

# Acetyl-coenzyme A

## A metabolic master regulator of autophagy and longevity

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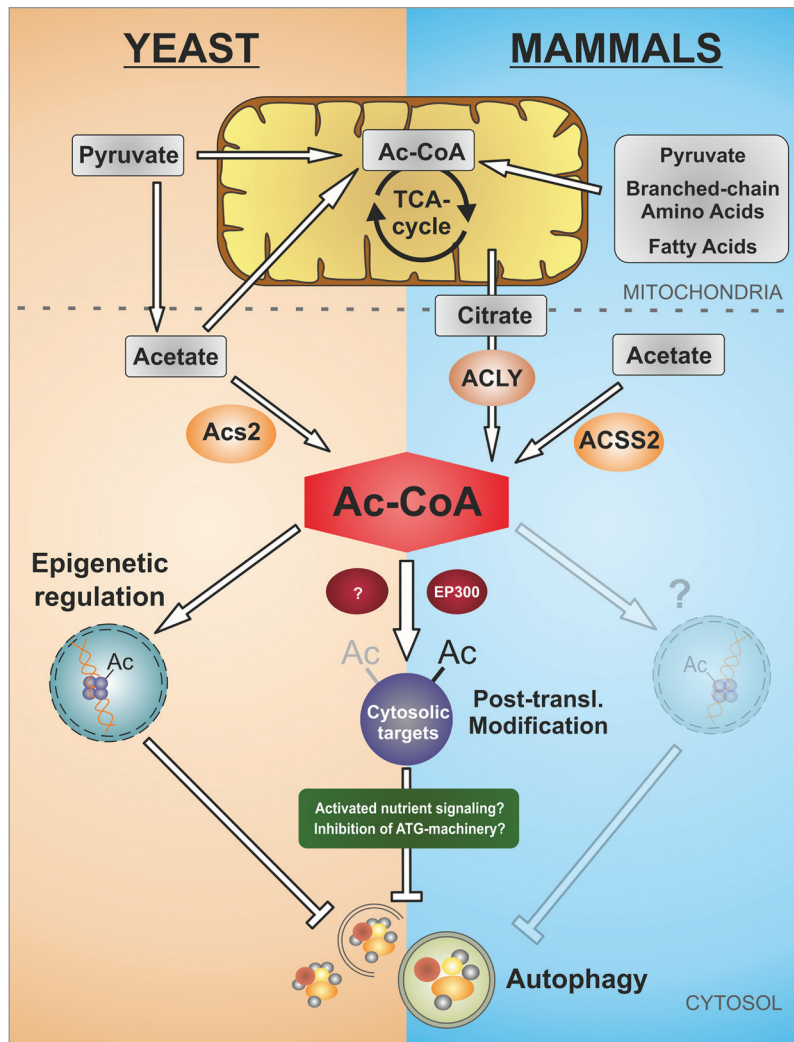
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**A**s the major lysosomal degradation pathway, autophagy represents the guardian of cellular homeostasis, removing damaged and potentially harmful material and replenishing energy reserves in conditions of starvation. Given its vast physiological importance, autophagy is crucially involved in the process of aging and associated pathologies. Although the regulation of autophagy strongly depends on nutrient availability, specific metabolites that modulate autophagic responses are poorly described. Recently, we revealed nucleocytosolic acetyl-coenzyme A (AcCoA) as a phylogenetically conserved inhibitor of starvation-induced and age-associated autophagy. AcCoA is the sole acetyl-group donor for protein acetylation, explaining why pharmacological or genetic manipulations that modify the concentrations of nucleocytosolic AcCoA directly affect the levels of protein acetylation. The acetylation of histones and cytosolic proteins inversely correlates with the rate of autophagy in yeast and mammalian cells, respectively, despite the fact that the routes of de novo AcCoA synthesis differ across phyla. Thus, we propose nucleocytosolic AcCoA to act as a conserved metabolic rheostat, linking the cellular metabolic

state to the regulation of autophagy via effects on protein acetylation.

Autophagy is regulated by nutrient- and energy-sensing pathways and the subsequent activity of protein kinases, such as the well-known aging regulators MTOR (mechanistic target of rapamycin; Tor in yeast), AMPK (AMP-dependent protein kinase; Snf1 in yeast) and AKT/protein kinase B (Sch9 in yeast). However, recent studies suggest that apart from phosphorylation, other posttranslational modifications, in particular acetylation of proteins, may also influence autophagy by targeting pathways that are, at least partly, different from that of known kinase regulators. Multiple regulators and core components of the autophagic machinery undergo changes in their acetylation status, and acetylation of histone lysine residues has been linked to different aspects of autophagy.

Besides peroxisomal  $\beta$ -oxidation that creates AcCoA by the breakdown of fatty acids, in the yeast *Saccharomyces cerevisiae* AcCoA is synthesized by a pathway that fuels 2 distinct, separate intracellular pools: the mitochondrial vs. the nucleocytosolic pool. The putative CoA transferase Ach1 and the mitochondrial AcCoA synthetase isoform Acs1 generate



**Figure 1.** Nucleo-cytosolic AcCoA determines the autophagic response in yeast and mammals. In *Saccharomyces cerevisiae* grown on glucose, the major source for nucleo-cytosolic AcCoA is the AcCoA synthetase 2 (*Acs2*), which uses acetate as a substrate. Mitochondria solely influence cytosolic AcCoA levels by removing acetate and its precursor pyruvate, fueling the tricarboxylic acid (TCA) cycle. In contrast, cytosolic AcCoA production in mammals requires a detour via mitochondria, since it depends on citrate derived from the TCA cycle, which is fed by pyruvate as well as by fatty acids and branched-chain amino acids. Citrate is exported from mitochondria and converted to AcCoA by the cytosolic ATP citrate lyase (*ACLY*). In both models, high concentrations of nucleo-cytosolic AcCoA favor protein acetylation by acetyltransferases. Protein hyperacetylation subsequently inhibits autophagy by epigenetic regulation of autophagy-related genes, by direct posttranslational inactivation of proteins engaged in the autophagic machinery, as well as by (direct or indirect?) modulation of nutrient-sensing kinase pathways.

AcCoA in mitochondria from acetate, while the pyruvate dehydrogenase complex converts pyruvate into mitochondrial AcCoA by oxidative decarboxylation. The predominantly nuclear-localized yeast AcCoA synthetase, *Acs2*, catalyzes the formation of AcCoA from acetate and CoA within the nucleo-cytosolic compartment. *Acs2* is essential for histone acetylation, when cells grow on glucose as a carbon source. Similarly, in mammals AcCoA is

produced in mitochondria from pyruvate, fatty acids or branched-chain amino acids. In contrast to yeast, the nucleo-cytosolic AcCoA production in mammals is mostly executed by *ACLY* (ATP citrate lyase), which ultimately converts citrate exported from mitochondria into AcCoA. However, it should be emphasized that in mammals, a cytosolic AcCoA synthetase contributes to the nucleo-cytosolic pool of AcCoA. Although the pathways through

which AcCoA is synthesized are largely species-dependent, we could demonstrate that the nucleo-cytosolic pool was more important for repressing autophagy than the mitochondrial pool, both in yeast and in mammals (Fig. 1).

We found that nucleo-cytosolic AcCoA production limited autophagy in aging yeast, meaning that even a partial knockdown of the *ACS2* gene sufficed to enhance autophagy upon chronological aging. In contrast, blockage of the mitochondrial route of AcCoA formation by deletion of the CoA-transferase *ACH1* or the mitochondrial pyruvate carrier *MPC1* caused an age-dependent defect in autophagic flux, as it hyperactivated the nucleo-cytosolic *Acs2* pathway, culminating in histone hyperacetylation and transcriptional repression of the autophagy essential gene *ATG7*. Corroborating this idea, simultaneous knockdown of *ACS2* in the *ACH1* deletion background reinstated the expression of *ATG7*, as it abolished the autophagy defect and the accelerated aging phenotype. This led us to conclude that the nucleo-cytosolic rather than the mitochondrial AcCoA pool suppresses autophagy. Interestingly, inhibition of nutrient-signaling, for example by additional deletion of *SCH9* or Tor complex 1 inhibition by rapamycin, failed to abolish the detrimental effects of increased nucleo-cytosolic AcCoA production on autophagy.

We also assessed the capacity of AcCoA to regulate starvation-induced autophagy in cell culture and mice by pharmacologically or genetically manipulating major routes of mammalian AcCoA formation, such as the pyruvate dehydrogenase complex or *ACLY*. Reduced or increased cytosolic AcCoA directly affected protein acetylation as it induced or suppressed autophagy, respectively. In line with the data obtained from yeast, changes in the nucleo-cytosolic fraction of AcCoA were finally causal for the observed impact on autophagy, since inactivation of purely cytosolic enzymes involved in AcCoA generation (e.g., *ACLY* or the mammalian AcCoA synthetase *ACSS2*) was sufficient to stimulate autophagy and, even more importantly, blockage of *ACLY* prevented autophagy suppression induced by mitochondrial AcCoA overproduction.

Of note, while autophagy regulation by AcCoA during aging seems to be independent of well-known kinase signaling pathways (such as those involving Tor and Sch9) in yeast, acute nutrient depletion was accompanied by dephosphorylation of MTOR substrates and the activation of AMPK in mammalian cells. Furthermore, high rates of AcCoA counteracted autophagy induction by rapamycin or AKT inhibitors in mammals. It is therefore tempting to speculate

that, at least in a short-term context, nucleo-cytosolic AcCoA depletion might activate energy-sensing kinase pathways via yet-to-be-explored mechanisms. Nevertheless, high AcCoA concentrations might also block the core autophagy machinery (e.g., by acetylation of *ATG* gene products) downstream of nutrient kinase targets. It remains to be explored to which extent (and on which time scale) cytoplasmic and epigenetic alterations explain the relationship between AcCoA

accumulation, protein hyperacetylation, and autophagy repression.

Our results clearly demonstrate the central importance of the nucleo-cytosolic AcCoA pool for autophagy regulation and suggest the possibility of inhibiting or stimulating autophagic flux by targeting AcCoA metabolism.

#### **Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.