Sorting nexins in protein homeostasis

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Abstract: Sorting nexins (SNXs) are a highly conserved membrane-associated protein family that plays a role in regulating protein homeostasis. This family of proteins is unified by their characteristic phox (PX) phosphoinositides binding domain. Along with binding to membranes, this family of SNXs also comprises a diverse array of protein-protein interaction motifs that are required for cellular sorting and protein trafficking. SNXs play a role in maintaining the integrity of the proteome which is essential for regulating multiple fundamental processes such as cell cycle progression, transcription, metabolism, and stress response. To tightly regulate these processes proteins must be expressed and degraded in the correct location and at the correct time. The cell employs several proteolysis mechanisms to ensure that proteins are selectively degraded at the appropriate spatiotemporal conditions. SNXs play a role in ubiquitin-mediated protein homeostasis at multiple levels including cargo localization, recycling, degradation, and function. In this review, we will discuss the role of SNXs in three different protein homeostasis systems: endocytosis lysosomal, the ubiquitin-proteasomal, and the autophagy-lysosomal system. The highly conserved nature of this protein family by beginning with the early research on SNXs and protein trafficking in yeast and lead into their important roles in mammalian systems. Underlying the importance of SNXs in protein homeostasis, genetic defects in SNXs have been linked with a variety of human diseases

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Introduction.

The integrity of the proteome is essential for maintaining homeostasis as well as coordinating stress response mechanisms. The job of maintaining homeostasis is handled by sophisticated protein quality control systems that ensure that malformed or excessive proteins are degraded at the appropriate time and location throughout the complete life cycle of a protein. Selective proteolysis is largely mediated by the ubiquitin-proteasomal pathway (UPS) and the autophagy-lysosomal pathway (ALP). In general, the UPS is the primary proteolytic route for short-lived, misfolded, or damaged proteins having essential functions in many critical cellular pathways, including cell cycle progression and transcriptional regulation [1]. The ALP tackles long-lived proteins, dysfunctional or superfluous organelles, and protein aggregates. As ALP is upregulated in response to cellular stress (nutrient deprivation, hypoxia, oxidative stress), it is considered a major adaptive mechanism, critical for cell survival following unfavorable environmental onslaughts [2, 3].

There are three major types of autophagy: macroautophagy, microautophagy, and chaperon-mediated autophagy (CMA) [4]. Macroautophagy (herein autophagy) is also further classified as being selective or non-selective. In non-selective bulk autophagy autophagic vesicles randomly engulf portions of the cytoplasm and various cytoplasmic components, predominantly in response to starvation signals. Selective mechanisms are predominantly utilized to maintain homeostasis under physiological conditions. Cargos include defective organelles including mitochondria, endoplasmic reticulum, peroxisomes [5], as well as cytoplasmic protein aggregates [6] and pathogenic intracellular invaders including RNA viruses like SARS-CoV-2 (COVID-19) [7-9]. In mammalian cells, ubiquitylation of the cargo is critical for recognition by the autophagic machinery, thereby linking UPS and ALP pathways [10, 11].

The third protein quality control system, the endocytosis-lysosomal pathway, is intricately linked to UPS and ALP [12]. Cargoes are transported from the plasma membrane to sorting endosomes where their fate is decided. In a ubiquitin and ESCRT dependent pathway, they

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can be selected for inclusion into intraluminal vesicles, that by budding away from sorting endosomes are ultimately delivered to the lysosome for degradation [13]. Alternatively, they are prevented from this degradative fate, and selected for enrichment in endosomal "retrieval" subdomains, namely the *trans*-Golgi network (TGN), or recycling endosomes. From here they are ultimately for recycled back to the plasma membrane [12, 14]. Retrieval of cargos is mediated through by cargo retrieval complexes (retromer, retriever, CCC (CCDC22, CCDC93, and COMMD) WASH (Wiskott–Aldrich syndrome protein and SCAR homolog)) and branched actin [15, 16]. Two of these complexes, the retromer, and the retriever, are aided by the cargo-adapted family SNX proteins, the subject of this review [17].

Although initially thought of as independent pathways, these systems are now known to be interconnected. Within these three pathways ubiquitin plays an important role in substrate targeting and specificity. In yeast *S. Cerevisiae* (unless stated otherwise), and mammalian systems alike, the evolutionarily conserved sorting nexin (SNX) family of proteins plays a role in regulating these protein homeostasis pathways (Figure 1). Underlying their importance, genetic defects in SNX proteins have been linked with a variety of human diseases including neurodegenerative, cardiovascular diseases, and cancer [18, 19]. In this review, we will discuss the growing evidence of SNXs in these three protein quality control pathways and as well as the interplay between SNXs and ubiquitin.

1. Outline of endocytosis pathways.

1.1 Overview.

The endosomal system is comprised of an interconnected set of organelles whose principal functions are nutrient acquisition, the control of protein and lipid turnover, and protection from pathogens. These vesicle networks also serve as membrane reservoirs supporting rapid changes in the plasma membrane surface area. The system is characterized by early/sorting Rab5 GTPase positive endosomes formed from primary endocytic vesicles that have undergone homotypic fusion or fused with a pre-existing endosome. These mature into late Rab7 positive endosomes by gradually acidifying the fluid within the endosomal lumen, ending with a pH. of 5.5. Both early and late endosomes are characterized by a vacuolar

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domain that contains intra-luminal vesicles (ILVs), formed by ESCRT (endosomal sorting complexes required for transport) complexes and enriched in proteins earmarked for lysosomal degradation. Early endosomes contain significantly fewer ILV's than late endosomes and are characterized by a tubular domain that buds from the endosome that ferries their contents to recycling pathways.

The fate of endocytosed proteins is decided upon reaching early endosomes [13]. If they are not an ESCRT-lysosome cargo, they are recycled directly from endosomes to the cell surface (outlined in Figure 2). Alternatively, cargos can passage through the TGN to their new destination [20, 21]. These cargoes include members of the Vps10 domain family cargo receptors (such as the Alzheimer's disease-linked proteins, sortilin, and SorLA/SorL1) [22, 23]. This endosomal sorting is crucial for maintaining cellular homeostasis and plays a critical role in development. Worth mentioning is the developmental role of the endopeptidase furin that proteolytically activates many proprotein substrates in secretory pathway. These substrates include include the pro-ß-nerve growth factor (pro-ß—NGF) pro-bone morphogenetic protein 4 and the insulin pro-receptor [24] as well as protease systems that control disease including Anthrax and Ebola [25].

1.2 The role of Snx proteins in maintaining protein homeostasis.

The trafficking of cargo from endosomes by degradative or retrieval pathways is mediated by distinct sorting nexins. Here sorting nexins can either promote or prevent cargoes from being destroyed. For example, three nexins (SNX27, SNX17, and SNX4) prevent lysosomal degradation as their role is to ferry cargo. e.g., β1 integrins [26], to the plasma membrane or recycling endosomes. Other nexins (SNX1, SNX2, SNX3, SNX5, and SNX6) mediate retrograde trafficking of cargo to the TGN from endosomes. Likewise, SNX4 interacts with β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) and prevents BACE1 trafficking to the lysosomes, increasing the half-life of BACE1 and production of β-amyloid [27]. In contrast, Snx11 promotes the trafficking of TRPV3 from the plasma membrane to lysosomes for degradation via protein-protein interactions [28]. Also, SNX1

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and SNX6 facilitate the fate of epidermal growth factor receptor (EGFR) and the tumor suppression p27Kip1 [29, 30].

Sorting nexins also interact with cargoes outside of endo-lysosomal-TGN pathways (see Figure 1). Here they play significant roles in both selective and non-selective autophagy pathways in yeast and mammalian cells alike [31-34]. This serves to emphasize the diverse regulatory roles sorting nexins play in maintaining protein homeostasis.

2. Classification of SNX proteins.

The SNX family of cargo adaptor proteins is a large family of proteins, with 10 and 33 members identified in yeast and mammals, respectively [35]. The members are classified into subfamilies based on the domain architecture of other conserved regions (Figure 3). They all contain a lipid binding phox homology (PX) domain that binds to phosphoinositide (PI) lipids decorating organelle membranes. This mediates their attachment to the cytoplasmic leaflets of endosomal compartments [35]. Most SNX family members also contain various other conserved structural domains, BAR and FERM domains being the most prevalent. These domains are targeted by the PX module to appropriate membranes within the endosomal network. Therefore, the SNX proteins are classified into different subfamilies based on the structural arrangements of their scaffolding, enzymatic, and regulatory domains [36]. This modularity confers a wide variety of functions to sorting nexins, from signaling to membrane deformation and cargo binding. Importantly sorting nexins are crucial modulators of endosome dynamics and as well as autophagic functions.

2.1 Lipid binding PX domain of SNX proteins.

Sorting nexins contain a canonical 100-130 amino acid phox homology (PX) domain that binds phosphoinositides (PtdIns) [37] (Figure 3). The PX domain was first identified in the NADPH phagocyte oxidase complex subunits p40phox and p47phox [38]. It is highly conserved and predominantly occurring in sorting nexins. Despite this, the PX domains show little sequence conservation across the SNX family members [39]. However, PX

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domains all possess the same core fold, consisting of three antiparallel β- strands (β1-β3), followed by three α-helices (α1–α3). Analysis of crystal structure has shown that the helices form a loop structure required for PtdIns3P binding. Mutations in the loop structure invariably result in the dissociation of PX domain proteins from endosomal compartments [40].

It is well established that different PtdIns (PtdIns3P, PtdIns(3,4)P2, PtdIns(3,5)P2, PtdIns(4,5)P2 and PtdIns(3,4,5)P3) decorate different membranes. This has resulted in the concept of a phosphoinositide code, that provides membrane identity within the endocytic system [41]. The PX domains of SNX family members predominantly bind phosphatidylinositol 3-monophosphate (PtdIns3P), a signaling lipid enriched in the early endosome membrane [42, 43]. However, SNX proteins can also bind to the other PtdIns phospholipids such as PtdIns(3,4)P2, PtdIns(3,5)P2, PtdIns(4,5)P2 and PtdIns(3,4,5)P3 [39].

2.2 *SNX-PX proteins.*

The SNX-PX subfamily consists only of a PX domain. This family includes the SNX3-**retromer,** which is multi-protein complex comprising of the core retromer (VPS26, VPS29, and VPS35) and SNX3. It retrieves transmembrane cargos from degradation by the lysosome by recycling them back to the cell surface via the TGN [44] (Figure 3). It is a multi-protein complex comprising of the core retromer (VPS26, VPS29, and VPS35) and SNX3. As such, retromer dysfunction impairs many cellular processes and underlies the pathogenesis of various neurodegenerative disorders. For example, a mutation in VPS35, encoding a subunit of the retromer complex, causes late-onset Parkinson disease [45], and microarray studies have implicated the retromer complex in Alzheimer's disease [18, 46].

Multiple SNX3-retromer cargos have been identified including Wnt sorting receptor Wntless [47, 48], the transferrin receptor [49] and the divalent metal ion transporter Dmt1-II [50]. In-depth structural studies revealed that retromer cargoes and Vsp26 and Vsp35 bind to the PX domain but at different surfaces. In short, *SNX3 binding to the retromer* exposes

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a binding site at the interface between SNX3 and VPS26 for cargo containing a Øx(L/M/V) sequence motif where Ø is a bulky aromatic residue) [51, 52]. The retromer trimer engages the Ankyrin-repeat protein ANKRD50 [53] and the actin-polymerizing Wiskott-Aldrich syndrome protein and SCAR homolog (WASH) complex, generating a branched actin networks on the endosomal surface (See Figure 2 and [54]). A different mechanisms is used for SNX16 association with E-cadherin. Here the PX domain of SNX16 is not used for membrane binding but instead is required for binding the cytoplasmic tail of E-cadherin [55].

2.3 SNX-FERM proteins (SNX17, SNX27 and SNX31).

The PX-FERM containing sorting nexins is a sub-group of the PX superfamily. This subfamily has PX domain and a C-terminal 4.1, ezrin radixin, moesin (FERM) domain with an atypical tertiary structure [56]. They are required for endosomal-to-cell-surface recycling of diverse transmembrane protein cargos [57]. PX-FERM nexins are further divided into two groups; SNX17 and SNX31 are cargo adaptor proteins for a retromer -independent complex called the retriever complex, whereas SNX27 associates with the retromer [58]. The retriever complex localizes to early endosomes and is primarily involved in driving the retrieval and recycling of its NPxY/NxxY-motif-containing cargo proteins [58] to the cell surface. Through quantitative proteomic analysis, over 120 cell surface proteins, including numerous integrins, signaling receptors, and solute transporters, that require SNX17–retriever to maintain their surface levels have been identified [58]. These include the LDL receptor, amyloid precursor protein and integrins [26, 56, 57, 59]. SNX17 also recognizes all Human Papillomavirus (HPV) L2 proteins. This interaction aids lysosomal escape thereby being crucial for HPV infection [60-62].

SNX27 is a unique SNX-FERM protein that contains an N terminal density 95/discs large/zonula occludens-1 (PDZ) domain. This domain binds PDZ-binding motif (PDZbm)-containing cargo such as the β2-adrenergic receptor [36, 63, 64]. Quantitative proteomics of the SNX27 interactome has provided an unbiased global view of SNX27-mediated sorting. Here over 100 cell surface proteins, including the glucose transporter GLUT1, the Menkes

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disease copper transporter ATP7A, various zinc and amino acid transporters, and numerous signaling receptors, were shown to require SNX27–retromer to prevent lysosomal degradation and maintain surface levels [65]. The FERM domain recognizes Asn-Pro-Xaa-Tyr–sorting signals in transmembrane cargos. Some of these cargo proteins need to be phosphorylated to facilitate binding to SNX27 as well [26, 56, 57, 59]. SNX27 is highly enriched in the brain. Consequently, cargos include proteins involved in neuronal signaling, such, AMPA receptors [66]. Deficiencies in SNX27 function are associated with Down syndrome [67] and epilepsy [68]. More recently, SNX27-mediated recycling of neuroligin-2 (NL2), a protein required for stabilization of synaptic inhibitory receptors contributes to the regulation of inhibitory synapse composition [37].

2.4 *SNX-BAR proteins.*

The SNX-BAR proteins contain 1-3 coiled-coil or BAR (Bin/Amphiphysin/Rvs) domains that have membrane-remodeling functions [69] (Figure 3). The BAR domains either homo or heterodimerize via extensive interactions between their BAR domains, generating a cup-shaped, concaved surface that sense high positive membrane curvature domains. This permits the BAR regions to induce membrane deformation, transitioning from flat membranes to tubular membrane surfaces [42]. Current models propose that both PX and BAR domains have to be engaged with the membrane to ensure specificity and efficient binding. Mammalian cells possess twelve SNX-BAR family members (SNX1, SNX2, SNX4, SNX5, SNX7 SNX8,SNX9, SNX18, SNX30, SNX32 and SNX33) [70]. The retromer-related SNX-BAR proteins (SNX1, SNX2, SNX5, SNX6, and the neuronal SNX32) form heterodimeric complexes. These are critical for both endosome–to–plasma membrane recycling and endosome-to-TGN retrieval [71]. More recently, they have identified as important players in autophagic processes, which is discussed in more detail below in section 5 [34, 72].

Despite holding extensive knowledge of the role of SNX-BAR proteins, how they recognize cargos has remained elusive. Recently, a conserved SNX-BAR-binding motif was

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identified using SNX5 and its lysosome-destined cargo the cation-independent mannose 6-phosphate receptor (CI-MPR) [70, 73]. In addition to the PX domain, SNX5 and CI-MPR is a bipartite motif, termed SNX-BAR-binding motif. This motif is also required for another SNX-BAR cargo, Insulin-like growth factor 1 receptor (IGF1R). Spurred on, by these results, the authors performed an *in silico* analyses of the human proteome and discovered over 70 putative SNX-BAR cargoes. Based upon these results, the authors propose a model in which SNX-BARs function as a direct cargo-selecting module for a large set of transmembrane proteins transiting the endosome.

Intriguingly, Snx-BAR proteins also recognize non-membrane proteins in yeast. For example, Vps5 (SNX1) interacts with the helicase enzyme Dhh1 (Miller, 2017). Mvp1 (SNX8) interacts with Sba1 a co-chaperone that binds and regulates the Hsp90 family of chaperones [74]. Also, the Snx4-Atg20 (Snx41) heterodimer to binds to the transcriptional regulator Med13 after TORC1 inhibition triggering its Snx4-Atg20 mediated autophagic degradation (see section 6.1 and [75]).

2.5 Other domains.

Some SNX proteins also contain additional domains including SH3 (Src homology 3), RA (RasGTP effector), and RGS (regulator of G-protein signaling) domains [76], [35] (Figure 3). These additional protein-protein binding domains enable SNXs to form homo- or heterodimers and associate with larger protein complexes such as the retromer or autophagy vesicles. In retromer mediated processes, SNX-BAR proteins that contain an extra Src-homology 3 (SH3) domain (SNX9, SNX18, and SNX33) homodimerize to coordinate actin polymerization with vesicle scission at sites of high membrane curvature [77, 78].

3. Snx cargo recognition in yeast.

3.1. The yeast endosome system.

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In yeast, in response to different physiological conditions, the plasma membrane's makeup is adjusted to maintain homeostasis. This is achieved by the internalization of plasma membrane proteins (cargoes) by endocytosis through a clathrin-dependent or independent mechanism. In clathrin-mediated endocytosis (CME), cargoes are first internalized in clathrin cages. Next the cages disassemble, thereby delivering cargoes to endosomes [79]. Some cargoes are recycled back to the cell surface either via the TGN or by a recycling pathway originating from endosome [80]. Others, tagged by ubiquitination, are degraded in the vacuole (the yeast equivalent of lysosomes) [81].

Recently it has been proposed that unlike other eukaryotic species, budding yeast lack early endosomes [82, 83]. Instead, cargo-carrying vesicles are initially targeted to the TGN. From here, cargoes are either recycled or transferred to late endosomes (also known as multivesicular bodies (MVBs) or pre-vacuolar endosome (PVE) compartments)[72]). PVE's contain cargo-laden intraluminal vesicles which require ESCRT pathways for their formation. Here ubiquitin plays a key role, as the transport of cargoes to the vacuole depends on ubiquitin linkages. Thus, ubiquitination serves both as a signal for endocytosis from the plasma membrane and a specific sorting signal for entry into the vacuolar lumen [13]. In the final step, late endosomes fuse with the highly acidic vacuoles that contain proteases for degradation of the endosomal contents. Here very elegant experiments using an engineered fluorescent vacuolar cargo and 4D microscopy have suggested that transfer of material from PVE compartments to the vacuole most likely involves "*kiss-and-run"* fusion events [82, 84].

3.2 The yeast retromer.

The highly conserved retromer (Vps35, Vps26 and Vps29), first identified in *S. cerevisiae*, generates cargo-selective tubulovesicular carriers from endosomal membranes [85, 86]. The best-characterized yeast retromer forms a pentameric structure with the Vsp1-Vsp17, Snx-BAR sorting nexin [44]. This pentameric complex is required for retromer endosome localization where it generates cargo-selective tubulovesicular carriers from endosomal membranes [71, 87]. Using cryo-electron tomography, the structure of the

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trimeric retromer assembled membrane tubules with Vps5 has been determined [88]. Here Vps5 homodimers attach the arch-like retromer structure to membranes. The arches extend away from the membrane surface, with Vps35 forming its legs and Vps29 siting at the apex. This allows Vps29 to interact with regulatory factors. The bases of the arches connect to each other and to Vps5 through Vps26. These studies provide significant insight into how the retromer is assembled on tubular membranes, which is important for understanding tubular-based cargo sorting.

Despite the high degree of conservation between the mammalian and yeast retromers, yeast do not recognize the mammalian retromer cargo recognition sequence [52]. This led to studies on Vsp10, the best characterized Vsp5 and Vsp17-retromer cargo which is the first member of the Sortilin receptor family. Vsp10, a transmembrane protein receptor for carboxypeptidase Y (CPY), sorts CPY into vesicles at the Golgi. Thereafter CPY-containing vesicles plus Vsp10 are transported to the endosome, which upon maturation, fuses with the vacuole, delivering soluble CPY to the vacuole lumen. Vps10 escapes this fate, being recycled from the endosome back to the Golgi by the retromer complex, making Vps10 available for additional rounds of CPY sorting. Remarkably, two distinct motifs on Vsp10 were identified which serve as a bipartite recycling signal, with each motif being recognized by the retromer subunits, Vps26 and Vps35. These striking results show that the retromer utilizes different binding sites depending on the cargo allowing this complex to recycle different proteins.

Other sorting nexins also contribute to retromer function [72]. Snx3 is an accessory protein that binds the retromer and recycles cargoes from endosomes to the TGN [89]. It recognizes relatively few cargoes, though a recent systematic genome-wide screen expanded its repertoire [33, 72]. One cargo, Neo1, deserves a special mention as its discovery uncovered a previously unknown role for the Snx3-retromer [90, 91]. Neo1 is an aminophospholipid flippase, that contributes to the phosphatidylethanolamine asymmetry of endosomal membranes [92]. The deletion of the Snx3 recognition site in Neo1 revealed that

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the Snx3-dependent sorting of Neo1 is required for the correct sorting of other Snx3 cargo protein [90]. Similarly, the packaging of human SNX3-retromer cargo, Wntless, also requires NEO1 [91]. Taken together, this suggests that the incorporation of Neo1 into recycling tubules may influence their formation.

Another less well understood sorting nexin that contributes to retromer function is the SNX-BAR protein Mvp1 [72, 93]. Mvp1 shares conservation with the mammalian SNX–BAR SNX8, whose function, like Mvp1, is unclear but is involved in endosomal sorting [94, 95]. Consistent with this, cells lacking Mpv1 exhibit defects retromer-dependent retrograde trafficking from the endosome to the TGN [96, 97]. Recent structural studies have revealed that the Mvp1 SNX-BAR protein exists as an autoinhibited tetramer in which the PX lipid-binding sites are occluded. The Mvp1 dimer retains membrane-remodeling activity and exhibits enhanced membrane binding. This suggests a model in which the unmasking of the PX and BAR domains is required for Mvp1 function. As most SNX–BAR proteins are invariably dimeric, this finding adds a layer of complexity to the regulation of SNX–BAR function.

3.3 Retromer-independent sorting nexin function in yeast.

Snx4, Snx41, Atg20 form two distinct retromer-independent complexes (Snx4-Snx41 and Snx4-Atg20) and are required for are required for endocytic recycling and selective autophagy. Consistent with these roles they co-localize to the endosome and the pre-autophagosomal structure (PAS) [32, 98, 99]. Moreover, we and others have shown that after nitrogen starvation, they sequester to the perinucleus where they transport nuclear cargos to the vacuole [31, 75](see section 5.1).

The most studied cargo of the Snx4-Atg20 complex is Snc1, which is a plasma membrane-directed v-SNARE, required for fusion of secretory vesicles with the plasma membrane [100]. More recently two distinct pathways have been defined which move Snc1 within the cell. Rcy1, which is an F-box protein interacts with Snc1 and is responsible for the

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delivery of endocytic plasma membrane to the TGN. Snx4-Atg20 are required for the retrograde pathway, delivering Snc1 back to the TGN from late endosomes [101]

Another important role in the function of Snx4-Atg20 heterodimer is to mediating endosome-to-Golgi transport of Atg9, an integral membrane component of the autophagy machinery, [102]. Atg9 is an essential protein required autophagosome biogenesis. Atg27 maintains a Golgi-localized pool of Atg9, which is critical for autophagosome formation [103]. In turn, Atg27 recycling and trafficking is regulated by the retromer and Snx4 [104, 105]. More recently, it has been shown that Atg27 is recycled from the vacuole membrane using a 2-step recycling process. First, the Snx4 complex recycles Atg27 from the vacuole to the endosome. Then, the retromer complex mediates endosome-to-Golgi retrograde transport. [104]. This is exciting as it represents the first physiological substrate for the vacuole-to-endosome retrograde trafficking pathway.

4.0 Ubiquitin and Endocytosis.

Ubiquitin (Ub) is a small molecule that covalently attaches to lysine resides on its targets. Ub itself can be conjugated to a second Ub molecule resulting in ubiquitin chains differing in linkage types and lengths [11, 106]. This wide variety of Ub modifications can have pleiotropic effects on its substrates [1]. K48-linked ubiquitin chains typically target proteins for degradation by the 26S proteasome [107]. On the other hand, K63-linked ubiquitination typically acts as a signaling event to modify function, such DNA repair, altering protein-protein interactions and protein trafficking [108].

During endocytosis, membrane proteins are identified as cargo either as part of a programmed biological response (such as ligand mediated receptor down-regulation) or as a way to remove aberrantly folded or damaged proteins from the cell surface as a quality-control mechanism. The proteins are decorated with Ub at plasma membranes and early endosomes to trigger their internalization and endosomal sorting respectively [109, 110]. A functional ESCRT pathway is also required. In short, cargoes are tagged with the ubiquitin

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sorting signal are recognized by ESCRT-0. These are then sequentially handed to ESCRT-I and -II or recruited to the ESCRT-I-II supercomplex before being incorporated into ILVs for delivery to lysosomes [81, 109].

4.1 Sorting nexins and E3 ligase activity.

Sorting nexins influence the regulation of proteasome activity and substrate degradation by a variety of different mechanisms. Some of the roles of SNXs in the UPS include blocking ubiquitination of protein substrates, inhibiting ubiquitin specificity factors, regulating protein stability of E3 ligase by either enhancing their recycling or degradation pathways and degrading inactive or excess proteasome complexes. These are summarized in Table 1. Intriguingly, there are several examples of the relationship of soring nexins proteins with E3.

In yeast, the E3 ligase specificity factor for Rsp5-dependent ubiquitination, Ear1 is recycled by Snx3. Snx3, therefore, enhances Ear1 protein stability protein thereby promoting the Rsp5 activity [33]. In mammalian cells, SNX18 is regulated by the E3 ligase Mib1, which indirectly promotes Notch signaling [111]. Likewise, Itch (atrophin-1 interacting protein 4), a member of the NEDD4 family of E3 ubiquitin ligases, ubiquitylates SNX9, thereby regulating intracellular SNX9 levels. [112]. In a seminal discovery, the E3 ubiquitin ligase partner of MAGE-L2, a protein that enhances E3 ubiquitin activity [113], was found to be the K48 E3 TRIM27 [113]. The MAGE-L2-TRIM27, localizes to endosomes through interactions with the retromer complex. The outcome of this interaction is K63 ubiquitination of the WASH complex, a known regulator of retromer-mediated transport. This action permits WASH to nucleate endosomal F-actin (see Figure 2). Moreover, this pathway is regulated by the deubiquitinating enzyme USP7 [114].

4.3 Sorting nexins and E2 enzymes.

Less is known about sorting nexins and E2 activity. In *Drosophila*, UBC-13, the E2 ubiquitin-conjugating enzyme that generates K63-linked ubiquitin chains, is essential for

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retrograde transport of multiple retromer-dependent cargoes, including MIG-14/Wntless. Here UBC-13 function is required for retrograde transport of SNX1 retromer-dependent cargoes [115].

4.4 Sorting nexins and deubiquitinases.

Sorting nexins also interact with deubiquitinating enzymes [116]. The hormone Vasopressin increases the expression of the USP10 that deubiquitylates and stabilizes SNX3 [117]. SNX27 interacts with the deubiquitinase OTULIN (OTU Deubiquitinase With Linear Linkage Specificity) that specifically hydrolyzes methionine1 (Met1)-linked ubiquitin chains. SNX27 association with OTULIN antagonizes SNX27-dependent cargo loading, binding of SNX27 to the VPS26A-retromer subunit, and endosome-to-plasma membrane trafficking. Moreover, these findings define a non-catalytic function of deubiquitinases in sorting nexin function [118].

4.5 Sorting nexins an proteasomes.

Following nitrogen starvation in yeast model system nuclear 26S proteasomes are first disassemble into 19S and 20S subcomplexes, transported through the nuclear pore complex (NPC) and targetted to autophagosomes for degradation [31, 119, 120]. The Snx-Atg20 and Snx4-Snx42 heterodimer both are required for transport of the 19S and 20S complexes after they emerge from the NPC [31]. How these complexes interact with the proteasome remains unknown, but it adds another example of how sorting nexins regulate the UPS machinery.

4.6 Oncogenic roles of sorting nexins and the UPS.

In recent years oncogenic roles of sorting nexins have been reported. Therefore, it comes as no surprise that many of these roles lead to the activation of well-characterized oncoproteins. Recently, TRIM27 has been classified as an oncoprotein. Consistent with this role, it is overexpressed in many cancers, including breast, endometrial, ovarian, lung, and colon [121]. TRIM27 association with the retromer and activates the cytoplasmic

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transcription factor, STAT3 [122]. This is an important discovery as STAT3 plays central roles in various physiological processes and its aberrant and persistent activation results in serious diseases, including cancer [123]. It is a cytoplasmic transcription factor as its activation and translocation to the nucleus is dependent upon its passage through the endosome system. [124]. In response to several cytokines or growth factors including interleukin-6 (IL-6) STAT 3 is phosphorylated [125]. This promotes its release from the endosome and translocation to the nucleus, resulting in the induction of downstream effector genes. Intriguingly, the E3 ubiquitin ligase activity of TRIM27 is dispensable for its ability to mediate STAT3 activation. Confirming a retromer linked role, knockdown of each of the retromer components significantly inhibited IL-6-induced transcription of STAT-dependent genes [122]. It is well established that endocytosis is an effective mechanism to downregulate cellular signaling events by internalizing receptors or ligand-receptor complexes [126]. Further studies are needed to address if other signaling proteins that are imprisoned by endocytosis, e.g., the promiscuous kinase glycogen synthase 3 beta (GSK3-β) are similarly regulated. This is important as this kinase has numerous phosphorylation targets in distinct pathways, including WNT, Hedgehog and MAPK signaling. [127].

Sorting nexins interaction with E3 ligases plays a role in oncogenesis in other cancers. In head and neck squamous cell carcinoma (HNSCC) SNX5 interacts with the E3 ligase F box proteins, thereby blocking FBW7 mediated ubiquitination of oncoproteins including c-Myc NOTCH and cyclin E1 [128]. SNX16 also has oncogenic properties in colorectal cancer, where it is significantly upregulated. This affects eEF1A2/c-Myc signaling, possibly by inhibiting proteasome-dependent ubiquitination of eukaryotic translation elongation factor 1 A2 (eEF1A2) [129]. As such, SNX16 has been implemented in the development of other tumors such as bladder and ovarian cancer [130, 131]. SNX10 also may be a tumor suppressor in mouse models of colorectal cancer. Here SNX10 deficiency prevents the degradation of LAMP-2A, the essential CMA lysosomal receptor [132 , 133]. Given the key role of soring nexins in many biological processes, there is no doubt that future work will reveal more links to cancer and other diseases.

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5.0 Sorting nexins in the autophagy-lysosomal pathway (ALP).

5.1 Autophagy mechanisms.

Autophagy is thought of as the first line of defense in response to many forms of extracellular stress [11]. In contrast to the sophisticated ubiquitin-proteasomal system (UPS), the lysosome was once thought to be a dumpsite for proteolysis, degrading bulk, non-selective cytosolic components. The rapid growth of the autophagy field has revolutionized this paradigm. Three forms of autophagy have been described: chaperone mediated autophagy (CMA), microautophagy and macroautophagy (selective and non-selcetive autophagy). CMA [134] and microautophagy [135] will not be discussed further here as their association with sorting nexins remains unknown. Sorting nexins have recently been identified as having a role in bulk autophagy and selective autophagy in yeast and mammalian cells.

5.2 Sorting nexins in non-selective autophagy in yeast.

In physiological conditions, SNXs primarily function within the endosomal pathway to maintain steady-state levels of membrane proteins. Following different stress cues such as nutrient depletion or starvation, SNXs engage in stress-induced regulatory roles. These stress-dependent functions require soring nexin cellular relocalization. This enables them to capture their cargos and engage the autophagic machinery. The role of sorting nexins in autophagy was first identified in yeast where it was found to be a component of the Atg1 initiation complex [98]. Here Snx4 binds the Atg17 scaffold complex which is required to localize the PAS to vacuole outer membranes [136, 137]. Consistent with this, deletion of Snx4 results in insufficient PAS formation and a delayed autophagic response [138]. In a more recent phosphoproteomics study, Snx4 was identified as a direct substrate for Atg1 [139]. This phosphorylation event may direct Snx4 away from its physiological functions in endosomal sorting and selective autophagy and towards its role in starvation-induced autophagy. Interestingly during selective autophagy. Snx42 interacts with the scaffold protein Atg11 that replaces Atg17 and initiates autophagosome assembly at the cargo site

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[136]. SNXs have also been implemented in later stages of autophagy involving autophagosome and vacuolar membrane fusion. It has been shown that Snx4 and Snx42 promote non-selective autophagy by exporting lipids from the vacuole which maintains the fusion competence of this organelle and allows autophagosome fusion [140].

5.3 Sorting nexins in non-selective autophagy in mammalian systems.

In mammalian cells, siRNA suppression of SNX4 or SNX7 results in a significant reduction in LC3 puncta suggesting that this SNX-BAR heterodimer is required for autophagosome assembly. The SNX4/SNX7 heterodimer affected autophagosome assembly rather than autophagic flux. influenced autophagosome assembly by controlling ATG9 trafficking [34]. In a similar study, SNX18 along with Dynamin-2 regulates ATG9 trafficking from recycling endosomes to autophagosomes [141]. In a complementary study, SNX18 positively regulates autophagosome formation by recruiting Atg16L1 and LC3-positive membranes to the autophagosome. Here SNX18 requires membrane binding capacity as well as direct LC3 interactions [141] (Figure 4A).

5.4 Sorting nexins in selective autophagy in yeast.

In yeast, Snx4 and Snx42 are required for several selective autophagy pathways. Cargos include mitochondria [142], peroxisomes [143, 144], proteasomes [31], ribosomes [31], fatty acid synthase complexes [145] and transcription factors [75] (Figure 4B). A role for sorting nexins in selective autophagy was first identified while studying the cytoplasm-to-vacuole targeting pathway (CVT). This pathway functions in physiological conditions to transport the aminopeptidase, Ape1, to the vacuole. Snx4 and Snx42 are required for the recruitment of proteins to the site for CVT formation [98]. Since then a role of Snx4 in other selective autophagy pathways has been uncovered. Despite being required for many pathways, the molecular details of Snx4's role in these pathways remain unclear.

5.5 TORC1 controls the endocytosis pathway.

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The endocytosis pathway is controlled by the target of rapamycin complex 1 (TORC1). TORC1 signaling is required to promote the endocytosis of specific plasma membrane proteins [146]. TORC1 inactivation, induced by starvation [147], however, initiates the destruction of another set of cargoes [148], providing the cell with an immediate source of amino acids. These are sufficient to uphold protein synthesis until other autophagy pathways are activated. These findings show that endocytosis and autophagy are highly coordinated in maintaining intracellular amino acid homeostasis, thereby promoting cellular survival during starvation [149].

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6. The interplay between soring nexins, lysosomal degradation and UPS-mediated degradation

In recent years, it has become apparent that the UPS and autophagy pathways are functionally interconnected [150-152]. Key findings from these studies have revealed that when the UPS is overwhelmed, autophagy pathways can step up to eliminate aberrant proteins [11]. Furthermore, ubiquitination is utilized as a degradation signal by autophagy pathways, being critical for removing damaged mitochondria by mitophagy in mammalian cells [153, 154]. Ubiquitin is required for autophagic degradation of protein aggregates [155, 156], peroxisomes [157]pathogens [158] and ribosomes [159, 160]. Here the Crosstalk between ubiquitination and autophagy is provided by autophagic adaptor proteins (or

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autophagy receptors), which bind both ubiquitin and autophagy-specific UBL modifiers (Atg8 and its homologues) [161, 162]. This has led to the more current hypothesis that the UPS and autophagy pathways constitute a single integrated degradation system, which together determine the fate of many proteins [136]. Consistent with this, following TORC1 inhibition, in yeast, nuclear proteasomes are disassembled and then destroyed by Snx4-Atg20 and Snx4-Atg42 mediated autophagy [31, 120, 163].

5.5 Snx4 OR the SCF^{Grr1} mediate the destruction of regulate Med13 following stress.

There are a limited group of proteins that are substrates for both lysosomal and proteasomal degradation. Our group has discovered that Med13, a conserved member of the cyclin-dependent kinase (Cdk) module of the mediator complex, is degraded either by a novel Snx4-mediated autophagy pathway or by the UPS in response to survival and cell death signals respectively (Figure 5) [75, 164, 165]. Med13 is a member of the conserved Cdk8 kinase module (CKM) that predominantly repress genes induced by environmental stress [166-168]. Activation of these genes is achieved by disrupting the CKM association with the mediator [167, 169]. Med13 and cyclin C are both targets of the UPS system following oxidative stress, but cyclin C is only destroyed after it has executed its “night job”. Here, cyclin C but not Cdk8, translocates to the mitochondria where it mediates stress-induced mitochondrial fission and promotes cell death in both yeast and mammalian cells [170-172]). In mammalian cells, mitochondrial located cyclin C also associates with Bax to promote its activation [171].

In contrast, following a survival cue (nitrogen starvation), cyclin C is rapidly destroyed by the UPS before its nuclear release that prevents mitochondrial fission, upregulates Autophagy genes *ATG* (AuTophaGy) genes promoting survival [173]. Here Med13 is removed from the nucleus by a Snx4-mediated autophagy pathway. After transitioning through the nuclear pore complex, Med13 is transported by the Snx4-Atg20 heterodimer to Atg17-initiated phagophores attached to PAS complexes originating at the vacuole [75]. Moreover, two transcriptional activators (Rim15 and Msn2) that regulate also

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ATG expression, are degraded upon nitrogen starvation by this mechanism. Taken together, this suggests a model in which Snx4-mediated autophagy of *ATG* transcriptional regulators allows fine-tuning of the autophagic response.

6.2 p27 is regulated by SNX6, proteasome degradation and endo-lysosomal pathways.

In mammalian cells the growth suppressor, kinase inhibitor p27 facilities cell cycle progression in arresting the cell cycle in response to a variety of environmental cues [174]. UPS mediated degradation of p27 by SCF^{Skp2} occurs in the nucleus in G1/S phase whereas the Kip1 E3 liagse mediates its 26S turnover in the cytoplasm in G(1). Together this ensures cell cycle progression by making S-phase entry irreversible [175, 176]. Cytoplasmic p27 is also directed for endo-lysosomal degradation SNX6. This is also important for cell cycle progression as silencing SNX6 delays S-phase entry on serum stimulation of starvation-synchronized NIH-3T3 cells [30]. Further studies are needed to understand if sorting nexins play key roles in coordinating these proteolysis systems.

7. Sorting nexins in disease

The etiology of several diseases such as cancer, cardiovascular and neurodegenerative diseases is linked to dysregulation of sorting nexin function. As deficiencies or dysregulation of sorting nexins results in protein homeostatic, current research is focusing on SNX-mediated regulatory mechanisms and their role in the pathophysiology of various disease states.

7.1 The role of sorting nexins in cardiovascular disease.

Sorting nexins are implemented in the development of cardiovascular diseases such as hypertension, coronary heart disease, and heart failure [19]. Here SNXs influence the maintenance of blood pressure by regulating the expression and function of GPCRs such as dopamine receptors, ion channels, and transporters [19, 117, 177].Consistent with this, knockdown/knockout animal models of SNX1, SNX5, and SNX19 correlate with hypertension. This has led testing if SNXs could potentially be a therapeutic target for

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hypertension. Therapeutic strategies have focused on expressing specific SNX subtypes within the kidney to decrease blood pressure [19].

SNXs also influence the pathogenesis of coronary artery disease by regulating lipid metabolism. SNXs interact with the leptin receptor and the low-density lipoprotein (LDL) receptor [37, 178] and decreasing SNX1 levels results in increased levels of triglycerides and cholesterol [37, 178]. SNXs may also influence coronary artery disease by regulating inflammation, which is linked to the etiology of vascular diseases [179]. SNX13 deficiencies also correlate with decreased heart function associated with cardiomyocyte apoptosis. SNX13 mediates the recycling of the apoptotic repressor, ARC. Loss of SNX3 results in the degradation of ARC and promotes cardiomyocyte apoptosis and heart failure [180].

Insulin insensitivity is a major hallmark of type 2 diabetes mellitus are characteristic feature of heart failure. Sorting nexins are linked to this pathophysiology as SNX5, SNX19 and SNX27 regulate of insulin degradation, secretion, and signaling. Silencing SNX5 in animal models increases blood insulin, decreases insulin excretion and causes insulin resistance [65, 181, 182].

7.2 The role of sorting nexins in neurogenerative diseases.

SNX dysregulation has been linked to several neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease, and Down's syndrome [183]. In neuronal cells, the composition of the cellular membrane is essential for responding to extracellular stimuli and neuroplasticity. SNX-mediated regulation of the cellular membrane composition influences several processes such as neuronal excitability, plasticity, neural development, signaling, psychostimulant response, and cellular drug resistance [184].

Best described is the role of sorting nexins in the pathogenesis of Alzheimer's disease (for details see [185]). This is characterized by brain accumulation of extracellular neuritic plaques containing deposits of β-amyloid (Aβ) peptide and neurofibrillary tangles

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compromised of microtubule-associated protein tau. One of the proteins, APP, innovled in regulating the (Aβ) peptide is SNX33 retromer complex.

SNX33 inhibits endocytosis of APP which in turn lead to retention at the APP at the plasma membrane which promotes to plaque formation [186]. SNX15 and SNX17 also regulate APP processing [187]. SNX4, SNX6 and SNX12 have been shown to regulate BACE1 trafficking which also controls Aβ peptide generation [27, 188]. In addition, SNX27 has also been shown to bind and inhibit Ɣ-secretases thereby decreasing Aβ peptide [189].

Parkinson's disease (PD) is defined by the loss of dopaminergic neurons and the accumulation of α-synuclein-enriched Lewy bodies. Genome-wide association studies have identified various mutations that increase Parkinson's disease susceptibility such as PINK1 and Parkin whose gene products regulate mitophagy [190, 191]. As such mitochondrial defects such as disruptions in mitochondrial fission and mitophagy are hallmarks of PD. Genetics relevance of SNXs in Parkinson's has not been determined however it can be speculated that dysregulation of SNXs may perturb autophagy pathways that are necessary to clear α-synuclein aggregates and damaged mitochondria. In support of this, the pathophysiology of Parkinson's disease in linked with mutation in VPS35, a member of the retromer complex [183]. This is relevant as mutations in VPS35 are linked to decreased association with the WASH complex which perturbs ATG9 transport, ultimately compromising autophagosome biogenesis [192, 193] Lastly, as VPS35 interacts with the mitochondrial fission regulator, Dynamin-1-Like Protein [194]. VPS35 mutations are also linked to increased mitochondrial fragmentation and cell death [195]. In neurons this particularly devastating as, mitochondrial fission directs mitochondrial transport to their potential docking sites in axons and dendrites [196].

SNX deficiencies have been implemented in Down's syndrome [67] as well as associated with epilepsy, developmental delays, and subcortical brain abnormalities [184].

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SNX27 knockdown/knockout animal models or human patients with non-functional SNX27 variants exhibit a wide range of neurological aberrations that may be associated with defects in cell surface receptors [68].Some of these receptors include neuroreceptors (AMPA, NMDA), ATPase copper transporters, glucose transporters, disintegrin /metalloproteinase, and adhesion proteins (NLGN2) [184]. For example, SNX27 expression is downregulated in human Down's syndrome brains. Mechanistically, SNX27 may regulate the retention of cell surface membrane proteins such as the myelination-related protein, GRP17 which play an important role oligodendrocyte development respectively [197]

7.3 Coronaviruses hijack the endosomal-lysosomal pathway for host cell entry and infection.

Coronaviruses (CoVs) are a group of enveloped, single-stranded positive genomic RNA viruses that are known to cause severe respiratory diseases in humans. Coronaviruses can be categorized into three groups: α-CoVs, β-CoVs, γ-CoVs. Two members of the β-CoVs group include the Middle East respiratory syndrome coronavirus (MERS-CoV) and the serve acute respiratory syndrome coronavirus (SARS-CoV) which shares high sequence identity with SARS-CoV-2 (COVID-19) [198].In the field of virology, viral entry has been extensively studied for its promising impacts in future therapeutic strategies. Endocytosis is thought to be a key regulator of viral host entry and infection. In support of this, CoVs were found to accumulate in the lysosomes of cells after infection. In addition, several studies have shown that different CoVs hijack the endosomal-lysosomal pathway by engaging the initiation of endocytosis thereby entering the host cell and using lysosomal machinery to release their RNA contents for subsequent replication [199]. The mode of endocytosis varies between different viruses and hot cell types. Coronaviruses can use several endocytosis mechanisms. For example, SARS-CoVs employs clathrin-dependent, caveolae-dependent, and clathrin- and caveolae-independent mechanisms involving lipid rafts [200].

The exact mode of endocytosis utilized by the SARS-CoV-2 has not yet been identified, however it is known that SARS-CoV-2 requires the same receptor SARS-CoV, which is angiotensin converting enzyme II (ACE2) for host cell entry. ACE2 is highly expressed in human epithelial cells of the lung and small intestine. ACE2 is located at the

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cell surface of many types of cells and regulates the renin-angiotensin-aldosterone system (RAAS) pathway. SARS-CoV-2 has been shown to directly interact with ACE2 via its spike-like protein on surface of the viral envelope which engages the endocytic pathway and thereby entering the host cell [199].

Currently it is unknown whether sorting nexins play a role in SARS-CoV-2 host cell entry. However other viral pathogens have been shown to exploit the trafficking role of sorting nexins for host cell entry and infection. One of the best examples of viruses hijacking the intracellular trafficking networks within the cell is the human papillomavirus (HPV) and SNX17 [60]. Here the PDZ domain of SNX17 and SNX27 interacts with the viral capsid protein L2. The capsid protein L2 ensures that the viral DNA enters the nucleus for propagation of viral transcripts by interacting with trafficking proteins within the cell. SNX17 and SNX27 therefore enhances HPV infection by trafficking L2 and the bound viral DNA from the late endosomes to the TGN and subsequently to the nucleus [201]. In a similar way, SNX2 traffics the human respiratory syncytial virus (HRSV) structural proteins to enhance viral infection [202]. Other pathogens have also evolved elegant mechanisms to inhibit the innate immune response roles of SNXs. SNX5 and SNX6 are inhibited by *Legionella* RidL and *Chlamydia* IncE to evade the lysosomal mediated degradation [203]. It would be of great interest to see if SARS-CoV-2 and other pathogenic viruses regulate SNX trafficking activity in order to enhance viral progeny production or evade the innate immune response.

Conclusions.

Cells sense and respond to various internal and external stimuli to regulate processes such as gene expression, cell cycle progression, metabolism, and protein homeostasis. In the cell, there are severe quality control mechanisms held in place to regulate protein degradation. The mode of protein degradation depends on several factors including size, localization, and timing of substrate proteolysis. For example, the large size of organelles and multiple subunit complexes requires lysosomal degradation. Localization

of proteins such as transmembrane proteins requires lysosomal degradation because these proteins are embedded within the membranes making proteasomal-mediated degradation unfavorable. For the cell to quickly turn genes on and off transcription factors are degraded via nuclear 26S proteasomes. This mode of degradation, therefore, relies on spatiotemporal factors because degradation needs to happen rapidly, and proteasomes are localized in close proximity within the nucleus. Understanding the molecular details behind SNX cargo recognition, membrane binding, and protein degradation provides insight into the diverse roles of SNXs in various biological processes. The growing evidence of SNXs in protein homeostasis will shed light on pathologies associated with perturbed proteolysis and provide innovative targets for therapeutics.

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COMPETING INTERESTS

The authors declare no competing or financial interests.

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Table 1. List of mammalian sorting nexins and associated proteins (WASH) that play a role in the UPS.

SNX	UPS activity	role	Ref
SNX3	ubiquitin-specific protease 10 (USP10)	Deubiquitylates and stabilizes SNX3	[117].
SNX5	Fbw7	SNX5 interacts with FBW7 and blocks FBW7-mediated ubiquitination of oncoproteins such as c-Myc, NOTCH1, and Cyclin E1	[128]
SNX9	Itch (atrophin-1 interacting protein 4, Nedd family member)	Itch regulates intracellular levels of SNX9	[112]
SNX16	indirect	Postulated that SNX16 interacts with and inhibits proteasome-dependent ubiquitination of eukaryotic translation elongation factor 1 A2 (eEF1A2), thereby activating c-myc signaling.	[129]
SNX18	Mib1 E3 ligase	Promote the endocytosis of Delta-like protein 1 (Dll1) which is the transmembrane ligand protein for the Notch proteins.	[111]
SNX27	Non-catalytic role of the deubiquitinase OTULIN	OTULIN antagonizes SNX27-dependent cargo loading, binding of SNX27 to the VPS26A-retromer subunit and endosome-to-plasma membrane trafficking.	[118]
retromer	TRIM27 E3 ubiquitin ligase (non-catalytic role)	Mediates the phosphorylation and activation of STAT	[122]
retromer	MAGE-L2-TRIM27 E3 ubiquitin ligase	The MAGE-L2-TRIM27 E3 ubiquitin ligase localizes to retromer-positive endosomes.	[204]
WASH	K63-linked ubiquitination and deubiquitinase USP7	WASH is activated by K63-linked ubiquitination of WASH K220 by MAGE-L2-TRIM27. USP7 regulates this activity.	[114, 204].

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Figure 1

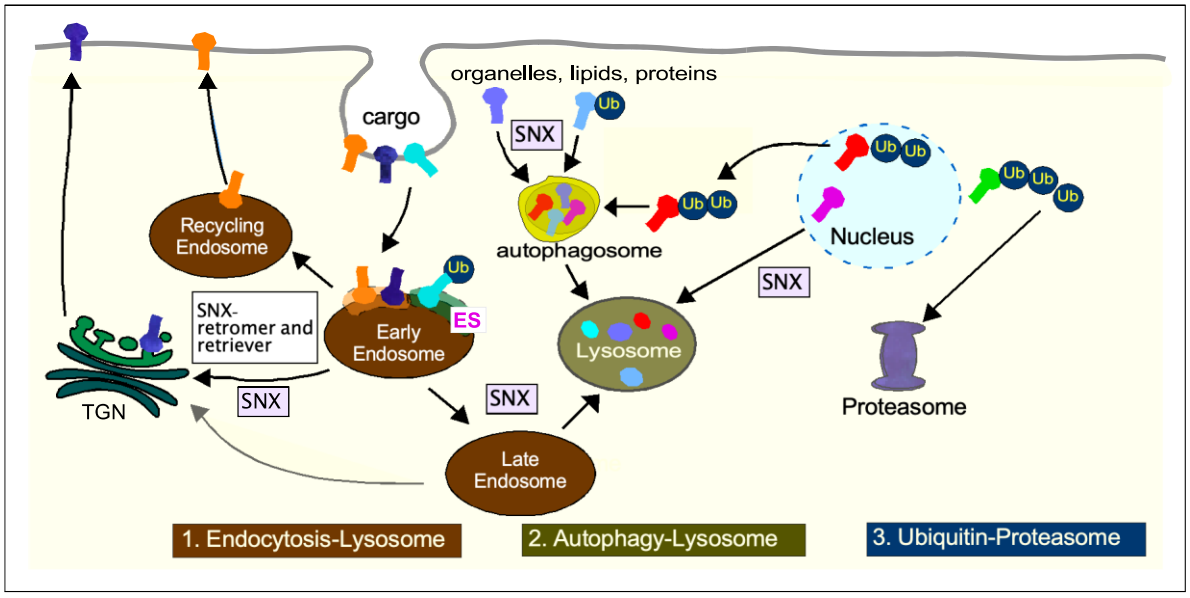

Figure 1. Sorting nexins (SNX) play a role in regulating protein homeostasis within three distinct but interconnected proteolysis pathways. The endocytosis-lysosomal pathway regulates steady-state levels of membrane proteins and consists of endosome vesicles, the *trans*-Golgi network (TGN), and the lysosome. Cargoes destined for delivery to the lysosome are ubiquitinated (Ub) and internalized into ILV's (not shown) using ESCRT pathways (ES). In the autophagy-lysosomal cargoes are sequestered to the vacuole by double-membraned vesicles called autophagosomes.. Cargoes include, but are not limited to, single proteins, protein aggregates, multi-subunit complexes, and organelles. In mammalian cells recognition of selective autophagy targets is dependent upon ubiquitination. Sequestration of these cargos usually requires the de novo synthesis of double-membraned vesicles termed autophagosomes. The third proteolysis pathway within the cell is the ubiquitin-proteasomal system (UPS). This mode of proteolysis targets short-lived regulatory protein that are selectively targeted and degraded. UPS-mediated degradation requires ubiquitination of substrates.

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Figure 2

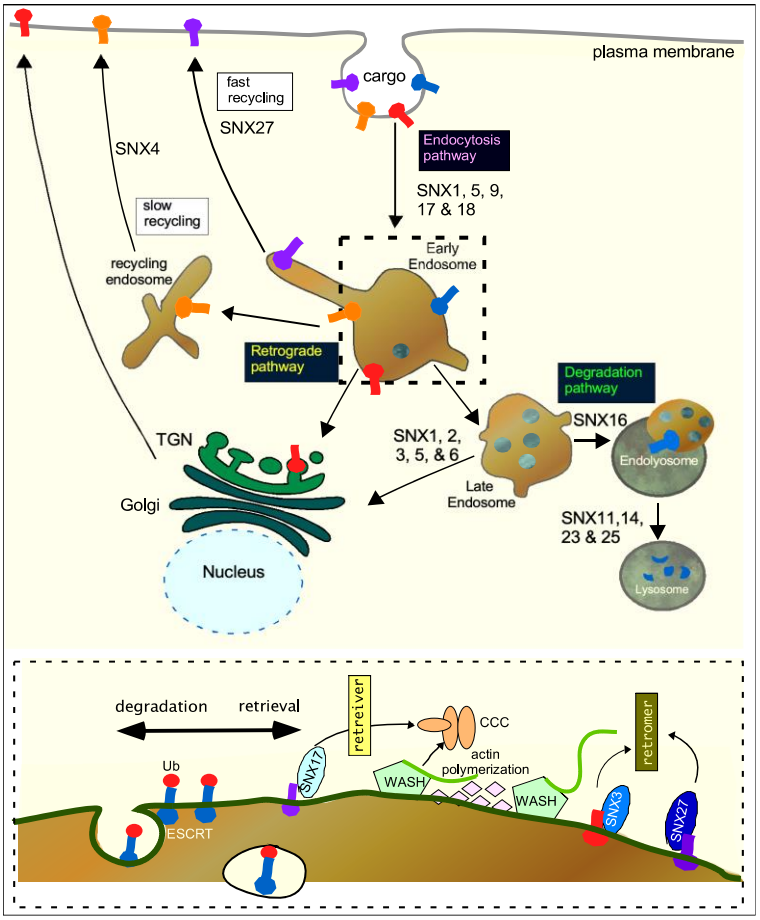

Figure 2. The role of SNXs in mammalian endocytosis. Mammalian cells have a complex and dynamic endomembrane network that consists of different three different endosome vesicles (early, late, and recycling). Retrograde transport of membrane proteins requires recycling endosomes and the TGN for delivery back to the plasma membrane. Degradation of membrane proteins requires late endosomes and the lytic organelle (lysosome). Different sorting nexins (SNX) are indicated in the different endocytosis pathways.

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Figure 3

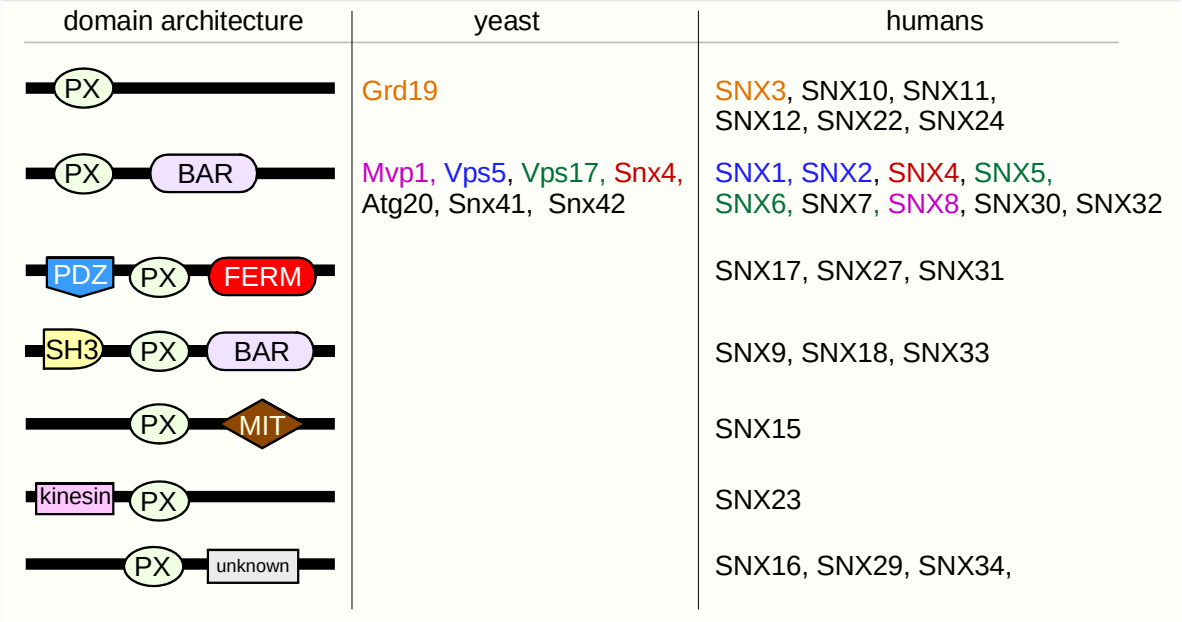

Figure 3. Structural classification of SNX subfamilies. Domain architecture describes the functional domains within different SNX subfamilies. PX denotes the highly conserved lipid binding domain that unifies the SNX protein family. SNX-BAR proteins are the largest subfamily and contain coiled-coil regions that enhance membrane binding, membrane remodeling, and protein-protein interactions. PDZ, postsynaptic density 95/discs large/zonula occludens domain; FERM, protein 4.1/ezrin/radixin/moesin domain; SH3, SRC homology 3 domain, MIT, microtubule interacting and trafficking domain, Kinesin motor domains.

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Figure 4

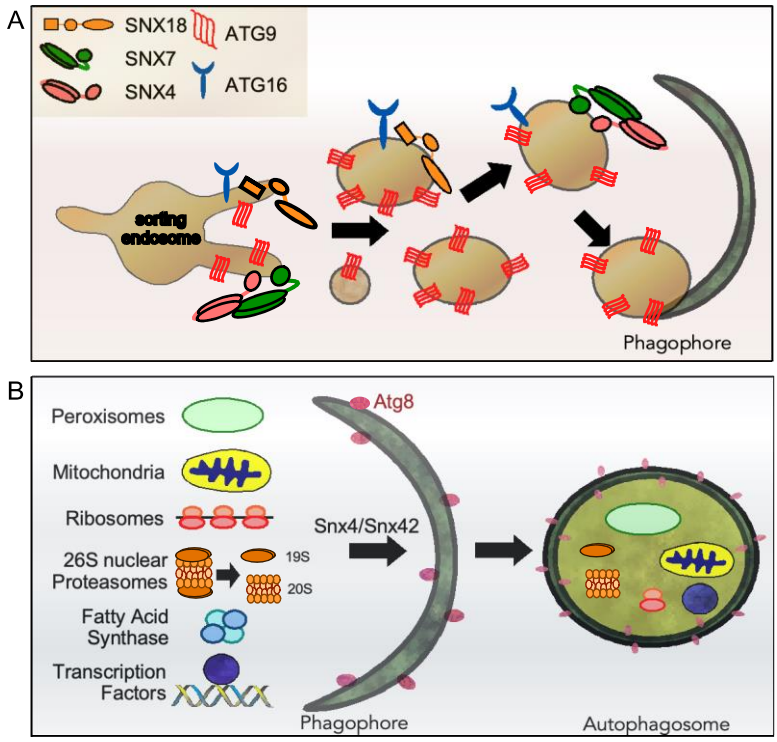

Figure 4. SNX play a role in autophagy following starvation stress. A) In mammalian cells SNXs play a role in autophagy-dependent localization of ATG16 and ATG9 from recycling endosomes to the pre-autophagosomal site (PAS) requires SNX4/SNX7 and SNX18. ATG9 containing vesicles are required for PAS formation and autophagosomes biogenesis. B) In yeast, Snx4 and Snx42 regulate many forms of selective autophagy including pexophagy, mitophagy, ribophagy, proteophagy, and degradation Fatty Acid synthase complexes as well as nuclear transcription factors.

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Figure 5

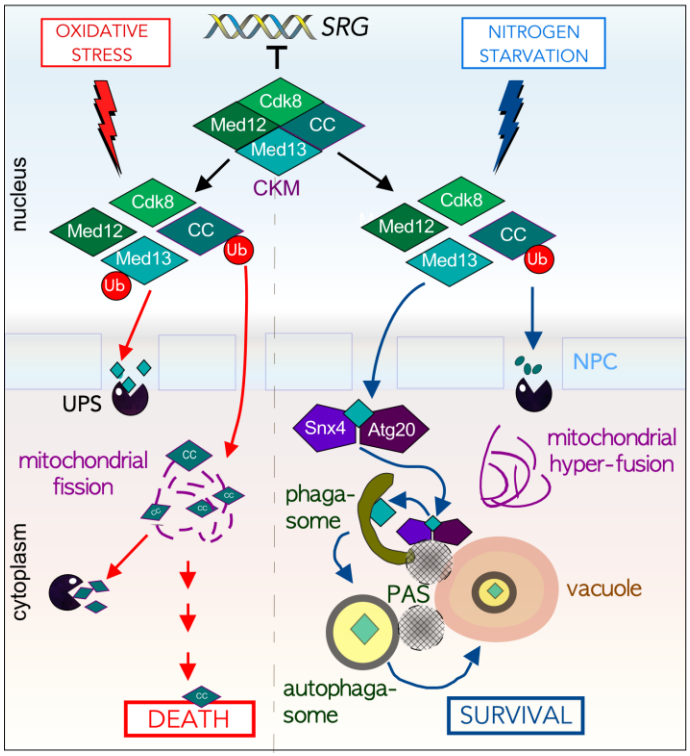

Figure 5. Med13 is destroyed either by the UPS or Snx4-Atg20 mediated autophagic degradation following cell death (left) or survival signals (right). Cartoon outlining stress-dependent fates of cyclin C and Med13, two members of the Cyclin Dependent kinase module (CKM). Here the subcellular address of cyclin C following stress mediates cell fate decisions by affecting mitochondria morphology. See text for details and [75, 164, 165, 173].