Journal Club

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Article

A non-canonical tricarboxylic acid cycle underlies cellular identity

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Check for updates

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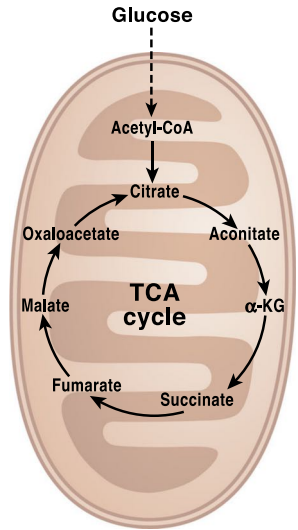
Aim

Tricarboxylic acid (TCA) cycle:

- Central hub of cellular metabolism imortant for both energy production and biosynthesis
- Series of chemical reactions to release stored energy through the oxidation of acetyl-CoA derived from carbohydrates, fats, and proteins
- Mammalian cells display diversity in TCA-cycle activity

→ **How is this diversity achieved?**

→ **Is the TCA cycle critical for establishing cell fate?**

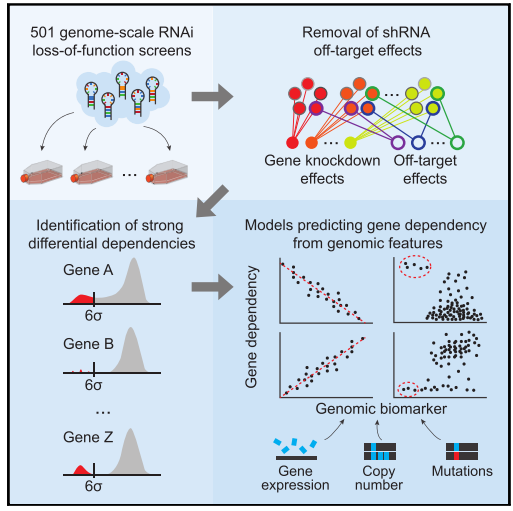


Martínez-Reyes & Chandel; *Nat. Commun.* (2020)

DepMap project

dependency map
Consortium

- **Project goal:** Systematic identification of cancer dependencies
- Genome-scale loss-of-function screens performed in diverse human cancer cell lines
- Dependency score: Function of both the magnitude of the differential dependency and its prevalence in cell line collection



Tsherniak *et al.* *Cell* (2017)

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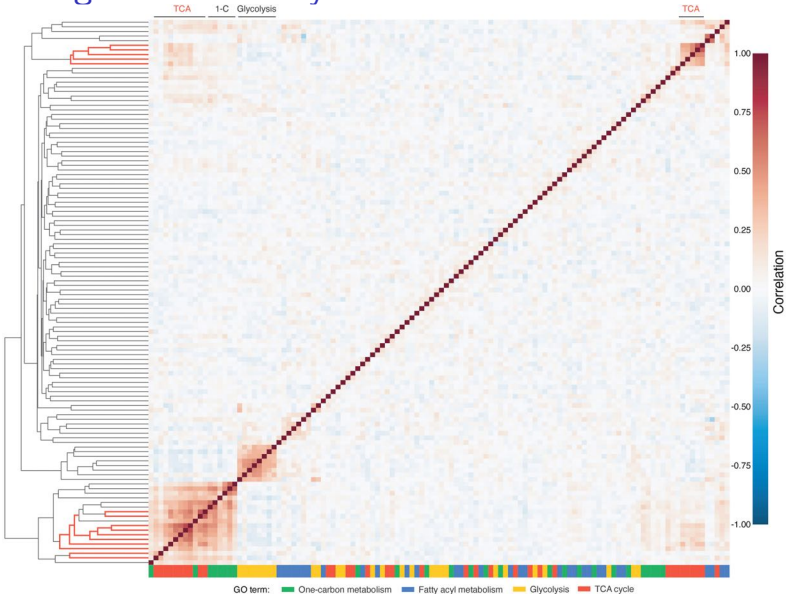
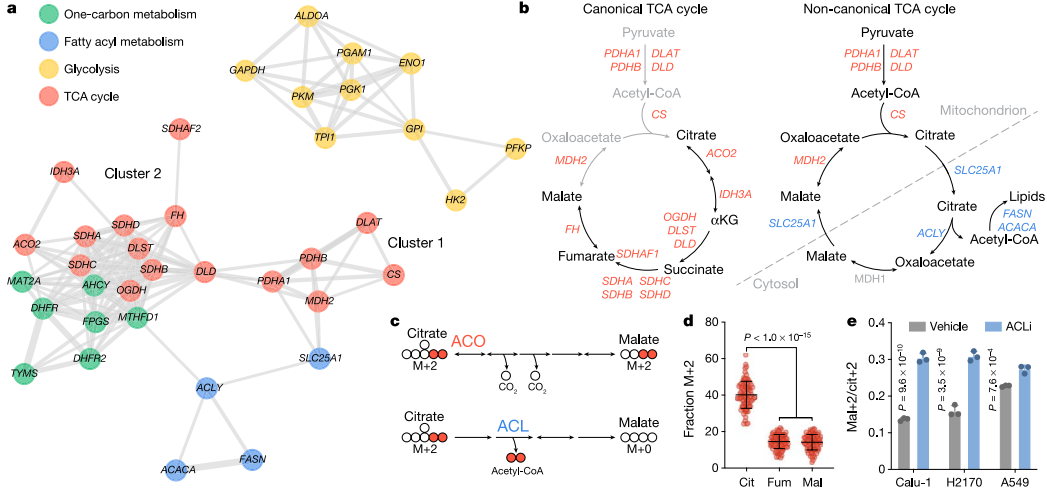
Metabolic gene essentiality correlations across cancer cell lines



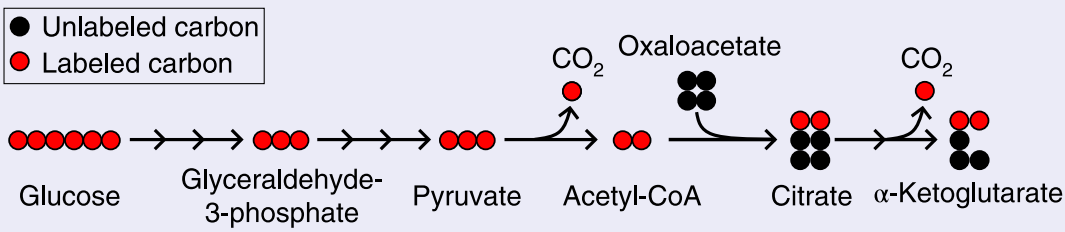
Two modes of TCA cycle metabolism



ACO: aconitase; ACL: ATP citrate lyase

Two modes of TCA cycle metabolism

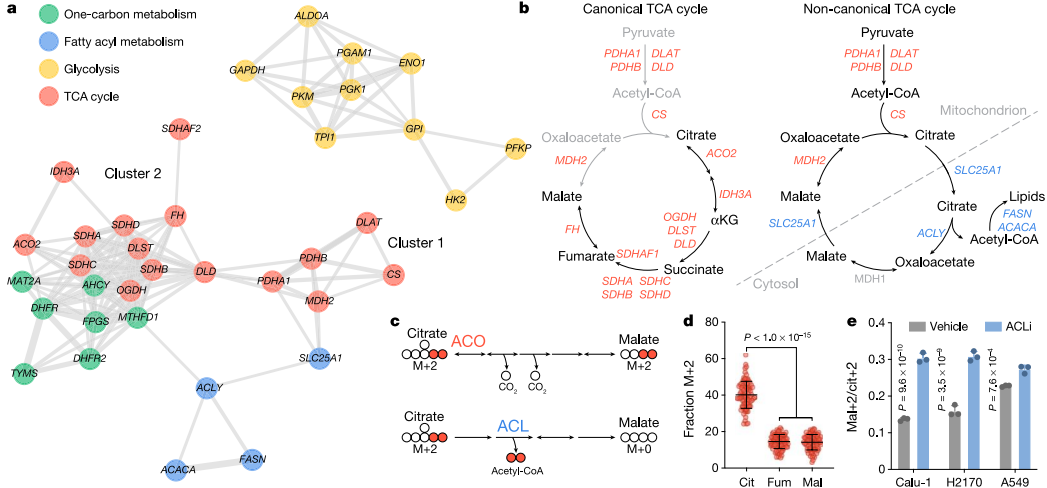
Stable isotope tracing:



Llufrio, Cho & Patti; *Nature Protocols* (2019)

ACO: aconitase; ACL: ATP citrate lyase

Two modes of TCA cycle metabolism



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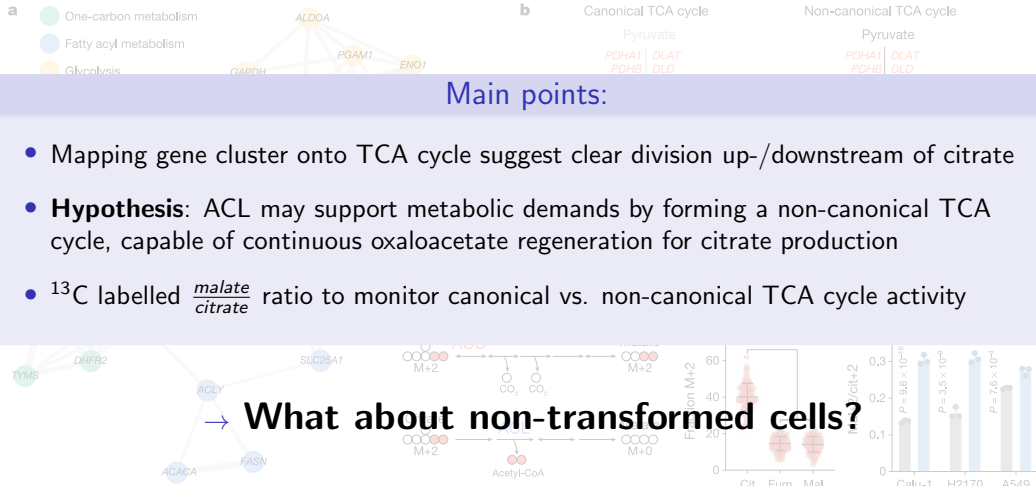
Two modes of TCA cycle metabolism



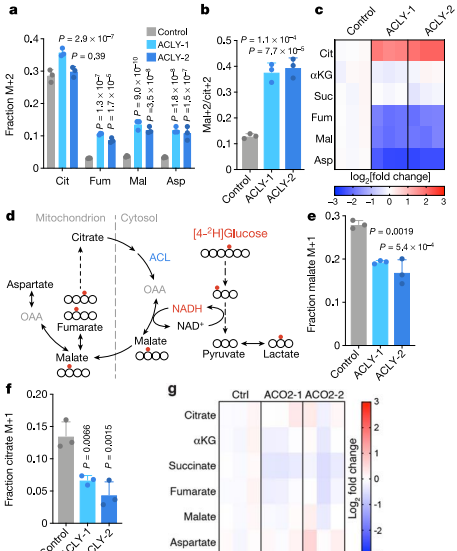
Main points:

- Mapping gene cluster onto TCA cycle suggest clear division up-/downstream of citrate
- **Hypothesis**: ACL may support metabolic demands by forming a non-canonical TCA cycle, capable of continuous oxaloacetate regeneration for citrate production
- ^{13}C labelled $\frac{malate}{citrate}$ ratio to monitor canonical vs. non-canonical TCA cycle activity

→ **What about non-transformed cells?**

ACO: aconitase; ACL: ATP citrate lyase

Embryonic stem (ES) cells engage a non-canonical TCA cycle

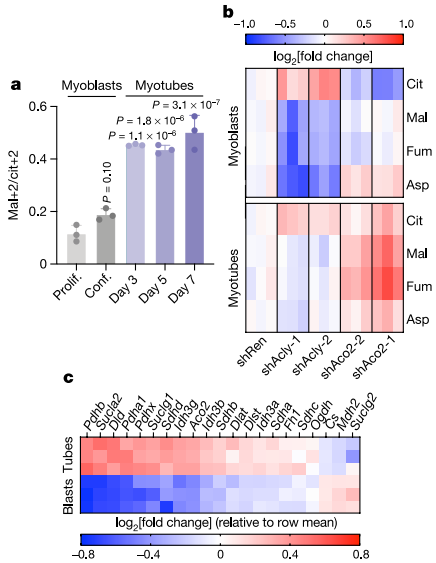


- ES cell line with genetic disruption of Acly and Aco2
- Acly mutation substantially alters levels of TCA cycle metabolites associated with cytosolic citrate processing

→ Does a portion of the TCA cycle flow through ACL?

- Deuterated [4-^{2}H] glucose tracing
- **Cytosolic malate is recycled back into the mitochondria for citrate regeneration**

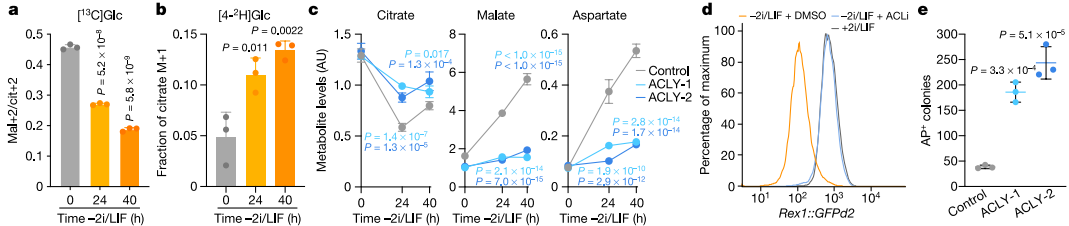
TCA cycle choice is cell-state dependent



- TCA cycle choice in myoblasts and differentiated myotubes
- **Myotubes:** increased incorporation of glucose-derived carbons into TCA cycle intermediates
- ACL inhibition effect stronger in myoblasts
- ACO inhibition effect stronger in myotubes

→ **When does the TCA cycle switch occur during differentiation?**

Exit from pluripotency requires ACL



- TCA cycle switch after pluripotency exit
- Differentiated cells rely on the non-canonical TCA cycle to maintain TCA cycle intermediates
- TCA cycle configuration plays a role in facilitating cell state transitions

Summary

→ Combining isotope tracing with genetic manipulation of Acly and Aco2 provide direct evidence of a non-canonical TCA cycle with differential activity across cell states

→ **Main advantages:**

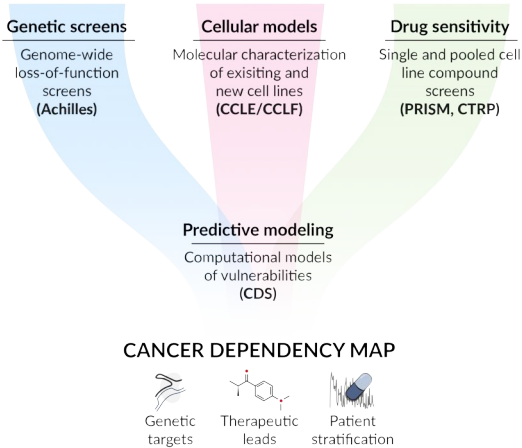
- Retain rather than combust reduced carbon and regenerate cytosolic NAD^+ required to sustain glycolysis
- Non-canonical TCA cycle maintains oxaloacetate regeneration by circumventing several TCA cycle steps which minimizes mitochondrial NADH production

→ Starting hypothesis based on genetic co-essentiality mapping database **DepMap**

Cancer Dependency Map

dependency map
Consortium

- **Project goal:** Systematic identification of genetic and pharmacologic cancer dependencies and the biomarkers that predict them



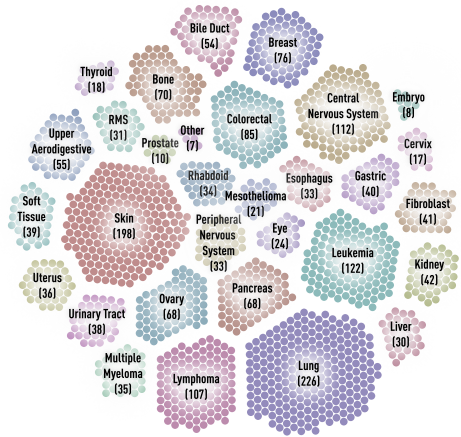
depmap.org

Cancer Dependency Map

- Achilles project 22Q2 release: 17,387 genes were screened in 1086 cell lines

→ **R** Bioconductor package: "depmap"

→ **Python** PyPI package: "depmap-downloader"



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DepMap Data Explorer