A person is standing on a rocky mountain peak in the foreground, looking out over a vast, golden-hued sea of clouds. In the background, a range of dark, rugged mountains with patches of snow stretches across the horizon under a clear sky.

Journal Club

Valentin Marteau

31.05.2022

Article

A non-canonical tricarboxylic acid cycle underlies cellular identity

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Check for updates

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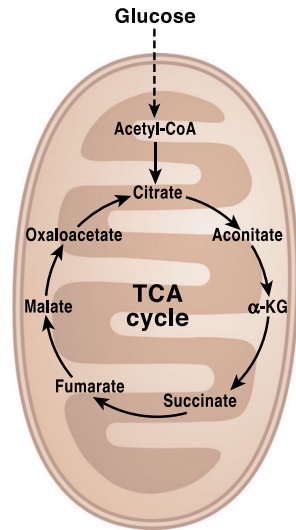
Aim

Tricarboxylic acid (TCA) cycle:

- Central hub of cellular metabolism important 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?**

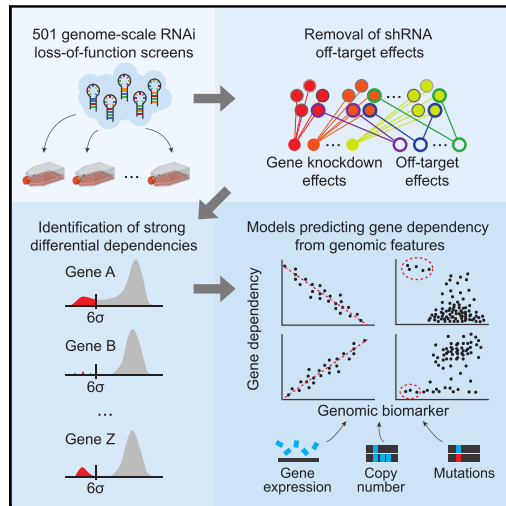


Martínez-Reyes & Chandel; *Nat. Commun.* (2020)

DepMap project

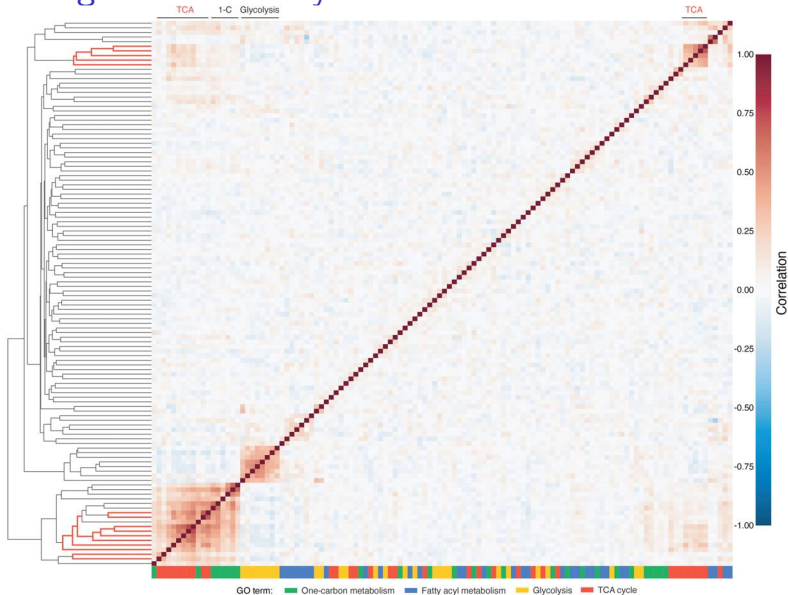


- **Project goal:** Systematic identification of cancer dependencies
- Genome-scale loss-of-function screens performed in diverse human cancer cell lines
- Dependency score: Function of both the magnitude of the differential dependency and its prevalence in cell line collection

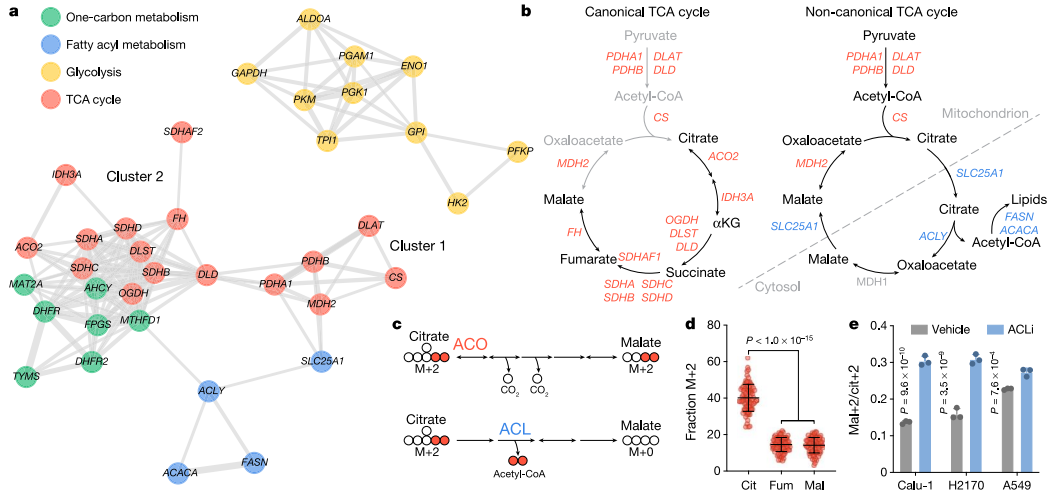


Tsherniak et al. Cell (2017)

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

- a**
- One-carbon metabolism
 - Fatty acyl metabolism
 - Glycolysis



b

Canonical TCA cycle

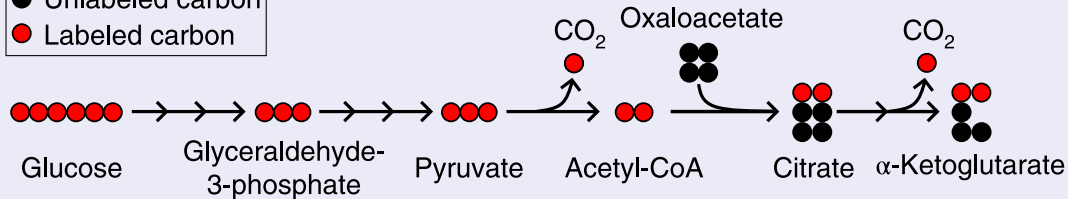
Pyruvate
PDHA1 PDHB
DLAT DLD

Non-canonical TCA cycle

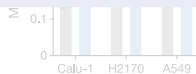
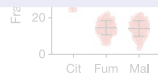
Pyruvate
PDHA1 PDHB
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Stable isotope tracing:

- Unlabeled carbon
- Labeled carbon

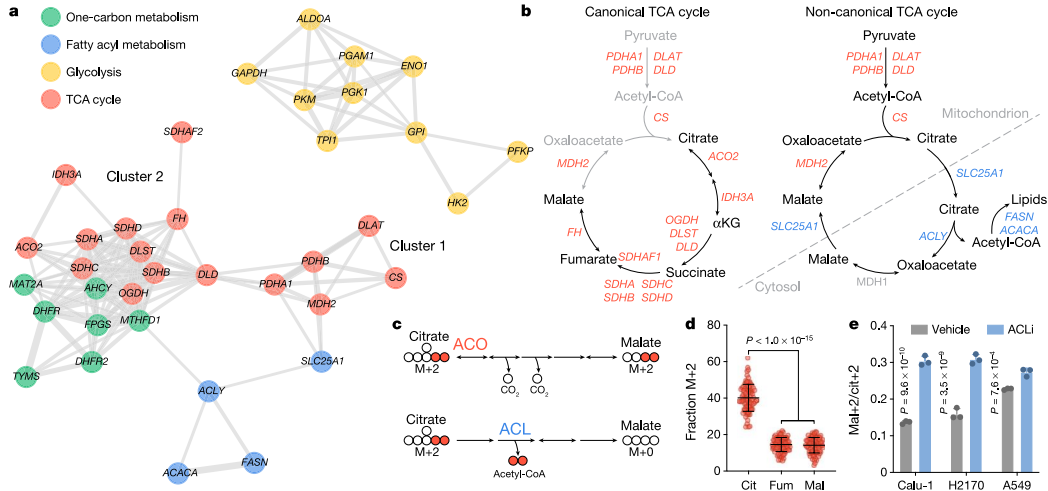


Llufrio, Cho & Patti; *Nature Protocols* (2019)



ACO: aconitase; ACL: ATP citrate lyase

Two modes of TCA cycle metabolism



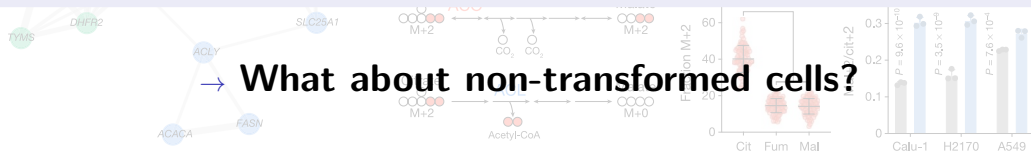
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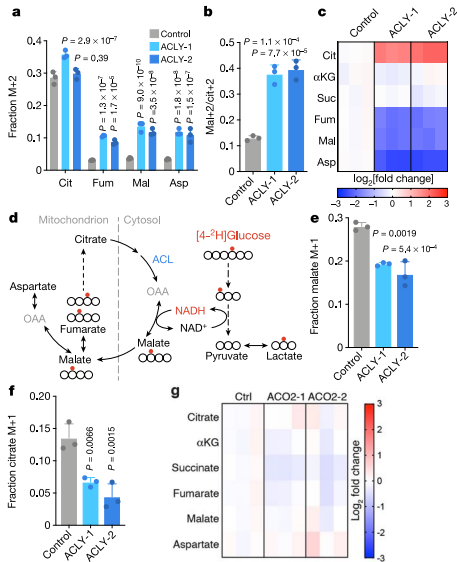


Main points:

- 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 $\frac{\text{malate}}{\text{citrate}}$ ratio to monitor canonical vs. non-canonical TCA cycle activity

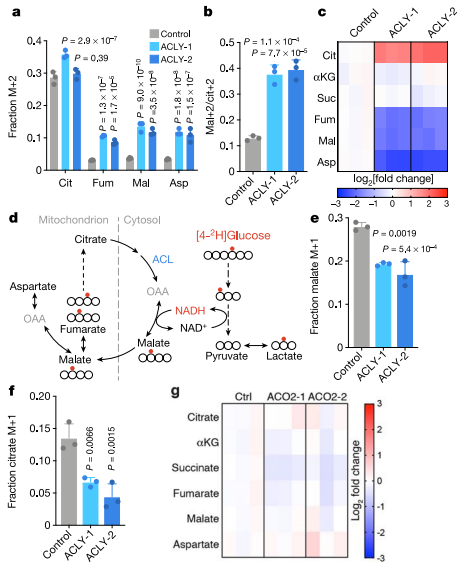


Embryonic stem (ES) cells engage a non-canonical TCA cycle



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

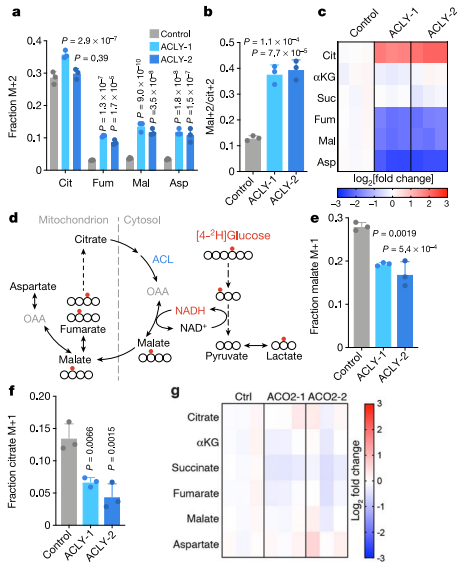
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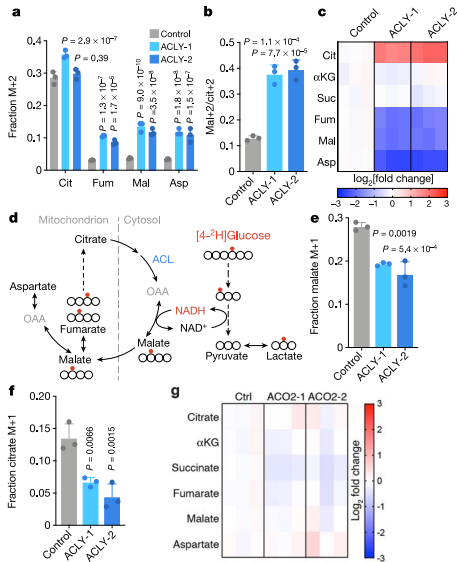
→ Does a portion of the TCA cycle flow through ACL?

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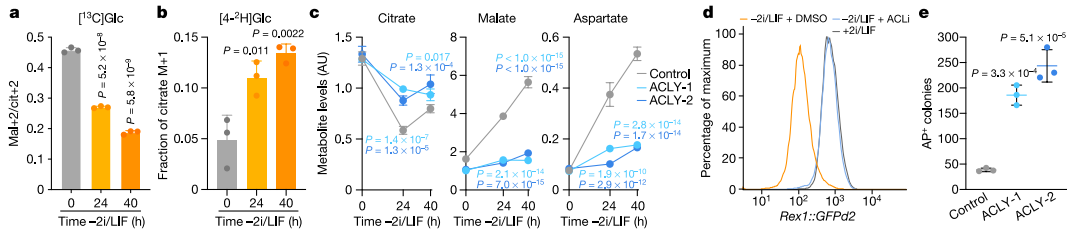
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- Deuterated $[4-^2\text{H}]$ glucose tracing

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

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

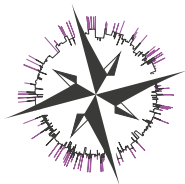
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- **Main advantages:**
 - Retain rather than combust reduced carbon and regenerate cytosolic NAD^+ required to sustain glycolysis
 - Non-canonical TCA cycle maintains oxaloacetate regeneration by circumventing several TCA cycle steps which minimizes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

Summary

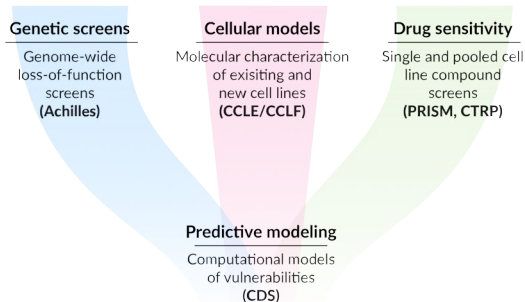
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- **Main advantages:**
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 - Non-canonical TCA cycle maintains oxaloacetate regeneration by circumventing several TCA cycle steps which minimizes mitochondrial NADH production
- Starting hypothesis 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



CANCER DEPENDENCY MAP



Genetic targets



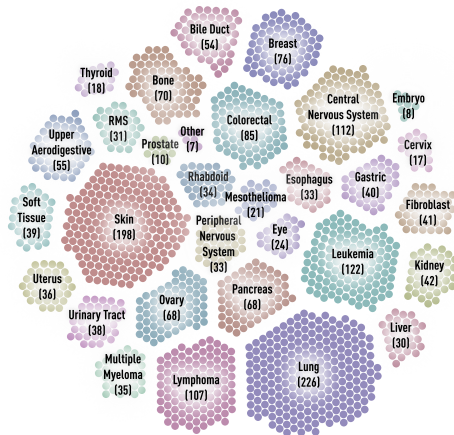
Therapeutic leads




Patient stratification

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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