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REVIEW

Modulation of the secretory pathway by amino-acid starvation

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As a major anabolic pathway, the secretory pathway needs to adapt to the demands of the surrounding environment and responds to different exogenous signals and stimuli. In this context, the transport in the early secretory pathway from the endoplasmic reticulum (ER) to the Golgi apparatus appears particularly regulated. For instance, protein export from the ER is critically stimulated by growth factors. Conversely, nutrient starvation also modulates functions of the early secretory pathway in multiple ways. In this review, we focus on amino-acid starvation and how the function of the early secretory pathway is redirected to fuel autophagy, how the ER exit sites are remodeled into novel cytoprotective stress assemblies, and how secretion is modulated in vivo in starving organisms. With the increasingly exciting knowledge on mechanistic target of rapamycin complex 1 (mTORC1), the major nutrient sensor, it is also a good moment to establish how the modulation of the secretory pathway by amino-acid restriction intersects with this major signaling hub.

Introduction

The secretory pathway in growing cells

The secretory pathway is highly conserved in the eukaryotic kingdom. It comprises a series of membrane-bound compartments that mediates the export of proteins and lipids from the lumen or the membrane of the ER to the extracellular medium, the plasma membrane, and nearly all cellular membrane-bound compartments. In the ER, newly synthesized proteins are folded, assembled, glycosylated, and exported from the ER in COP II-coated transport vesicles that bud at specialized cup-shaped regions of the ER, the ER exit sites (ERES). Proteins reach the Golgi complex, where they are further processed, sorted, and dispatched to their correct destination (Bonifacino and Glick, 2004; Gomez-Navarro and Miller, 2016).

COPII vesicle formation is initiated through activation of the GTPase Sar1 (GTP-bound state) by the guanine nucleotide exchange factor (GEF) Sec12, which mediates Sar1 recruitment and insertion at the ERES membrane. Sar1-GTP recruits the inner coat Sec23/Sec24 heterodimer. This recruitment is coupled to cargo selection and recruitment through binding to Sec24 either directly (Miller et al., 2002; Barlowe, 2015) or indirectly via the cargo receptor (Otte and Barlowe, 2004; Dancourt and Barlowe, 2010). This is followed by the recruitment of the outer coat Sec13/Sec31 heterotetramer, leading to the formation of a COPII-coated bud and the release of a COPII-coated vesicle that is loaded with cargo (Miller and Schekman, 2013).

The large hydrophilic ERES protein, Sec16, plays an important role in COPII dynamics. Sec16 directly binds nearly all COP II subunits, and it contributes to ERES stability (Sprangers and Rabouille, 2015). As shown in *Pichia pastoris* (Connerly et al., 2005), mammalian cells (Bhattacharyya and Glick, 2007; Hughes et al., 2009), *Caenorhabditis elegans* (Witte et al., 2011), and *Drosophila melanogaster* (Ivan et al., 2008), Sec16 acts as a scaffold required for proper COPII coat assembly at ERES. In addition, studies in *Saccharomyces cerevisiae* (that do not exhibit clear focused ERES) have shown that Sec16 also regulates the rate of budding and uncoating of COPII vesicles by inhibiting the Sec23 GTPase-activating protein activity toward Sar1 (Kung et al., 2012; Bharucha et al., 2013).

In mammalian cells, 30% of open reading frames use the secretory pathway to reach their cellular destination (Sharpe et al., 2010). It is therefore considered a major anabolic pathway. To adapt to the ever-changing environment, the secretory pathway must also respond to extracellular cues (Farhan and Rabouille, 2011). In this context, the early secretory pathway is directly regulated by proliferative signaling pathways. For instance, Sec16 is a protein required for cell proliferation (Tillmann et al., 2015) and has been shown to be phosphorylated on threonine 415 by ERK2 downstream of Ras signaling that is stimulated by EGF. This phosphorylation increases Sec16 recruitment to ERES and enhances the rate of COPII vesicle budding (Farhan et al., 2010).

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The early secretory pathway under cellular stress

Conversely, the early secretory pathway is also sensitive to cellular stress as demonstrated by the Golgi fragmentation observed upon heat stress (Petrosyan and Cheng, 2014), DNA damage (Farber-Katz et al., 2014), oxidative stress (Catara et al., 2017), and cancer (Makowski et al., 2017). Less is known, however, about the regulation of the secretory pathway and secretion in general during changes in metabolism.

In this review, we focus on nutrient stress (i.e., starvation of amino acids and serum) and report on how it modulates and/or redirects secretion, with a focus on ER export via COPII-coated vesicles. First, we discuss the ever-growing relationship between autophagy and the COPII-coated vesicles. Second, we outline how serum and amino-acid starvation also trigger a large remodeling of ERES, at least in *Drosophila*, and blocks protein trafficking in the secretory pathway. In the third part, we discuss sensing of amino-acid starvation in vivo and how secretion is modulated in response to this stress.

Autophagy requires COPII-coated vesicle budding

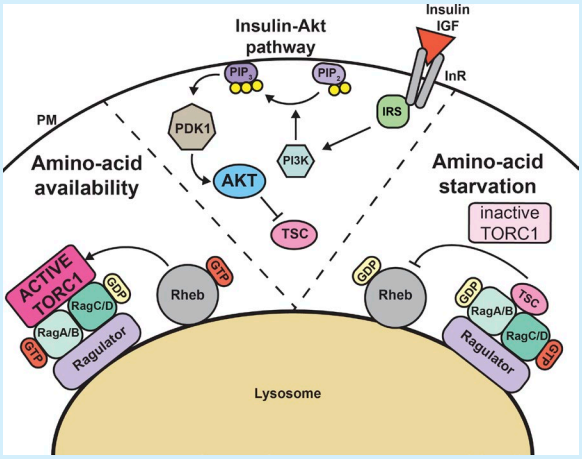
A major response to amino-acid starvation is the stimulation of the degradative pathway, macroautophagy (hereafter referred to as autophagy) resulting from mechanistic target of rapamycin complex 1 (mTORC1) inhibition. mTORC1 is a central regulator of nutrient-sensing pathways and is activated by nutrient availability (see mTORC1 activation text box). Active mTORC1 stimulates anabolic pathways leading to cell and organismal growth (Saxton and Sabatini, 2017). Conversely, mTORC1 inhibition (such as upon nutrient deprivation) leads to a series of compensatory events (Huang and Fingar, 2014) including the activation of autophagy (see Molecular players of autophagy text box). Autophagy is a complex catabolic process involved in degradation of cytoplasmic materials comprising several biochemical phases supported by 40 gene products (autophagy-related genes [*Atgs*]; Mizushima et al., 2011). Autophagy starts at the phagophore assembly site (PAS) with the formation of an isolation membrane that expands into a phagophore, a flattened cisterna that starts engulfing a portion of the cytoplasm. It then expands and eventually closes to form a double membrane autophagosome. This fuses with lysosomes, leading to the degradation of the inner membranes and their content (Suzuki et al., 2007; Kawamata et al., 2008; Suzuki and Ohsumi, 2010; see Molecular players of autophagy text box and enclosed figure).

ERES contribute to autophagosome biogenesis via COPII vesicle budding both in yeast and mammalian cells

Even though the nature of the phagophore is still under debate, COPII-coated vesicle budding is clearly necessary for the autophagic process (Ge et al., 2017). The general consensus is that COP II-coated vesicles help build the phagophore or lead to its expansion and maturation into an autophagosome (Farhan et al., 2017).

The first indication of an interaction between autophagy and COPII vesicle budding comes from *S. cerevisiae*. When these harbor defective mutations in COPII subunits, they also display impaired autophagosome formation upon nutrient starvation (Ishihara et al., 2001). This was confirmed using a temperature-sensitive *Sec12-4*, where the autophagosome flux was

mTORC1 activation on the lysosome by amino acids and the AKT pathway



The evolutionary conserved protein kinase mTORC1 is a major nutrient sensor, and as such is a key regulator of growth ensuring that cells adapt to different environmental and nutritional cues like amino-acid availability (Wolfson and Sabatini, 2017). mTORC1 is activated via multiple pathways, including cholesterol (Castellano et al., 2017), growth factors (Inoki et al., 2002) such as the insulin-AKT pathway, and, consistent with its role as a key integrator of the cellular nutrient status, amino acids (Hara et al., 1998). Overall, when amino acids are present, mTORC1 is active and drives anabolic pathways such as translation and growth. When amino acids are absent, mTORC1 is inactivated, anabolic pathways are inhibited, and catabolic pathways, such as autophagy, are stimulated (Wolfson and Sabatini, 2017). Inhibition of mTORC1 leads to activation of nutrient stress–responsive transcription factors in yeast (Crespo et al., 2002). mTORC1 inhibition leads to increased lifespan, translation inhibition, inhibition of lipid synthesis, inhibition of lysosome biogenesis, inhibition of ribosome biogenesis, and down-regulation of transcription (Huang and Fingar, 2014).

At the molecular level, the kinase AKT is activated when insulin/IGF binds to the insulin receptor (InR). Activation of InR subsequently activates phosphoinositide-dependent kinase 1 via PI3K. Phosphoinositide-dependent kinase 1 activates AKT, which phosphorylates and inhibits the tuberous sclerosis complex (TSC). TSC is a GTPase-activating protein for Rheb, a small GTPase that is active in the GTP-bound state (Long et al., 2005; Buerger et al., 2006). As a result, the nucleotide-loading state of Rheb is controlled by both Akt and the TSC complex (Wullschleger et al., 2006). When Rheb is activated, it activates mTORC1 (Dibble and Cantley, 2015).

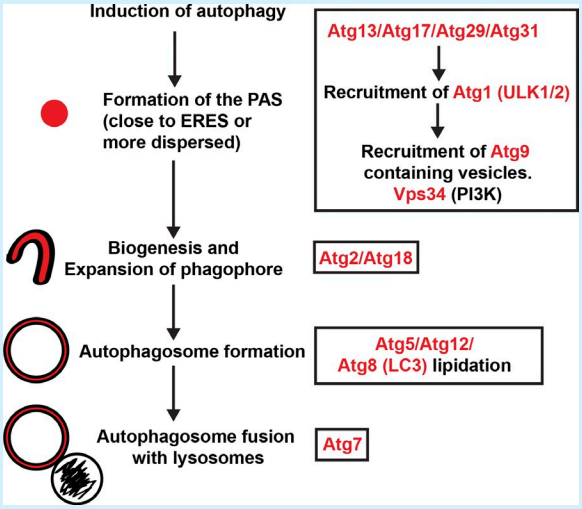
Parallel to the insulin–Akt pathway, amino-acid levels (in the extracellular medium, the cytoplasm, and/or the lysosome) also minutely modulate the activity of the mTORC1 pathway as they promote the formation of a heterodimer of active RagA^{-GTP}/C^{-GDP} or RagB^{-GTP}/D^{-GDP}. Rag GTPases form a complex with Ragulator, which is anchored to the lysosomal membrane (Kim et al., 2008; Sancak et al., 2008, 2010). This leads to the lysosomal recruitment of mTORC1 (Sancak et al., 2010; Manifava et al., 2016) and to its activation (see enclosed figure).

Interestingly, Rag GTPases further modulate Rheb activity. Ultimately, the nucleotide loading state of the Rag heterodimer determines whether mTORC1 or TSC is recruited to the lysosome and whether mTORC1 is active (Sancak et al., 2010; Demetriades et al., 2014; see enclosed figure).

Of note, Rags are regulated by several amino-acid sensors, such as Sestrin2 (leucine) and CASTOR1 (arginine), of which the crystal structures have recently been elucidated (Kim et al., 2008; Chantranupong et al., 2016; Saxton et al., 2016a,b; Wolfson et al., 2016).

Conversely, when amino acids are absent, the nucleotide status of the Rag heterodimer switches to RagA^{-GDP}/C^{-GTP} or RagB^{-GDP}/D^{-GTP}, mTORC1 dissociates from the lysosome, and TSC is recruited to the lysosome by the Rag complex. There, TSC, which is inhibited by the PI3K–Akt pathway (Manning et al., 2002; Dibble and Cantley, 2015), is activated and catalyzes the hydrolysis of Rheb-GTP to Rheb-GDP (Demetriades et al., 2014), and therefore abrogates activation of mTORC1 by Rheb (Inoki et al., 2003).

Molecular players of autophagy



Autophagy is a catabolic process involved in degradation of cytoplasmic materials to maintain cellular homeostasis. It is efficiently triggered by amino-acid starvation and mTORC1 inhibition to quickly obtain new building supplies.

Autophagy is a conserved complex process compromising several biochemical phases supported by 40 gene products of the so-called *Atg* genes (Mizushima et al., 2011). It starts at the PAS and the formation of a phagophore (Suzuki et al., 2007; Kawamata et al., 2008; Suzuki and Ohsumi, 2010). As it engulfs portions of cytoplasm tagged for degradation, phagophores expand and eventually close to form a double membrane autophagosome. These fuse with lysosomes, leading to degradation of the inner membranes and their contents.

In yeast, the autophagic pathway is very well understood. The kinase Atg1 (ULK1/2 in mammals) is recruited at the PAS through its interaction with upstream proteins Atg13, Atg17, Atg29, and Atg31. This results in the formation of a phagophore (Suzuki et al., 2007; Kawamata et al., 2008; Suzuki and Ohsumi, 2010). Thereafter, the catalytically active Atg1 recruits Atg9 and the class III PI3K, the Vps34 complex (Kawamata et al., 2008). Atg9 is a transmembrane protein that is transported from the Golgi to the phagophore in Atg9-containing vesicles. Vps34 phosphorylates phosphatidylinositol phosphate to PI(3)P. Atg18 (WIPI in mammals) and Atg2 bind to PI(3)P and play a role in Atg9 turnover (Reggiori et al., 2004). The expanded phagophore acquires Atg8 (LC3 in mammals) that is lipidated and matures into an autophagosome. Atg proteins disassemble (Cebollero et al., 2012) before they fuse with lysosomes, forming an autolysosome (see enclosed figure).

found to be markedly reduced (Graef et al., 2013). Similar to the role of COPII vesicles in yeast, knockdown of COPII subunits in HeLa cells by siRNA also resulted in loss of autophagosomes (Ge et al., 2014).

The notion that the COPII machinery is necessary for the initiation of autophagy was reinforced by the finding that yeast Atg proteins bind COPII subunits (Sar1, Sec23, and Sec24) and Sec16 after treatment with the mTORC1 inhibitor rapamycin (Graef et al., 2013). The functional relevance of these interactions was further demonstrated using tagged Atg8 (marking the PAS, phagophores, and autophagosomes; see Molecular players of autophagy text box) that was visualized very close to ERES markers (Sec16 and Sec13) in rapamycin-treated cells (Graef et al., 2013; Suzuki et al., 2013; Fig. 1). Collectively, these data suggest that COPII vesicle budding contributes to phagophore formation.

In mammalian cells, COPII-coated vesicles are also required to feed the expanding phagophore (or contribute to its formation). However, they appear to bud from the ER–Golgi intermediate compartment (ERGIC), not from the ERES as during the transport to the Golgi during normal growth (Fig. 1). Indeed, upon starvation, Sec12 appears to be relocated from the ERES to the ERGIC as observed by superresolution microscopy (Ge et al., 2017). This is modulated by CTAGE5, an ERES scaffold protein, and FIP200, a subunit of the ULK1 protein kinase complex. Depletion of either prevents ERES remodeling upon starvation and COPII complex assembly at ERGIC. The relocation of the COPII budding activity to the ERGIC appears to be regulated by a phosphoinositide 3-kinase (PI3K), the activity of which is stimulated by starvation. This leads to an enrichment of phosphatidylinositol 3-phosphate (PI(3)P) on the ERGIC leading to the recruitment of the COPII coat. This is in agreement with earlier data that showed that autophagosomes form at PI(3)P-rich sites (Axe et al., 2008). The ERGIC-derived COPII vesicles recruit LC3 and lead to its lipidation, a key event in phagophore expansion (Ge et al., 2014, 2015; Fig. 1).

The yeast TRAPPIII complex appears to redirect COPII vesicles to the PAS to mediate phagophore expansion

The second mechanism supporting the role of ERES activity in phagophore formation involves the yeast TRAPP complexes. These are GEFs of the small yeast Rab GTPase Ypt1 that is present on COPII vesicles and is necessary for the tethering of COPII vesicles, through Uso1p, to the Golgi during secretion (Cao et al., 1998; Weide et al., 2001; Cai et al., 2007). Three TRAPP complexes have been described. TRAPPI would play a role in ER–Golgi trafficking (Cai et al., 2007), TRAPPII would mediate intra-Golgi trafficking (Cai et al., 2007; Yamasaki et al., 2009), and the TRAPPIII complex would be required for autophagy (Lynch-Day et al., 2010), but not in ER–Golgi transport.

However, this view has recently been challenged. Tandem affinity purification of the TRAPP complexes from log-phase yeast revealed the absence of TRAPPI. Yeast appear to only display TRAPPII and TRAPPIII (Thomas et al., 2018). The use of transport assays in the early secretory pathway lead to the conclusion that TRAPPIII functions as the GEF for Ypt1 in ER–Golgi trafficking (Thomas et al., 2018). Interestingly, a very similar conclusion was drawn from studies in *Drosophila* (Riedel et al., 2018). CRISPR/Cas9 knockout mutants for *TRAPPC1* encoding a subunit of the TRAPPIII complex in metazoa, leads to the absence of Rab1 (orthologue of yeast Ypt1) activation in the secretory pathway (Riedel et al., 2018). Thus, TRAPPIII is required for the ER–Golgi transport, both in yeast and metazoa (Fig. 1).

In conditions that trigger autophagy, yeast TRAPPIII also acts as a GEF for Ypt1 (Lynch-Day et al., 2010; Tan et al., 2013). Both the loss of function of Ypt1 and Trs85 (a key subunit of TRAPPII) result in impaired autophagy (Meiling-Wesse et al., 2005; Nazarko et al., 2005). In agreement, TRAPPIII and Ypt1 localize to the PAS upon starvation (Lynch-Day et al., 2010). Furthermore, TRAPPIII recruitment to the PAS appears dependent on Atg17 (Wang et al., 2013). Once at the PAS, activated Ypt1 recruits the Atg1 kinase complex that triggers phagophore formation (Wang et al., 2013), perhaps via tethering and fusion of Golgi-derived Atg9 vesicles (Rao et al., 2016).

As mentioned, Ypt1 is present on COPII-coated vesicles, and in vitro, TRAPPIII also interacts with Sec23 via its Trs33 subunit (Tan et al., 2013). The model proposed, at least in yeast, is that

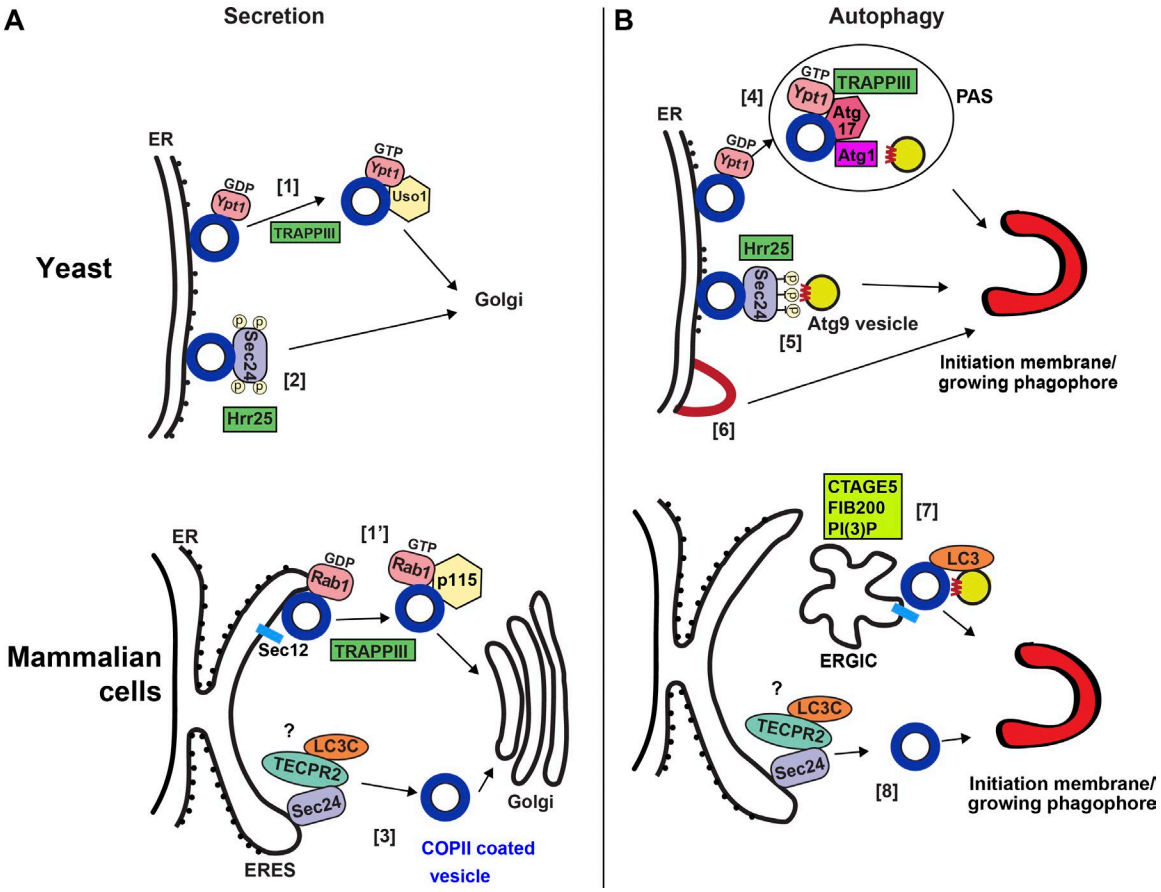


Figure 1. **The dual role of COPII-coated vesicles in secretion and in autophagy. (A)** Upon growing conditions, COPII-coated vesicles transport cargo from the ER (in yeast) or ERES (metazoa) to the Golgi. (1 and 1') In yeast (1), Ypt1 is present on COPII vesicles in an inactive GDP-bound state. TRAPPIII catalyses the nucleotide exchange on Ypt1 (GTP-bound) that recruits Uso1p. In metazoa (1'), TRAPPIII has the same role on Rab1 that recruits p115. (2) In yeast, Hrr25-dependent Sec24 phosphorylation is necessary for fusion of the COPII-derived vesicle with the Golgi. (3) In mammalian cells, COPII vesicle formation is regulated by TECPR2, which protects Sec24 against proteasomal degradation and helps maintain ERES integrity. Interestingly, TECPR2 interacts with lipidated LC3C. **(B)** Under conditions in which autophagy is activated, ERES provide membranes for phagophore biogenesis and extension. (4) In yeast (*S. cerevisiae*), TRAPPII-activated Ypt1 present on COPII-coated vesicles recruits Atg17 and the Atg1 complex. This allows the binding of Atg9-containing vesicles that in yeast derive from the Golgi. Collectively, this forms the PAS that matures into a phagophore. (5) Upon autophagy stimulation, Sec24 is specifically phosphorylated on three threonines in a Hrr25-dependent manner. This phosphorylation allows the binding to Atg9 vesicles and redirects COPII-coated vesicles to the growing phagophore. (6) Phagophores (Atg8-positive) have also been observed in close proximity to Sec13 and to Sec16. (7) In mammalian cells, autophagy induction leads to the remodeling of ERES and the relocalization of Sec12 to ERGIC in a CTAGE5- and FIB200-dependent manner. This leads to COPII vesicle budding from ERG IC, recruitment of LC3, and binding to Atg9-containing vesicles. Together, they form and/or feed the growing phagophore. (8) In mammalian cells, the TECPR2 interaction with LC3C somehow leads to the redirection of COPII vesicles to the phagophore.

TRAPPIII binds to COPII vesicles to activate Ypt1, which directs COPII vesicles either toward the Golgi or to the PAS depending on whether the cell is in a growing condition or undergoing autophagy. COPII vesicle redirection to the PAS provides the membranes required for phagophore expansion (Fig. 1).

COPII vesicle redirection by the yeast kinase Hrr25 provides membrane for phagophore expansion

A third mechanism supporting a role for COPII-coated vesicles in phagophore expansion in yeast involves the kinase Hrr25 that is found necessary for both ER–Golgi trafficking and autophagy (Murakami et al., 1999; Lord et al., 2011; Wang et al., 2015) depending on the growing conditions. On one hand, Hrr25 phosphorylates Sec23 and Sec24 in vitro (Lord et al., 2011) and loss of Hrr25 kinase activity results in impaired fusion of COPII vesicles with the Golgi. This indicates that Hrr25-mediated phosphorylation of Sec23/Sec24 is essential for ER–Golgi trafficking (Fig. 1). On the other hand, Hrr25 is also involved in autophagosome formation (Wang et al., 2015).

How does Hrr25 activity regulate both processes? When the Sec24 threonines T324, T325, and T328 (the latter being also conserved in mammals) are mutated to alanine, autophagy is inhibited but not ER–Golgi transport (Davis et al., 2016), indicating that these specific phosphorylation events are critical for the autophagic process. Indeed, this form of phosphorylated Sec24 binds Atg9, resulting in an increase in the autophagosome number upon nutrient starvation (Fig. 1). Conversely, expression of a kinase-dead Hrr25 lowers the interaction between Sec24 and Atg9 (Davis et al., 2016). Of note, Hrr25 does not appear to directly phosphorylate Sec24 whether in growing conditions or upon autophagy induction, and it is also unclear how it is activated.

Collectively, the specific Hrr25-dependent phosphorylation of Sec24 is proposed to mediate the redirection of COPII vesicles to the nascent phagophore, leading to an increase in autophagosome number (Fig. 1).

Mammalian TECPR2 stabilizes ERES and positively regulates autophagy

The fourth mechanism involves mammalian TECPR2, which localizes at ERES, where it protects Sec24D against proteasome degradation (Stadel et al., 2015; Fig. 1). Consequently, TECPR2 depletion reduced the number of ERES. Furthermore, TECPR2 is also necessary for autophagy as it interacts with lipidated LC3C (Behrends et al., 2010; Oz-Levi et al., 2012, 2013), and TECPR2-depleted cells showed a reduced number of LC3C-positive autophagosomes (Stadel et al., 2015).

In addition, the level of the autophagosome marker protein WIPI2 (Atg18) is also decreased upon TECPR2 depletion (Stadel et al., 2015). At the moment, it is not clear whether TECPR2 is directly involved in autophagosome formation or indirectly by maintaining an efficient COPII vesicle budding.

Collectively, data from yeast and mammalian cells show a strong cross talk between the early secretory pathway and autophagy. Evidence indicates that COPII vesicles are necessary for the early step of autophagy. Upon nutrient starvation or mTORC1 inhibition, COPII vesicles are proposed to be redirected and provide membranes for phagophore expansion using different mechanisms. How those are coordinated remains to be better understood.

Effects of amino-acid starvation on the early secretory pathway in *Drosophila*

As discussed, amino-acid starvation triggers autophagy via mTORC1 inhibition. However, amino-acid starvation is sensed as a stress and as such also triggers other pathways. The hallmarks of stress are the stalling of anabolic pathways (for instance, protein translation [Aguilera-Gomez et al., 2017]), transcriptional and translational up-regulation of factors helping the cells to deal with stress such as Hsp (Ashburner and Bonner, 1979) and ATF4 (Harding et al., 2000), posttranslational modifications elicited by stress signaling (Duncan and Hershey, 1989), changes in cytoplasmic biophysical properties (Munder et al., 2016), and the formation of membraneless stress assemblies (Rabouille and Alberti, 2017).

The best-documented stress assemblies are stress granules (Anderson and Kedersha, 2009), reversible coalescences that form upon liquid–liquid phase separation with features of liquid droplets (Molliex et al., 2015; Patel et al., 2015; Protter and Parker, 2016; Franzmann et al., 2018) in a manner that depends both on low complexity sequences (Boeynaems et al., 2017; Franzmann et al., 2018) and on mRNA (Van Treeck et al., 2018). Stress granules form in response to protein translation inhibition or stalling imposed by many stresses, such as heat shock, oxidative stress, and ER stress (Aulas et al., 2017). As a result, untranslated mRNAs accumulate and bind RNA-binding proteins that coalesce (Anderson and Kedersha, 2002; Aulas et al., 2017). Interestingly, the stress of amino-acid starvation leads to the formation of stress granules in mammalian cells (Damgaard and Lykke-Andersen, 2011) and in *Drosophila* (Zacharogianni et al., 2014; Aguilera-Gomez et al., 2017).

In addition, at least in *Drosophila*, amino-acid starvation triggers the formation of a novel stress assembly, the Sec body. Indeed, amino-acid starvation inhibits not only protein translation but also protein transport in the secretory pathway (Zacharogianni et al., 2014), and Sec bodies provide a cytoprotective mechanism for ERES components. There is therefore an interesting parallel between the inhibition of protein translation and the formation of stress granules, and the inhibition of protein transport through the early secretory pathway and the formation of Sec bodies.

Sec bodies are membraneless assemblies that form after 3–4 h of amino-acid starvation and are quickly and efficiently reversible upon refeeding. They are in part made of COPII subunits as well as the ERES component Sec16, the level of which increases during starvation. Sec bodies appear to be associated with ER membranes but are distinct from COPII-coated vesicles, autophagosomes, endosomes, and lipid droplets. Instead, Sec bodies have liquid droplet properties and appear to form by phase separation that is driven by proteins exhibiting low complexity sequences, such as Sec16 and Sec24. As such, they have properties very similar to those of stress granules except that they are not RNA-based (Zacharogianni et al., 2014). Sec bodies appear to protect COPII subunits against degradation, and they are necessary for survival during stress and fitness upon stress relief (Zacharogianni et al., 2014).

Sec body formation and autophagy

How is Sec body formation compatible with the induction of autophagy that, as discussed above, requires ERES function and COPII vesicle budding? First, autophagosome formation in *Drosophila* S2 cells occurs earlier than Sec bodies. It peaks ∼1.5 h after starvation and appears to slow down after 3–4 h, at least in S2 cells (Zacharogianni et al., 2014). Second, Sec body formation does not appear to interfere with autophagy. Conversely, inhibiting autophagy leads to the premature formation of Sec bodies (Zacharogianni et al., 2014). Autophagy leads to the replenishment of amino acids in the cytoplasm (Carroll et al., 2015), and the amino-acid level in the cytoplasm may need to drop below a certain threshold for ERES components to coalesce into Sec bodies.

Because amino starvation leads to strong inhibition of mTORC1 (see mTORC1 activation text box), we tested whether mTORC1 inhibition on its own (via rapamycin and depletion of its Raptor subunit) would result in Sec body formation. However, this is not the case (Zacharogianni et al., 2014). This shows that although it happens during amino-acid starvation, mTORC1 inhibition is not sufficient to trigger Sec body formation. Collectively, we propose that the ERES remodeling into Sec bodies is compatible with the initiation of autophagy, at least in *Drosophila* S2 cells, and that Sec body formation is mediated by signaling pathways that do not solely depend upon mTORC1 inhibition.

Posttranslational modifications on Sec16 and Sec body formation

As discussed, formation of membraneless liquid droplets (and more generally, phase-separated higher assemblies) is driven

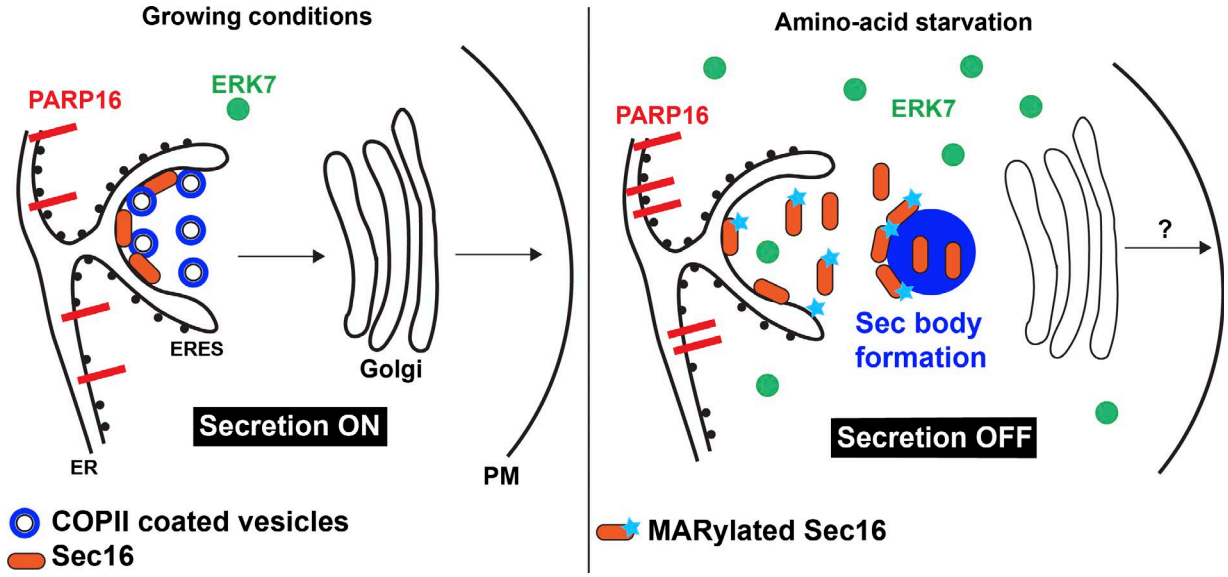


Figure 2. **Amino-acid starvation of *Drosophila* cells leads to protein transport inhibition and Sec body formation.** In growing conditions, COPII-coated vesicles in *Drosophila* S2 cells bud from the ERES in a Sec16-dependent manner, leading to active transport through the secretory pathway. ERK7 levels are low and dPARP16 is inactive. Upon amino-acid starvation, protein transport from the ERES to the Golgi is inhibited. ERK7 level increases, leading to Sec16 release away from the ERES membrane. dPARP16 is activated and mono-ADP-ribosylates Sec16 on its SRDC, leading to the coalescence of Sec16 and COPII subunits into Sec bodies after 3–4-h starvation. MARylated, mono-ADP-ribosylated.

by stress that changes the properties of the cytoplasm, such as a drop in yeast cytoplasmic pH upon energy depletion (Alberti, 2017). In addition, stress can elicit posttranslational modifications of key proteins, leading to their coalescence through their low complexity sequences that can engage into multivalent low-affinity interactions (Rabouille and Alberti, 2017).

One of the interesting posttranslational modifications involved in the formation of stress assemblies is ADP-ribosylation. ADP-ribosylation (either mono or poly) is catalyzed by 17 poly-ADP-ribose-polymerases (PARPs) in mammals and 3 in *Drosophila* (Hottiger et al., 2010), and has been shown to be involved in the composition of stress granules during oxidative stress of mammalian cells (Leung et al., 2011; Leung, 2014; Catara et al., 2017).

Interestingly, ADP-ribosylation via dPARP16 is involved in Sec body formation in *Drosophila*, as dPARP16 overexpression in the absence of stress leads to Sec body formation and its depletion prevents their formation upon amino-acid starvation. Furthermore, dPARP16 is a survival factor during amino-acid starvation and is required for cell fitness upon stress release (Aguilera-Gomez et al., 2016). During amino-acid starvation, dPARP16 is proposed to mono-ADP-ribosylate Sec16 on a small 44-residue conserved sequence localized toward its C terminus, SRDC (serum starvation domain conserved). Interestingly, overexpression of this sequence leads to the formation of Sec bodies in a dPARP16-dependent manner, suggesting a model whereby dPARP16 dependent Sec16 mono-ADP-ribosylation leads to the coalescence of ERES components into Sec bodies (Aguilera-Gomez et al., 2016; Fig. 2).

It is still unknown whether yeast and *C. elegans* form Sec bodies during amino-acid starvation. Furthermore, although Sec bodies have so far not been reported in mammalian cells, amino-acid starvation of mammalian cells induces remodeling of their ERES and formation of structures that are reminiscent of Sec bodies (unpublished data). Which features of mammalian Sec16 and PARP16 are necessary for Sec body formation needs to be further investigated. Furthermore, the response to amino-acid starvation in mammalian cells might be more geared toward autophagy. Because this process requires COPII vesicle budding, this might impede the formation of ERES components–based cytoprotective assemblies.

ERK7: Another pathway intersecting with Sec bodies?

Serum starvation also remodels the early secretory pathway, in both *Drosophila* and mammalian cells (Zacharogianni et al., 2011), as it also changes the dynamics of Sec16. However, during serum starvation, Sec16 disperses away from the ERES instead of forming Sec bodies.

The unconventional ERK kinase ERK7 appears to have a role in the serum starvation response, with ERK7 overexpression leading to dispersion of Sec16 away from ERES akin to serum starvation (Zacharogianni et al., 2011). Consistently, ERK7 is necessary for cells to sense serum starvation (in both *Drosophila* and HeLa cells), and ERK7 is strongly stabilized by starvation (Fig. 2). This leads to the model that upon nutrient starvation, ERK7 protein levels increase leading to Sec16 release from ERES and cessation of protein transport in the secretory pathway (Zacharogianni et al., 2011). Whether ERK7 is involved in Sec body formation remains to be defined. This will help specify if ERK7 and dPARP16 cooperate.

Collectively, these results indicate that amino-acid starvation does more than stimulate autophagy, at least in *Drosophila* cells. It leads to the cessation of secretion and to the strong remodeling of ERES into a membraneless Sec body. The signaling events

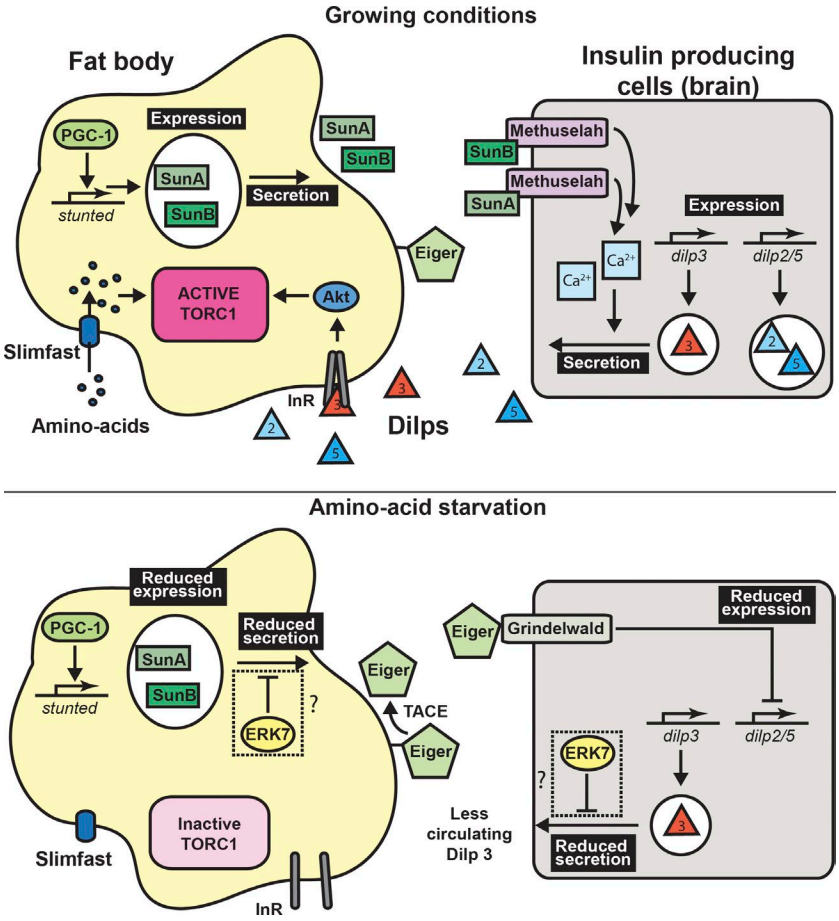


Figure 3. **The secretion-based signaling between the *Drosophila* fat body and IPCs upon sensing the level of circulating amino acids.** Top: When amino acids are available, they are transported into the fat body cells by Slimfast, where they activate mTORC1 (see mTORC1 activation text box). The nutrient responsive coactivator PGC-1 stimulates *stunted* expression, giving rise to the SunA and SunB peptides that are secreted into the hemolymph. SunA and SunB subsequently bind to their receptor Methuselah on the plasma membrane of IPCs in the brain. The IPCs transcribe the *Dilp* genes, leading to the accumulation of Dilps 3, 2, and 5. Activation of Methuselah triggers intracellular calcium release, which stimulates Dilps secretion to the hemolymph. In turn, Dilps activate the InR in the fat body, which increases mTORC1 activity further via the insulin-Akt pathway (see mTORC1 activation text box). Bottom: Upon amino-acid starvation, mTORC1 activation is reduced, leading to a reduction of SunA and SunB. It is also possible that their secretion is inhibited through the increase of the ERK7 level. Decreased SunA and SunB secretion leads to a decreased stimulation of Methuselah, resulting in a reduced Dilp secretion from IPCs where they accumulate. ERK7 level in the IPCs is increased, possibly contributing to secretion inhibition. Reduced Dilp secretion during amino-acid starvation effectively reduces mTORC1 activation and overall growth.

triggered by amino-acid starvation resulting in the activation of both ERK7 and dPARP16, as well as their interplay, will be important to discover.

Amino-acid starvation modulates secretion in vivo via humoral and cell-autonomous responses

Nutrient availability needs to be sensed at the organismal level to couple nutrient availability to organismal growth. Accordingly, nutrient restrictions in vivo affect secretion, and this is well understood in *Drosophila*, especially in larvae. In brief, *Drosophila* embryogenesis is followed by three larval molts during which larvae mostly feed and grow. The third-instar larvae then stop feeding and start the process of pupation that ultimately leads to the eclosion of a new fly.

Feeding *Drosophila* larvae possess a sensory mechanism that measures amino-acid availability and integrates it into systemic insulin signaling, which adjusts the growth rate of the larva. This sensory mechanism is made of two very important tissues that communicate with one another, the fat body and the insulin-producing cells (IPCs). The insulin/insulin-like growth factor–signaling pathway is conserved from *Drosophila* to vertebrates, but it became functionally differentiated: in *Drosophila*, *Drosophila* insulin-like peptides (Dilps) control both metabolism and growth, whereas in vertebrates, insulin controls metabolism and insulin-like growth factors control growth (Nakae et al., 2001; Garofalo, 2002; Wu and Brown, 2006).

The Drosophila *fat body senses the circulating amino-acid level through the amino-acid transporter Slimfast*

The fat body is a larval organ with features of both mammalian liver and adipose tissue. Nutrients originating from the gut, including amino acids, are imported into fat body cells, where they are stored and stimulate mTORC1, resulting in larval growth.

Conversely, the larval fat body serves as an amino-acid sensor and detects the level of dietary circulating amino acids. Larvae that are deprived of amino acids are smaller and give rise to significantly smaller adults (Colombani et al., 2003). Strikingly, ablation of the amino-acid transporter encoded by the *slimfast* gene solely in the fat body is sufficient to phenocopy the effects of starvation on wild-type larvae—that is, smaller larvae and smaller adults. This strongly suggests that Slimfast is the major amino-acid transporter in fat body cells (Colombani et al., 2003; Fig. 3). Accordingly, Slimfast activity impacts mTORC1 signaling. In the presence of circulating amino acids, mTORC1 is activated in a Slimfast-dependent manner (Colombani et al., 2003).

When amino acids are available, fat body–secreted SunA and B trigger Dilp secretion from IPCs

The sensing of amino-acid availability by the fat body is transduced into a systemic growth response. When the amino-acid level is high, the fat body secretes fed signals, such as SunA/B. SunA and SunB are two peptide isoforms encoded by the *Drosophila stunted* gene, their expression and secretion in the fat

body being strongly stimulated by amino acids (Delanoue et al., 2016). Interestingly, Sun secretion depends on mTORC1 activity, but not *stunted* expression that is enhanced by the nutrient-responsive transcription coactivator PGC-1, independently of mTORC1 (Delanoue et al., 2016).

After being secreted into the hemolymph by fat body cells, SunA/B binds to the Methuselah receptor at the surface of IPCs. Activation of Methuselah by SunA/B triggers the release of intracellular calcium, which leads to the fusion of Dilp-containing vesicles with the plasma membrane, releasing Dilps into the hemolymph (Delanoue et al., 2016; Fig. 3). Dilps, in turn, bind to the fat body cell plasma membrane and enhance/reinforce mTORC1 activation via Akt (see mTORC1 activation text box).

The loop described above clearly establishes that fat body cells secrete a signal in response to amino acids, which triggers voltage-dependent Dilp secretion from IPCs. This relay mechanism allows the secretion of Dilps from IPCs when amino acids are available, which therefore has a permissive effect on systemic growth. The homology of the fat body with mammalian liver and adipose tissue emphasizes the need of assessing how amino-acid starvation modulates secretion by these two tissues.

In addition to Dilp secretion from IPCs, Dilp expression by IPCs is regulated at the transcriptional level. Upon starving conditions, the transcriptional regulation of *dilp* genes is inhibited in the *Drosophila* larvae by the release of fat body factors, including TACE-dependent cleaved Eiger, the *Drosophila* TNF-α that is sensed by the IPC receptor, Grindelwald (Agrawal et al., 2016; Fig. 3). Interestingly, Eiger function is conserved in mice, as TNF-α inhibits insulin gene expression in mouse insulinoma-derived cells and islets (Agrawal et al., 2016). This suggests that the physiological response to amino-acid starvation in *Drosophila* is conserved and has become pathological by causing insulin resistance in obese mammals (Agrawal et al., 2016). However, whether the TNF-α–dependent reduction of insulin gene expression in mice is specific for amino-acid starvation remains unclear.

Upon amino-acid starvation, IPCs stop secreting Dilps in both a cell-nonautonomous and -autonomous manner

Conversely, amino-acid starvation leads to a reduced secretion of SunA/B by fat body cells, leading in turn to a diminished stimulation of Methuselah on the IPCs. Dilp release from stored secretory vesicles is diminished, leading to their accumulation. A reduced amount of circulating Dilps would prevent mTORC1 activation and allows the larvae to resist starvation by reducing growth (Agrawal and Subramani, 2016; Agrawal et al., 2016; Delanoue et al., 2016; Fig. 3).

In addition, the IPCs also inhibit Dilp secretion in a cell-autonomous manner. Indeed, similar to amino acid–starved *Drosophila* S2 cells (see Effects of amino-acid starvation on the early secretory pathway in *Drosophila*), the level of ERK7 in the IPCs increases when larvae are starved (Hasygar and Hietakangas, 2014). This ERK7 increase is proposed to lead to a cessation of Dilp2 secretion in a cell-autonomous manner. Whether the ERK7 increase specifically inhibits Dilp secretion or secretion in general needs to be established (Fig. 3). Whether ERK7 also works in fat bodies to slow down SunA/B secretion remains to be investigated.

In conclusion, it appears that the humoral loop originating from the fat body to control Dilp release by the IPC is backed up by an ERK7-dependent cell-autonomous response. When the amino-acid level is high, the fat body secretes fed signals (humoral signals) in the hemolymph that reach the brain, where they activate the secretion of Dilps. Dilps, in addition to amino-acid levels, are then sensed in the fat body, stimulating mTORC1 and the release of more fed signals. mTORC1 is also overall stimulated to allow larvae to grow (Géminard et al., 2009; Delanoue et al., 2016; Fig. 3). Conversely, when the amino-acid level is low, the fat body stops secreting fed signals, and Dilp release is inhibited in the IPCs. This is accompanied by an increase in ERK7 levels that down-regulates secretion efficiency. Whether this is linked to the dispersion of Sec16 or ERES remodeling occurs remains to be elucidated.

Conclusions and perspectives

Over the last 10 years, research on the early secretory pathway upon nutrient starvation has brought to light the extent of its modulation. One lesson that has been learned is that nutrient starvation remodels the ERES and their function to stimulate survival and adaptive strategies. First, the ER–Golgi transport machinery and COPII vesicle budding appear to be diverted to fuel the growth of phagophores, the first membrane compartment of the autophagic pathway, a clear survival pathway aiming to replenish the amino-acid level in the cell cytoplasm. At least three distinct mechanisms governing this redirection have been described, but how they are integrated and what the additional layers of controls are still need to be investigated. It is also unclear whether the ERES also contribute to autophagy in other ways.

Interestingly, at least in *Drosophila* cells, amino-acid starvation also stimulates autophagy and leads to an inhibition of protein export from the ER. Given that autophagy requires trafficking out of the ER via COPII-coated vesicles, this raises the question of whether a pool of ERES is marked at the molecular level and dedicated to the phagophore expansion, whereas the ERES functioning in the classical secretory pathway are shut down. This remains to be further studied as it would help reconcile this apparent contradiction.

Nutrient starvation also results in ERES remodeling and coalescence of ERES components into membraneless Sec bodies, where they are protected against degradation, thus an adaptive response providing a fitness advantage upon stress relief. The question is whether Sec bodies form in vivo and in mammalian cells and whether they also provide a fitness advantage there. In *Drosophila* larvae, it is possible that Sec body formation might function as a mechanism regulating secretion of signaling peptides. As discussed, ERK7, a factor that modulates Sec16 dynamics, is a negative regulator of Dilp secretion from IPCs in response to amino-acid starvation. How it cooperates with dPARP16 in vivo also remains to be investigated.

Overall, the regulation of secretion upon nutrient stress is essential for cellular fitness, and as such it is an exciting field for future research in the context of whole-organism fitness and tumor growth.

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