

## Unconventional Protein Secretion in Animal Cells

Fanny Ng and Bor Luen Tang

### Abstract

All eukaryotic cells secrete a range of proteins in a constitutive or regulated manner through the conventional or canonical exocytic/secretory pathway characterized by vesicular traffic from the endoplasmic reticulum, through the Golgi apparatus, and towards the plasma membrane. However, a number of proteins are secreted in an unconventional manner, which are insensitive to inhibitors of conventional exocytosis and use a route that bypasses the Golgi apparatus. These include cytosolic proteins such as fibroblast growth factor 2 (FGF2) and interleukin-1 $\beta$  (IL-1 $\beta$ ), and membrane proteins that are known to also traverse to the plasma membrane by a conventional process of exocytosis, such as  $\alpha$  integrin and the cystic fibrosis transmembrane conductor (CFTR). Mechanisms underlying unconventional protein secretion (UPS) are actively being analyzed and deciphered, and these range from an unusual form of plasma membrane translocation to vesicular processes involving the generation of exosomes and other extracellular microvesicles. In this chapter, we provide an overview on what is currently known about UPS in animal cells.

**Key words** Animal cells, Autophagy, Exosomes, GRASP, Unconventional protein secretion (UPS)

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### 1 Introduction: Protein Secretion—Conventional and Unconventional

Eukaryotic cells are characterized by an elaborate intracellular membrane system, and protein secretion or exocytosis is classically viewed as occurring by multiple rounds of sequential budding and fusion of membranous vesicles or carriers from the endoplasmic reticulum (ER), the Golgi apparatus, and the trans-Golgi network (TGN), which ultimately fuse with the plasma membrane [1]. All cells secrete a range of proteins in a constitutive manner, but for specialized cells with regulated exocytosis, another classical route to the plasma membrane exists for specific cargoes such as hormone and neurotransmitters. Proteins destined for secretion (or those to be transported to the plasma membrane) are first targeted to the ER by N-terminal or internal signal sequences as the nascent polypeptide emerges from the ribosome. Secretory proteins exit the ER via coat protein complex II (COPII) vesicles nucleated by the small GTPase Sar1 [2, 3]. Many secretory proteins acquire a carbohydrate group-based post-translational modification, namely core

N-linked glycosylation at the ER, and undergo subsequent modifications to their N-linked carbohydrate groups by glycosyltransferases as they traverse through the Golgi/TGN. The conventional exocytic transport process at the Golgi requires another GTPase, the ADP ribosylation factor 1 (Arf1), which nucleates the coat protein I (COPI) complex. COPI-mediated transport is known to be inhibited by a host of compounds, including the fungal metabolite brefeldin A (BFA), which inhibits guanine nucleotide exchange of Arf1 [4]. Viotti provides a more detail discussion on the conventional mode of protein secretion in a separate chapter.

Amongst proteins that are secreted from cells, a small number were released from cells in ways that appear independent of the conventional or canonical secretory pathway or mechanisms. Prominent in this regard are proteins such as FGF1/2 [5], cytokines like IL-1 $\beta$  [6], the *Drosophila*  $\alpha$ -integrin [7], the nuclear protein amphoterin/high motility group protein B1 (HMGB1) [8], extracellular matrix proteins like galectins [9], yeast heat-shock protein 150 (Hsp150) [10], the neuropathogenic protein  $\alpha$ -synuclein [11, 12], and more recently the *Dictyostelium* and yeast acyl-CoA-binding protein [13–15] as well as the membrane protein cystic fibrosis transmembrane conductor (CFTR) [16]. Evidently, both soluble and membrane-bound proteins located at various cellular compartments can undergo unconventional protein secretion (UPS). There is no distinct commonality between these in terms of identity and function.

There appears to be different modes by which UPS can take place. At least three different transport modes are apparent, depending on the nature and cellular location of the cargoes involved. Firstly, for proteins that are absolutely cytosolic and are never enclosed by membranous vesicles (such as FGF2) secretion would require some specific membrane translocation processes that bring them across the plasma membrane [17]. Secondly, cytoplasmic proteins could become membrane encased prior to secretion, and these processes may involve the generation of exosomes or ectosomes [18–23]. In the third scenario, there are cargoes, both soluble and membrane bound, that could initially enter the canonical secretory pathway through ER translocation as they possess ER-targeting signals. However, these could be subsequently transported to the cell surface or be secreted in a manner that is independent of COPII-mediated ER budding, and bypassing the Golgi apparatus. Some modes of UPS have been more extensively investigated, and some aspects of UPS are better known than others. For example, while the mechanism of generation of exosomes by the endosomal sorting complexes required for transport (ESCRT) complexes has been extensively examined [24], the mode of unconventional secretion that is dependent on autophagy is less clear in mechanistic terms [25, 26].

UPS appears to occur in organisms across the entire eukaryotic domain. In this chapter, we provide a brief overview of what is currently known about this process in animal cells, and also draw results from some lower eukaryotes. In the paragraphs that follow, we first describe some representative cargoes undergoing unconventional secretion or exocytic cell surface transport, followed by a discussion on the mechanisms involved.

## 2 Proteins Known to Undergo Unconventional Protein Secretion

We outline in this section a few prominent examples of proteins that are unconventionally secreted by animal cells. They are rather loosely categorized as below, and listed in Table 1. This list is far from being exhaustive. The reader is referred to more extensive and dedicated reviews in the literature for other examples [27–29].

### 2.1 Growth Factors: Fibroblast Growth Factors 2

Fibroblast growth factor 2 (FGF2, also known as basic FGF (bFGF)) is a member of the heparin-binding FGF family, which are key regulators of proliferative and differentiation processes in a wide spectrum of

**Table 1**  
**Proteins known to be secreted by unconventional secretion**

Protein	Cellular localization	Known mechanistic insight
Fibroblast growth factor 2 (FGF2)	Cytosolic	Direct plasma membrane crossing with unique mechanism dependent on phosphoinositides and extracellular heparin sulfate proteoglycans
Interleukin 1- $\beta$ (IL1- $\beta$ )	Cytosolic	Autophagy- and GRASP-dependent UPS, secretory lysosomes, exosomes
Acyl-CoA-binding protein (ACBP)		Autophagy- and GRASP-dependent UPS
Galectin	Extracellular matrix	
$\alpha$ -Integrin	Plasma membrane (and internal membranes)	dGRASP dependent
Cystic fibrosis transmembrane conductor (CFTR)	Plasma membrane (and internal membranes)	Autophagy- and GRASP-dependent UPS
$\alpha$ -Synuclein	Cytosolic	Exosomal secretion
$\gamma$ -Synuclein	Cytosolic	Exosomal secretion
Tau	Cytosolic/microtubule associated	Exosomal secretion

See text for details

tissues. FGF2's known roles in tumor angiogenesis and wound healing and as a key factor in maintenance of renewability in stem cell cultures are particularly prominent [30]. FGF2 has several isoforms of high and low molecular sizes, and the 18 kDa small isoform is known to be secreted extracellularly in an unconventional manner [31]. A related FGF family member, FGF1, is likewise unconventionally secreted, particularly under the condition of stress and starvation [32, 33]. FGF2 has no signal peptide or leader sequence and is expected to be exclusively cytoplasmic, but its non-cell-autonomous activity is of vital physiological and pathological importance. FGF2's unconventional secretion represents a unique pathway as secretion appears to occur via direct crossing of the plasma membrane [5, 34], which is discussed further below.

## **2.2 Cytokines: Interleukin-1 $\beta$ (IL-1 $\beta$ )**

IL-1 $\beta$  belongs to the IL-1 family of pro- and anti-inflammatory cytokines [35], which unlike most other cytokines do not have an ER-targeting signal peptide and thus have no access to the conventional secretory pathway. Many if not all members of the IL-1 family are secreted unconventionally, but IL-1 $\beta$  is the best studied in this regard [36]. IL-1 $\beta$  is synthesized as a pro-peptide in monocytes, which is proteolytically processed by activated caspase-1 of the inflammasome complex upon infection, injury, and other forms of stress [37]. Cleaved/mature IL-1 $\beta$  could then exit the cells if these are lysed during pyroptosis, a mode of lytic cell death driven by caspase-1 or caspase-11 [38]. Otherwise, its release is not inhibited by perturbation of the secretory pathway with drugs such as BFA or monensin [39]. Unconventional secretion of IL-1 $\beta$  could occur by a variety of mechanisms that include plasma membrane translocation, exosomes, or other forms of secretory micovesicles [18, 25, 28, 36]. Caspase-1 is also apparently a driver for the unconventional secretion of damage-associated molecular patterns (DAMPs) or alarmins, such as the nuclear HMGB1 [40]. Recent findings suggest that IL-1 $\beta$  secretion is dependent on autophagy and the Golgi reassembly stacking protein 55 (GRASP55) [41]. This "GRASP and autophagy-dependent" (GAD) pathway/mechanism [25] is further discussed below.

## **2.3 Non-Cell-Autonomous Modulator: Acyl-CoA-Binding Protein**

The evolutionarily conserved acyl-CoA-binding protein (ACBP) [42, 43] has both a cell-autonomous activity of binding to medium- and long-chain acyl-CoA esters [44], as well as non-cell-autonomous functions as a secreted protein. The *Dictyostelium* *AsbA* product is secreted as the sporulation factor spore differentiation factor 2 (SDF-2) [45], while the mammalian ACBP is the precursor of benzodiazepine-binding inhibitor (BDI) [46]. A series of recent studies have revealed that ACBP orthologues in *Dictyostelium* and yeast are unconventionally secreted in a GRASP and autophagy-dependent manner [13–15].

## **2.4 Extracellular Matrix Components: Galectin-1 and Integrin- $\alpha$**

The evolutionarily conserved galectin-1 is a nuclear/cytoplasmic  $\beta$ -galactoside-binding protein that has cell adhesion, immune suppression, and neuroprotective activities [47–49]. Lacking an ER-targeting signal peptide, it is nonetheless secreted into the extracellular matrix

[9, 50]. Integrin subunits are known to go through conventional exocytosis en route to the cell surface. However, during certain stages of *Drosophila* wing imaginal disc epithelia development nascent integrin- $\alpha$  subunits are transported to the specific plasma membrane domains in contact with the basal membrane via a mechanism that appears to bypass the Golgi, but is dependent on the *Drosophila* dGRASP [7, 51]. Whether integrins could be unconventionally secreted in mammalian cells has not yet been clearly demonstrated.

**2.5 Membrane  
Proteins with  
Conventional  
Exocytosis: The Cystic  
Fibrosis  
Transmembrane  
Conductance  
Regulator**

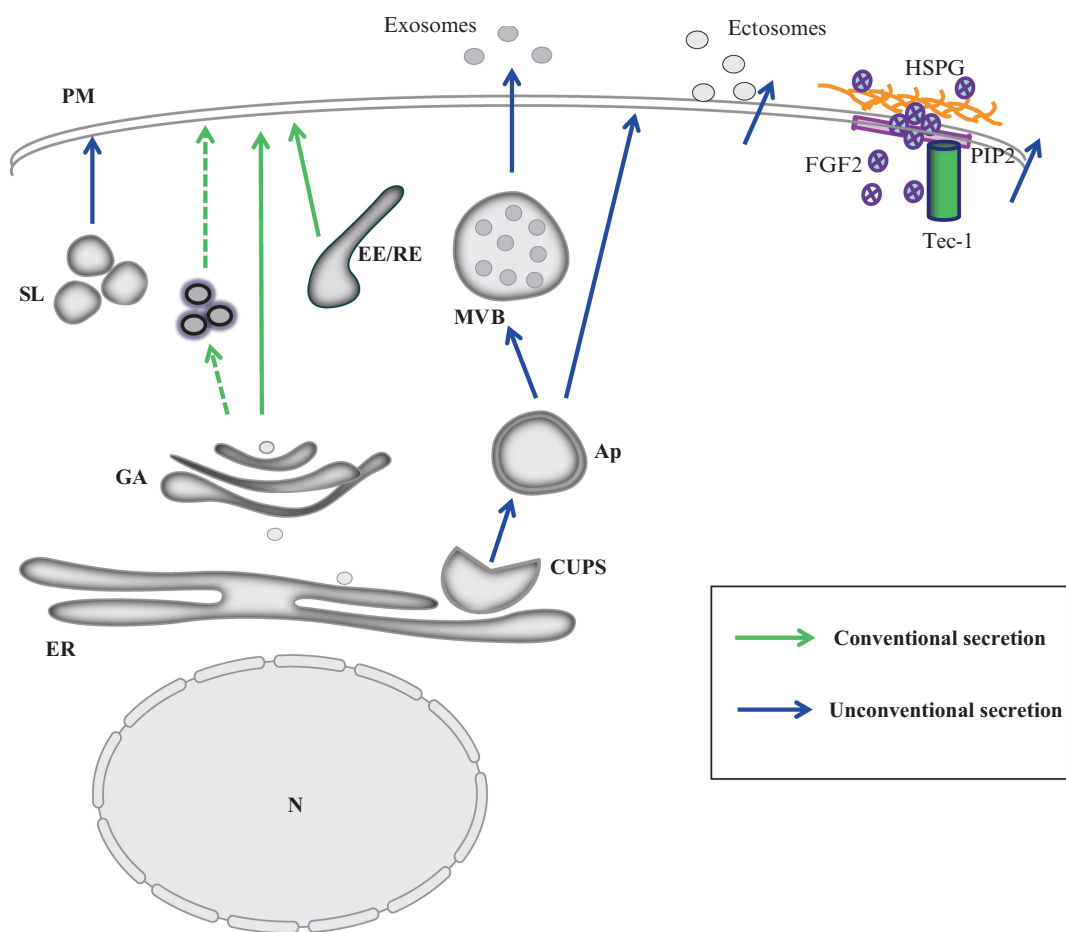
The multi-membrane-spanning cystic fibrosis transmembrane conductance regulator (CFTR) is the protein mutated in cystic fibrosis [52]. It is ER targeted and transported via conventional exocytosis to the cell surface, which is critical for its function as a chloride channel. Surface CFTR also undergoes endocytic recycling and its surface transport was shown to be dependent on the TGN SNARE syntaxin 16 [53, 54]. The most common and prominent class of CFTR mutations,  $\Delta F508$ -CFTR, has a defect in ER exit and exocytosis. Both wild-type and mutant CFTR are however capable of undergoing some form of unconventional ER to cell surface transport [55, 56]. Such a mode of exocytosis is not inhibited by BFA or by silencing of COPI and COPII components, and the transported CFTR retains its ER core-glycosylated forms [56]. This unconventional surface trafficking process of core-glycosylated CFTR is dependent on GRASP55 and autophagy, and appears to be enhanced by stress. The discovery of an unconventional transport mode for integrin- $\alpha$  and CFTR (particularly ER-entrapped  $\Delta F508$ -CFTR) suggests that even proteins that usually undergo conventional exocytosis could be engaged in unconventional exocytosis under certain conditions, and this finding has important implications.

**2.6 Neuropathogenic  
Proteins:  $\alpha$ -Synuclein  
and Tau**

Many neurodegenerative diseases are characterized by intra- or extracellular accumulation of protein aggregates with a dominant etiological component. The small molecule  $\alpha$ -synuclein encoded by the *SNCA* gene has the propensity to form toxic aggregates, which is a major component of the pathological feature of Lewy bodies (LB) in brains of Parkinson's disease (PD) and other LB disease [57].  $\alpha$ -Synuclein mutations could enhance its propensity to aggregate, and give rise to the juvenile onset form of PD.  $\alpha$ -Synuclein pathology could spread in a prion-like manner from neuron to neuron [58], and evidence has accumulated to suggest that  $\alpha$ -synuclein could be unconventionally secreted by an exosome-based mechanism [11, 59, 60]. The microtubule-binding protein tau, whose pathological hyperphosphorylated form is found in intracellular fibrillary tangle in Alzheimer's disease and other tauopathies [61], is likewise secreted in an unconventional manner [62, 63], likely through the generation of microvesicles.

### 3 Mechanisms of Unconventional Protein Secretion

In this section, we review what is currently known about the mechanisms underlying unconventional secretion in animal cells. It should be noted that some proteins have possibly more than a single mode of unconventional secretion (such as IL-1 $\beta$ ) while some others could be secreted by both canonical and unconventional means (such as CFTR). A schematic summary of the UPS pathways is presented in Fig. 1.



**Fig. 1** Conventional versus unconventional secretion. A schematic diagram depicting paths and compartments known to be involved in UPS. The conventional secretory pathways are marked by *green arrows* while the unconventional pathways by *blue arrows*. For simplicity, the endocytic pathways are not depicted. FGF2 UPS requires phosphatidylinositol 4,5-bisphosphate (PIP2) and extracellular heparin sulfate proteoglycan (HSPG), and is regulated by the Tec-1 kinase. The underlying molecular components for other modes of UPS are less clear. *N* nucleus, *ER* endoplasmic reticulum, *CUPS* compartment for unconventional protein secretion, *Ap* autophagosome, *GA* Golgi apparatus, *MVB* multivesicular bodies, *EE/RE* early endosome/recycling endosome, *SL* secretory lysosomes, *PM* plasma membrane

### **3.1 Crossing the Plasma Membrane: The FGF2 Chronicle**

Proteins that are absolutely cytosolic and have no access to any form of membranous vesicles would not be able to exit an intact cell unless there are ways to negotiate the plasma membrane (PM). In theory, peptides could penetrate a barrier of lipid bilayer via hydrophilic channels and pumps, such as the ATP-binding cassette (ABC) transporters and the mitochondrial translocon complexes. Membrane-penetrating peptides could interact with and perturb the structure of the phospholipid bilayer and form inverted micelles [64]. In most if not all of these mechanisms, however, the polypeptide is translocated in a denatured or misfolded form.

Work from the laboratory of Walter Nickle has shed light on a rather unique mode of plasma membrane translocation that is exhibited by FGF2 [5, 34], which appears to translocate directly through the PM in a fully folded form without relying on protein-conducting channels. FGF2 is able to interact with phosphoinositides at the inner leaflet of the PM [65]. Membrane-recruited FGF2 could oligomerize to form a membrane pore [66]. Phosphorylation of Tyr 81 of FGF2 by Tec kinase, a non-receptor tyrosine kinase which contains a pleckstrin homology (PH) domain that binds phosphoinositides, enhances the lipidic membrane pore formation [67]. FGF2 has a high affinity for heparin sulfate proteoglycans enriched at the outer leaflet of the PM bilayer [68], and the latter could literally extract FGF2 from their prior interaction with phosphoinositides, thus completing the translocation. At the moment it is yet uncertain whether this mode of PM translocation is utilized by other proteins. However, the HIV-TAT protein is known to require phosphoinositides for secretion [69] and a direct PM translocation mechanism has also been proposed for FGF1 and IL-1 $\beta$ .

### **3.2 Exosome, Ectosomes, and Other Microvesicles**

A more common mode of unconventional secretion is one that utilizes some form of membranous vesicles. The unconventional secretion of IL-1 $\beta$ , for example, is perhaps largely through a vesicle-mediated mechanism. Amongst these, exosomes from multivesicular bodies (MVBs) are perhaps the best understood. These 40–100 nm endosome-derived vesicles [70–72] are formed in MVBs by an unusual luminal budding of vesicles from the limiting membrane of late endosomes. The mechanism involves the generation of intra-MVB vesicles by the ESCRT complexes [73, 74], and these vesicles are released as exosomes when MVBs move towards the cell periphery and fuse with the plasma membrane. Other than proteins, exosomes also contain RNA and DNA molecules, and these are potential mediators of intercellular communication.

Another possible mode of unconventional secretion of IL-1 $\beta$  involves the secretory lysosomes, or lytic granules [75, 76]. Lysosomes are traditionally viewed as lytic compartments for the terminal destruction of cellular materials. However, in some cases, modified lysosomes could undergo regulated secretion in response to intracellular Ca<sup>2+</sup> elevation, for example resulting from the



activation of P2X7 purinergic receptors on monocytes [77], which are ATP-gated ion channels [78]. When secretory lysosomes fuse with the plasma membrane, likely facilitated by a set of syntaxin 11-based [79] fusion machinery akin to that used by lytic granules [80], their contents could be released into the extracellular space. In human monocytes and dendritic cells,  $\text{Ca}^{2+}$  elevation triggers secretion of both IL-1 $\beta$  and the lysosomal hydrolase cathepsin D [81–83], which suggest that IL-1 $\beta$  could be co-released from the same compartment as a lysosomal marker.

Another possible mode of extracellular release of cytosolic materials is plasma membrane shedding of microvesicles. These plasma membrane-derived microvesicles have been given several different names, ranging from ectosomes to shedding microvesicles [23, 84]. These microvesicles are generally somewhat larger than the MVB-derived exosomes (100 nm to 1  $\mu\text{m}$  in size), and are enriched in the inner leaflet phospholipid phosphatidylserine on their outer surface. The mechanism for microvesicle formation is yet unclear. Like the case for secretory lysosomes, there is evidence that P2X7 receptors expressed in the membrane of microvesicles may be involved in the regulation of IL-1 $\beta$  release [85, 86].

### **3.3 The GRASP and Autophagy-Dependent (GAD) Pathway of UPS**

A particularly interesting recent development is emerging evidence for the involvement of GRASPs and the autophagy machinery in unconventional secretion. Both the unconventional secretion of the cytoplasmic ACBP [14, 15] and the membrane-bound CFTR [16] involve GRASP and autophagy. In fact, it was recently showed that the secretion of IL-1 $\beta$  [41] also requires these to be functional. GRASP orthologues act in Golgi cisternae stacking and Golgi ribbon formation [87–89] by forming oligomers through the N-terminal PDZ domains. Two paralogues exist in the mammalian genome GRASP65 and GRASP55; both are peripheral membrane proteins at the *cis*- and medial-*trans*-Golgi cisternae. Silencing of both GRASP proteins leads to disassembly of the Golgi stack [89].

The involvement of GRASPs in unconventional secretion may superficially appear paradoxical, as cargoes unconventionally secreted (including CFTR which also follows the canonical secretory route) could bypass the Golgi apparatus in reaching the plasma membrane. In that case why should the Golgi cisternae stacker GRASP be involved? It is possible that GRASPs' function in Golgi stack maintenance is unrelated to its role in unconventional secretion. It has been proposed that GRASPs are primarily membrane tethers, as GRASPs oligomerization through their PDZ motifs could bring opposing membranes in close proximity [90]. Thus, they could possibly act in membrane tethering of a specific subset of ER-derived vesicles with the PM. While this is an interesting hypothesis, the PM-targeting mechanism based on GRASP alone is not yet well defined. It is possible that canonical secretory pathway components such as Rabs and SNAREs are involved in this



tethering and fusion step. Acb1 secretion in *S. cerevisiae* requires the plasma membrane t-SNARE, Sso1p [14], and that in *P. pastoris* requires PM SNAREs of *Pichia* [15].

The apparent requirement for components of the autophagy pathway for the unconventional secretion of quite a range of cargo types is intriguing. Autophagy is an evolutionarily conserved process in which cytosolic materials and membranous organelles like the mitochondria are encased within a double-membrane autophagosome, which eventually fuses with the vacuole or lysosome for the degradation of its contents [91]. The roles of autophagy in cellular and systemic physiology as well as pathology have been extensively studied [92–97]. A particularly interesting point to note is that autophagy is typically induced under conditions of nutrient or growth factor starvation, or during various conditions of stress [98]. For the cases of ACBP, CFTR, and IL-1 $\beta$ , autophagy-dependent unconventional secretion of these proteins does indeed become apparent under conditions of stress. It is thus conceivable that during times of stress, the cell may resort to unconventional secretion of cytosolic proteins (such as IL-1 $\beta$  and ACBP) to elicit a non-cell-autonomous signal. For the case of proteins already targeted to the ER (such as CFTR), activation of UPS by stress could help relieve the accumulation of unfolded protein in the ER, or bypass of trafficking defects in the canonical secretory pathway to allow some degree of secretion to occur.

Exactly how autophagy leads to UPS is not yet clear, and several possibilities have been proposed. Interestingly, autophagy components that are important for unconventional secretion are largely those involved in the early stages of generation of autophagosome and for endosomal fusion, but not for the final lysosomal fusion. Secretion of the Acb-1 in yeast, for example, does not require the vacuole/lysosomal SNARE VAMP7p or the Rab Ypt7p that are important for vacuole fusion [14]. Possible intermediates for autophagy-mediated unconventional secretion may therefore be autophagosomes (specifically created or otherwise) that fuse with endosomes/MVBs to form amphisomes [99] that subsequently fuse with the PM [25, 26, 29], and not lysosomes. In the former case, exosomes are presumably generated from the intraluminal vesicles in the amphisome or MVB.

In yeast, a novel compartment for UPS, known as the compartment for unconventional protein secretion (CUPS), is induced by nutrient starvation and the GRASP orthologue Grh1p as well as autophagy proteins initiating autophagosome formation are recruited into these structures [100]. Whether the autophagosome generated by CUPS differs from other sites of autophagosomal origin is not yet clear, and structures analogous to CUPS have not yet been reported in mammalian cells. While amphisome-PM fusion may be akin to MVB-PM fusion, direct fusion between autophagosome and the PM has not been clearly documented. It

is however conceivable that autophagosomes generated by CUPS-like structures carrying GRASPs could dock with the PM. What happens after this fusion is unclear. It should be noted that other than exosomes, microvesicles known as ectosomes could be generated by direct budding from the PM [101]. These microvesicles are known to be shed from the cilium and flagellum [102, 103]. At the moment the mechanism of ectosome generation remains unknown and any connection with autophagy remains speculative.

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## 4 Studying Unconventional Protein Secretion in Animal and Yeast/Fungal Cells/Tissues

UPS, far from simply a curious set of biological phenomena, has important implications in health and disease. Its mechanism and functions have been rigorously tackled by workers in various research niches. A lot of the work is cargo centered, with important molecules such as FGF2 [34], IL-1 $\beta$  [36], and CFTR [16] being investigated as part of efforts to understand their basic biology. Another major aspect of the work pertains to investigations done on microvesicle-based secretion, which are of pathological interest [104, 105]. Biophysical and biochemical characterization of extracellular microvesicles is actively pursued in various contexts [106, 107]. With advances in high-throughput screening approaches, it is anticipated that more holistic analysis such as genome-wide siRNA screens will be performed to decipher components and pathways underlying the various modes of unconventional secretion. Genetic screens with model organisms such as *S. cerevisiae* should be ongoing, and are likely to reveal more mechanistic insights into the near future. Although unconventional protein secretion tends to bypass some, if not most, of the needs of the conventional secretory machinery, it is likely that some vesicular transport components responsible for conventional secretion are still involved. A bunch of Rab proteins, for example, are critically involved in various aspects of autophagy [108–110], and are therefore likely to influence UPS that rely on autophagy. UPS processes that rely on fusion of amphisomes, MVB, or other membranous carriers with the plasma membrane would likely need the participation of SNAREs. As proposed earlier, secretory lysosome may use a bunch of cell surface syntaxins or the atypical syntaxin 11 [79, 80] to facilitate plasma membrane fusion.

From a translational perspective, exosome and other extracellular microvesicles are being developed as specific sources for disease biomarkers [111] and as biomimetic drug delivery vehicles [112, 113]. Unconventional secretion appears to be a major contributor to the secretome of cancer cells and tissues [114], and some of the proteins that are unconventionally secreted could

promote tumorigenesis and metastasis [115, 116]. UPS, particularly in the mode of exosomal release, has also been extensively implicated in neurodegenerative diseases [117, 118]. Causative agents of neurodegeneration such as prion protein [119] and tau [62, 63] could potentially spread from diseased neurons to healthy ones via UPS. On the other hand, discovery of  $\Delta F508$ -CFTR's unconventional exocytosis to the cell surface opens up new therapeutic possibilities for cystic fibrosis [16] and potentially other diseases that result from impaired surface transport of mutated and misfolded proteins. A thorough understanding of both the modes and mechanism of UPS would therefore be of tremendous academic and clinical interest in the coming years.

In this collection, methods and approaches for investigating many of the proteins undergoing unconventional secretion shall be presented and discussed. Methods for preparation and analysis of the tissue secretome from tumor interstitial fluid (TIF) are presented by Gromov and colleagues. Amaral and co-workers examine unconventional transport of CFTR, Lacazette and colleagues discuss studies on FGF2, Shou and colleagues look at synuclein- $\gamma$  in cancer cells, while Beer and colleagues examine the role of caspase-1 in unconventional secretion. Yeast and fungal genetics shall provide useful handles for dissecting mechanisms and identification of mechanistic components of UPS, and could be used for the preparation of molecules of biological interests via UPS. Schipper and colleagues present the use of UPS to express sugar-free heterologous proteins in the plant fungal pathogen *Ustilago maydis*. On UPS-related pathogenicity, MacLean and colleagues look at the hydrophilic acylated surface protein B of *Leishmania*, and Reynard and colleagues discuss the role of unconventional matrix protein VP40 secretion in Ebola pathogenicity. Shedding of microvesicles may underlie a large fraction of all UPS, and in their respective chapters, Hajj and colleagues discuss microvesicle-based UPS of the co-chaperone stress-inducible protein 1, while Rodrigues and co-workers examine extracellular vesicles from yeast.

Bellucci, Zhang, Goring, and Pocsfalvi discuss methods for preparation and isolation of exosome-like vesicles or secretome, derived from various plant materials, and chemical modulation of secretory pathway in protoplasts.

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