Review

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A review of ADP-ribosylation factor-like 1's role in vesicular trafficking and autophagy in *Saccharomyces cerevisiae*

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Abstract

ADP-ribosylation factor-like protein 1 (Arl1p) is a GTPase that functions in organelle recycling and membrane trafficking pathways within *Saccharomyces cerevisiae (S. cerevisiae)*. By disturbing Arl1p's protein interactions and creating *arl1Δ* mutants, researchers observed a loss of *trans*-Golgi network (TGN) structure, the unregulated production of endosomes, and an inability to move proteins to the cell membrane and recruit tethering proteins to the TGN for vesicle tethering and formation. Specifically, Arl1p was found to be necessary in the stress induced tethering of retrograde vesicles originating from early endosomes. This function in stress reduction is further seen through the role of Arl1p in macroautophagy. The GTPase has been found to work as a facilitator and as an independent pathway to Ypt6p, a GTPase protein with an independent pathway facilitating phagophore synthesis and vesicle fusion with Arl1p; with evidence suggesting Arl1p functions to promote central vacuole synthesis. These studies place Arl1p as a component of *trans*-Golgi structure, vesicle trafficking and autophagy pathways in *S. cerevisiae.*

Introduction

ADP-ribosylation factor (ARF) proteins are a family of GTPases that work in membrane trafficking pathways in *Saccharomyces cerevisiae* (*S. cerevisiae)* (D'Souza-Schourey & Chavrier, 2006). ARF's were first characterized by their role in activating cholera-toxin catalyzed ADP-ribosylation of the adenylate cyclase G (Moss & Vaughan, 1995). Proteins that are part of the ARF family but do not activate cholera toxin are categorized as ARF-Like (ARL) proteins (Kahn et al., 2014). These proteins contain an amphipathic N-terminal alpha helix that has gone through myristoylation, a lipid modification, allowing for strong membrane interactions (Kahn et al., 2014; Donaldson & Jackson, 2011). Once activated by guanine nucleotide exchange factors (GEF's), which facilitate GTP binding, ARF's are able to interact with effectors (Donaldson & Jackson, 2011). These effectors can include coat and tethering proteins that enable the movement of macromolecules by vesicle transport (Kahn et al., 2014). After activation, ARL's are hydrolyzed by GTPase activating proteins (GAP's), allowing for the exchange of GTP to GDP and the inactivation of the GTPase (Donaldson & Jackson, 2011). Of the ARL's, ADP-ribosylation factor-like protein 1 (Arl1p) shares the most similarities with ARF's. Through studies observing

the effects of *arl1's* deletion and *S. cerevisiae*'s exposure to various stressors, Arl1p was established as a component of vesicular trafficking, *trans*-Golgi membrane integrity, and selective and non-selective macroautophagy in yeast.

Arl1p's Role in Trans-Golgi Network Integrity and Vesicle Trafficking

Malena Heinrichs

While its functions have not yet been confirmed, studies have been conducted to narrow down Arl1p's role in *trans*-Golgi network (TGN) structure and vesicle trafficking (Figure 1). Benjamin et al. (2011) investigated the exposure of *S. cerevisiae* to low temperatures and its effect on Gcs1p, a GAP necessary for Arl1p regulation. A loss of function in this GAP was observed and resulted in unregulated and continuous endosome formation at the TGN, tying Arl1p to vesicle budding and endosome traffic. Arl1's role in vesicle formation and TGN structure was further supported by a study conducted by Lu et al. (2001). Through the creation of constitutively expressed, permanently GTP-bound and GDP-bound *arl1* mutants, these researchers studied how the Golgi network was regulated by Arl1p. The GDP mutant cell saw deterioration of the Golgi network and vesicle formation, most likely caused by the inability of Arl1p to bind to an effector. The GTP mutant saw a continuous growth of the TGN, once again most likely linked to the continuous recruitment of effector proteins. Tsai et al. (2012) determined that Arl1p was necessary for the function of the Drs2p, a flippase at the TGN membrane responsible for moving phosphatidylserine (PS) from the luminal side of the TGN to the cytosolic side. Although it may seem unrelated to vesicle trafficking, Arl1p's interaction with the Drs2p flippase allowed for the investigation of Gea2p, a GEP for Arl1p's regulation. The Arl1p-Gea2p-Drs2p complex was confirmed to be needed for the recruitment of the Arlp1 specific effector responsible for vesicle tethering and formation at the TGN: Imh1p. While it still remains under examination, Sebastian et al. (2013) also noted this relationship between TGN membrane flippases and vesicle budding.

Certain studies were also able to narrow down Arl1p's function through *arl1's* deletion and the subsequent disturbance of its involved pathways.

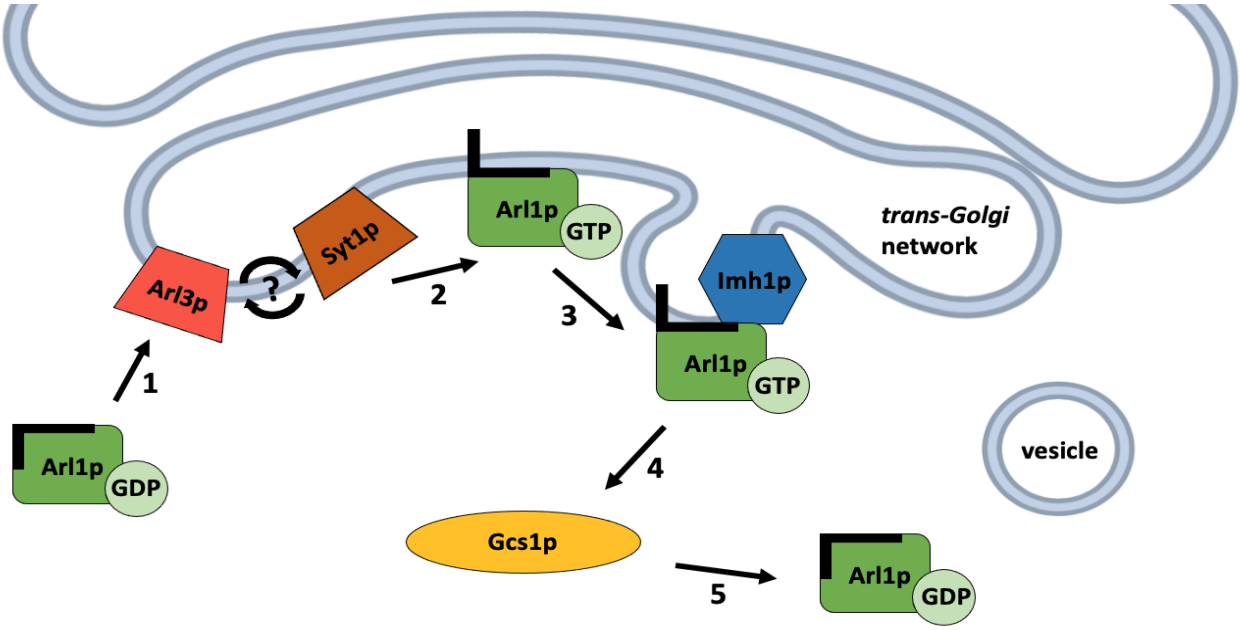

Figure 1. Arl1p's interactions at the *trans*-Golgi network (TGN) during vesicle formation. (1) GDP-Arl1p is activated by the possible GEP's, Arl3p and Syt1p, whose order of interaction are still under investigation. (2) GTP-Arl1p is able to dock at the TGN due to its amphipathic helix (black corner bracket). (3) GTP-Arl1p recruits its effector, Imh1p, for vesicle tethering. (4) GTP-Arl1p is hydrolyzed by the GAP, Gcs1p, and (5) is consequently deactivated and released back into the cytoplasm.

Rosenwald et al. (2002) were able to confirm Arl1p's role in membrane trafficking when the lone removal of Arl1p resulted in a loss of plasma membrane proteins and the improper localization of carboxypeptidase Y. As both are trafficked by vesicles from the TGN, this malfunction reinforced Arl1p's function in vesicle formation and trafficking. Setty et al. (2003) further proved Arl1p's role in vesicle transport and TGN structure through the GTPase's relationship with the Imh1p golgin. Golgins are coiled proteins that often participate in vesicle tethering. Without the presence of Arl1p, Imh1p was localized in the cytosol nowhere near the TGN, demonstrating Arl1p's necessary presence at the TGN for vesicle formation and transport. Though Arl1p's place in vesicle trafficking pathways have been shown through protein interactions, Liu et al. (2006) researched trafficking of glycosylphosphatidylinositol (GPI) anchored plasma membrane resident proteins from the TGN, with the same goal of identifying Arl1p's function. They found that the Arl1p's removal from a yeast cell resulted in the accumulation of these GPI proteins in the Golgi network. A non-mutant cell did not see any accumulation, most likely due to the fact that Arl1p was able to work in trafficking these proteins to the plasma membrane.

While Arl1p has been identified as a GTPase, its interactions with other proteins and definitive functions still remain unknown. As researchers slowly piece together and identify the functions of Arl1p, such as maintaining TGN structure (Lu et al., 2001), vesicle trafficking, and membrane integrity (Liu et al., 2006; Setty et al., 2003c), Arl1p's regulation and interaction with other proteins becomes more complicated. For instance, as only one effector, Imh1p, and one GAP protein are known for Arl1p, its relationship to the possible GEF's Arl3p and Sysd1p becomes unclear (Liu et al., 2006; Yu et al., 2017). Through the clarification of these regulation pathways and the associations of specific regulation proteins to Arl1p's different functions, the role of this GTPase in yeast will become clearer and deepen our understanding of vesicle formation at the TGN.

Arl1p's Role in Vesicle Maturation and Autophagy via Anterograde Trafficking

Emilie Kaye

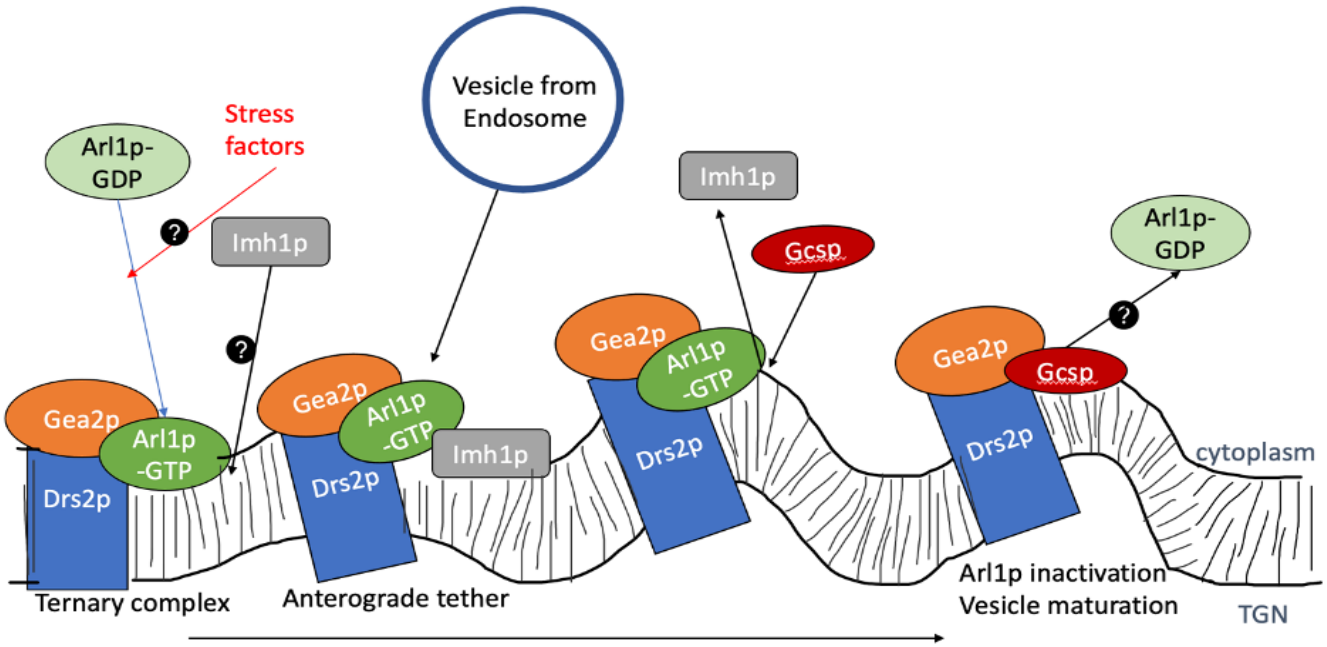

Figure 2. Arl1p activity in regulating vesicular formation and anterograde traffic at the TGN. Flippase Drs2p and GEF Gea2p form a ternary complex with Arl1p-GTP – which can be activated by stress factors. The ternary complex recruits Imh1p to activate membrane curvature initiation and can also act as a tether for anterograde vesicles from the endosome. Membrane-bound Imh1p inhibits the binding of Gcsp. When the membrane curvature has reached a mature state, Imh1p is released which opens a space for Gcsp to bind. Gcsp hydrolyzes Arl1p to its inactive GDP-bound form, which is released into the cytoplasm.

Arl1p has been found to function in the trans-Golgi network (TGN) and is primarily responsible for vesicular traffic regulation and cytoskeleton reorganization (Figure 2) (Rosenwald et al., 2002; Yu & Lee, 2017). The curvature of the membrane in the TGN plays a large role in vesicle formation and maturation (Tsai et al., 2013). When active, Arl1p forms a stable complex with ARF-guanine-nucleotide-exchange factor (GEF) Gea2p and flippase Drs2p. This ternary complex recruits Imh1p to the TGN membrane, which is required for membrane curvature initiation. When the membrane curvature is sufficient, Imh1p is released, which allows room for Gcsp to bind to the membrane and inactivate Arl1p. This process, regulated by Arl1p, allows sufficient curvature of the membrane for vesicle maturation (Tsai et al., 2013; Yu & Lee, 2017).

Another important function of Arl1p is to promote stress resistance to allow cells to continue to grow and function in the presence of extracellular stress (Yang & Rosenwald, 2016). Arl1p is responsible for creating a GRIP domain with Imh1p to function as a tether for retrograde vesicles coming from the early endosome to the TGN (Marešová et al., 2012). This anterograde movement of vesicles seems to be key in stress resistance as it influences the cation fluxes (Marešová et al., 2012, 8). Arl1p's regulation in anterograde traffic is necessary for autophagy, which is the removal of unnecessary or dysfunctional components to recycle the required macromolecules (Parzych et al., 2018; Yang & Rosenwald, 2016). Anterograde trafficking and autophagy, regulated by Arl1p, is essential for the cell's survival in high stress environments such as starvation and high temperatures (Yang & Rosenwald, 2016).

The *ARL1* gene was found to be non-essential for cell viability as *arl1Δ* mutant cells, which produce little to no Arl1p, seem to survive (Lee et al., 1997). However, *arl1* deletion affects the physiological function of the cell through vesicle mislocalization and autophagy defects resulting in increased sensitivity to stress factors (Lee et al., 1997; Yang & Rosenwald, 2016; Yu & Lee, 2017). Arl1p that controls membrane trafficking is necessary to induce autophagy in high-stress environments. *arl1Δ* mutants show mislocalization of carboxypeptidase Y to the vacuole, which is a key protease implicated in the final degradation steps of autophagy (Yu & Lee, 2017; Parzych et al., 2018). As a result, defects in autophagy occur at high stress temperatures of 37°C but normal autophagy occurs at normal growth temperatures of 30°C (Yang & Rosenwald, 2016). This is because without functioning *arl1* to control endosome-*trans*-Golgi traffic, essential proteins that form the autophagosome won't be properly localized at restrictive temperatures (Yang & Rosenwald, 2016). As a result of autophagy defects, the *arl1Δ* mutant cells show an increase in sensitivity to salts, high temperatures and high pH compared to the wild-type *arl1* cells which supports the role of *arl1* in stress tolerance (Yu & Lee, 2017). Therefore, it should be expected that *arl1Δ* mutants would show vesicle mislocalization, autophagy defects in the presence of stress factors and a decrease in stress resistance.

While it is known that Arl1p plays a role in the structure and function of the TGN, some mechanisms of the pathway should be further investigated (Lee et al., 1997; Rosenwald et al., 2002). The regulation of GEFs Gea2p on Arl1p, the mechanism of which stress factors lead to the activation of Arl1p, and the factors responsible for the recruitment of Imh1p in the presence of active Arl1p remains unknown. Arl1p-GDP and Imh1p are recycled in the cytosol for later use, however, the point at which the hydrolysis of Arl1p-GTP occurs is still being questioned. A feedback mechanism has been proposed: where Imh1p determines the inactivation of Arl1p through hydrolysis by Gcsp (Yu & Lee, 2017). While many functions of Arl1p have been studied, the regulation of these mechanisms in the pathway involving Arl1p is yet to be determined.

Arl1p's Role in Autophagy via Phagophore Assembly, Docking, and Tethering

Aashwan Dhaliwal

In the context of macroautophagy, *S. cerevisiae* mutants lacking Arl1p expression will depict no change in function at permissive temperatures during periods of stress but will be unable to continue growth at non-permissive temperatures. The signaling cascade of the Ras protein, Rapamycin, induces inhibition of TORC1p (a negative regulator consisting of

the TOR protein complex 1) to inform the cell of a nutrient-starved state (Yang & Rosenwald, 2018) (Figure 3).

Ras proteins such as RhoAp (Yang & Rosenwald, 2018), ROCK1 (Yang & Rosenwald, 2018), Ras/PKA signaling pathway (a negative regulator) (Inoue & Klionsky, 2010), and the Rac/Rho/Cdc42 family (Yang & Rosenwald, 2018) are all involved in signaling within macroautophagy. Their individual contributions are still not exactly known. The inhibition of TORC1p initiates the starvation state. Sys1p (a transmembrane protein) activates Arl3p at the trans-Golgi network (TGN); the activation of Arl3p allows for the activation of Arl1p at the TGN via regulation by Syt GEF (Wang et al., 2017) (Figure 3). Arl1p cycles between being GTP-bound (active) and GDP-bound (inactive) to facilitate the synthesis of phagosomes at the PAS and the fusion of autophagosomes to the vacuole (Wang et al., 2017). However, the exact pathway for the activation of Arl1p and Arl3p, the guanine nucleotide exchange factors (GEFs), and the GTPases that initiate macroautophagy are not known. Arl1p and Ypt6p function independently in their use of effectors and cascades to promote the macroautophagy pathway (Benjamin et al., 2011), but work in conjunction to facilitate macroautophagy.

Arl1p and Ypt6p facilitate the synthesis of phagophores (a double-membrane vesicle) at the Phagophore Assembly Site (PAS), which facilitates the fusion of the autophagosomes (proceeds from the phagophore) at the vacuole (Yang & Rosenwald, 2016) (Figure 3). Fusion of autophagosomes via anterograde traffic of Atg9p (macroautophagy mediator proteins (Inoue & Klionsky, 2010)) with the vacuole is facilitated by the binding of Arl1p and the "Golgi-associated retrograde protein" (Yang & Rosenwald, 2016) GARP complex subunit Vps53, and the binding of Ypt6p and the GARP complex subunit Vps52 (Rosenwald et al., 2016), to initiate

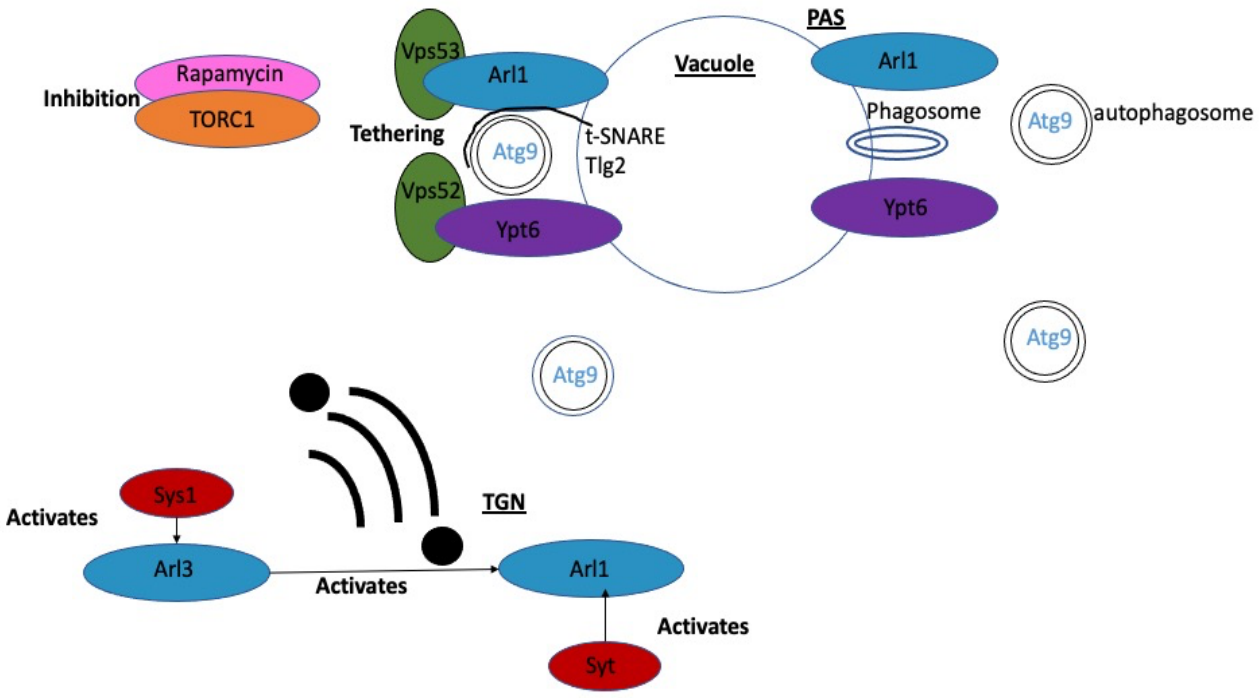

Figure 3. Biochemical pathway of Arl1p in macroautophagy. Rapamycin inhibits TORC1p to initiate macroautophagy. Arl3p is activated at the TGN by Sys1p. Arl1p is activated at the TGN by Arl3p and Sytp, and proceeds to the PAS to facilitate synthesis of phagophores. These photophores proceed to autophagosomes. Autophagosomes containing Atp9p are fused into the vacuole using t-SNARE Tlg2, facilitated by Arl1p/Vps53 complex and Ypt6p/Vps52 complex. The full function of all GTPases and signaling cascades are not currently known.

tethering via t-SNARE Tlg2 (Yang & Rosenwald, 2016).

Arl1p has different conformations, and it is known that Arl1p Q72L is responsible for the fusion of Atp9p-containing autophagosomes with the vacuole at non-permissive temperatures (Yang & Rosenwald, 2016). The Atg9p protein is essential for recognition of the autophagosomes with the Arl1p and Ytp6p proteins; recognition initiating fusion into the vacuole, hydrolysis of proteins and organelles, and release of the recycled macromolecules back into the cytosol (Inoue & Klionsky, 2010). Arl1p and Ypt6p are both GTPases, but Ypt6p can facilitate the synthesis and fusion of autophagosomes independently without the expression of Arl1p, depicting that expression of Arl1p is not crucial within yeast (Yang & Rosenwald, 2018).

The effects of depleted expression of Arl1p on macroautophagy will result in a reduced size of the *arl1Δ* mutant cells to the wild type. Mutant *arl1Δ* cells are unable to grow at temperatures of 37°C due to a lack of organelle and protein recycling from the diminished macroautophagy pathway (Yang & Rosenwald, 2016). Moreover, without the expression of Arl1p during periods of growth within *S. cerevisiae,* the large central vacuole is unable to be synthesized which also prevents the cell from using macroautophagy to initiate cell death at periods of high stress (Abudugupur et al., 2002). The Ypt6p cascade is able to bind with the GARP complex at a strong affinity at permissive temperatures, 30°C, thus no difference in the wildtype and *arl1Δ* mutant cells would be seen. However, at increasing temperatures Ypt6p is no longer able to bind the GARP complex with an affinity that dominates the missing Arl1p protein, and the macroautophagy pathway is unable to continue fusion of Atg9p protein-mediated autophagosomes (Yang & Rosenwald, 2018). Thus, at non-permissive temperatures of 37°C the *arl1Δ* mutant cell can grow at only half the rate of the wildtype cell (Yang & Rosenwald, 2018), which is consistent with the previous finding by Yang & Rosenwald (2016) where Ypt6p is able to compensate for the lack of expression by Arl1p in the macroautophagy pathway at permissive temperatures (30°C). An *arl1Δ* mutant cell lacking expression of the Arl1p Q72L protein would be unable to facilitate the phagophore formation at the PAS, and phagophore fusion at the vacuole at high temperatures (Yang & Rosenwald, 2016).

To guide future studies there needs to be a focus on the mediation of the Arlp family on autophagy pathways within *S. cerevisiae*. There remains much uncertainty regarding the GTPases and GEFs involved in activation. Understanding the interactions involved in the pathways of the Arlp family may deduce findings of the autophagic cell death pathway within yeast cells and the involvement of Arl1p. This is important because it may indicate the role of Arl1p in macroautophagy being essential over non-essential and may provide further insight as to why no change in size is seen at permissive temperatures. Moreover, there needs to be further research regarding the role Arl1p plays in vacuole synthesis and whether there will be uncontrolled growth of Arl1p depleted colonies, and the affinity of Atg9p proteins with Arl1p. Understanding this affinity may provide reason for the presence of Ypt6p as essential and Arl1p as non-essential within the cell for the macroautophagy pathway. This may expand our understanding of why the *S. cerevisiae* can continue growth and macroautophagy at permissive temperatures but not at non-permissive temperatures without expressing Arl1p Q72L.

Mutant *arl1Δ* cells will remain smaller in size at non-permissive temperatures, and they will be unable to undergo macroautophagy-facilitated cell death. This will cause an increase in the smaller Arl1p deficient colonies, and perhaps uncontrolled growth. The mechanisms behind the activation of Arl1p within macroautophagy remain unknown but there is support for the role of Arl1p expression not being essential nor detrimental to the survival of the cell at permissive temperatures. Studies regarding the different variations of Arl1p need to be further studied, as the expression of Arl1p Q72L at high temperatures allows for the fusion of autophagosomes with the vacuole at high temperatures.

The presence of Atg9p is required within the autophagosomes to mediate the fusion of autophagosomes with the vacuole via Arl1p and Ypt6p pathways and t-SNAREs. Moreover, Arl1p and the GARP complex Vsp53 are required to form a complex to facilitate the fusion of the autophagosomes.

Without Arl1p expression, there may be no formation of a central vacuole, which would inhibit *S. cerevisiae* from initiating cell death in stressful environments, resorting to the Arl1p mutants to continue to bud and colonies to form of smaller size. Many questions regarding the activation of Arl1p remain unanswered, but research should focus on the Arlp family and the interactions among them. This will increase our understanding of how Arl1p functions at high temperatures and whether a conformational change (such as Arl1p Q72L) is required for *S. cerevisiae* to overcome temperature stress, and how Arl1p may function in the formation of the central vacuole.

Summary and Conclusion

Arl1p has been found to work in vesicular trafficking, *trans*-Golgi membrane integrity, and selective and non-selective macroautophagy in *S. cerevisiae*. Though its relationship with Syt1p and Arl3p remains unclear due to disputing literature, Arl1p's presence in the vesicular trafficking pathway can be confirmed as a deletion of *arl1* resulted in the improper localization of carboxypeptidase Y (Rosenwald et al., 2002). In addition, Arl1p's role in vesicle formation and regulation of anterograde traffic from the endosome has been proposed (Yu & Lee, 2017). However, the regulatory mechanisms acting on Arl1p and its pathway at the TGN are still under examination. Arl1p has been found facilitating an independent macroautophagy pathway to the dominant Ypt6p pathway, permitting no difference in colony sizes between wildtype and *arl1Δ* mutant cells at permissive temperatures (Yang & Rosenwald, 2018). Furthermore, literature proposes at non-permissive temperatures the independent Arl1p and Ypt6p macroautophagy pathways present a compensatory relationship to maintain wildtype colony size, because the Arl1p Q72L protein conformation compensates for the reduced affinity of Ypt6p during the fusion of autophagosomes (Yang & Rosenwald, 2016). While Arl1p's clear role in yeast has not yet been confirmed, its role in macroautophagy and the *trans*-Golgi network is evident. Thus, future studies should be directed towards understanding the proposed compensatory relationship between Arl1p and Ypt6p.

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