

The Mystery Behind Unconventional Protein Secretion and Secretory Autophagy

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Proteins, one of the building blocks in every organism, are synthesised inside a cell in a compartment called Endoplasmic reticulum (ER) and gets targeted to different regions within and outside the cell based on their unique “zip-code”. Some proteins get secreted out of the cell for several physiologically important reasons. This can happen *via* a conventional mode or an unconventional mode. The canonical secretory proteins follow the strict protocol route of ER-Golgi and to the exterior due to the presence of the specific zip-code called the leader peptide whereas the unconventional secretory proteins lack this code but still secretes out of the cell and is called the unconventional protein secretion (UPS), mostly under cellular stress such as inflammation, nutrient stress, ER stress, mechanical stress.

Research reports suggest that there are multiple routes the unconventional secretory proteins take up to get out of the cell and are classified as Type I, II, III and IV. Based on this recent classification, type I is a pore-mediated translocation across the plasma membrane, type II is an ABC transporter mediated secretion, type III is an autophagosome/endosome-based secretion and the type IV is a Golgi bypass mechanism. Our lab is interested in understanding the type III system where there is a crosstalk between the unconventional protein secretion (UPS) and a process known as autophagy. Autophagy, as the name suggests, is a self-eating process. Autophagy machinery

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can be compared to a vacuum cleaning system that clears the damaged or redundant organelles, proteins and other cargoes. This homeostatic machinery works by encapsulating these defective items in double-membrane vesicles called autophagosomes and then ferried *via* microtubules to the lysosomes, the compartment containing acidic hydrolytic enzymes where they get chewed up. In the lysosomes, the proteins and other cargoes are broken down into simpler forms and the nutrients are recycled back. This form of autophagy is called as degradative autophagy (see Figure 1).

Besides degradative autophagy, there is another type of autophagy called the secretory autophagy (Figure 1). The involvement of autophagy machinery in UPS of a small subset of proteins has been reported (secretory autophagy). We try to understand the crosstalk of secretory autophagy with unconventional protein secretion in a detailed manner using multidisciplinary approaches. We are interested in identifying the molecular players involved in this process. Many UPS proteins are found to be pivotal and are detected in pathophysiological conditions with dysfunctional autophagy such as neurodegeneration. Thus, autophagy intersects with protein trafficking and secretion thus playing a broad role in the constitutive biosynthetic pathway, regulated exocytosis and alternative routing of integral membrane proteins to the plasma membrane.

In the conventional protein secretion (marked by orange arrow), the proteins synthesised from ER containing the leader peptide undergoes post-translational modifications in Golgi apparatus and gets secreted outside the cell whereas in unconventional protein secretion (marked

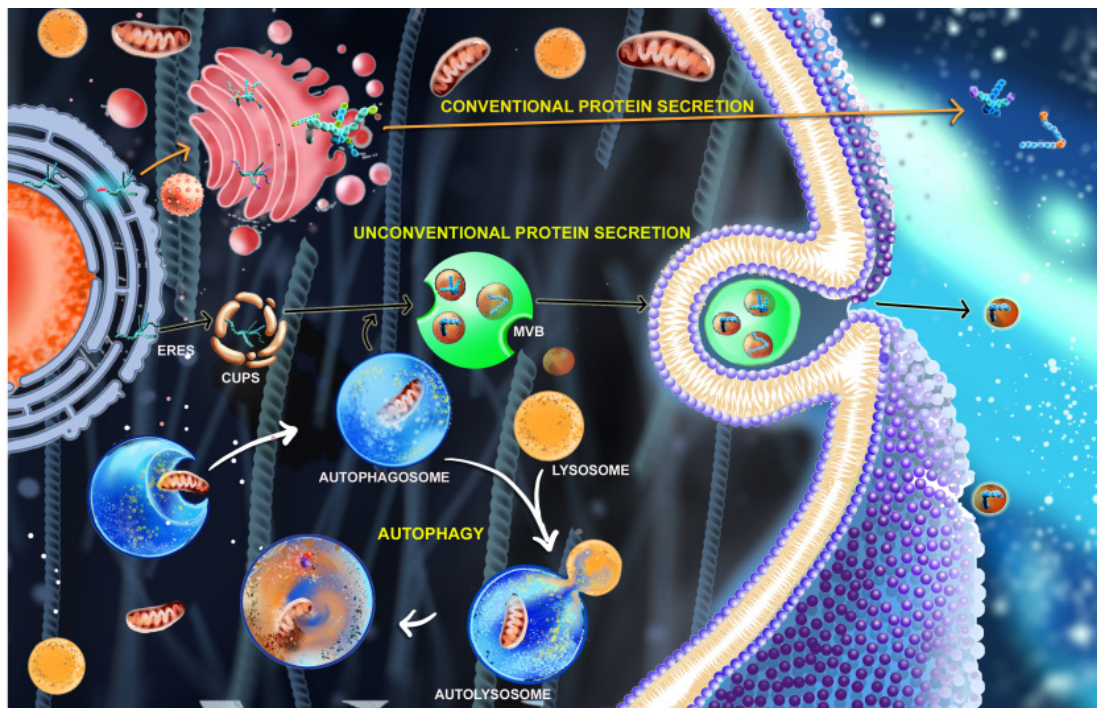


Figure 1: Secretory pathways in cell involving autophagy process.

by black and blue arrows), the proteins bypass the Golgi route or get trapped in CUPS near the ERES (ER exit site) and gets secreted outside that are mediated by autophagosomes (blue arrow) and multivesicular bodies (MVBs; black arrow). The process of degradative autophagy involving phagophore expansion, autophagosome formation, lysosomal fusion and degradation are showed using white arrow.

In a neurodegenerative disease like Parkinson's, the aggregation of proteins such as alpha-synuclein forms the main cause of the disease. This protein misfolding also affects many other PD-linked genes such as parkin, PINK-1 etc., compromising their function at different stages. The gene mutations in alpha-synuclein, by which the protein association with Lewy bodies happen, are the main pathological observation in these diseases. It is reported that dysfunctional lysosomal functions promotes secretion of such aggregates outside the cell. We are interested in understanding the secretory modes and the molecular machinery involved in the secretion of such aggregates using various biophysical methods. Exosomes, small-sized vesicles ranging between 30 and 200 nm are

found to be one of the modes of secretion. The main caveat in the field is to identify potent UPS cargoes and also in delineating their molecular machinery in the protein trafficking. Unravelling the close nexus between autophagy and UPS in aggregate clearance pertaining to neurodegenerative diseases mediated by exosomes has potent therapeutic interventions. We are also employing a theoretical approach to determine /classify the UPS cargoes based on context dependence of the specific 'DE' motif (Sreedevi P *et al.*, <https://www.biorxiv.org/content/early/2018/01/18/250076.1>)

In layman's language, an analogy can be drawn of a cell with a utopian kingdom "Cellpuria". In the distant microland of cellpuria lives the emperor DNA in the palace of Nucleus. He is closely associated with his consort histone. The bond between the emperor and the queen is inseparable. The kingdom is ably run by his eminent ministers heading various departments – Home affairs minister (ER), mitochondria (power minister), communication department (Golgi), department of law and justice maintenance (lysosomes), foreign

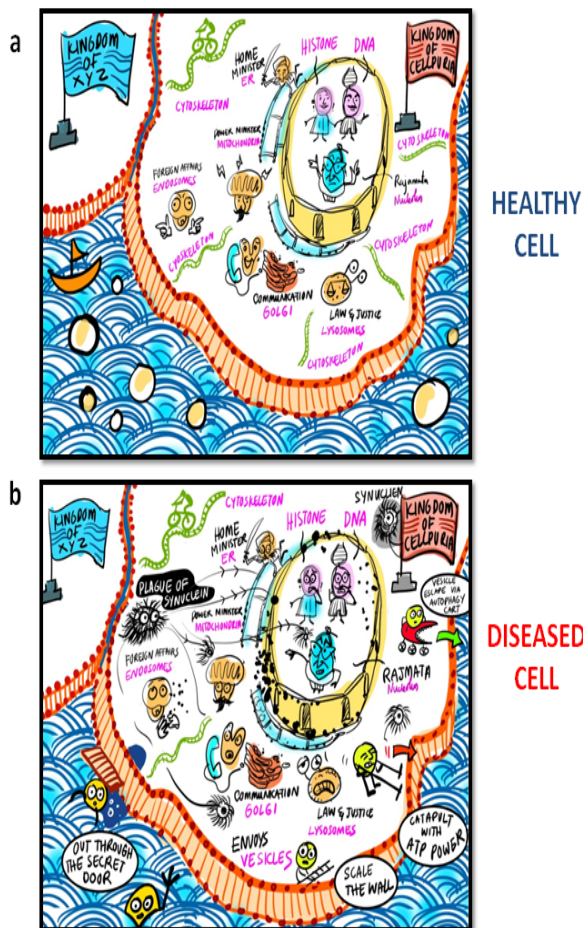


Figure 2. Depiction of protein trafficking in a healthy and diseased cell.

affairs (endosomes). The palace is surrounded by two giant layers of fence (nuclear membrane) where trespassing is prohibited. The atmosphere (cytoplasm) is clean, free of pollution. The kingdom is maintained nicely with well-paved roads (cytoskeleton) and well-defined boundaries (plasma membrane). All the basic amenities are taken care by the government (homeostasis) (Figure 2a).

In the ideal prudent kingdom of Cellpuria, when there is a stroke of evil spirit (mutation)/ natural disasters (environmental cues), outburst of plague (aggregate formation and spreading), it collapses the entire kingdom. Faulty production/revolt of young men such as alpha-synuclein, huntingtin, and amyloid set-up outbursts in many parts of the kingdom. These eruptions, as huge explosions (Lewy bodies), cause havoc in the kingdom (Figure 2b). The vesicles which are like envoys may get (secreted) out of the kingdom *via* different routes as suggested earlier (Type I – secret door; Type II – ABC transporter, ATP-power driven catapults; Type III – Autophagy machinery; Type IV – Golgi bypass).

The figure (2a) is of a healthy cell with proper homeostatic machinery while (2b) is a diseased cell affected with neurodegenerative disease causing aggregate such as alpha-synuclein.

Our interests have been in understanding these routes and probable way to clear these aggregates and thereby stop the spread the transmission of aggregates that are mediated by secretory autophagy. Future work should reveal selectivity of the unconventionally secreted vesicles and identification of critical modulators of this underexplored unconventional secretory pathway that would pave a way in treating the disease specifically.

As rightly said by the famous American poet, Robert Frost,
“Two roads diverged in a wood, and I—
I took the one less travelled by,
And that has made all the difference.”

UPS is also one such journey of the intracellular proteins that is non-canonical which would pave way to understand its nature of secretion in a comprehensive manner.