Metabolic Dynamics at the Transition Between Growth and Non-Growth (all *E. coli*)

- pulsing glucose at or below growth supporting rates
- role of glycogen

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Collaboration:
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Roman Stocker (ETH)

ETH Zürich

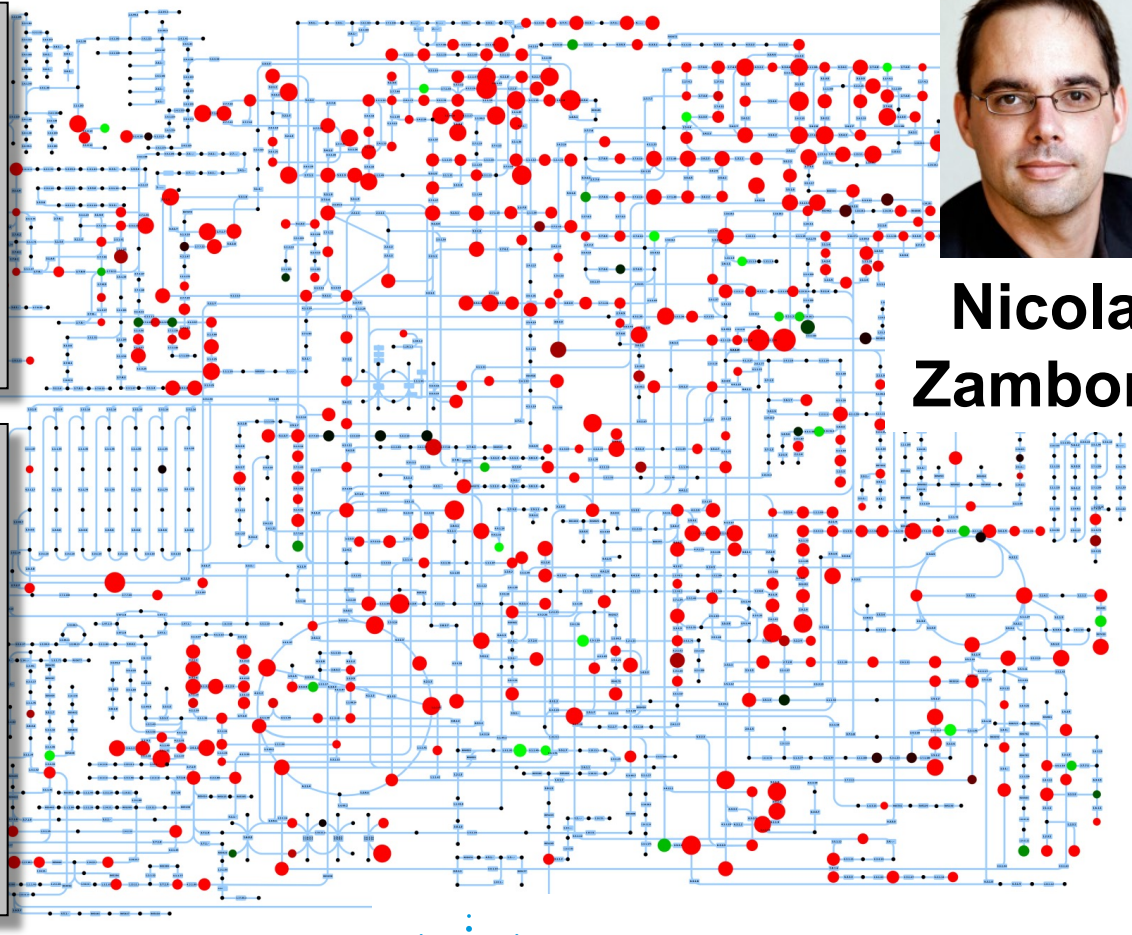
High-Throughput Metabolomics FIA-TOF
(400-800 metabolites per sample/minute)

HTP Metabolomics

- 2000 samples/day
- semi-quantitative
- 400-800 annotated metabolites

Shortcomings

- matrix must be constant
- annotation purely mass based >0.001 Da
- cannot distinguish compounds with identical mass



Nicola Zamboni

Supported by **Agilent Technologies**

Exploiting Dynamic Data
Automated Real-Time Metabolomics

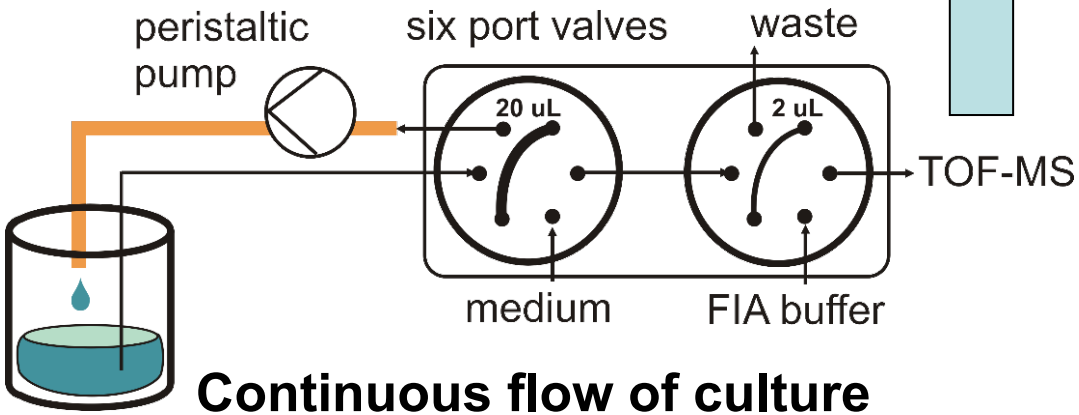
6550 Q-TOF

- **200-300 metabolites**
- **dynamic resolution 6x per minute**

2 µl

cells + medium

must be low salt!



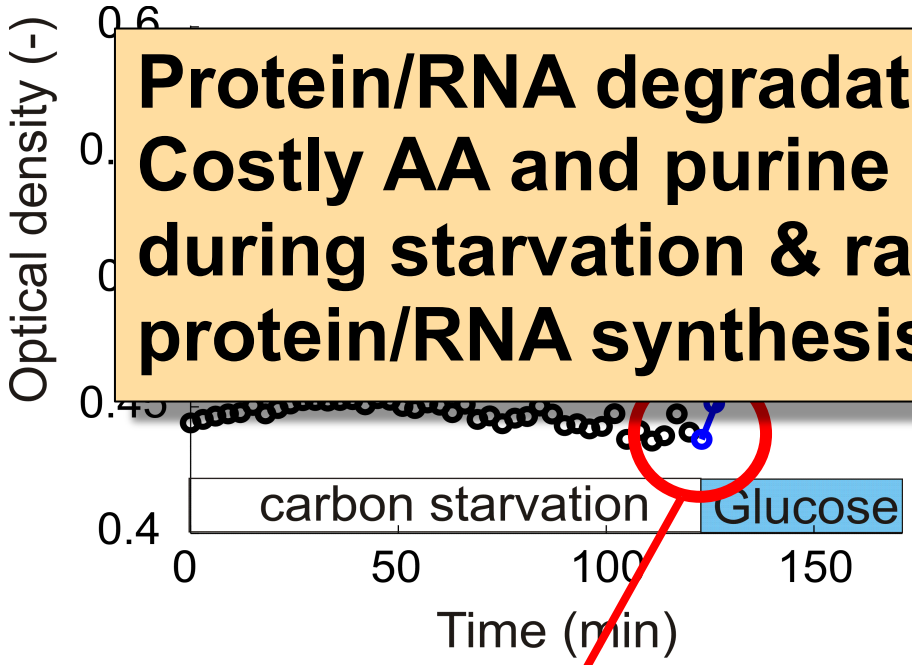
Continuous flow of culture through 20 µl loop

Hannes Link

Tobias Fuhrer

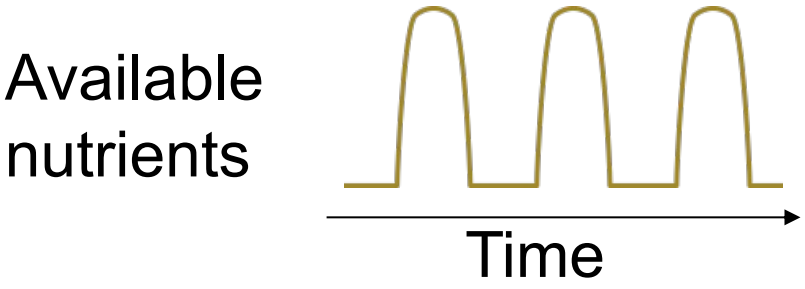
How Does *E. coli* Exit Rapidly from Stationary Phase ?

Protein/RNA degradation:
Costly AA and purine nucleotides "stored" during starvation & rapidly utilized for protein/RNA synthesis upon growth.



Role of metabolites in the switch from starvation to growth?

Sporadically Available Nutrients are a Reality for Microbes

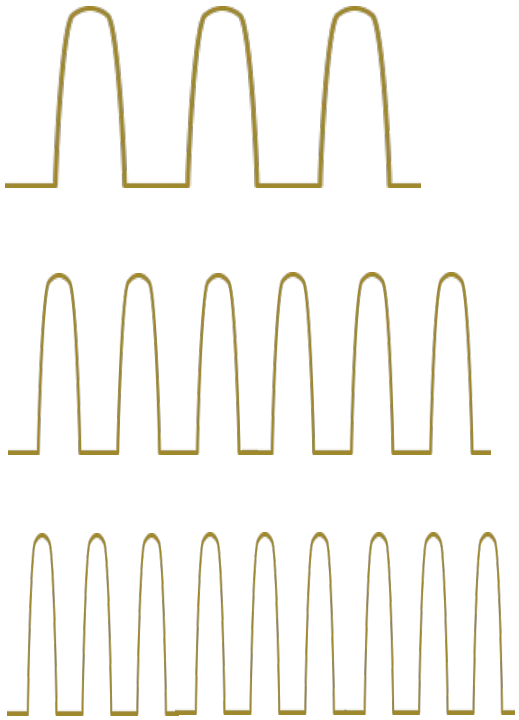


What happens to incoming nutrient pulses ?

How do cells store intermittent carbon in absence of growth?

Karthik Sekar

Experimental Design



modulate glucose feed by pulse frequency

Grow to OD 0.8-1.0

↓

Resuspend in diluted minimal medium w/o glucose

↓

starve for 2 h

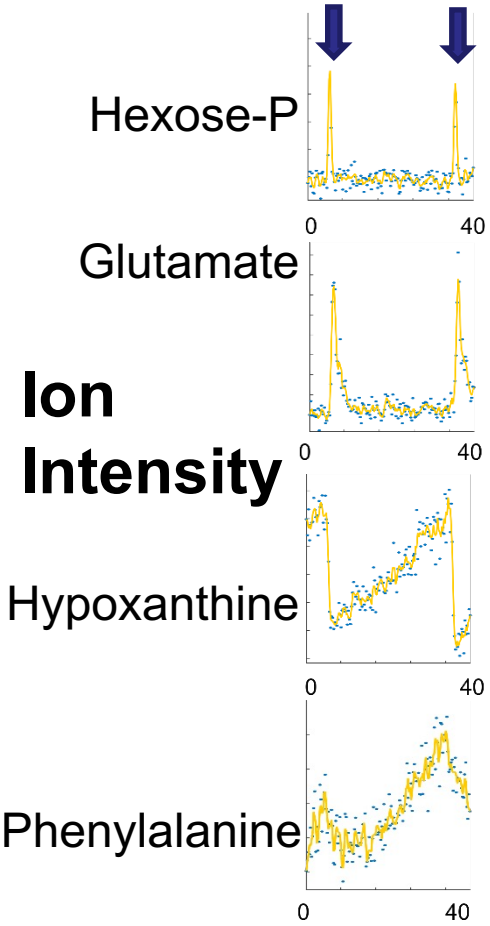
↓

Pulse glucose

Glucose Pulses to Non-Growing *E. coli*

Low frequency glucose pulses that do NOT support growth (**> 0.23 mmol g^{-1} h^{-1}**) build up biomass precursor

Pulses 0.06 0.12 0.18 mmol/g_{cells} h



Hypothesis:

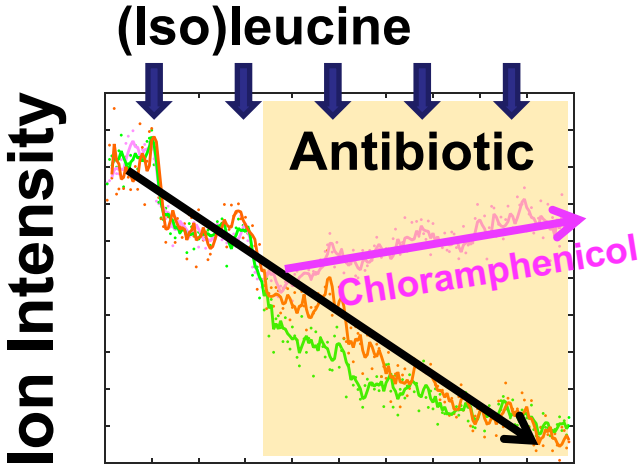
Carbon pulses

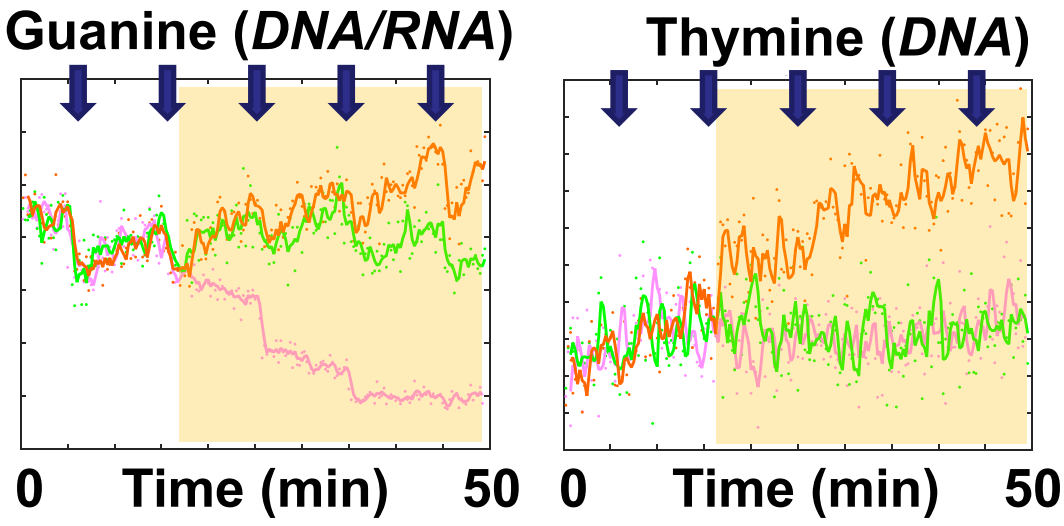
- gush through central metabolism
- accumulate as cellular macromolecules – even without growth !

Protein synthesis

Antibiotics Confirm Macromolecule Formation w/o Growth

Pulsing at 0.18 mmol/g/h (non-growth regime)

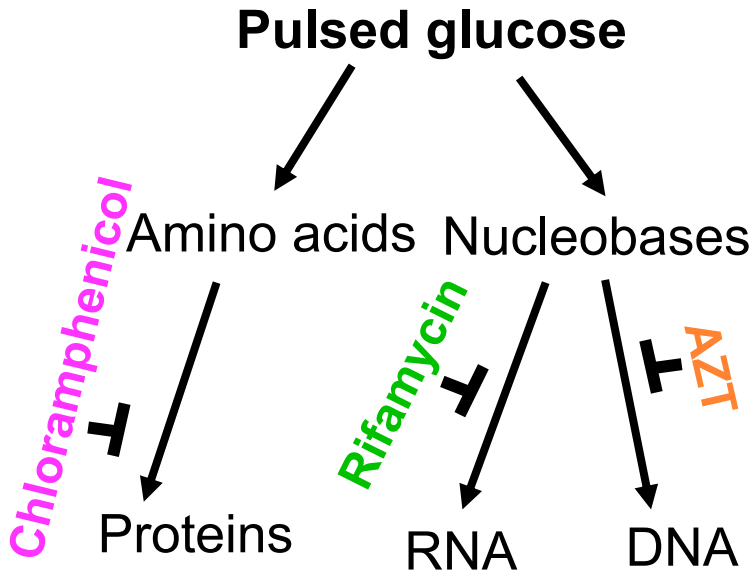




Chloramphenicol

Rifamycin

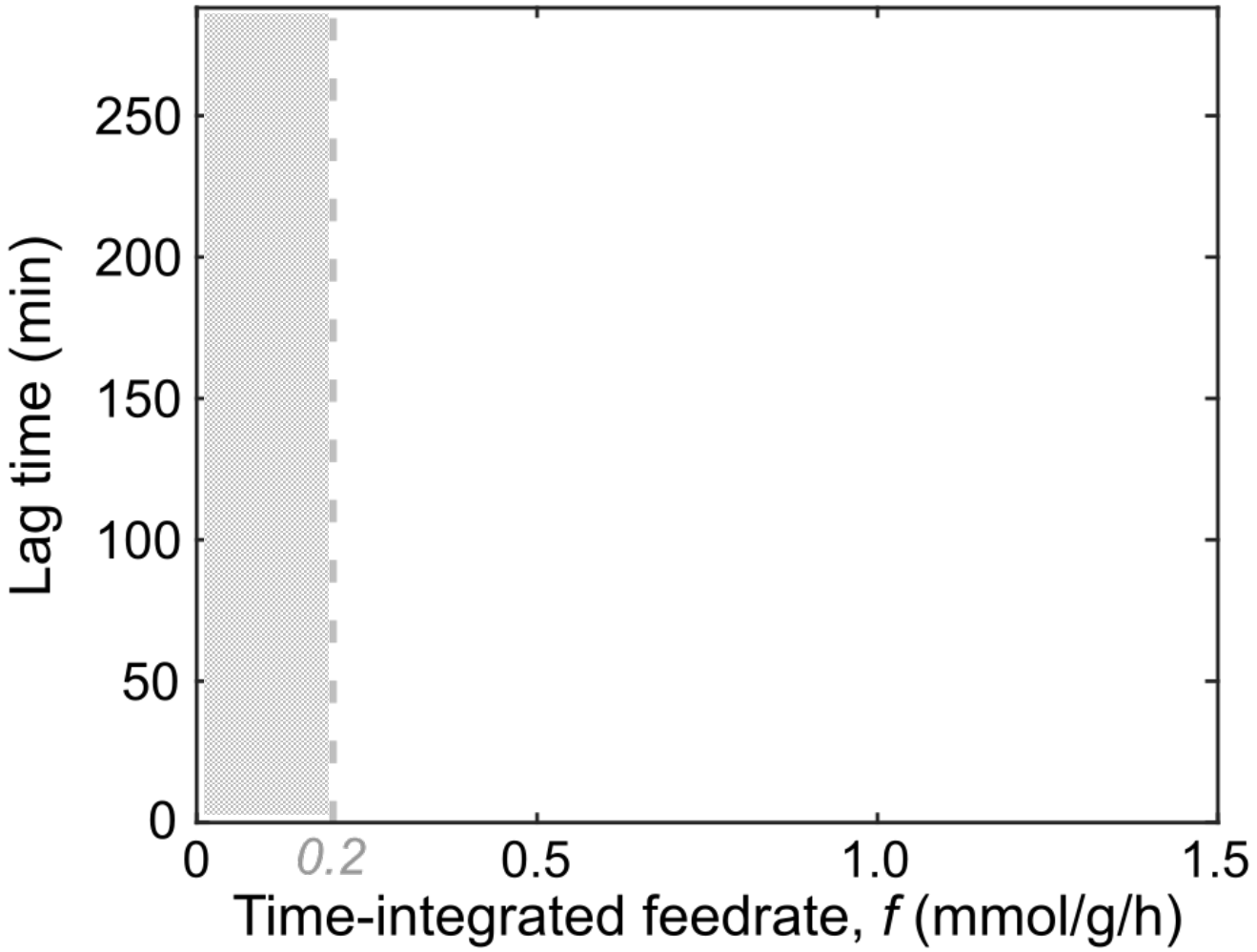
AZT



- Non-growing *E. coli* funnel pulse-fed glucose immediately into **AA/nucleotide synthesis**
- At higher pulse frequency (well before initiation of growth) **protein and RNA/DNA synthesis** starts
  - because AA/nucleotides decrease with each pulse
  - blocking synthesis prevents the monomer decrease
  - we observed ^{13}C-label incorporation into DNA & protein (data not shown)

What happens when we pulse faster?

Lag phase depends on time-integrated (pulse) feedrate



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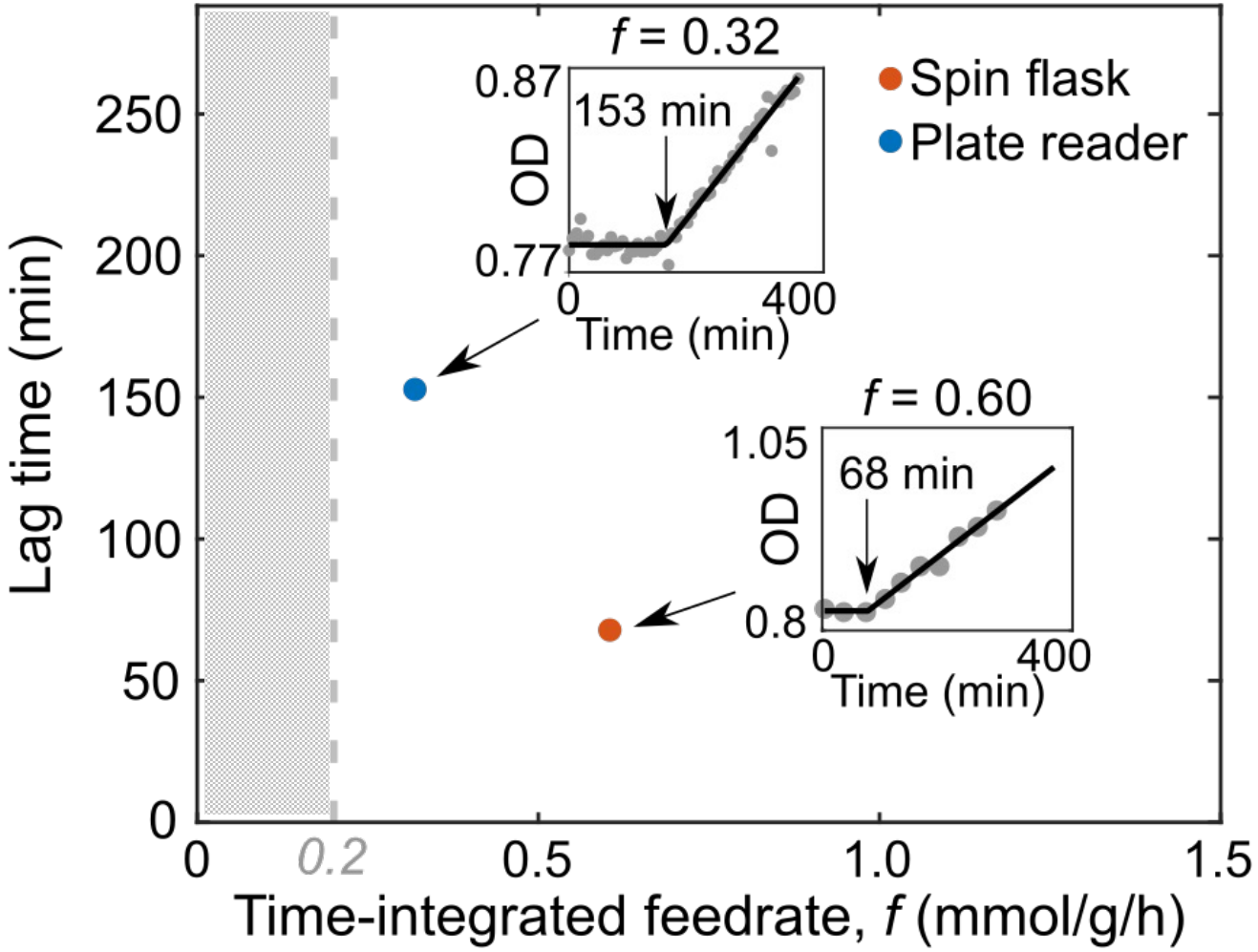

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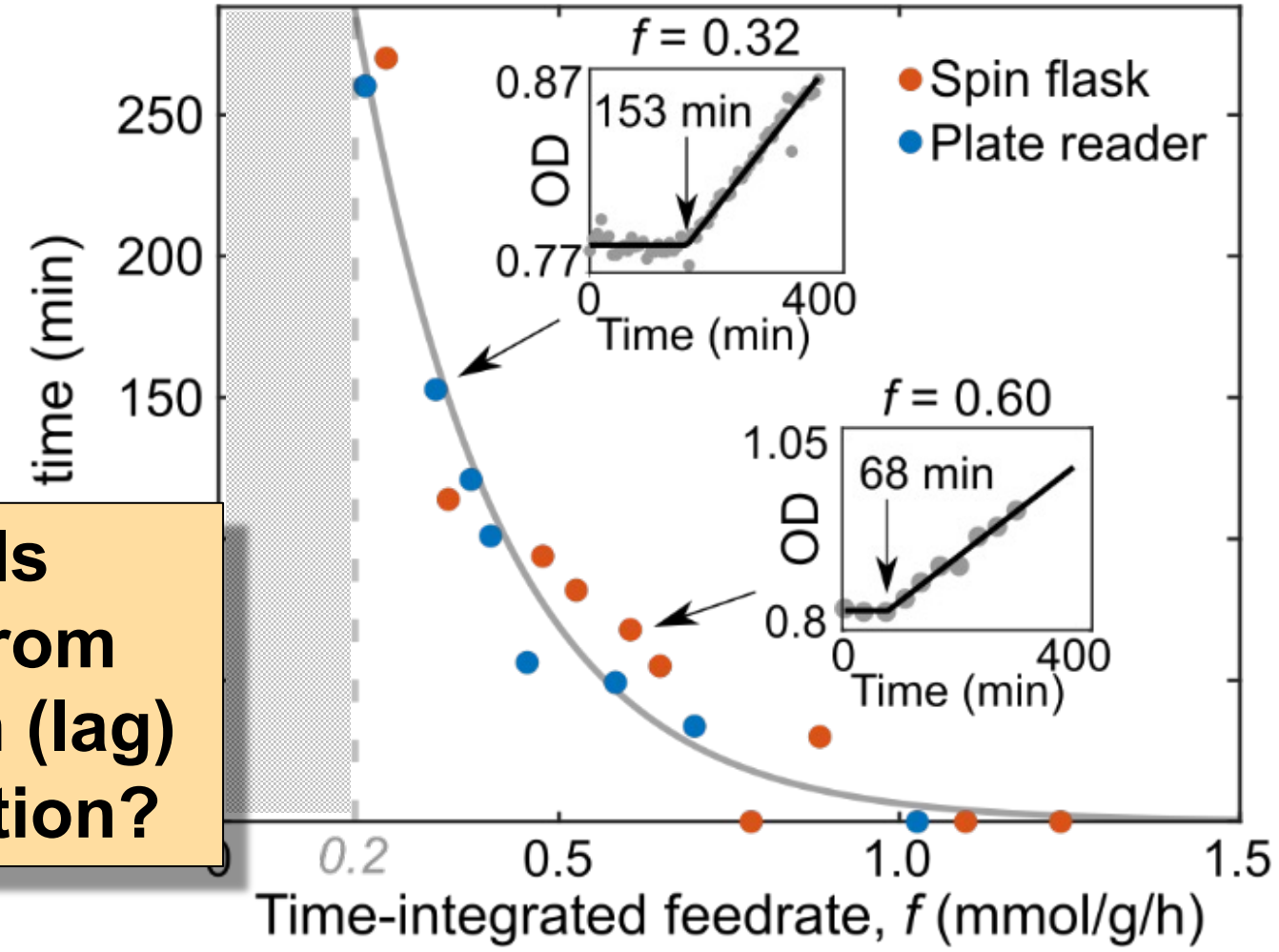


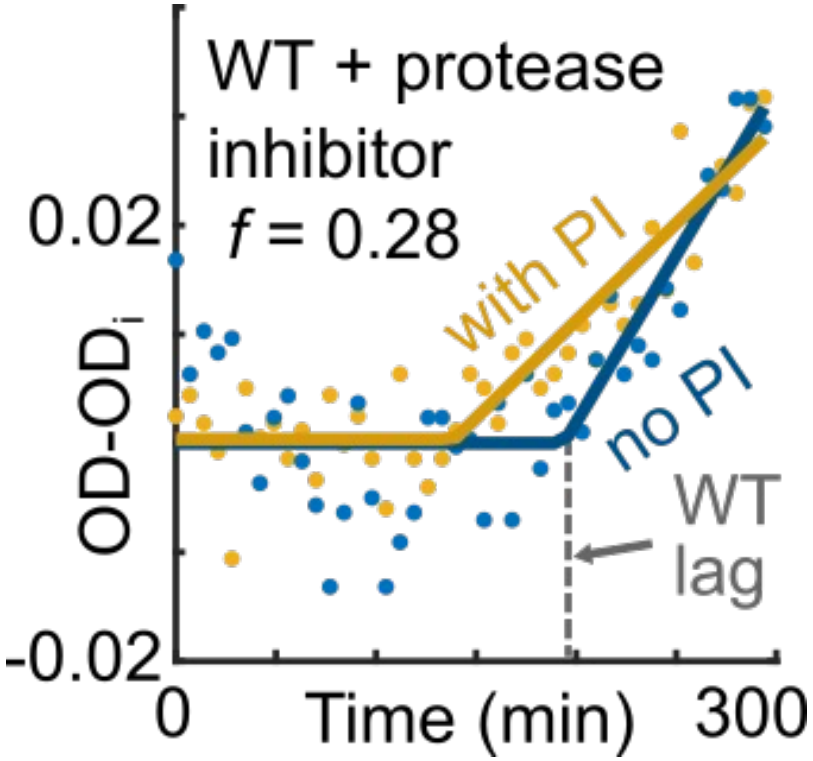
How do cells transition from non-growth (lag) to proliferation?

How do cells transition from non-growth (lag) to proliferation?

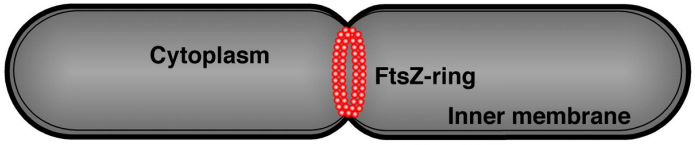
- There is already active macromolecule synthesis even w/o growth!
- HYPOTHESIS: one or few macromolecule species limit cell division stoichiometrically!
- Since proteins comprise largest share of carbon it is likely a protein ... (?)
- Most parsimonious explanation: the hypothetically limiting protein is **synthesized after pulse** and **degraded in intervening starvation** ....

Inhibiting protein degradation shortens lag phase!

Adding cocktail of protease inhibitors at onset of pulse-feeding

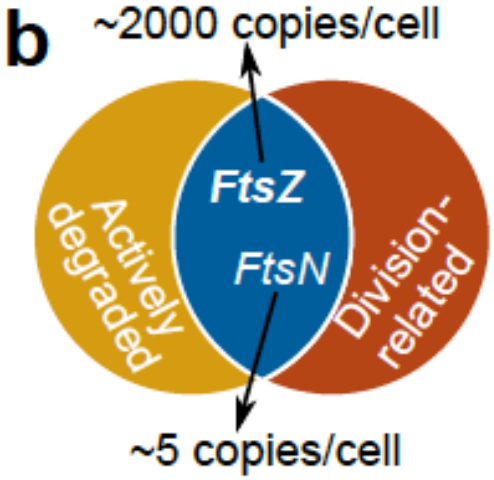


of all cell division proteins only FtsZ and FtsN are subject to active degradation

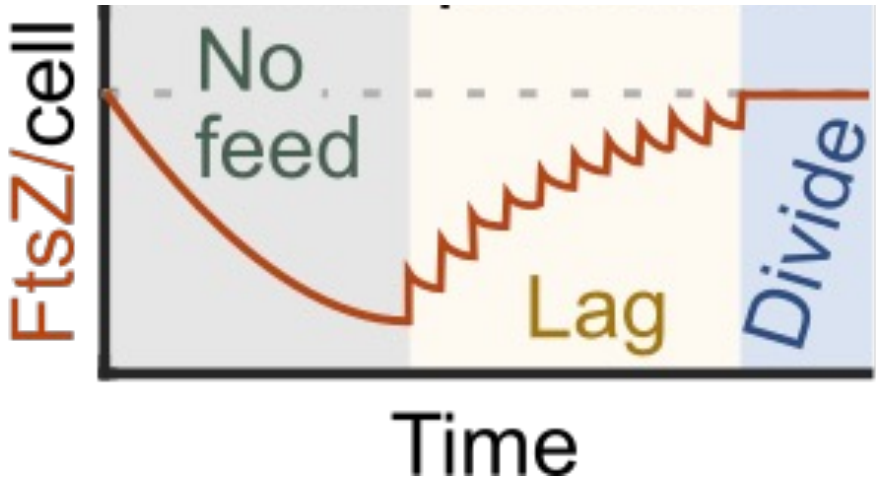


FtsZ ring

Ganhui Lan et al. PNAS 2007;104:16110-16115

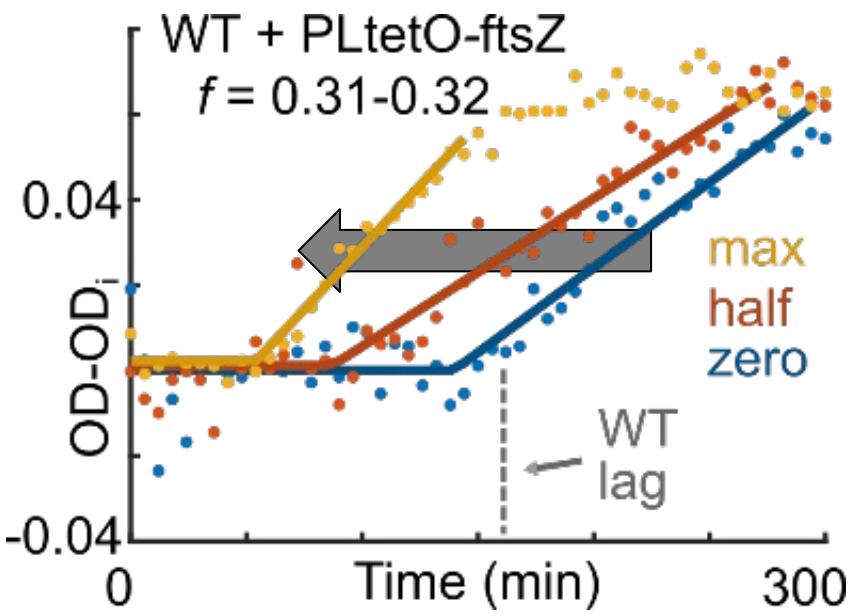


Hypothesis:
FtsZ is synthesized during pulses and slowly degraded between pulses

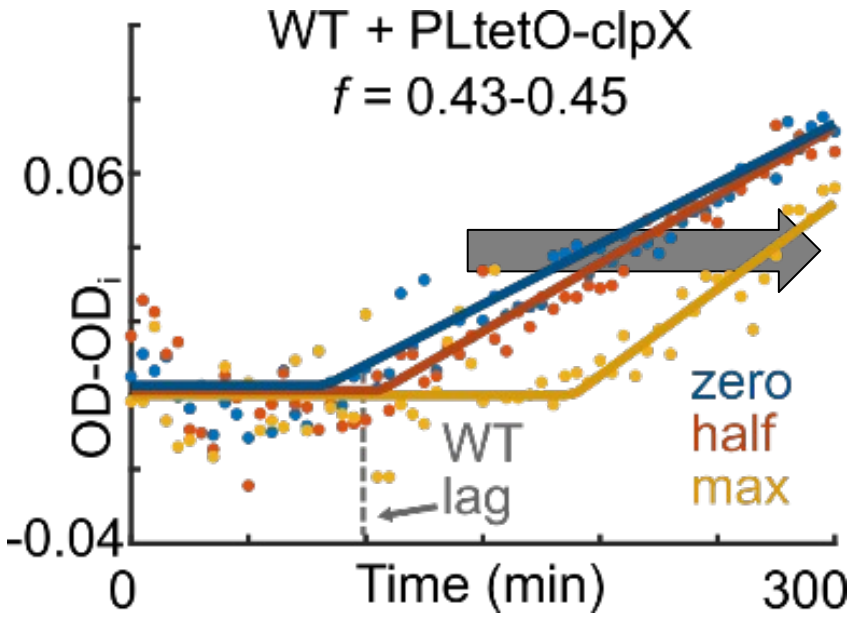


FtsZ or Protease Overexpression Shorten/Lengthen Lag Phase

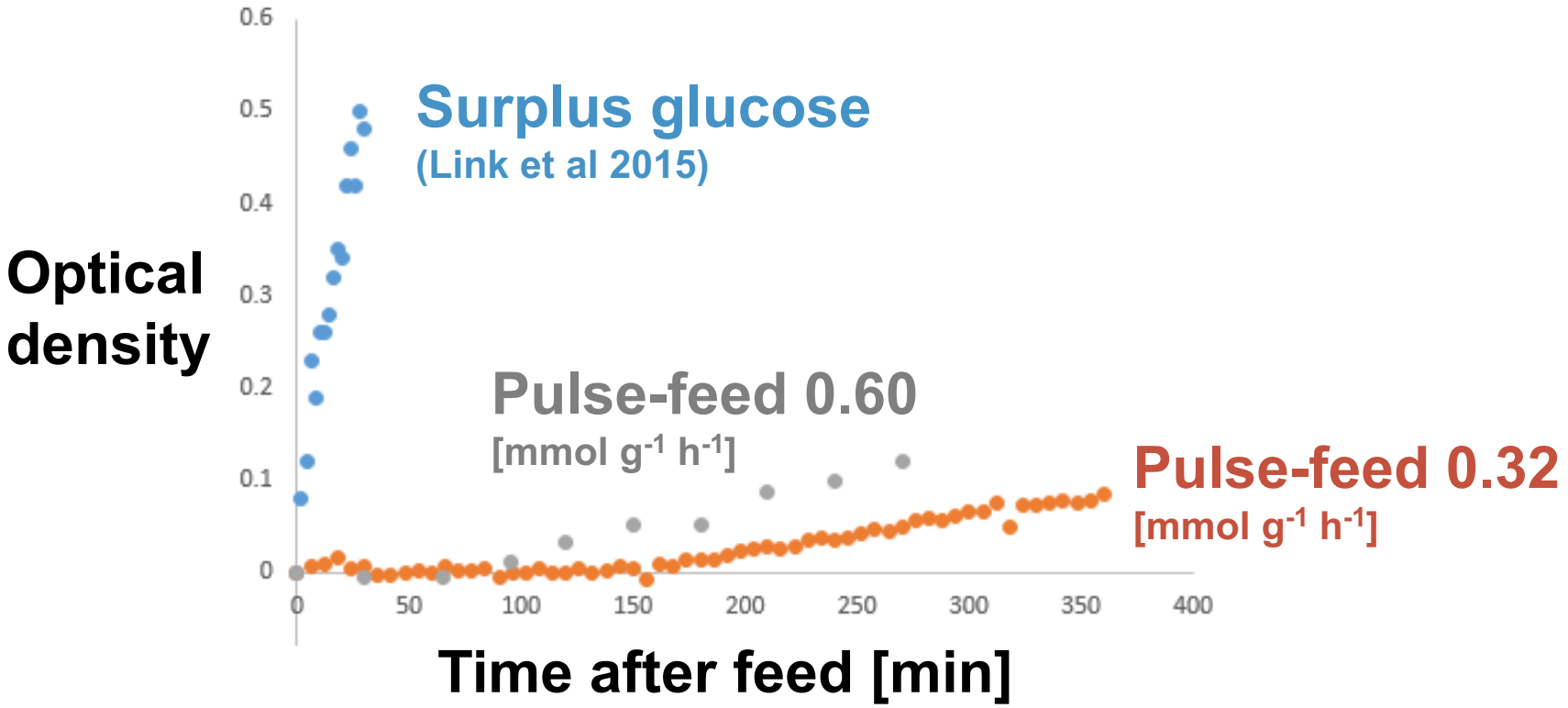
FtsZ overexpression



ClpX protease overexpression

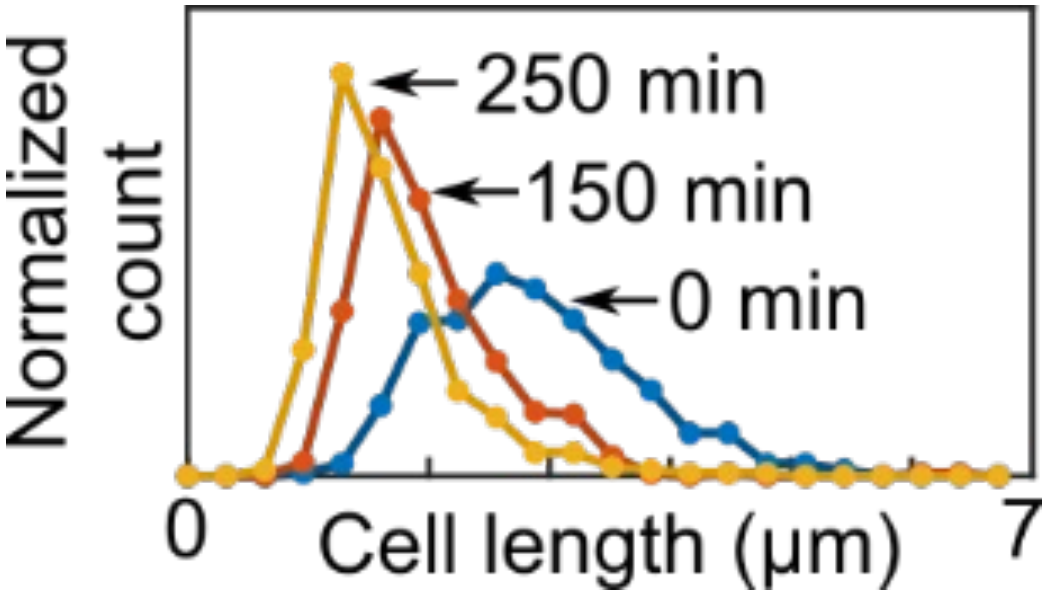


How do Cells Divide With (little) Pulse-Fed Carbon?



Essentially no increase in biomass .....

Average Cell Size Decreases After Lag Phase



Flow cytometry shows a shift from 2 N DNA population to 1 N.

Low, pulse amplitudes that just support some OD increase enable started cell divisions to conclude.

Conclusions on pulsing glucose at or below growth supporting rates

- Even minute carbon pulses gush through central metabolism into biomass monomers (eg AA)
- Pulse-fed carbon is assimilated into protein, RNA, DNA even **well before growth**
- FtsZ is the decisive element for **FIRST** cell division
  the adder model works only for exponential growth
- Once FtsZ synthesis exceeds the protease-based degradation, proliferation is initiated at the critical threshold!
- The FtsZ threshold model holds also for other C or N pulses (they are also shortened by FtsZ overexpression)
- FtsZ is ubiquitous in microbes!

Many habitats are characterized by periods of nutrient starvation, intermittently punctuated by nutrient availability. Maintaining **rapid growth capacity gives competitive advantage.**

General short-term strategies involve accumulation of unused resources that can be quickly activated, eg:

- maintaining a pool of ribosomes that become translationally active only as available nutrients become more abundant (*Metzl-Raz & Barkai 2017 eLife*);
- maintaining surplus enzymatic capacity beyond what is immediately required (*Davidi & Milo 2017 Curr Opin Biotech*);
- protein degradation and β-oxidation;
- accumulating costly amino acids from protein degradation during starvation (*Link et al 2015 Nat Meth*);
- ……
- **storage polymers**

The Role of Storage Polymers under Sporadic Nutrient Availability: Glycogen

- well established energy storage in mammals;
- in *E. coli* both long-term energy storage and temporary ressource are discussed;
- prevailing view: a nutrient “bank” from which cells withdraw and deposit energy (*Bertrand 2019 J Bacteriol*);
- **but for how long can glycogen supply energy and for which physiological processes is it used?**

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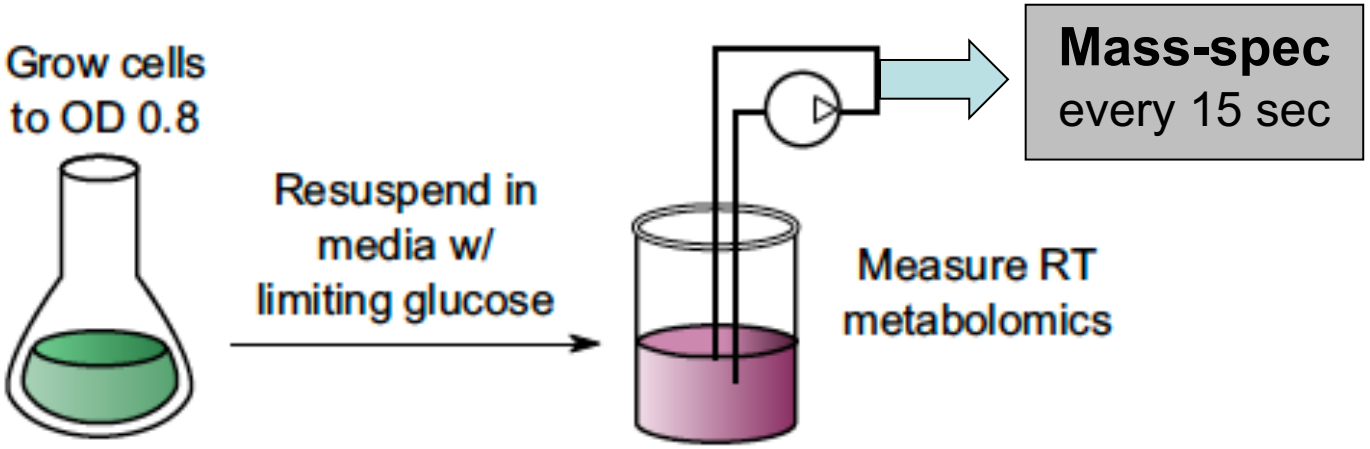
Gradual Glucose Starvation Experiment



mid-expo *E. coli* transferred into medium with glucose for 30-40 min

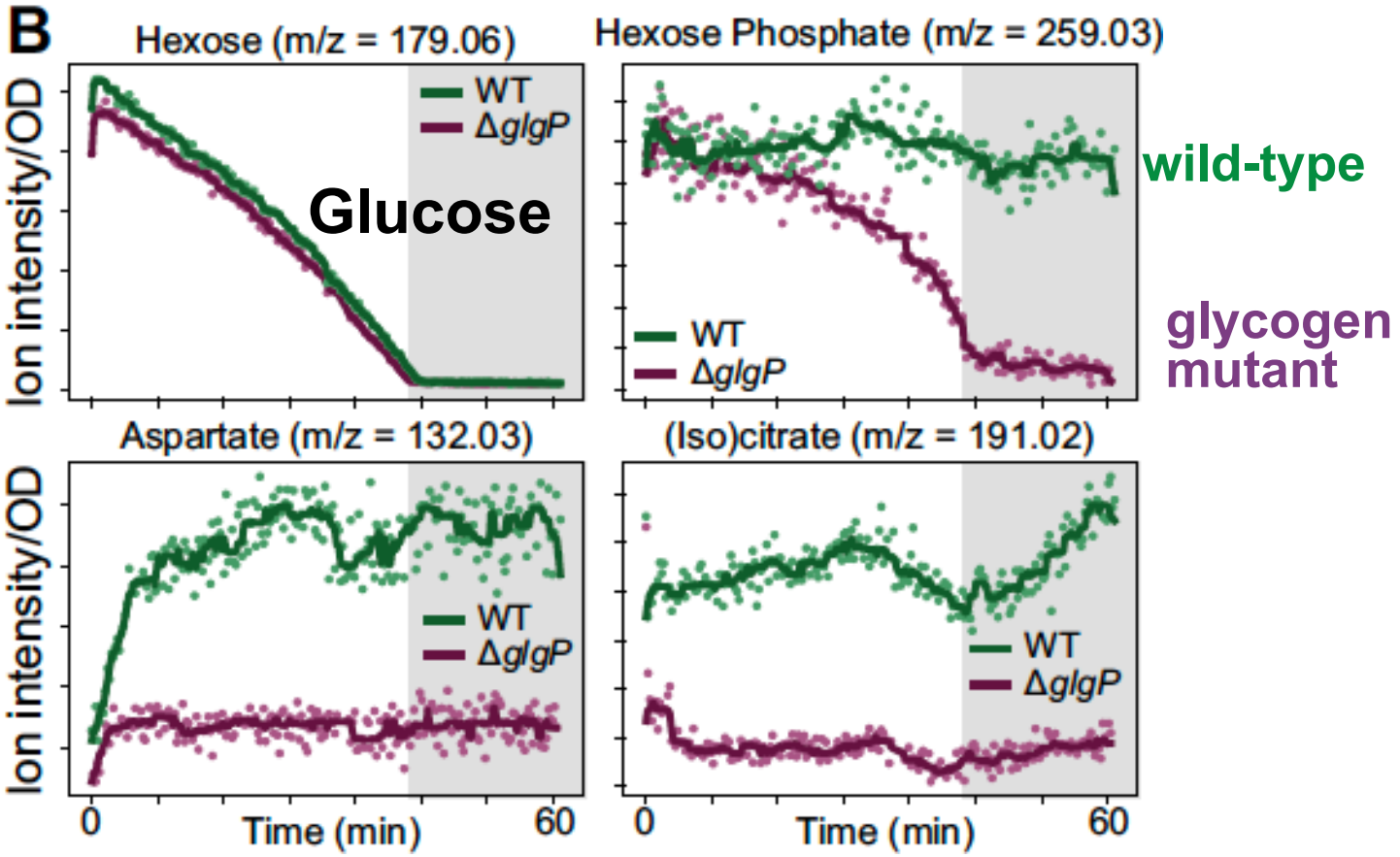
- wild-type
- Δ*glpP* unable to utilize glycogen

Many central metabolites drop right upon glucose depletion, but not all.

Big differences btw WT glycogen minus mutant.

Real-time Metabolome Response at Entry into Gradual Glucose Starvation

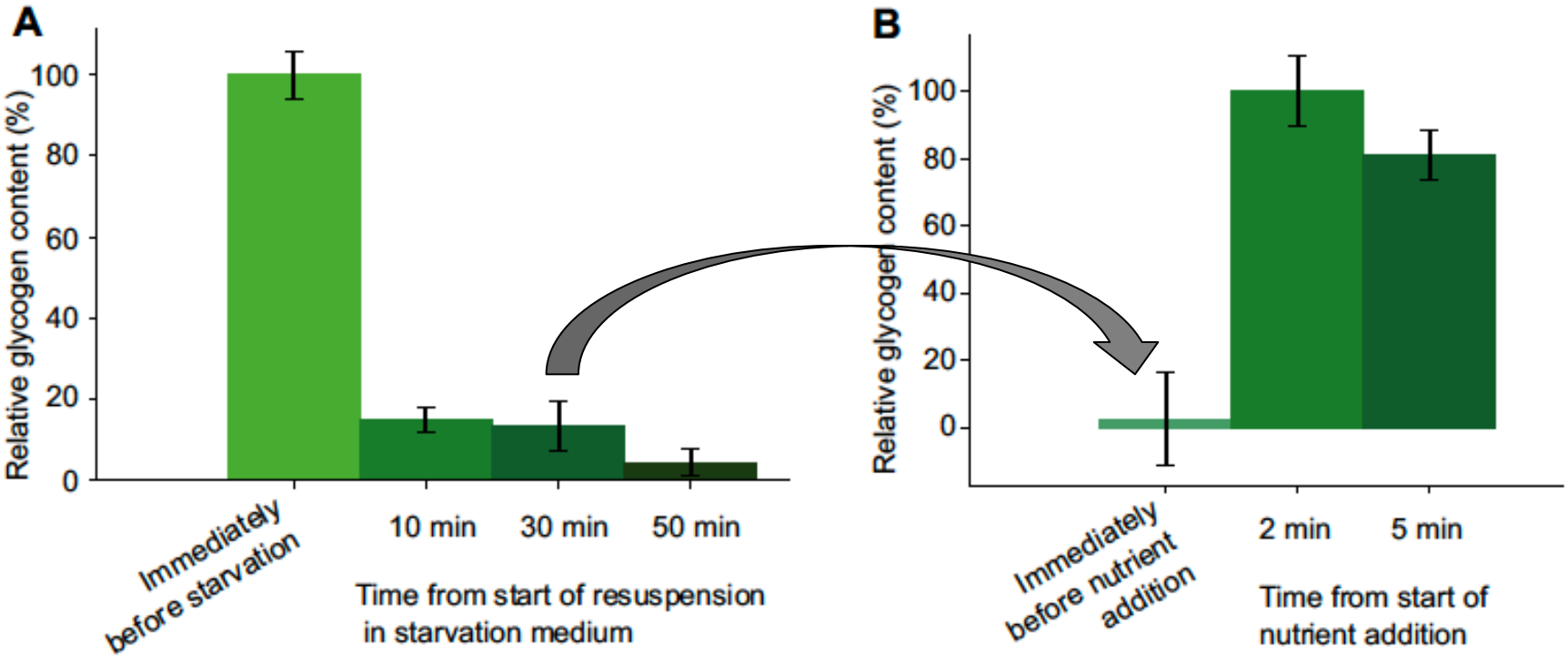
could be G6P, F6P ….. and/or G1P (from glycogen degradation)



Rapid Depletion and Synthesis of Glycogen: Short-term Energy Buffer

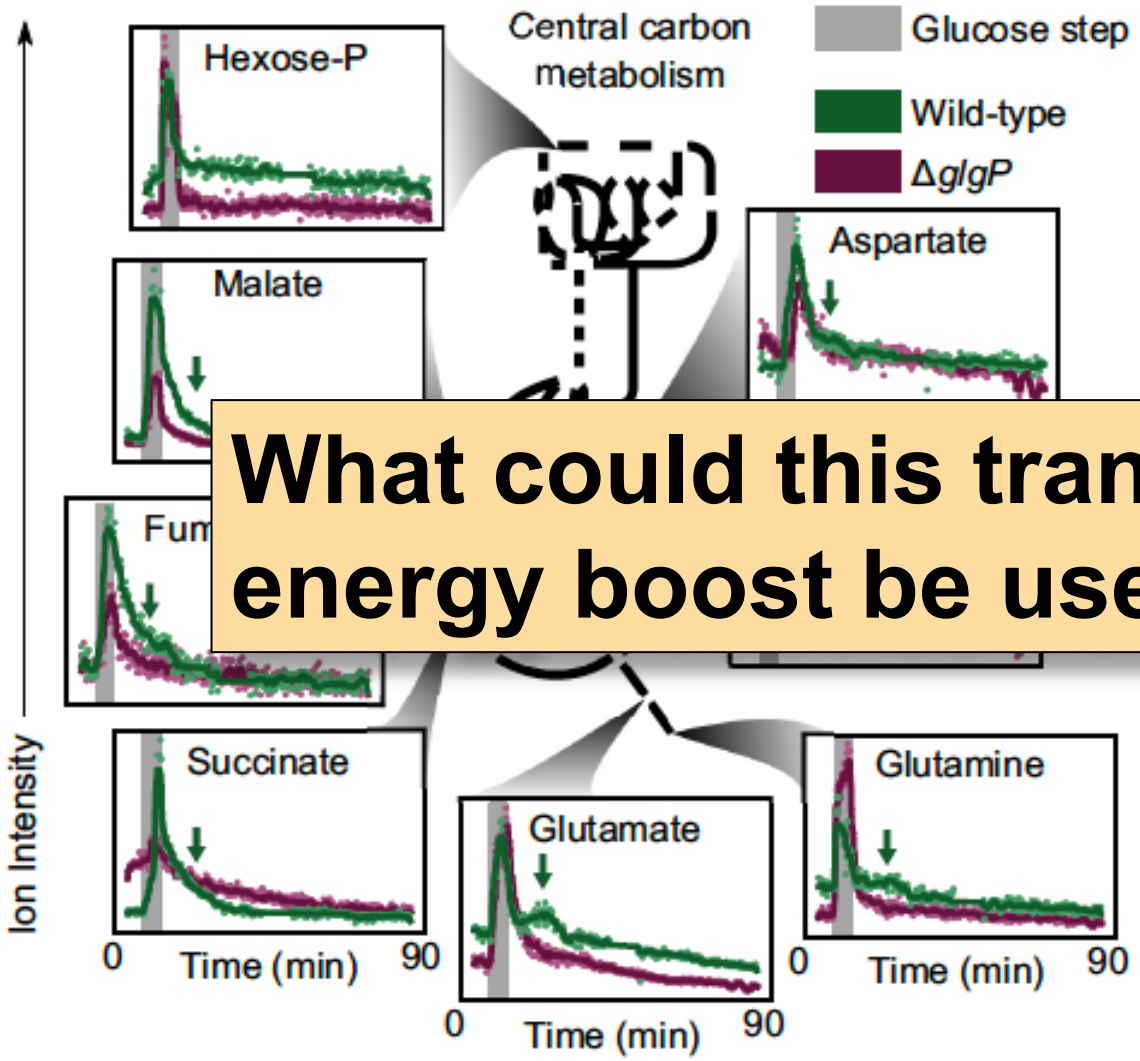
Within 10 min, 80% of glycogen depleted

Within 2 min, glycogen pool replenished



upon fructose addition to cells C-starved for 30 min

Sudden Glucose Depletion: Glycogen Seems to Fuel Respiration?



What could this transient energy boost be used for?

After 30 min glucose starvation, 5 min glucose feed below max uptake rate (i.e. 8 mmol/g_{cdw}) ***> nearly instantaneous glucose depletion.***

followed in 80 min.

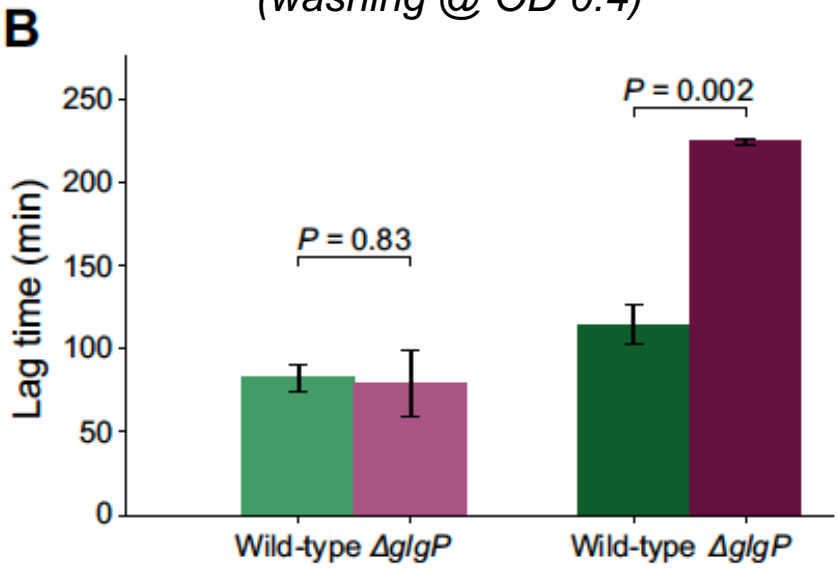
ter stop of ptake, many metabolites close to TCA-cycle bounce up again!

Transition btw Different Nutrients: Glycogen Shortens Lag

Steady state growth rate of all glycogen mutants +/- 10% of wild-type

Abrupt glucose -> acetate shift

(washing @ OD 0.4)



w/o starvation

with 30 min starvation

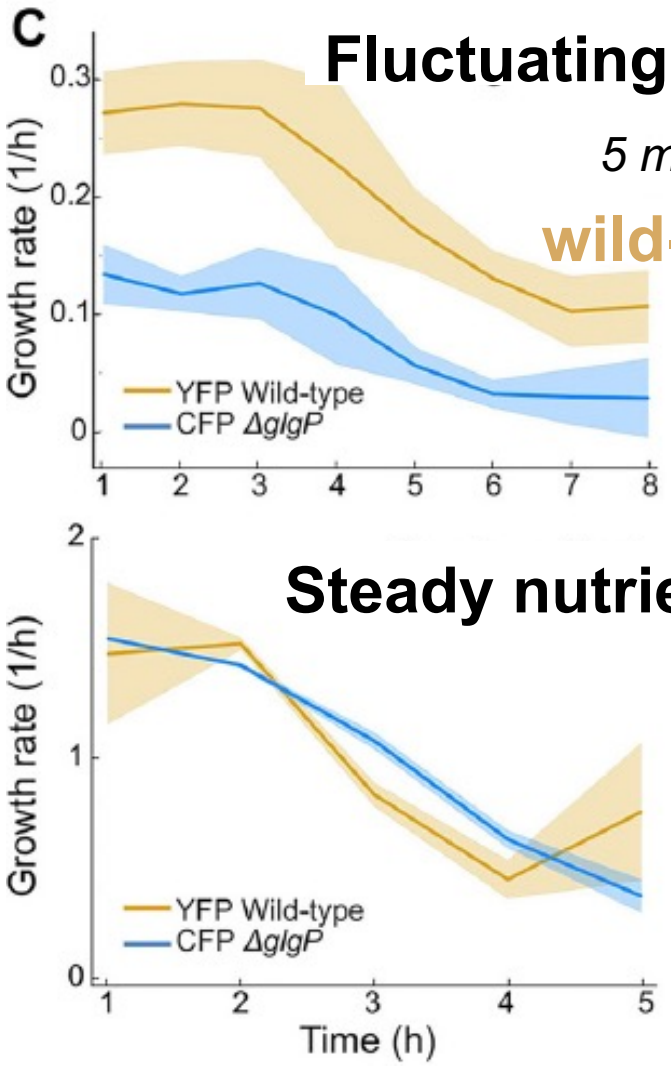
Cells unable to use glycogen are slower in completing molecular adaptations for full growth under new conditions.

Fluctuating LB-Medium Conditions

Single-cell growth rates
(ie expo cell volume doubling)

μ_{max} **0.28 h^{-1}**

μ_{max} **0.13 h^{-1}**



Experiment: Stocker lab, ETH
Microfluidics and time-lapse imaging to monitor volumetric growth of individual *E. coli* cells. Medium flow over surface-attached cells.

Glycogen utilization confers growth advantage in **dynamic** environments by providing energy and carbon in nutrient-poor transition phases.

Besides biofilm formation, which cellular functions benefit from glycogen carbon supply?

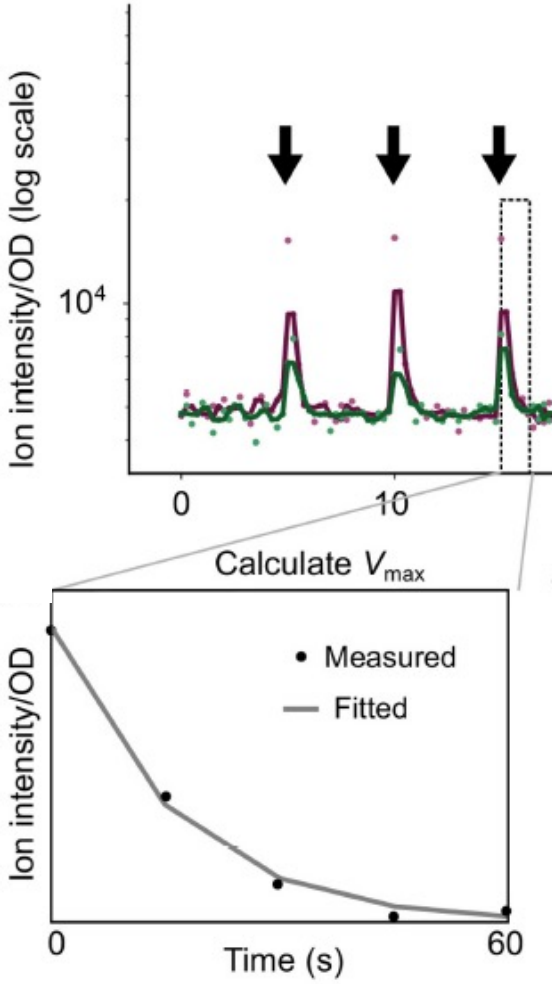
Maintaining sugar uptake capacity?

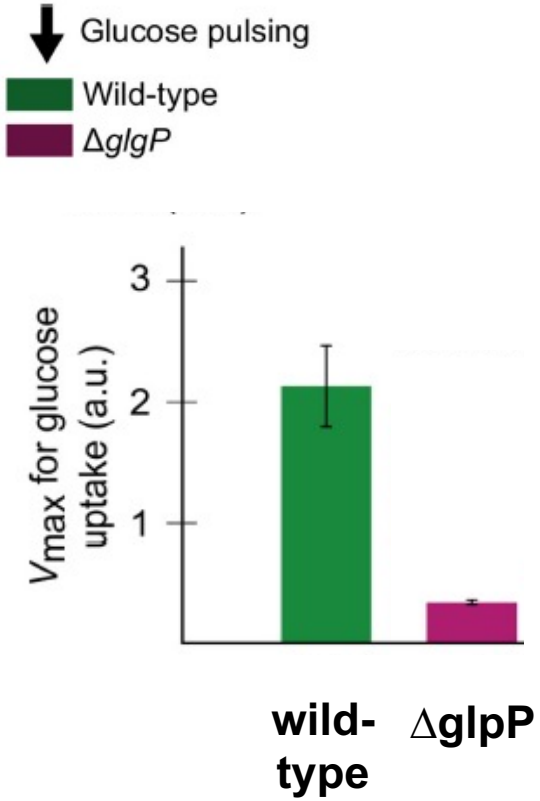
Glucose Uptake Rate during Pulses to Starving *E. coli*

Pulses of ≈ 0.4 mM glc/g_{cells} every 5 min to starving cells (freshly centrifuged at OD 0.8)

Hexose ion from real-time metabolomics per OD

Michaelis-Menten mode fit to data ($v_{max} = q_{glc}$)

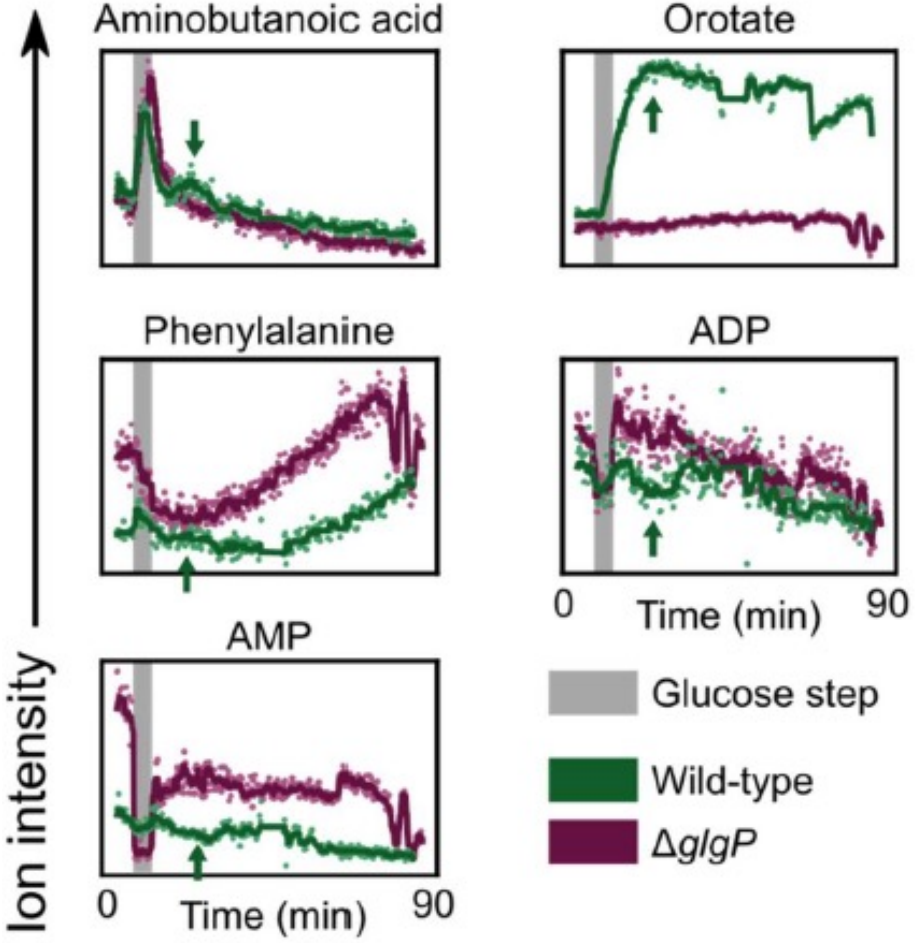




Carbon/Energy Supply from Glycogen Maintains Higher Sugar Uptake Capacity. But How?

Real-time metabolomics has many shortcomings such as background effects and missing metabolites.

PEP as the key driver of glucose uptake (PTS system) is not detected, but higher AMP and ADP levels in the glpP mutant suggest a lower energy charge and thus lower PEP levels in the mutant.



Glycogen Conclusions

- Rapid dynamics of glycogen synthesis/breakdown:
  - 80% depleted within 10 min starvation
  - replenished within 2 min.
- Glycogen confers advantages under nutrient fluctuations or transitions (ie faster growth, shorter lag).
- Glycogen maintains higher glucose uptake capacity during fluctuations and (short-term) starvation – presumably by fueling respiration.

THANKS

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