RESEARCH ARTICLE | CELL BIOLOGY

Lysosomal cystine mobilization shapes the response of TORC1 and tissue growth to fasting

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SCIENCE • 18 Feb 2022 • Vol 375, Issue 6582 • DOI: 10.1126/science.abc4203

Cystine as lysosomal fasting signal

Communication between the lysosome and mitochondria appears to help maintain control of metabolism in fruit flies deprived of food for prolonged periods. When food is limited, the target of rapamycin complex 1 (TORC1) protein kinase complex is inhibited, which promotes catabolism and autophagy to provide nutrients. Newly supplied amino acids could reactivate TORC1, but Jouandin *et al.* implicated cystine released from lysosomes in allowing continued catabolism during prolonged fasting. Cystine, when reduced to two molecules of cysteine, may promote the transient storage of remobilized amino acids in the form of tricarboxylic acid cycle intermediates in the mitochondria, thus limiting TORC1 reactivation during a prolonged fast. —LBR

Structured Abstract

INTRODUCTION

Adaptation to changes in diet involves a complex cellular response controlled by interacting metabolic and signaling pathways. During fasting periods, this response remobilizes nutrients from internal stores through catabolic programs. In *Drosophila*, the fat body, an organ analogous to the liver and adipose tissue in mammals, functions as the organism's main energy reserve, integrating nutrient status with energy expenditure. How the fat body sustains its own needs and balances remobilization of nutrients over the course of a starvation period during developmental growth is unclear.

RATIONALE

The target of rapamycin complex 1 (TORC1) signaling pathway is a master regulator of growth and metabolism. Activated when nutrients are replete, TORC1 promotes biosynthesis and represses catabolic processes such as autophagy. In the fat body of fasting animals, however, TORC1 activity is dynamic. Activated to a maximum in feeding animals, TORC1 is acutely down-regulated at the onset of fasting, followed by partial and progressive reactivation through the amino acids generated by proteolysis during autophagy. This reactivation hints at a model in which TORC1 reaches a specific activity threshold allowing for minimal anabolism to occur concomitantly with catabolism, such as autophagy. To analyze how TORC1 dynamics is achieved, we used

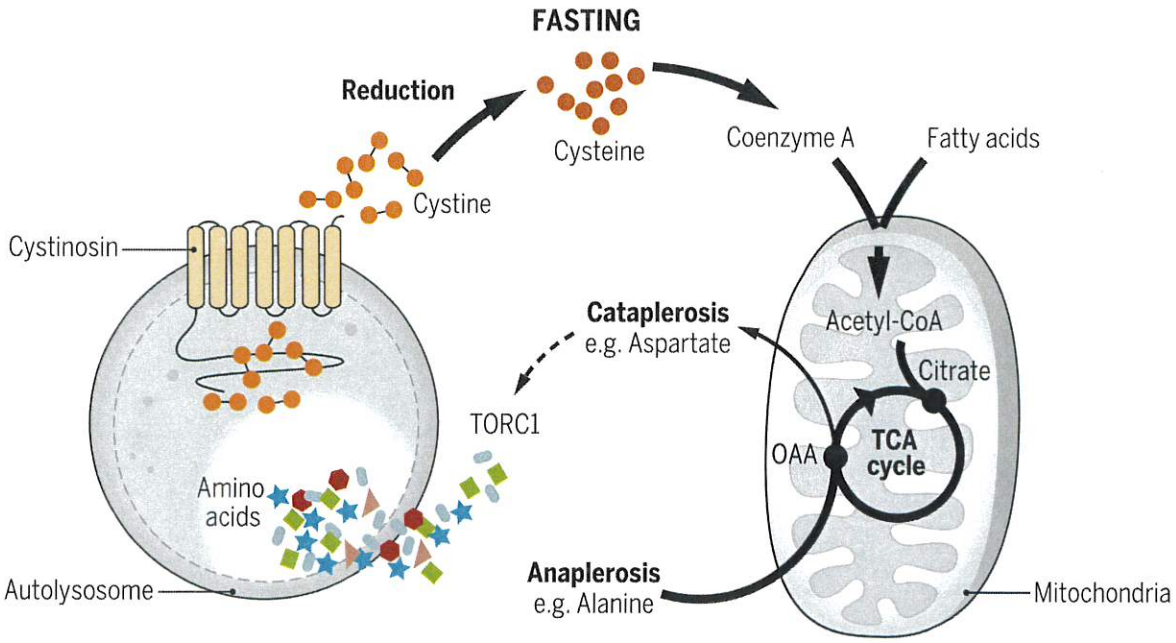
screening approaches, combined metabolomics with genetics, and developed specific heavy isotope–tracing methods in intact animals.

RESULTS

A screen to test the role of amino acids on animal fitness when starved on a low-protein diet identified cysteine as a potent suppressor of growth. During fasting, cysteine concentration was elevated through lysosomal cystine export through dCTNS, the mammalian ortholog of which, cystinosin, is responsible for the lysosomal storage disease cystinosis. dCTNS depletion and overexpression, respectively, lowered and elevated cysteine concentration in fasted animals, providing us with a genetic means with which to manipulate cysteine levels in vivo. Parallel metabolomics profiling of fasting animals revealed an increased concentration of tricarboxylic acid (TCA) cycle intermediates during fasting. Moreover, heavy isotope cysteine tracing demonstrated cysteine metabolism to coenzyme A (CoA) and further to acetyl-CoA, a process that was coupled to lipid catabolism in the fat body during fasting. Acetyl-CoA appeared to facilitate incorporation of additional substrates in the TCA cycle, with dCTNS overexpression increasing the entry of a heavy isotope alanine tracer in the TCA cycle. The elevation of TCA cycle intermediates by cysteine metabolism could be linked to the reactivation of TORC1. dCTNS overexpression dampened the reactivation of TORC1 during fasting, and it was sufficient to suppress TORC1 activity and cause ectopic autophagy in the fat body of fed animals. By contrast, dCTNS deletion did not affect TORC1 activity nor autophagy in fed animals but elevated the reactivation of TORC1 above a threshold suitable to halt autophagy during fasting. Finally, we show that cysteine metabolism regulates anaplerotic carbon flow in the TCA cycle and the level of amino acids, in particular aspartate. Combinatorial amino acid treatments rescued TORC1 activity upon fasting when cysteine levels were high. This suggests that the balance between cysteine metabolism and amino acid synthesis by the TCA cycle ultimately controls TORC1 reactivation, and thereby autophagy, during fasting. As a consequence, dCTNS depletion shortened life span during fasting, which could be restored by dietary cysteine, highlighting the central role of cysteine in the metabolism of fasted animals.

CONCLUSION

We found that in developing animals, adipose cells control biosynthesis during fasting by channeling nutrients between the lysosome and the mitochondria. After autophagy induction, amino acids are released from the lysosome, and some serve as substrates for the TCA cycle to replenish carbons in the mitochondria. Nutrients appear to be transiently stored in the form of TCA cycle intermediates and then extracted for the synthesis of amino acids that promote the reactivation of TORC1. We uncovered a new regulatory role for the metabolism of cysteine to acetyl-CoA during this process. By facilitating the incorporation of carbons into the TCA cycle and limiting amino acid synthesis, cysteine appears to regulate the partitioning of carbons in the TCA cycle. We propose that cysteine acts in a negative metabolic feedback loop that antagonizes TORC1 reactivation upon fasting above a threshold that would compromise metabolic homeostasis and animal fitness.



Cysteine metabolism acts in a negative feedback loop to maintain autophagy during fasting. The lysosomal transporter Cystinosin exports cystine, which is further reduced to cysteine in the cytosol. Cysteine is metabolized to coenzyme A (CoA) and fuels acetyl-CoA metabolism. Cysteine metabolism drives anaplerotic substrates into the TCA cycle and limits biosynthesis from oxaloacetate (OAA). This process is particularly important during fasting to regulate the reactivation of TORC1 and control autophagy.

Abstract

Adaptation to nutrient scarcity involves an orchestrated response of metabolic and signaling pathways to maintain homeostasis. We find that in the fat body of fasting *Drosophila*, lysosomal export of cystine coordinates remobilization of internal nutrient stores with reactivation of the growth regulator target of rapamycin complex 1 (TORC1). Mechanistically, cystine was reduced to cysteine and metabolized to acetyl-coenzyme A (acetyl-CoA) by promoting CoA metabolism. In turn, acetyl-CoA retained carbons from alternative amino acids in the form of tricarboxylic acid cycle intermediates and restricted the availability of building blocks required for growth. This process limited TORC1 reactivation to maintain autophagy and allowed animals to cope with starvation periods. We propose that cysteine metabolism mediates a communication between lysosomes and mitochondria, highlighting how changes in diet divert the fate of an amino acid into a growth suppressive program.

Supplementary Materials

This PDF file includes:

Supplementary Text S1 and S2

Figs. S1 to S15