molecular systems biology

Bacterial persistence is an active σ^S stress response to metabolic flux limitation

汇报人：潘奇

Abstract

Introduction

目录

CONTENT

Result

Material

Discussion

Abstract

1.Persisters are a health threat due to their transient antibiotic tolerance, but little is known about their phenotype and what actually causes persistence.

2.The persister proteome is characterized by σ^S-mediated stress response and a shift to catabolism, a proteome that starved cells tried to but could not reach due to absence of a carbon and energy source.

3.Metabolism of persisters is geared toward energy production, with depleted metabolite pools. We developed and experimentally verified a model, in which persistence is established through a system-level feedback.

4.The vicious cycle is stabilized and modulated by high ppGpp levels, toxin/anti-toxin systems, and the σ^S-mediated stress response.

σ^S: RNA聚合酶的α亚基，是一般胁迫反应的主控调控因子
ppGpp：鸟苷四磷酸 细胞内缺乏氨基酸时做的应急反应

Materials

Bacterial strains

Strain	Source
BW25113	Obtained from (Baba *et al*, 2006)
BW25113 Δ*rmf*	Obtained from (Baba *et al*, 2006)
BW25113 Δ*relA*	Obtained from (Baba *et al*, 2006)
BW25113 Δ*rpoS*	Obtained from (Baba *et al*, 2006)
BW25113 Δ*rpoS* + pNT3-rpoS	BW25113 Δ*rpoS* transformed with pNT3-rpoS plasmid from (Saka *et al*, 2005)
BW25113 + pBAD-LacY-EYFP	BW25113 transformed with pBAD-LacY-EYFP plasmid (gift from Jonas van der Berg)
MG1655	Obtained from (Maisonneuve *et al*, 2011)
MG1655 Δ10	Obtained from (Maisonneuve *et al*, 2011)
MG1655 Δ10Δ*rpoS*	MG1655 Δ10 with rpoS knockout transduced with P1 phage from BW25113 Δ*rpoS*
MG1655 Δ10Δ*rpoS* + pNT3-dctA	MG1655 Δ10Δ*rpoS* transformed with pNTR-SD-dctA plasmid from (Saka *et al*, 2005)

Media and cultivation

Escherichia coli K12 strain BW25113 was used for the phenotypic characterization.

Parts of the model validation were done with the strain MG1655.

All experiments were performed using M9 minimal medium

The carbon source stock solutions were made by dissolving the carbon source in demineralized water, adjusting the pH to 7 with NaOH or HCl,and filtering through a 0.2 lm PES filter.

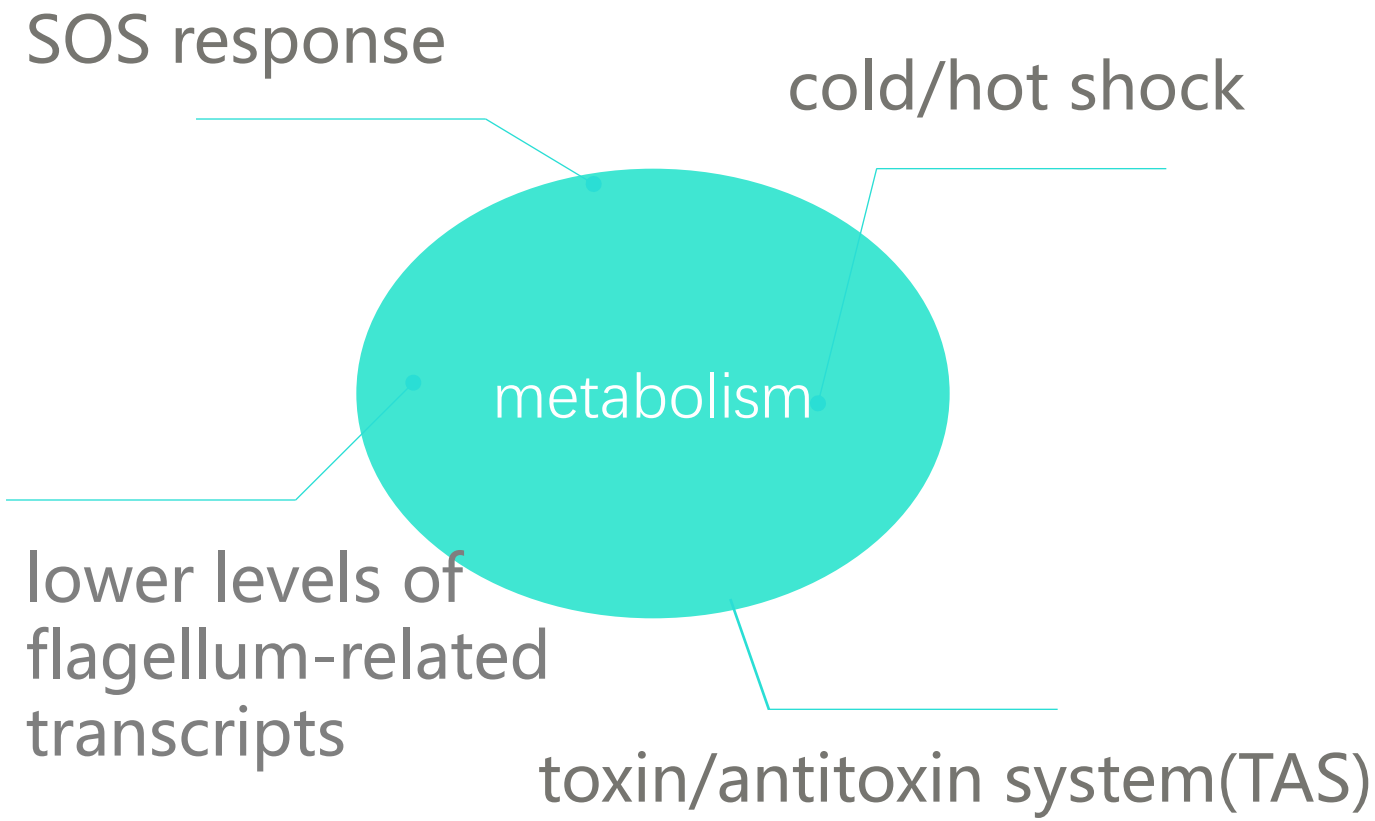
Cultivations were done in 50 ml of M9 medium in a 500-ml Erlenmeyer flask closed with a 38 mm silicone sponge closure (Bellco Glass) at 37°C, 300 rpm, and 5 cm shaking diameter.

keep the cells in mid-exponential phase.

ampicillin treatment or FACS and through performing transcriptome analyses.

higher abundances of persisters

The involvement of various mechanisms could explain the recently observed heterogeneity between persister cells.



FACS:流式细胞术是一种在功能水平上对单细胞或其他生物粒子进行定量分析和分选的检测手段，它可以高速分析上万个细胞，并能同时从一个细胞中测得多个参数，与传统的荧光镜检查相比，具有速度快、精度高、准确性好等优点，成为当代最先进的细胞定量分析技术。

Introduction

Three in vitro models:
the antibiotic-tolerant cells that are formed stochastically in growing cultures.

starved cells which have diminished or absent antibiotic target activity due to the absence of nutrients.

after certain nutrient shifts (i.e. abrupt shifts or gradual shifts resembling diauxie) a large number of non-/slow-growing and antibiotic-tolerant cells emerge in nutrient-rich conditions

these findings suggest that the metabolic state of a cell and persistence might be closely tethered

Result

switched Escherichia coli cells from glucose to fumarate medium

with a carbon source

an extremely small fraction of cells (0.1±0.05%, SD) adapted and started to grow on fumarate.

The other cells entered a state of non-/slow growth resembling the one of persister cells

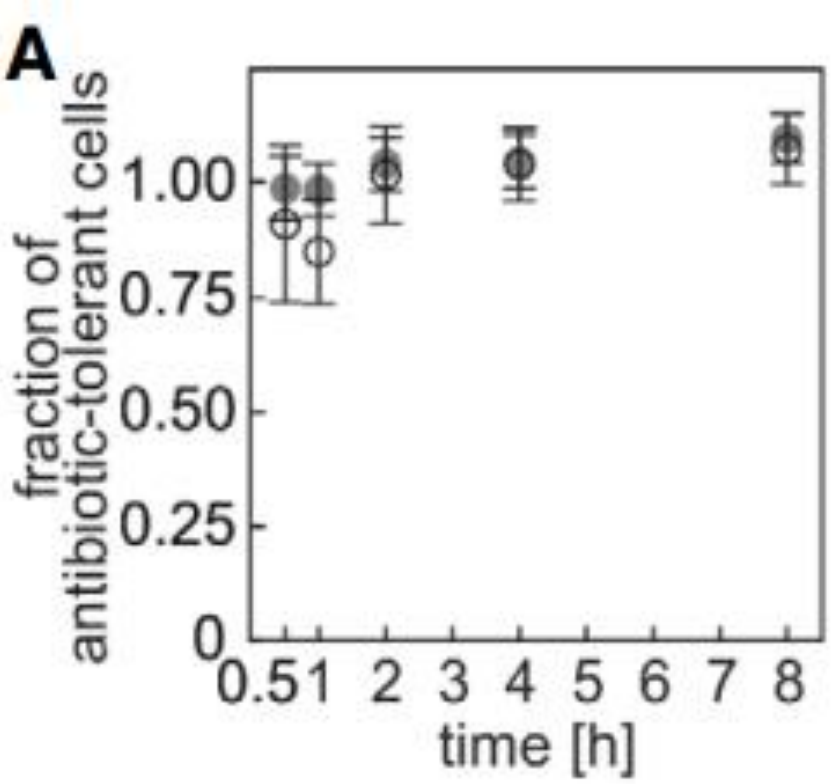
without a carbon source

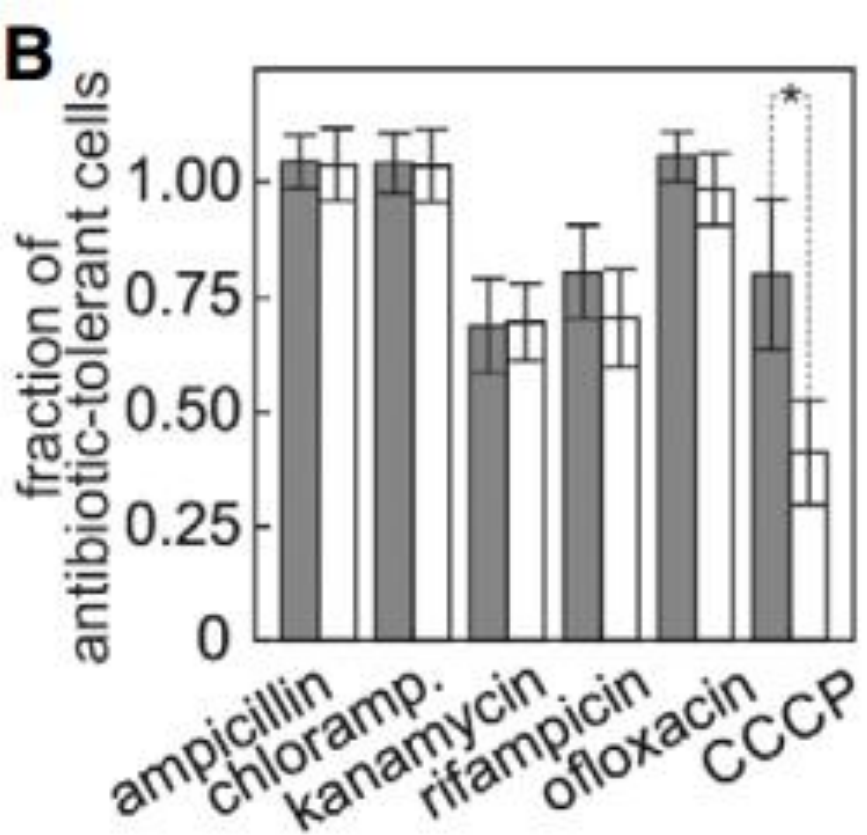
generating starved cells, which allowed us to investigate the effect of nutrient presence on the persister phenotype.

Nutrient shifts

ampicillin

virtually all cells becar
after the nutrient shift
conditions obtained a
stochastically induced



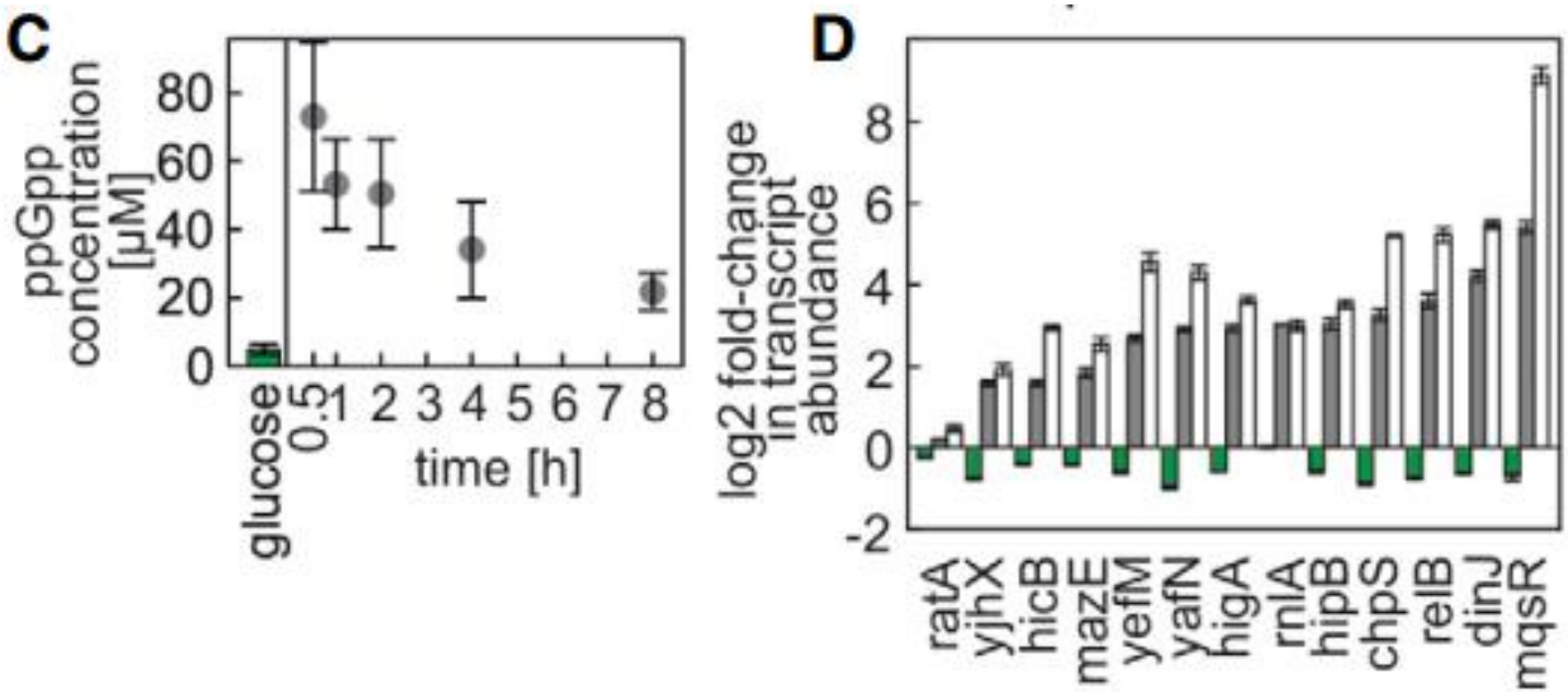


show that non-/slow-growing cells in nutrient-rich conditions and starved cells are tolerant to numerous antibiotics at concentrations that killed fumarate-growing cells. However,
the observed difference
the higher tolerance of non-/slow-growing cells in nutrient-rich conditions against CCCP compared to starved cells.
persister cells in nutrient-rich conditions must exploit specific tolerance mechanism that enhance their survival over that of persisters generated by starvation.

CCCP (proton gradient disruptor)

ppGpp levels are also inc
cells obtained after the n

the shifts to fumarate ar
increased abundance of
exponentially on glucose



Persisters are metabolically acti

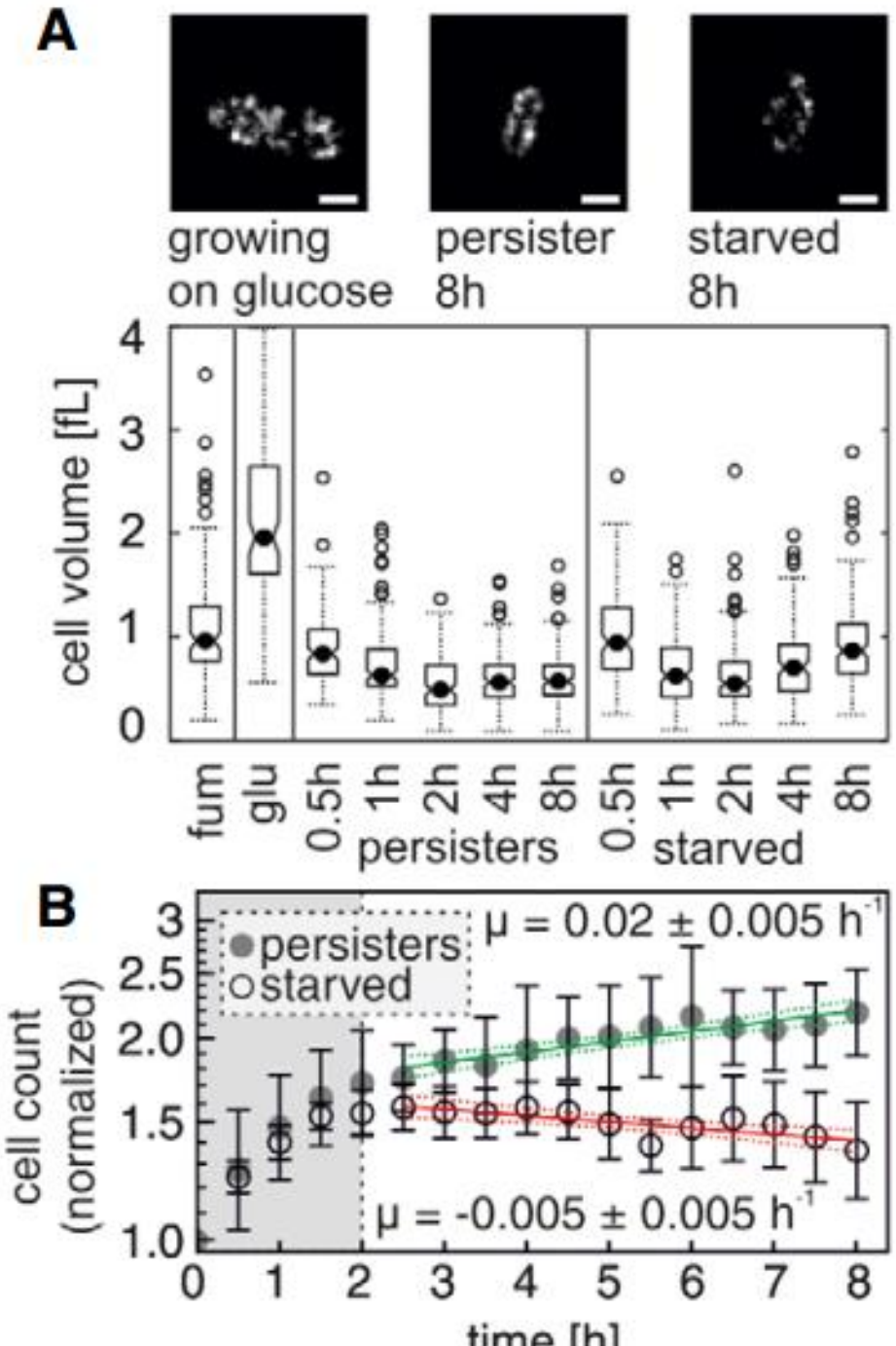
In the first 2 h after the switch to fumarate or to medium without a carbon source, cells underwent a reductive division characterized by a decrease in cell volume and an increase in cell count.

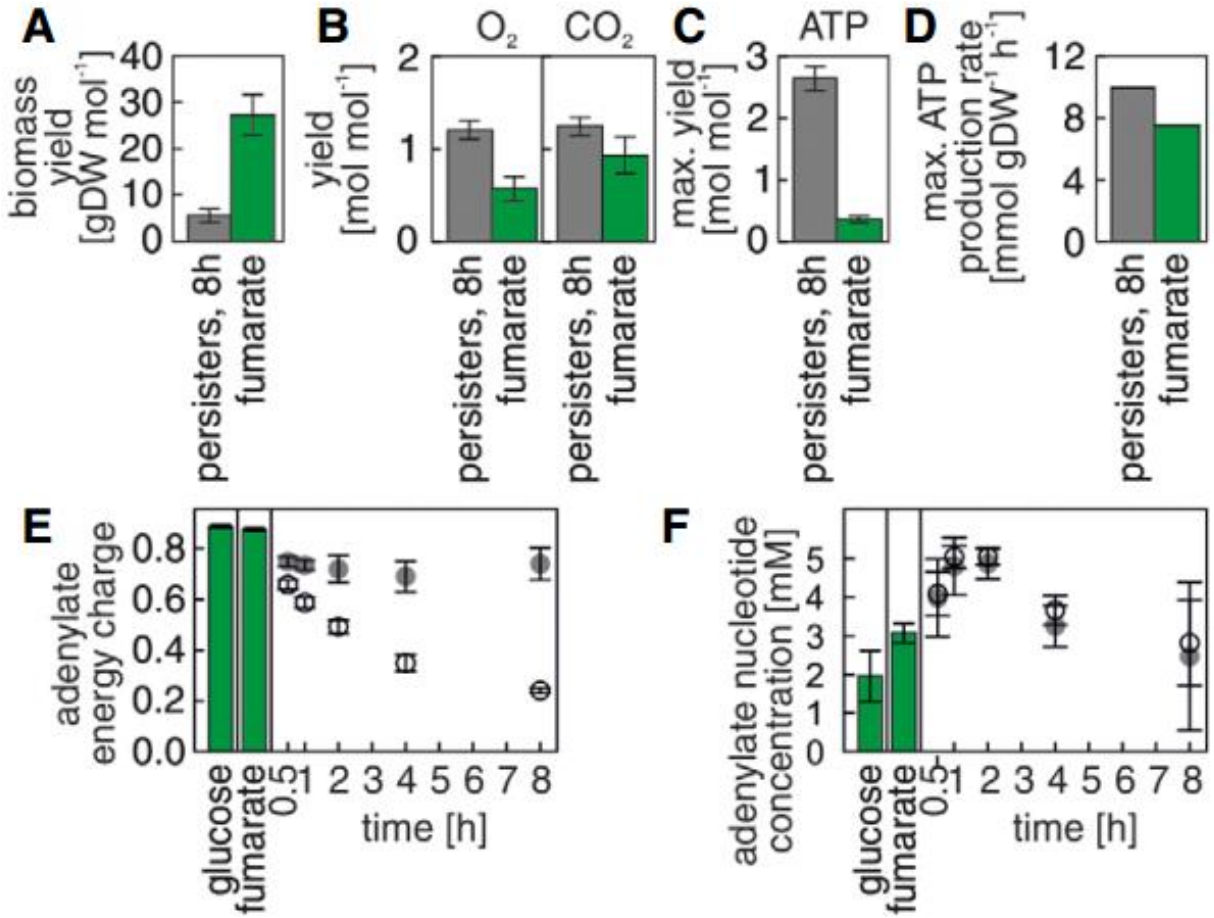
the persisters grew at a rate of 0.02±0.005 h^{-1} (95% confidence interval)and their volume remained constant .

the starved cells did not grow in number , but they did in volume for the following 6 h

persister cells must have metabolic activity to sustain their slow growth, and possibly to maintain their membrane potential.

Green line (persister cells)
red line (starved cells)





Focusing on the nutrient and gas exchange rates in the persister cells, we found that they took up fumarate and oxygen and produced carbon dioxide, all at rates (per cell) approximately one order of magnitude lower than cells growing on fumarate.

persisters consumed less fumarate,exchanged more O2 and CO2 and produce more ATP per mol of consumed carbon source than fumarate-growing cells.

persisters operate their metabolism in a way that is optimized for energy generation, in contrast to biomass production and growth.

persisters in nutrient-rich conditions are able to maintain high energy charges, eventually contributing to the observed enhanced antibiotic tolerance compared to starved cells.

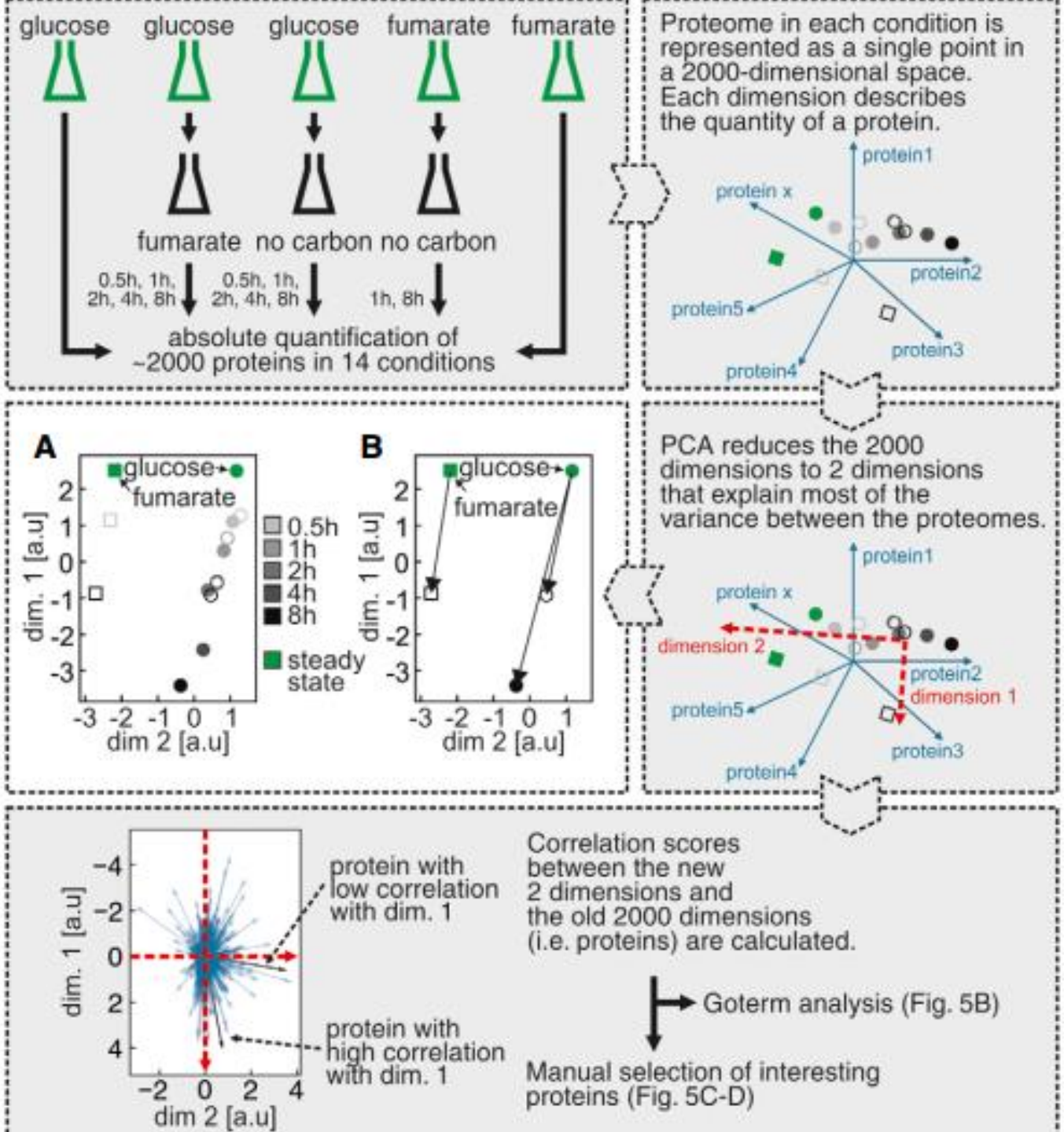
Persisters achieve a proteome state that starved cells fail to attain

measured levels of about 2,000 proteins through liquid chromatography/mass spectrometry proteomics during entry into persistence from glucose.

although all cells were switched to fumarate, the proteome of persister cells did not approach the one of fumarate-growing cells. In fact, the proteomes of fumarate- and glucose-growing cells were more similar to each other than the proteome of persister cells compared to either the proteome of glucose-growing cells or fumarate-growing cells.

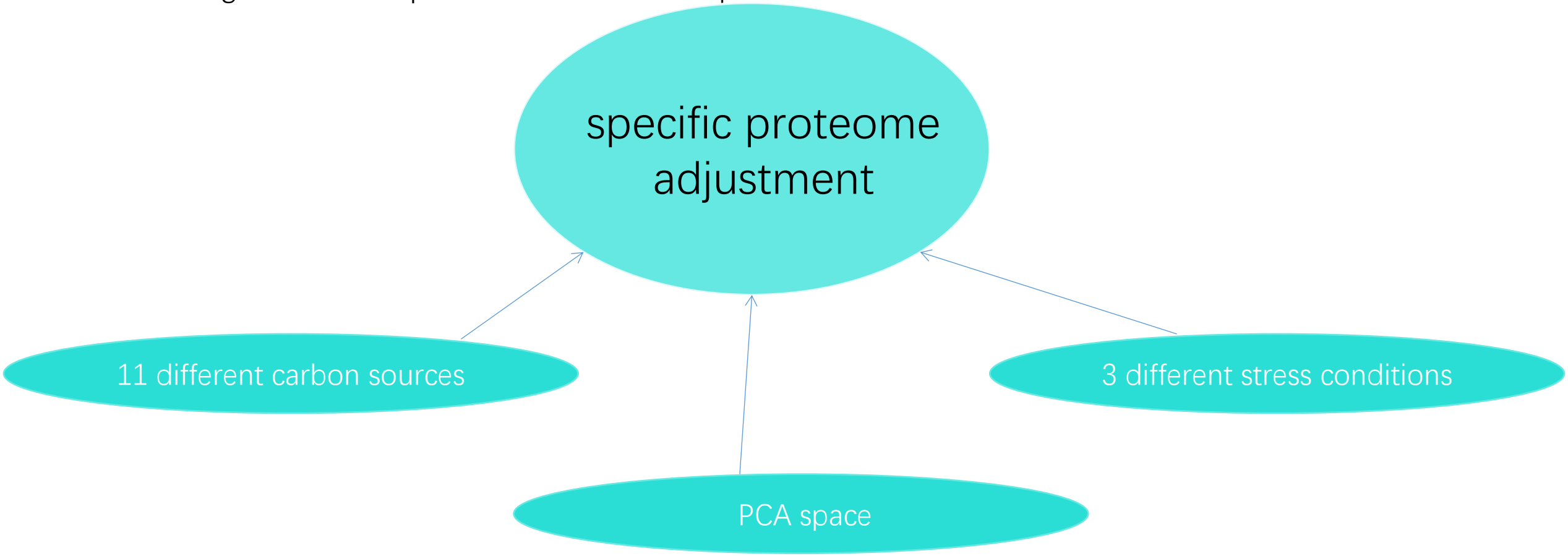
the changes in the proteome of starved cells, which almost exclusively occurred during the first 2 h after the nutrient shift, followed the same trajectory as the proteome changes in the persister cells

the proteome adjustments in both starved and persister cells must be caused by a common cue,which is not specific to the availability of a carbon source in the medium.



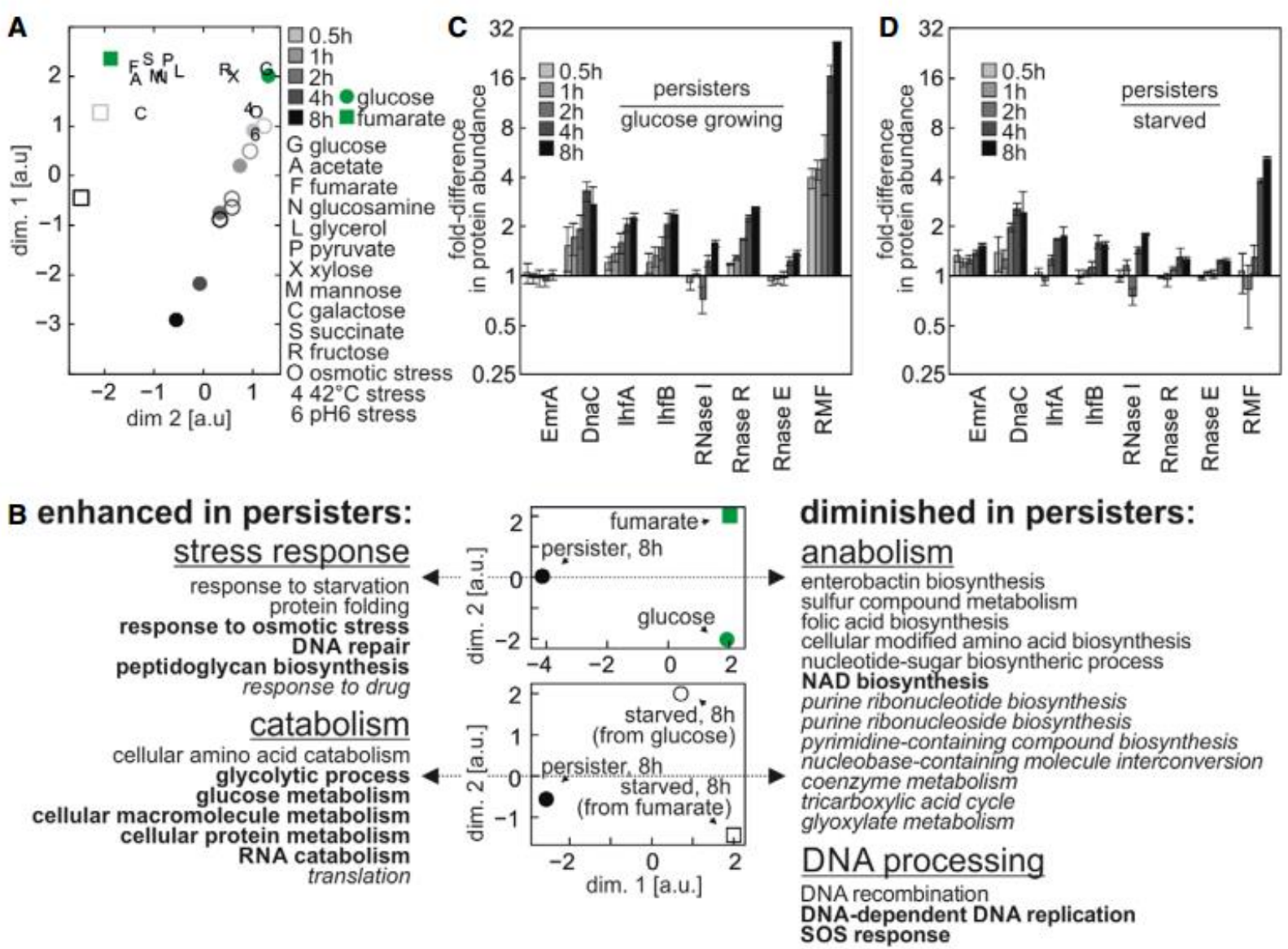
Persister proteome is characterized by enhanced catabolism and σ^S-driven stress response

To decipher whether the observed changes are a mere reflection of growth rate changes or whether the observed changes resemble specific characteristics of persisters.



A: the proteomes of persister cells and starved cells moved along the dimension 1,under the stress conditions

B: persister proteome was characterized by lower levels of proteins for DNA replication, recombination, and SOS response .



the main difference between the proteomes of persister cells and starved cells is caused by availability of nutrients and the rudimentary metabolic activity of persisters.

performed a hypergeometric test using known sigma factor–gene interactions, transcription factor (TF)-gene interactions, and regulatory RNA-gene interactions.

the proteome changes in persister cells were largely controlled by σ^S

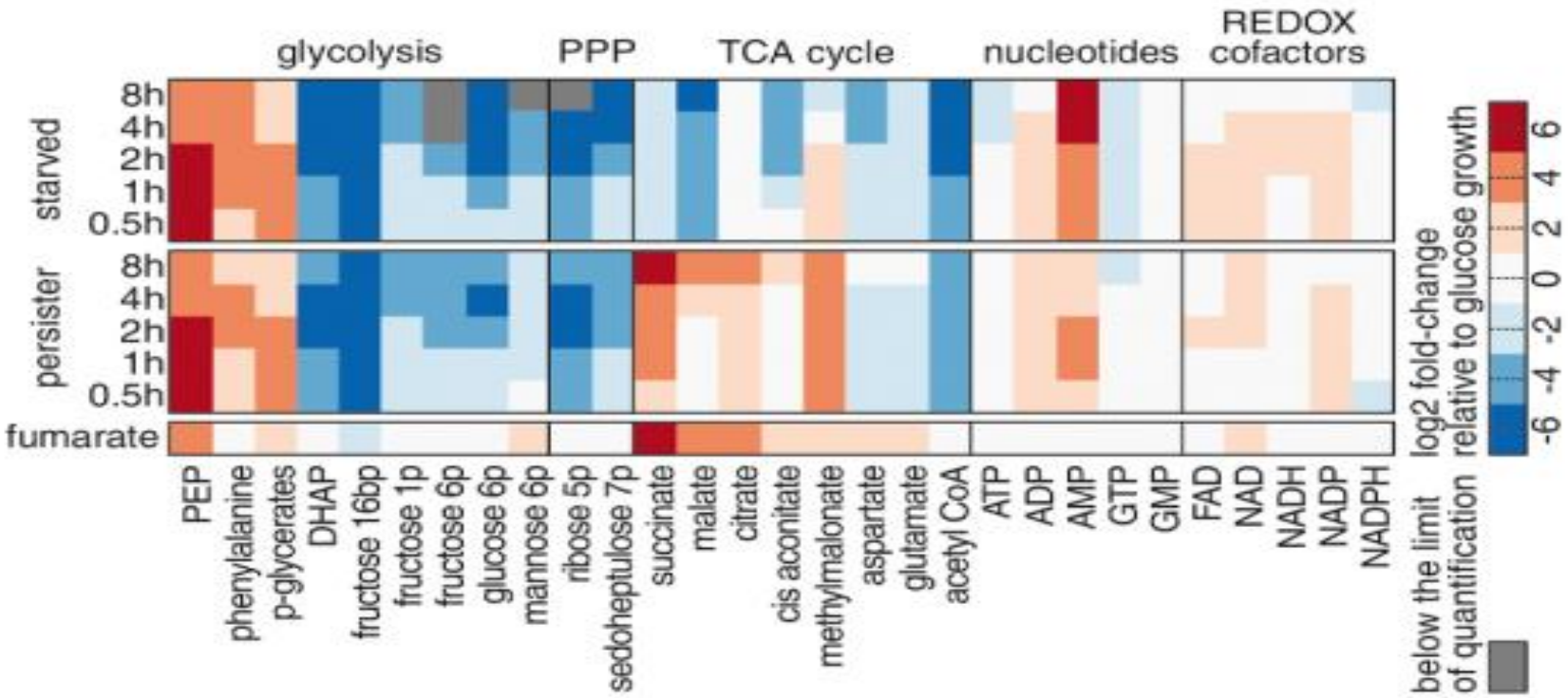
The ppGpp levels in persisters elevated , these increased ppGpp concentrations could be the cause for the higher abundance of $\boldsymbol{\sigma^S}$ in persister cells.

it is still a question which mechanism would cause ppGpp levels to be increased in persisters, and what is the cue triggering this mechanism.

Persister cells and starved cells share a distinct metabolite pool pattern

measured the concentrations of 29 metabolites of central carbon metabolism as well as of energy and redox cofactors at different time points during entry into persistence and starvation, and in cells growing exp

The distinct concentration pattern extremely low levels with the excep and phosphoglycerates), similar in could contain the signal for the en response.

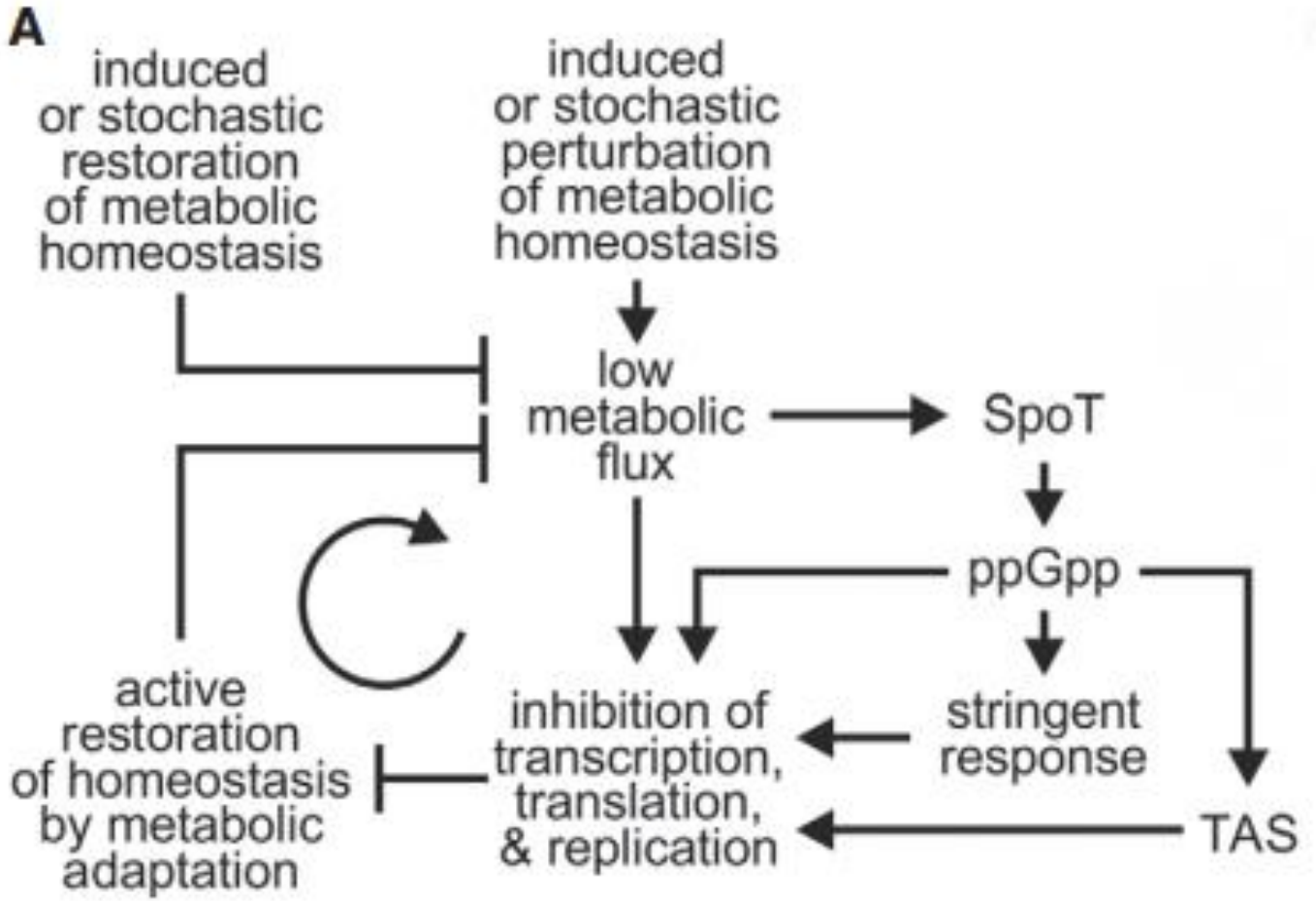


Low metabolic flux causes persister formation withoutσ^S or TAS action

a conceptual model for the entry into and the sustenance of the persister state

These feedback-enhancing would likely ensure bacterial survival during strong perturbations of metabolic homeostasis, by prohibiting that cells engage in low metabolic fluxes

TAS, σ^S, or ppGpp synthase perturbations should only modulate the fraction of persister cells, but not eliminate them.

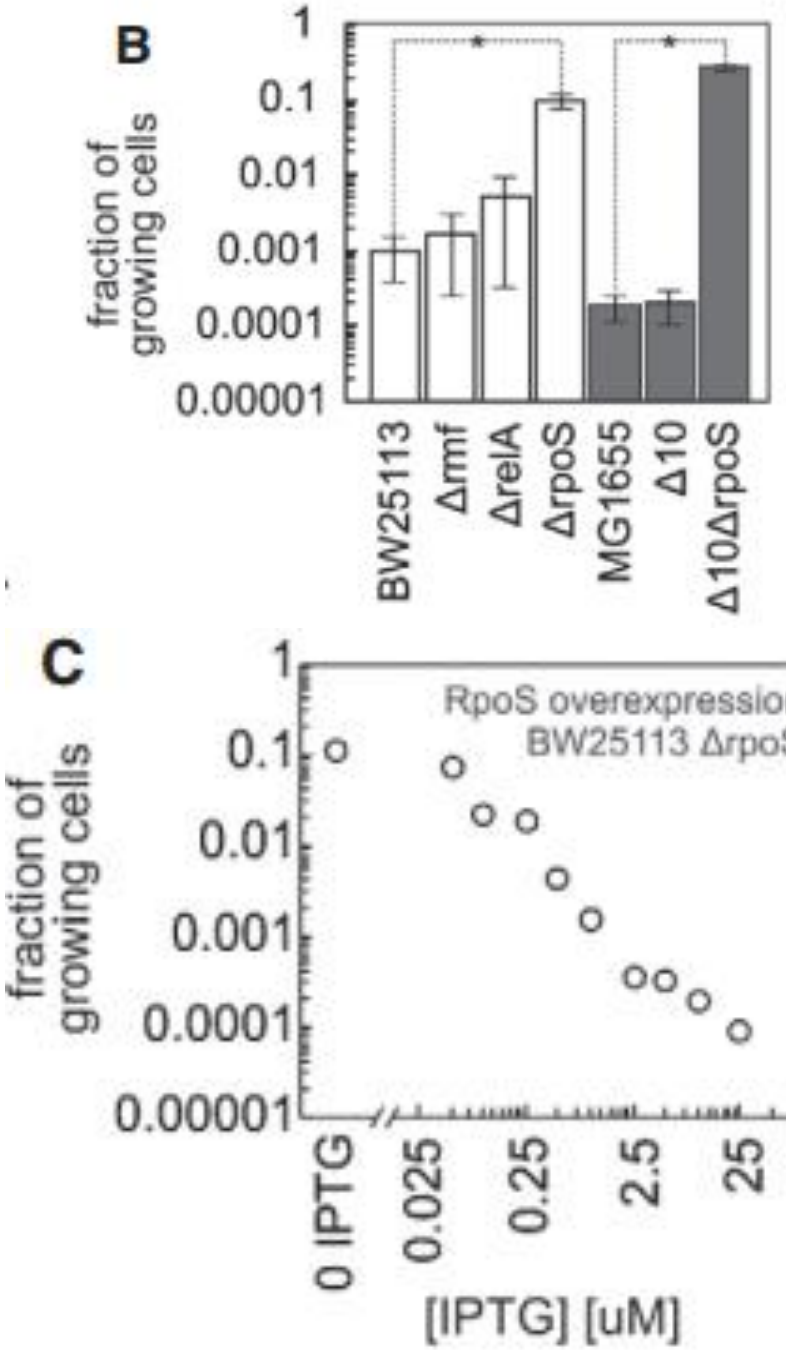


Δrmf (ribosome modulation factor, inhibitor of protein synthesis)、 ΔrpoS

σ^S modulates the strength of the feedback and thus the amount of persisters, most probably as a response to SpoT activity.
σ^S is not essential, but still plays a role in establishing the persister state.

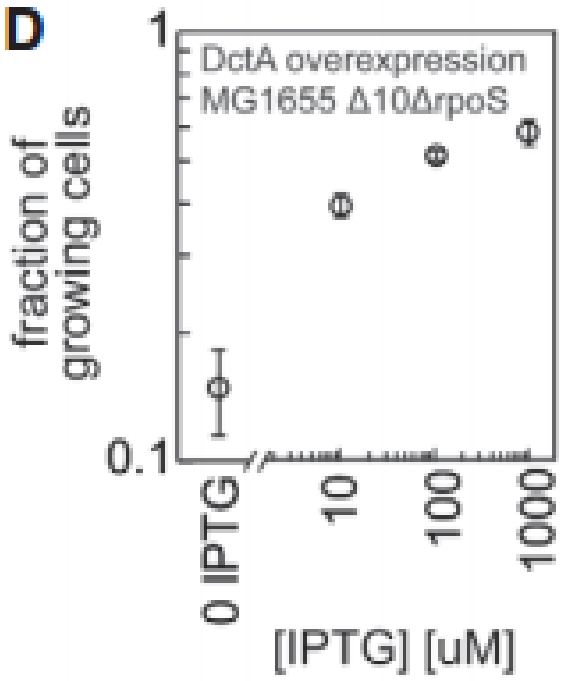
metabolic flux-dependent primitive vicious cycle forcing cells into persistence

the fraction of persisters inversely correlated with glucose influx , and with acetate or fumarate influx



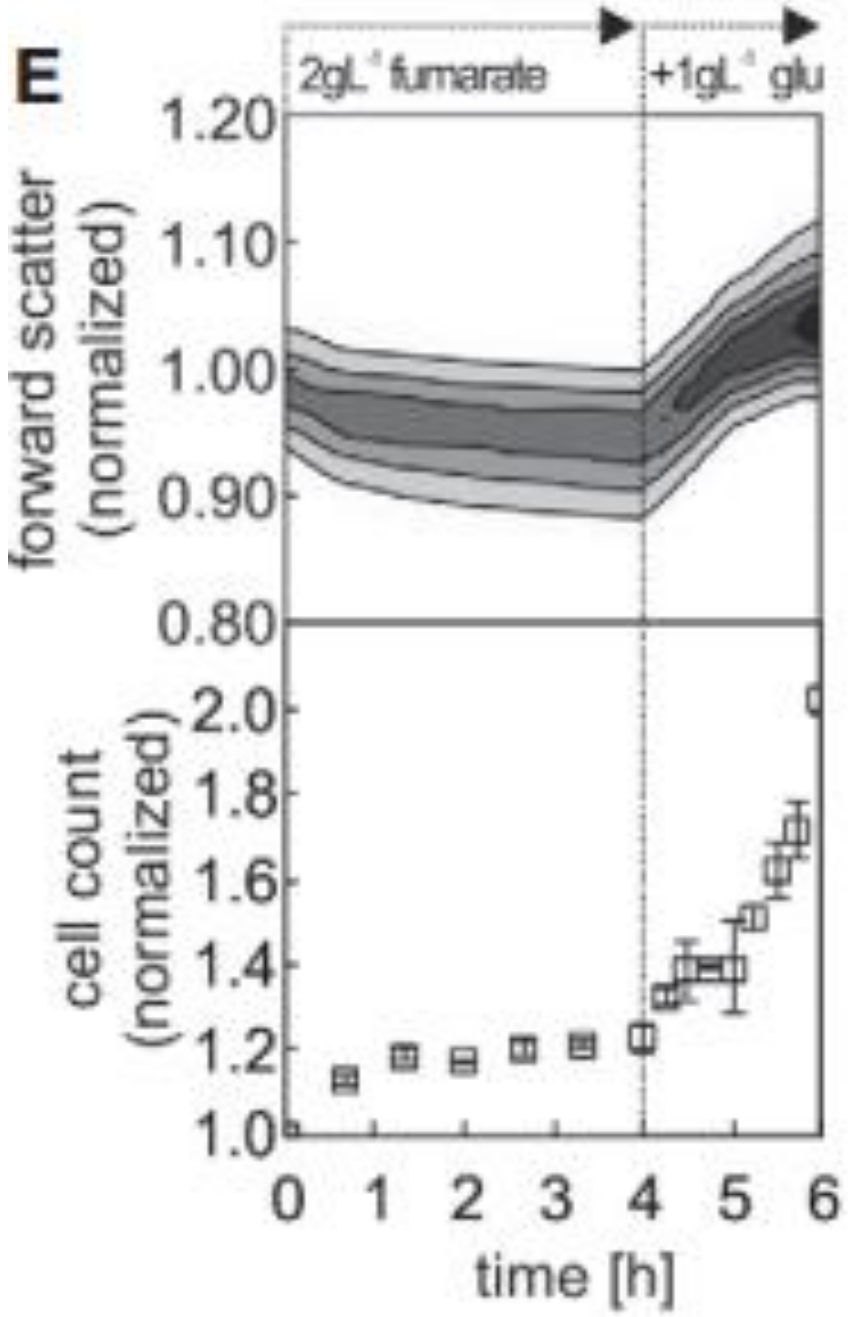
To test whether the entry into persistence is still metabolic flux dependent in a strain that lacks TAS and σ^S

transformed the Δ10 ΔrpoS strain with a plasmid for IPTG-inducible expression of the fumarate transporter DctA

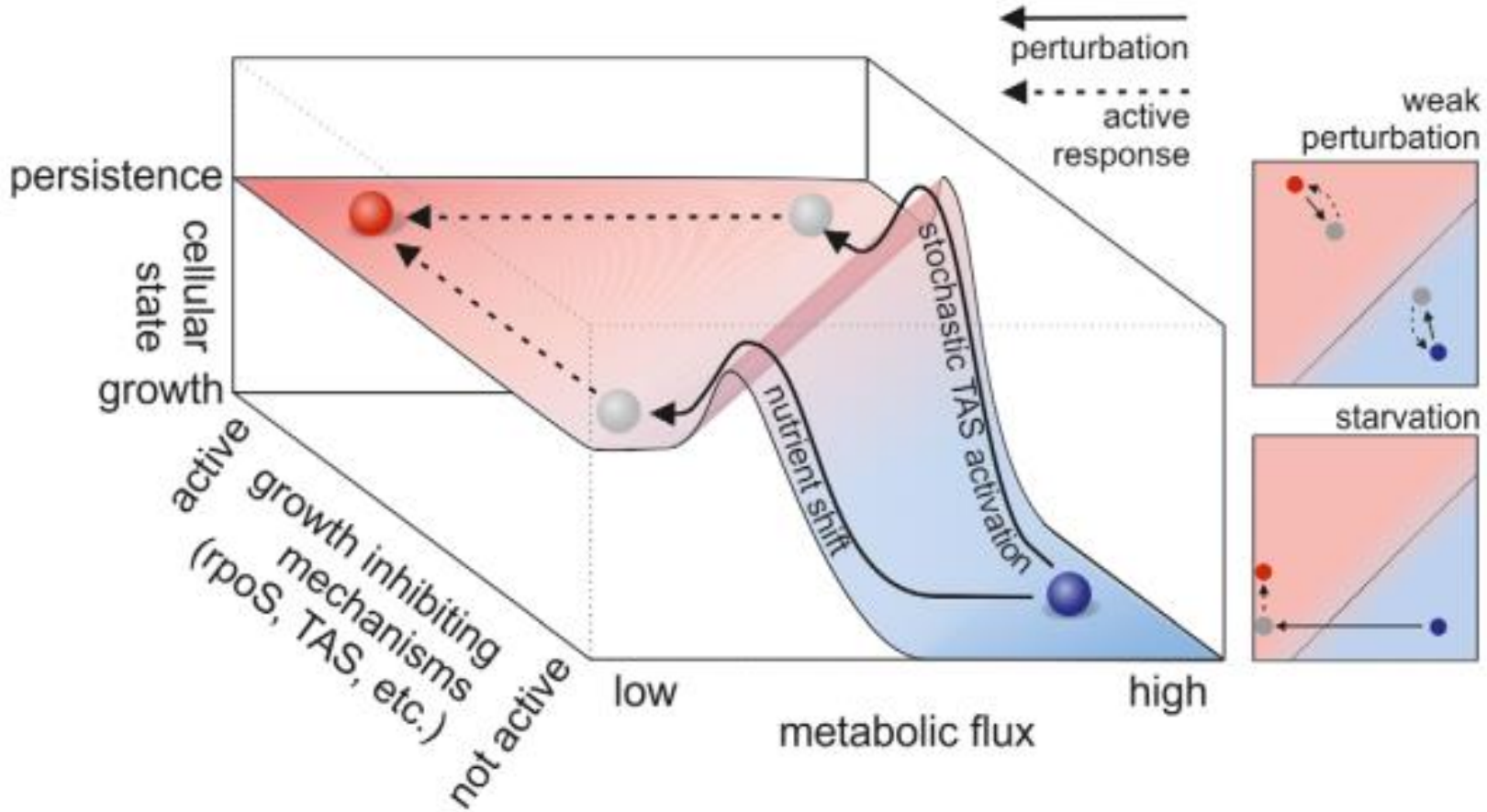


the metabolic flux is the basic factor in establishing persistence, while other mechanisms enhance the feedback.

added glucose to the persister cells 4 h after the shift to fumarate



This finding suggests that the factors inhibiting persister growth can be removed on a very short timescale.



If a cell is on the right side of the watershed , it will move toward the attractor indicated by the blue disk and achieve normal growth in metabolic homeostasis. If a cell happens to be on the left side of the watershed, it will become a persister cell.

Persistence and growth are two attractor states on a phenotypic landscape with the dimensions “metabolic flux” and “activity of growth-inhibiting mechanisms”

Discussion

Advertisement

根据现象，大胆假设

根据实验结果，谨慎推测

进一步实验，得出结果

Disadvertisement

实验材料仅为大肠杆菌，没有研究多种细菌，模型可信度较低

没有提出完全消除耐药菌株的方法

1

善于发现问题，探索规律，透过现象看本质

2

学会总结，将自己的实验结果归纳，总结为一般规律

THANKS