

Review Article

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Advances in Understanding Unconventional Protein Secretion Pathways

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Abstract

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Conventional protein secretion follows the endoplasmic reticulum (ER)–Golgi–plasma membrane route, in which proteins bearing N-terminal signal peptides are co-translationally translocated into the ER lumen, processed in the Golgi, and then released by exocytosis. However, an increasing number of secreted proteins lack signal peptides yet are nevertheless released into the extracellular milieu. Collectively termed unconventional protein secretion (UPS), these pathways bypass classical secretory compartments and employ diverse cellular machineries, including direct plasma-membrane translocation, vesicle-mediated routes involving autophagosomes or multivesicular bodies (MVBs), and Golgi-bypass mechanisms mediated by GRASP proteins and the compartment for unconventional protein secretion (CUPS). Over the past decade, major mechanistic advances—spanning work in yeast, Dictyostelium, and mammalian cells—have elucidated the molecular machines that underlie these unconventional routes and their physiological relevance to inflammation, development, and cancer. This expanded review integrates discoveries up to 2016, emphasizing molecular mechanisms, evolutionary conservation, and disease relevance while identifying open questions and future research directions.

1. Introduction — Why “Unconventional” Matters

The cell’s capacity to secrete proteins is central to intercellular communication, immune signaling, and tissue organization. Canonical secretion, as first delineated by Palade (1975), involves the cotranslational targeting of signal peptide-containing proteins to the ER, their maturation through the Golgi, and vesicular trafficking to the plasma membrane for release. However, many extracellular proteins lack signal peptides yet perform essential biological functions. Examples include interleukin (IL)-1 family cytokines, fibroblast growth factor 2 (FGF2), acyl-CoA-binding proteins (Acb1/AcbA), galectins, thioredoxin, and leaderless enzymes such as

superoxide dismutase (SOD1) and peroxiredoxins [1–4].

Such proteins bypass the ER–Golgi axis entirely, utilizing alternative routes collectively referred to as *unconventional protein secretion* (UPS). These routes have profound physiological consequences: they underpin inflammation (e.g., IL-1 β , IL-18), tissue repair and angiogenesis (FGF2), starvation-induced differentiation (Acb1), and tumor–stroma interactions. Understanding UPS therefore provides insights into fundamental aspects of cell biology, homeostatic stress responses, and disease pathogenesis.

2. Conceptual Framework and Classification of UPS

UPS pathways can be organized into four broad mechanistic categories [3,4,6,12]. This classification aids in understanding the diverse molecular machineries employed and the overlapping regulatory modules that integrate vesicular and non-vesicular trafficking.

Type I: Direct Translocation Across the Plasma Membrane

Certain folded cytosolic proteins can cross the plasma membrane directly without entering vesicular intermediates. This process involves transient pore formation and specific lipid-protein interactions (exemplified by FGF2).

Type II: Transporter- or Channel-Dependent Export

Although less defined in higher eukaryotes, this class includes ABC transporters and other membrane-associated export systems capable of mediating substrate-specific release.

Type III: Vesicle-Mediated (Autophagy/MVB/Exosome) Secretion

In this mode, leaderless proteins are sequestered into vesicular intermediates such as autophagosomes or multivesicular bodies that fuse with the plasma membrane or release exosomes.

Type IV: Golgi Bypass via GRASP and CUPS

Certain secretory proteins bypass the Golgi through specialized compartments formed under stress, called *CUPS* (Compartment for Unconventional Protein Secretion). These compartments require Golgi reassembly stacking proteins (GRASP55/65) and intersect with autophagy and ESCRT machineries.

While this framework offers clarity, in practice the boundaries between categories are fluid, with significant mechanistic overlap—for example,

GRASP and ESCRT components can function in both vesicular and non-vesicular contexts [3,4,7,12].

3. Type I UPS — Direct Membrane Translocation: The Paradigm of FGF2

Among all known UPS cargos, FGF2 provides the best-characterized example of direct plasma-membrane translocation. Unlike most growth factors, FGF2 lacks a signal peptide and is not processed through the ER or Golgi [5–7]. Instead, it directly crosses the plasma membrane through a series of orchestrated steps.

3.1 Molecular Steps of FGF2 Export

FGF2 binds phosphatidylinositol-4,5-bisphosphate (PI(4,5)P₂) on the inner leaflet of the plasma membrane, oligomerizes, and inserts into the bilayer. This interaction triggers transient pore formation that allows the folded protein to translocate [6,8]. On the extracellular side, FGF2 is captured by heparan sulfate proteoglycans (HSPGs), which stabilize it and facilitate its accumulation in the extracellular matrix.

3.2 Accessory Factors and Regulation

Genome-wide RNAi and biochemical screens identified auxiliary factors supporting FGF2 export: Na⁺/K⁺-ATPase subunits, the non-receptor tyrosine kinase Tec, and cytosolic chaperones [6]. These factors modulate the oligomerization and membrane insertion steps. Importantly, this process is tightly regulated—only a small fraction of cytosolic FGF2 is exported at any time, preventing cytolytic leakage.

3.3 Broader Implications

The FGF2 mechanism established that folded, signal-peptide-less proteins can traverse membranes via controlled translocation rather than vesicular transport [5,8]. This challenged long-standing dogmas regarding protein topology and secretion, introducing new paradigms of lipid-mediated protein export.

4. Type III UPS — Vesicular and Autophagy-Linked Secretion

4.1 The Autophagy Connection

Autophagy, classically viewed as a degradative process, can also mediate secretion. Starvation-induced autophagy in yeast revealed a non-degradative role in Acb1 export [9–12]. The process assembles a novel organelle—the *Compartment for Unconventional Protein Secretion* (CUPS)—which integrates early secretory and autophagic machinery.

4.2 Formation and Function of CUPS

CUPS formation requires GRASP proteins (particularly Grh1 in yeast), autophagy proteins (Atg8, Atg9), and ESCRT components [9–13]. These components reorganize endomembrane compartments to produce vesicles that package leaderless cargos like Acb1 for export. Electron microscopy and live imaging have shown that CUPS possess hybrid features of the Golgi and endosomes, representing a “repurposed” trafficking organelle for secretion under stress [11–13].

4.3 MVBs and Exosomes as UPS Carriers

In mammalian cells, many leaderless proteins—including cytokines and metabolic enzymes—are released in *exosomes*, small extracellular vesicles derived from multivesicular bodies [14–17]. The ESCRT machinery orchestrates the formation of intraluminal vesicles within late endosomes, which then fuse with the plasma membrane to release exosomes [25,26]. Exosomal UPS can thus serve both signaling and waste-disposal functions, exporting misfolded or aggregation-prone proteins (e.g., α -synuclein) and mediating intercellular communication.

4.4 Distinguishing True UPS from Classical Exocytosis

Because many exosome cargos originate from endosomes that interact with the Golgi,

distinguishing genuine UPS from conventional secretion remains challenging. Rigorous experiments combining pulse-chase labeling, Brefeldin A sensitivity assays, and live-cell imaging are necessary to define truly Golgi-independent routes [14,15].

5. Type IV UPS — GRASP-Dependent Golgi Bypass

5.1 The GRASP Proteins: Structure and Function

GRASP55 and GRASP65, first identified as Golgi stacking factors, are now recognized as central components of UPS [23–26]. Each contains an N-terminal PDZ-like domain that mediates oligomerization and a C-terminal serine/proline-rich region subject to phosphorylation. These modifications regulate GRASP tethering activity and its ability to form secretion-competent compartments.

5.2 GRASP in Golgi Bypass and CUPS Formation

During nutrient stress, GRASPs relocate from the Golgi to the CUPS, where they scaffold autophagy and ESCRT components, promoting the generation of vesicles that bypass the Golgi [9–13,23–25]. This mechanism operates in yeast (Grh1-dependent Acb1 secretion) and in mammalian cells (GRASP55-dependent stress secretion of Acb1 homologs and misfolded cytosolic proteins).

5.3 GRASP and Cellular Stress Responses

Beyond structural roles, GRASPs integrate stress signaling pathways, linking mTORC1 activity, lysosomal signaling, and the UPS machinery [22,23]. Phosphorylation of GRASP by kinases such as ERK1/2 modulates its relocation, enabling dynamic adaptation between conventional and unconventional routes during ER stress or starvation.

6. ESCRT Complexes: Architects of Membrane Remodeling

6.1 ESCRT Machinery Overview

ESCRT complexes (ESCRT-0 through ESCRT-III and Vps4) orchestrate topologically unique membrane scission events—budding away from the cytoplasm—critical for endosomal sorting, cytokinesis, and viral budding [25–29]. Their involvement in UPS underscores the convergence of degradation, repair, and secretion pathways.

6.2 ESCRT Function in UPS

During CUPS maturation, ESCRT-III subunits (Snf7/Vps32 and Vps24) mediate membrane constriction and scission to form secretion-ready vesicles [13,29]. Similarly, ESCRT components facilitate resealing of gasdermin D–induced membrane pores during IL-1 β secretion, linking membrane repair and cytokine export [18–22].

6.3 Mechanistic Integration

The interplay between ESCRT and autophagy is now recognized as pivotal. Autophagic intermediates can recruit ESCRT proteins to remodel limiting membranes, thereby controlling whether vesicles fuse with lysosomes for degradation or with the plasma membrane for secretion [9–13,30]. This fine balance dictates the cell's response to stress—recycling versus release.

7. Inflammasome-Linked Secretion: IL-1 Family Cytokines

IL-1 β and IL-18 are archetypal leaderless cytokines secreted via UPS [18–22]. Synthesized as cytosolic precursors, they require cleavage by caspase-1 within inflammasome complexes. Once processed, their secretion proceeds through several interrelated routes:

1. **Gasdermin D–dependent pores** formed during pyroptosis can directly release mature IL-1 β [19].

2. **Secretory lysosomes and microvesicle shedding** contribute to cytokine export in certain immune cell types [18,20].
3. **Autophagy/ESCRT-mediated exocytosis** can facilitate non-lytic release, coupling innate immunity with membrane-trafficking systems [19,21].

The precise route depends on the activation stimulus and cell type. These findings illustrate how UPS pathways integrate immune signaling, membrane repair, and cell-death processes into a unified stress-response network.

8. The Role of Autophagy Machinery in UPS

8.1 Autophagy as a Secretory Route

Core autophagy proteins (Atg5, Atg7, Atg8/LC3) participate not only in degradation but also in “secretory autophagy,” redirecting cargo to the extracellular space [9–13,30]. Such cargoes include IL-1 β , Acb1, and misfolded proteins.

8.2 Determinants of Secretory Fate

The choice between degradative and secretory autophagy is influenced by nutrient status, membrane lipid composition, and interactions with scaffolding proteins like GRASP [11,23]. ESCRT-mediated membrane remodeling can further determine whether autophagosomes fuse with lysosomes or the plasma membrane.

8.3 Functional Significance

Secretory autophagy contributes to immune surveillance, tissue remodeling, and clearance of damaged cytosolic material. It also intersects with neurodegenerative mechanisms, as exophagic pathways may mediate extracellular release of aggregation-prone proteins such as α -synuclein [14–17].

9. Physiological and Pathological Relevance of UPS

9.1 Physiological Roles

UPS fulfills crucial physiological roles:

- **Development and differentiation:** AcbA secretion in Dictyostelium triggers prespore differentiation under starvation [9–12].
- **Tissue repair and angiogenesis:** FGF2 release drives endothelial proliferation and migration [5–7,31].
- **Innate immunity:** IL-1 β and IL-18 release coordinate inflammatory cascades [18–22].
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9.2 Disease Contexts

Aberrant UPS contributes to:

- **Chronic inflammation,** through dysregulated IL-1 β secretion in autoinflammatory diseases.
- **Tumor microenvironments,** where leaderless factors (e.g., galectins, annexins) support tumor–stroma communication and angiogenesis [7,31,32].
- **Neurodegeneration,** as exosomal pathways disseminate misfolded proteins between neurons, facilitating prion-like propagation [14–17].
- **Pathogen exploitation,** since viruses and bacteria co-opt host ESCRT and autophagy machinery for their own release or effector export [33].

Thus, UPS represents both a physiological necessity and a potential vulnerability in disease states.

10. Technical Advances (2010–2016)

Technological innovations have propelled the field forward:

- **High-resolution live-cell imaging** visualized real-time secretion of FGF2 and IL-1 β .
- **Yeast genetics** enabled identification of CUPS and conserved autophagy/ESCRT factors [9–12].

- **Genome-wide RNAi and CRISPR screens** uncovered novel translocases and accessory proteins.
- **Advanced biochemical assays** improved quantification of leaderless protein release and exosome isolation [6,9,11,13,34].

Together, these advances consolidated UPS as a bona fide cellular process rather than an experimental artifact.

11. Outstanding Questions (as of 2016)

Despite major progress, critical questions remained unresolved:

1. **Universality of translocation mechanisms:** Is there a conserved translocon for folded proteins, or do cargo-specific machineries dominate [5–8]?
2. **Fate determination of autophagic vesicles:** What factors decide whether autophagosomes fuse with lysosomes or the plasma membrane [9–13,30]?
3. **Cargo selection rules:** Are there specific structural motifs or post-translational marks that define UPS cargos [3,4,12]?
4. **Intersection with membrane repair:** How do gasdermin D pores and ESCRT-mediated resealing intersect with UPS of inflammatory cytokines [18–22]?
5. **Physiological diversity:** How is UPS differentially regulated across tissues, developmental stages, and stress conditions [14–17,31]?

Addressing these questions requires integration of structural biology, live-cell imaging, and system-level proteomics.

12. Translational and Therapeutic Prospects

Understanding UPS provides opportunities for therapeutic intervention.

- **Inflammation:** Modulating IL-1 β release could ameliorate autoinflammatory and autoimmune diseases.
- **Cancer:** Inhibiting leaderless secretion of pro-tumorigenic factors may suppress metastasis and angiogenesis.
- **Biotechnology:** Harnessing UPS allows engineered secretion of proteins incompatible with the ER–Golgi system.
- **Drug delivery:** Manipulating exosome biogenesis and cargo loading holds promise for targeted therapies [14–17,31–33].

Thus, the boundary between basic cell biology and translational application continues to blur as UPS pathways are elucidated.

Conclusion

Unconventional protein secretion expands the classical secretory landscape by enabling the export of leaderless proteins through diverse mechanisms. Research through 2016 has delineated at least four mechanistic types—direct membrane translocation, vesicle-mediated (autophagy/MVB/exosome) secretion, and GRASP-dependent Golgi bypass—each employing conserved modules such as GRASP, ESCRT, and autophagy machinery.

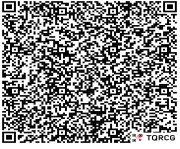
These pathways underpin essential physiological functions in inflammation, development, and tissue repair, but also contribute to disease when dysregulated. The UPS field stands at the intersection of membrane biology, autophagy, and immunology, revealing a fundamental adaptability in cellular trafficking systems. Continued mechanistic and structural exploration promises to uncover new principles governing secretion, homeostasis, and intercellular communication.

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