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SPOTLIGHT

# Slowing down to take it in: Endocytosis during cellular aging

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**Aging cells functionally decline and accumulate damage through poorly understood mechanisms. In this issue, Antentor et al. (https://doi.org/10.1083/jcb.202412064) find that increased vacuolar pH in older yeast cells slows clathrin-mediated endocytosis. These findings have broad implications in aging-related plasma membrane protein quality control.**

## Cellular aging, molecular damage, and functional decline

Aging is an inevitable process in most organisms, leading to declining function and decreased viability over time at the cell, tissue, organ, and whole-organism levels. In cells, accumulation of molecular damage to proteins, lipids, DNA, and metabolites drives aging-related phenotypes (1). Unsurprisingly, declining function that accompanies aging is not limited to multicellular organisms. Indeed, yeast and even bacteria undergo aging, and, in those that divide asymmetrically, selective partitioning of damaged components to the mother cell makes these cells particularly useful in studies of aging (2). The budding yeast *Saccharomyces cerevisiae* is a well-established model for replicative aging, where mother cells progressively accumulate and retain damage with each cell cycle while producing "young," essentially damage-free daughter cells. Eventually (after ~25 rounds of budding), mother cells lose replicative potential, stop dividing, and ultimately die. The mechanisms governing accumulation of molecular damage and functional decline during replicative aging are poorly understood and are thus an area of considerable interest.

## A link between cellular aging, pH regulation, and endocytosis

In this issue, Antenor and colleagues explore the relationships between endocytosis and replicative aging (3). The plasma membrane (PM) represents the point of contact between a cell and its environment and is thus the first site to encounter toxic environmental insults that cause molecular damage, even though aging-related damage accumulates throughout the cell. Endocytosis plays critical roles in PM quality control by removing damaged proteins and membranes from the cell surface and targeting them to the vacuole (the lysosome equivalent in yeast) for degradation. Clathrin-mediated endocytosis (CME) is by far the best-studied endocytic pathway, using modules or "waves" of proteins to recruit and concentrate membrane-associated cargo at sites where clathrin polymerizes to support membrane deformation and generation of a clathrin-coated vesicle (Fig. 1) (4, 5). Endocytosis via CME is critical for maintaining PM protein and lipid homeostasis and is important for PM quality control (6).

Currently, the relationship between pH regulation and CME during cellular aging is ill defined. During cell division, the $H^+$ ATPase Pma1 preferentially partitions to the mother cell PM, likely causing increased proton export and elevated cytosolic pH in the mother that becomes more pronounced during aging. In turn, elevated cytoplasmic pH triggers disassembly of the vacuolar ATPase (V-ATPase), the molecular complex that maintains vacuolar acidity, into cytosolic ($V_1$) and membrane-associated ($V_0$) subcomplexes, thereby reducing V-ATPase function (7). The resulting increase in vacuolar pH is associated with numerous aging-related phenotypes, including metabolic and mitochondrial dysfunction (1).

Antenor and colleagues demonstrate for the first time that CME progression is slower in aging mother cells than in younger cells; delayed CME is observed in mothers that have produced as few as three daughter cells and slows further with successive rounds of budding (3). In contrast, daughter cells retain consistent, faster endocytic kinetics even when arising from aged mother cells. This slowing of CME during replicative aging seemingly occurs due to an extension of early phases of coated pit assembly, which are variable in duration (~1–2 min) and linked to cargo loading. Both Ede1 (homologous to Eps15), an early arriving CME protein that binds to cargo-enriching adaptors, and Sla1, a coat-associated protein that participates in regulation of actin polymerization, have extended lifetimes and slower recruitment kinetics at CME sites in aged cells. In contrast, late-arriving proteins like the myosin Myo5, the Arp2/3 complex, and the scission effector Rvs167 each show consistent lifetimes at endocytic sites regardless of replicative age. Unlike the variable phase of CME associated with Sla1 and Ede1, late-arriving proteins act in a temporally consistent "regular" phase that completes vesicle formation after the maturation-checkpoint of cargo loading is completed (8).

Delayed CME progression is strongly linked to elevated pH in the cytosol and/or vacuole, which occurs during replicative

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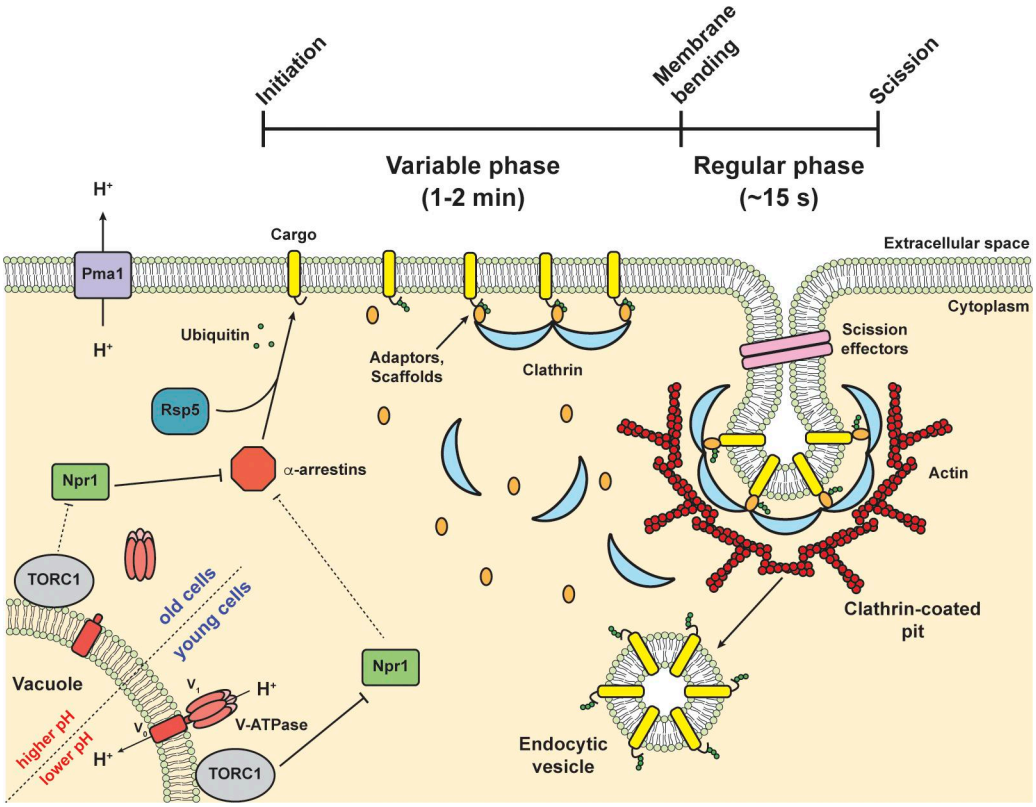


Figure 1. **CME during aging.** CME involves recruitment of adaptors, scaffolds, and clathrin during a "variable" phase, whose timing likely depends on cargo. Actin, myosins, and scission effectors arrive in a shorter, regular phase of predictable duration, generating a coated vesicle. During aging, $H^+$ ATPase Pma1 accumulation leads to elevated cytoplasmic pH, V-ATPase disassembly, and increased vacuolar pH that inhibits TORC1 signaling. With reduced TORC1 signaling, the kinase Npr1 phosphorylates endocytic factors such as α-arrestins to inhibit their activity. This could ultimately delay endocytosis by reducing cargo ubiquitination and loading into vesicles, causing the prolonged variable phase of endocytosis observed in old mother cells.

aging (1, 7). Intriguingly, genetic and pharmacological manipulation of cytosolic and vacuolar pH influences endocytic rates: lowering pH in mother cells restores young endocytic dynamics, while raising pH in daughters slows CME progression, as occurs in older cells (3). Mechanistically, reduced V-ATPase function impedes target of rapamycin complex 1 (TORC1) kinase activity, as evidenced by reduced phosphorylation of key substrates. While TORC1 regulates many aspects of nutrient sensing and cellular function, a critical signaling output for CME is its inhibitory phosphoregulation of the protein kinase Npr1. Under conditions of reduced TORC1 activity, Npr1 phosphorylates and inhibits α-arrestins, which function as cargo-specific adaptors for the ubiquitin ligase Rsp5 (9). Since ubiquitination is a key marker for cargo recruitment to CME sites, reduced α-arrestin function may delay a CME cargo-loading checkpoint, thus explaining the lengthened variable phase of CME during replicative aging.

## Is declining endocytosis a conserved feature of cellular aging?

Studies in yeast and mammalian cells revealed that most of the proteins needed for CME, and indeed the modular, stepwise assembly and maturation of clathrin-coated pits at the PM, are remarkably well conserved (4, 5). Moreover, lysosomal pH dysregulation is a common theme in many models of cellular aging (1), supporting the idea that a conserved link between aging, pH, and endocytosis exists. Decreased endocytic capacity may well be associated with aging-related diseases. For example, Alzheimer's disease is associated with toxic accumulation of β-amyloid fibrils, and CME is involved in internalization and lysosomal degradation of β-amyloid oligomers (10). During aging, reduced endocytosis may thus lead to accumulation of potentially toxic protein aggregates, which in turn could potentiate cellular dysfunction in aging-related diseases and neurodegeneration.

## Future implications: Rejuvenating endocytosis

By linking lysosomal pH, TORC1 signaling, and CME, these findings represent an important advance in our mechanistic understanding of how PM quality control and membrane traffic are impacted during aging. Moreover, they raise new questions about the reciprocal impact of endocytosis and aging. Which components of the CME machinery are especially important for age-dependent changes in endocytosis? Might a subset of cargos be key for passing through a maturation checkpoint? Does delayed internalization of specific cargos exacerbate pH dysregulation during replicative aging, leading to further decreases in endocytosis? Clearly, there are many areas to explore in the young field of aging-dependent regulation of protein trafficking.

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