve consideration of additional, but thus far unidentified players. This could be, for example, additional interacting
partner(s) or a posttranslational modification of a retromer subunit or endosomal cargo vesicle component.

2.4 Retromer and sorting in concert

Biochemical and immunohistochemical studies have revealed the subcellular localization of retromer and retromer subunits in the post-endocytic pathway. Although subunits of the cargo-selective complex were enriched in early endosomes (EEs), levels of SNX2 were greater in sorting endosomes. An estimated 25% of total Vps26 and SNX2 was localized in EEs, with negligible portions in recycling endosomes as well as in late endosomes and lysosomes. Although Vps26 was found in structures of more heterogeneous size and shape than SNX2, these markedly overlapped. As a consequence, the two retromer subcomplexes mostly colocalized. Remarkably, retromer was found preferentially as part of the transcytotic pathway. Other studies have shown that pharmacological inhibition of phosphoinositide 3-kinase affected the co-distribution of retromer with pIgA and the cation-independent mannose 6-phosphate receptor (CI-MPR), delaying pIgA progress to the apical surface (Mellado et al., 2014; Vergés, 2016; Kvainickas et al., 2017).

Retromer as an evolutionarily conserved protein complex required for endosome-to-Golgi transport is necessary also for the retrieval of lysosomal hydrolases receptors (McGough and Cullen, 2011; Gallon and Cullen 2015; Abubakar et al., 2017). A dimer of two sorting nexins, typically SNX1/SNX2 or SNX1/SNX5 or SNX1/SNX6, deforms the membrane and thus cooperates with retromer to ensure cargo sorting. Wassmer et al. (2009) established that retromer activity depends on a dynamic spatial organization of the endosomal network regulated via the association of SNX5/SNX6 with the “glued” p150 component of dynactin, which serves as an activator of the minus-end directed microtubule motor dynein. The spatial
organization of the retromer activity is directed through the association of SNX1 with the proposed TGN-localized Rab6-interacting protein-1 (RIP-1). The stepwise sequence of these interactions is fundamental for retromer-mediated transport and strongly suggests that a critical element required for efficient retromer-mediated sorting is defined by the spatial organization of the retromer network. The role of retromer as well as SNXs in endosomal protein (re)cycling and protein targeting to specialized plasma membrane domains in polarized cells, including transcytosis, adds further complexity and has implications in growth control, the establishment of developmental patterns, cell adhesion, migration, etc.

However, it needs to be stressed here that some proteins may be targeted to their ultimate destination immediately after endocytosis via a less circuitous route that can involve direct movement between the basal/basolateral and apical domains (Kobayashi et al., 2002; Rojas and Apodaca, 2002; Hoessli et al., 2004; Fuchs and Ellinger, 2004). This type of simplified transcytosis is considered to be clathrin-independent uptake which occurs via caveolae or other membrane microdomains. Caveolae are small flask-shaped plasma membrane invaginations that contain the protein caveolin-1. They are relatively static structures, but a number of factors can stimulate caveolae uptake, creating caveosomes (Parton et al., 1994; Pelkmans and Helenius, 2002; Thomsen et al., 2002; Mukherjee et al., 2006). The uptake of caveosomes is likely to be similar to clathrin-mediated endocytosis because caveolae are enriched in proteins that function in membrane docking and fusion events (Schnitzer et al., 1995; Oh et al., 1998). Caveolae may be a subgroup in a broader class of membrane microdomains that are involved in facilitating endocytic events (Johannes and Lamaze, 2002; Laude and Prior, 2004; Alfarah et al., 2005; Stan, 2005; Cheng et al., 2006).
3. Transudation

3.1 A link between transudation and apocrine

Historically, the transudation process has mainly been associated with the exudation of various serum components (e.g. immunoglobulins, albumins, numerous enzymes) into mucous body fluids including nasal mucus, saliva, tears, ascitic fluid, pulmonary fluids, etc. and often shown to be exacerbated during injury and inflammation (Auerswald et al., 1952; Schorn et al., 1975; Malmendier and Amerijckx, 1976; Selinger et al., 1979; Gachon et al., 1982; Janssen and van Bijsterveld, 1983; Kuhn et al., 1983; Bard et al., 2002; Bours et al., 2005). In addition to these body fluids, the process has also been observed in association with cervical fluid (or cervicovaginal secretions) (Schumacher, 1970; Schwarz et al., 2010; Petäjä et al., 2011), middle ear fluid (MEF) or cerumen (Ishikawa et al., 1972; Howie et al., 1973; Jones et al., 1979; Bernstein, 2002; Kaur et al., 2012), bronchoalveolar fluid (Osebold et al., 1975), seminal plasma (Tauber et al., 1975; Lizana et al., 1987), colonic mucosa (Prigent-Delecourt et al., 1995), sputum (Vogel et al., 1997), corporeal fluid (Bélec, 2002), oviductal secretions (Buhi et al., 2000), and peritoneal fluid (as a result of endosalpingeal secretion) (Hunter et al., 2007).

In the great majority of cases the common feature of these transudates or exudates is the presence of the same or closely related proteins such as serum albumin, immunoglobulins (IgG1, IgG2, IgA, and IgM), lactoferrin, transferrin, alpha 1-antitrypsin (AAT), lysozyme, C3-component of complement, ceruloplasmin, fibrinogen, eosinophil cationic protein (ECP), tumor necrosis factor α (TNF-α), transforming growth factor (TGF)-beta1 (TGF-β1), alpha 2-macroglobulin. Consequently, some of these proteins e.g. serum albumin, secretory immunoglobulin A (S-IgA), AAT, lysozyme, lactoferrin and transferrin were then frequently used as transudation markers (Dufour et al., 2005). The mucosal regions of the body, where these proteins are secreted, are responsible for defense against environmental pathogens. Particularly in the lumen of the vas deferens, intestinal and colonic lumens, airway lumen,
oviductal and vaginal lumen the antibody- and defense proteins-mediated immune responses are critical for preventing invasion by pathogens (Gonnella and Neutra, 1984; Hermo and de Melo, 1987; Oshikawa et al., 1996; Parr et al., 1998; Buhi et al., 2000; Hermo et al., 2000; Macpherson and Uhr, 2004; Hamburger et al., 2006). Interestingly, all of the listed proteins are also markers of apocrine secretion, as previously compiled and defined (Farkaš, 2015). This list of apocrine markers is now longer and continuously growing; however, there is no doubt that all listed tissues or organs displaying the capability of transudation are also polarized epithelia using apocrine secretion to deliver intracellular proteins to mucosal regions of the body where they serve as a barrier at the exterior interface.

3.2 Apocrine mechanism

The definition of apocrine secretion as a non-canonical and non-vesicular transport and secretory mechanism was possible due to its serendipitous discovery in prepupal salivary glands of Drosophila (Farkaš et al., 2014). This genetically well defined model organism provided us with an exceptional opportunity to elucidate the mechanistic aspects of this fundamental process through molecular, cellular, and developmental analyses. It was also a chance to undertake a critical reappraisal and comprehensive review of our knowledge on apocrine secretion in order to place new findings in a historical context and, at the same time, develop new concepts that encapsulate the basic features of apocrine secretion using insights from the most recent data. In addition to the already established phrases and definitions which have been used in the description of apocrine secretion for almost a century, the most intense phase of apocrine extrusion in the Drosophila salivary gland is accompanied by the release of large cellular fragments including entire organelles or their structurally compact nondisintegrated parts: mitochondria, microsomes, Golgi zone and areas of the ER (Farkaš et al., 2014; Farkaš,
Thus, apocrine secretion is composed of membranous, cytoskeletal, microsomal, mitochondrial, ribosomal, and most surprisingly, nuclear and even nucleolar proteins. Apocrine secretion is characterized by massive protein release, rather than being solely devoted to the secretion of oily substances, as was previously incorrectly defined (Purkyně, 1833b; Schiefferdecker, 1917, 1921; Richter, 1932; Kuno, 1938; Hurley and Shelley, 1954; Gesase et al., 1996; Gesase and Satoh, 2003).

Preliminary and current proteomic analyses of the apocrine secretory material have revealed that it is a highly complex mixture consisting of hundreds to thousands of proteins from different subcellular locations and with highly diverse functions. These proteins include enzymes, structural proteins, signaling molecules, membrane and intracellular receptors, chaperones, storage proteins, transcription factors, coactivators and corepressors, chromatin and nucleolar components (Farkaš, 2015, 2016). Although numerous nuclear proteins are released, nuclear DNA and nuclei appear completely intact (Farkaš et al., 2014). By isolating freshly secreted material directly from the lumen of the *Drosophila* salivary glands and evaluating the presence of specific proteins using a broad panel of antibodies in western blotting, we demonstrated that proteins are released undegraded, and most probably fully functional (Farkaš et al., 2014). Even though many of the proteins that we analyzed are actively released to undetectable levels inside cells, they start to slowly reappear at their original subcellular locations within about an hour after the completion of apocrine secretion. In addition, the tissue, even though it has undergone massive apocrine secretion, continues to live and produce gene products as shown by the incorporation of radioactive precursors into newly transcribed RNA and newly synthesized proteins. When the fluorescent signal from secondary antibodies or GFP-/RFP-/YFP- fusion proteins that were released into the lumen was quantified, more than 90-95% of these proteins were found to be secreted from the cells,
suggesting that the remaining few percent of a cell’s proteome is sufficient to maintain tissue viability and allow for its rapid regeneration (Farkaš et al., 2014).

3.3 Heavy endosomal trafficking, vacuolation and resecretion in an apocrine organ

Equally unanticipated as apocrine secretion was the finding that *Drosophila* salivary glands during the early prepupal period are remarkably active in endocytosis, generating high numbers of small acidic vacuoles that continuously fuse to larger ones. Midway through the prepupal period, there is an abundance of late endosomal trafficking and vacuole growth, later followed by vacuole neutralization and disappearance via membrane consolidation (Farkaš et al., 2015). All this endosomal activity, in which a crucial role is played by dynamin, clathrin, Rab5, Rab11, syntaxins, vacuolar ATPases Vha36-1, Vha55, Vha68-2, etc., takes place a few hours prior to apocrine secretion. The final phases of this process, involving vacuole disappearance and their fusion with membranes of ER and TGN, are controlled by *Drosophila* retromer constituent, Vps35 (Farkaš et al., 2015). It has been determined that among apocrine secretory products which are released by salivary glands there are several proteins which are not produced by themselves, but must be taken up from the surrounding hemolymph by endocytosis (Farkaš et al., 2014).

It should be emphasized that apocrine secretion is characterized by massive protein release, which is in striking contrast to exocytosis when only a single product or a few proteins are released from vesicles, homotypically fusing with the cell membrane. Thus, in the majority if not all above mentioned cases, when immunoglobulins, albumins, various enzymes, lactoferrin, transferrin, AAT, lysozyme, alpha 2-macroglobulin, ceruloplasmin, fibrinogen, ECP, C³3-component of complement, TNF-α, and many others that are not synthesized by a secretory organ, are released into exocrine mucous regions and body fluids of mammals, apocrine
secretion is involved. All these data suggest that early endocytosis as well as further cargo sorting and selection for the post-endocytic pathway using retromer (Vergés et al., 2004, 2007; Vergés, 2008, 2016; Bonifacino and Hurley, 2008; Kleine-Vehn and Friml, 2008; van Weering et al., 2010; Burd and Cullen, 2014; Gallon and Cullen, 2015) might be common to both the re-release of engulfed proteins by exocytosis (transcytosis) and apocrine secretion (transudation). However, at present we cannot conclude which of the retromer subcomplexes are preferentially used in transcytosis and which are used in transudation, or whether they are distinct at all.

3.4 Retromer or its vicinity - potential intersection point for transcytosis and transudation

Due to the non-vesicular character of apocrine transport and the secretory mechanism, differences at the post-endocytic pathway and later stages at the level of cargo recognition, sorting and/or selection will exist which will allow bulky recruitment of the specified cargo to this massive externalization pathway. This conclusion opens up new vistas for investigation in the future. There is no exocytosis during or after apocrine secretion in Drosophila salivary glands (Farkaš et al., 2014, 2016) and thus proteins taken up from circulation via endocytosis during the early prepupal stages can be released or secreted only by the apocrine pathway. The involvement of retromer in this preceding endocytosis has been already evidenced (Farkaš et al., 2015). This finding poses an important question: in the case of apocrine secretion, does any preceding endocytosis for the purpose of re-secretion, require clathrin-dependent retromer-associated sorting machinery and automatically exclude caveolae-mediated endocytosis?

Transcytosis may be either unidirectional or bidirectional. Unidirectional transcytosis may occur selectively in the luminal to abluminal direction, or in the reverse direction (abluminal to luminal). However, apocrine secretion occurs exclusively on the apical pole and therefore this
type of transport and re-secretion is only unidirectional providing grounds to consider these as two separate mechanisms, another reason to clearly differentiate transudation from transectosis (see Figures 1 and 2). This differentiation opens up several new and interesting avenues of enquiry, such as the existence of crosstalk or the interconnection between an endosomal (vesicular) system and apocrine secretion which in principle is a non-vesicular mechanism, or in other words, how endocytosed material of foreign origin becomes integrated into apocrine machinery or whether this endocytosed material needs a specific sorting mechanism to escape the vesicular pathway on the route to being released. The above described system of Drosophila salivary glands can easily be used to explore these and similar questions by designing biochemical protein-protein interaction studies or screens for genetic interactions focused directly on key trafficking components and the apocrine machinery itself.
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**Figure Legends**

**Figure 1** Schematic illustration of transcytosis. In principle, endocytosis takes place on the basal or basolateral pole of the cell; endocytosed cargo captured in vesicles, which occasionally can fuse, is drawn across the cell, and ejected on the apical side of the cell by exocytosis. There is well-documented evidence that retromer (comprising of Vps26, Vps29, Vps35 and two sorting nexins *e.g.* SNX1/SNX2, SNX1/SNX5 or SNX5/SNX6) are chiefly involved in the mediation of this clathrin-dependent (gray vesicles) transport via the trans-Golgi network or ER system (yellow vesicles). This route strikingly differs from endosomal trafficking leading to multivesicular bodies (MVB) and lysosomes (brown and dark brown). However, ejection of the vesicles on the apical side can also involve clathrin-independent and caveolin-associated less circuitous route (pink vesicles) that is achieved by direct or close-to-direct movement between basal/basolateral and the apical domains, thus representing a simplified form of transcytosis. Under these circumstances, the cargo is re-exocytosed quickly and without any change. At the same time, the cell can produce its own, distinct secretory vesicles (dark violet) targeted for exocytosis. Large red numbers indicate the three main routes of transcytosis: 1. and 2. utilise clathrin-dependent endocytosis, while 3. is caveole-linked and clathrin-independent. L = lumen.

**Figure 2** Schematic illustration of transudation. Material endocytosed on the basal or basolateral pole of the cell, in a similar manner as during transcytosis, is released on the apical pole by apocrine secretion, allowing the massive externalization of many more proteins than were endocytosed, and often together with hundreds, if not thousands of the cell own products. In
comparison to transcytosis, many more products are re-released than during transcytosis. The initial phases of the process (endocytosis) can utilize the same vesicular mechanisms for internalization on the basal and basolateral pole as during transcytosis, but the subsequent stages are directed towards massive apocrine secretion which is a completely non-vesicular pathway; many of its steps remain enigmatic at the molecular level. Nevertheless, experimental evidence suggests that numerous foreign proteins, produced by distal cells and tissues that are internalized from circulation by endocytosis, are detected among the plethora of products manufactured by the epithelial secretory cell which release them en masse via the apocrine mechanism which involves various cellular components including entire or parts of organelles such as ER, Golgi, mitochondria (orange), microsomes and even unsorted vesicles if they were present at the moment of secretion. L = lumen.
FIGURES