Coordination of bacterial proteome with metabolism by cyclic AMP signalling

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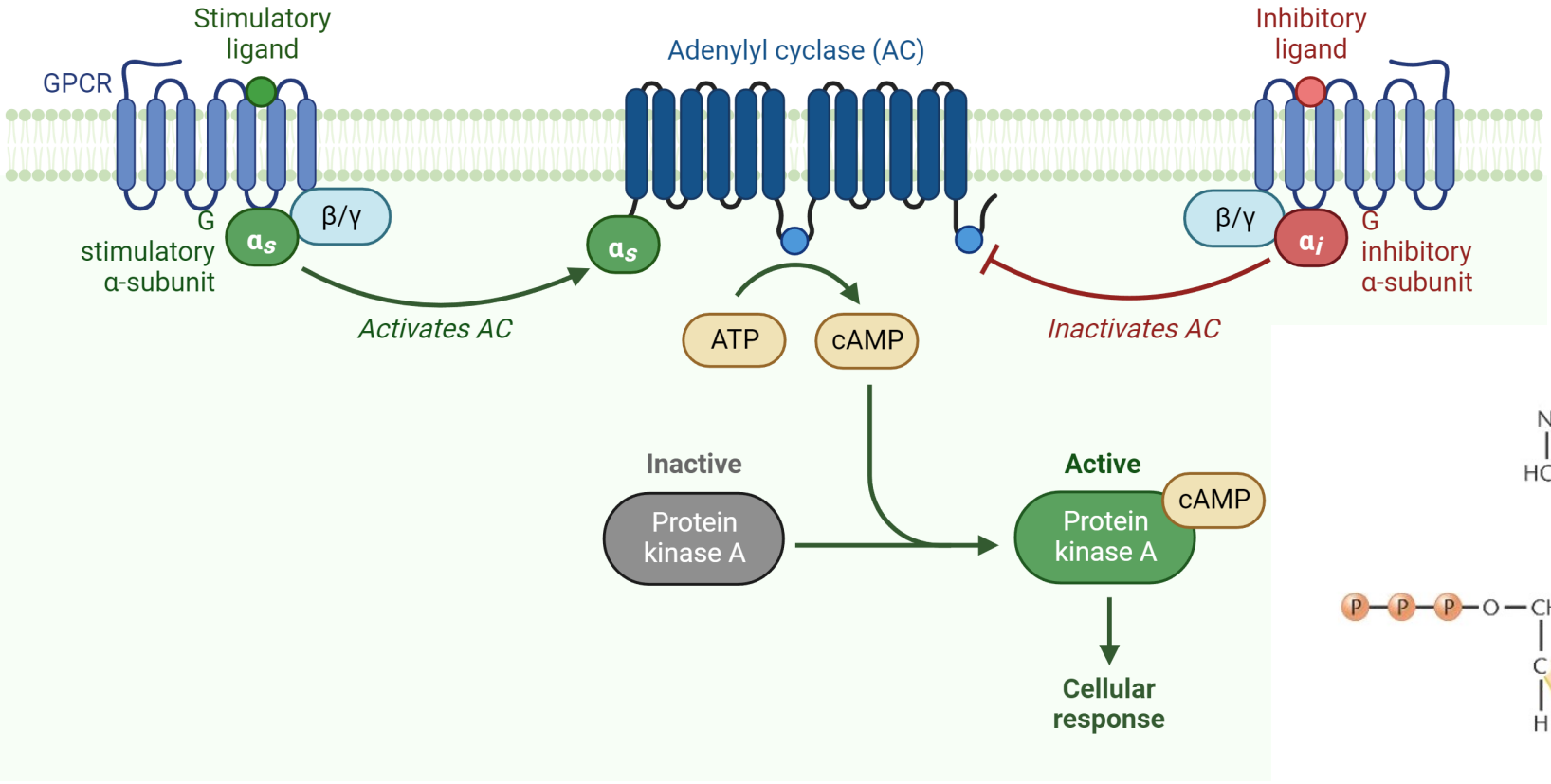
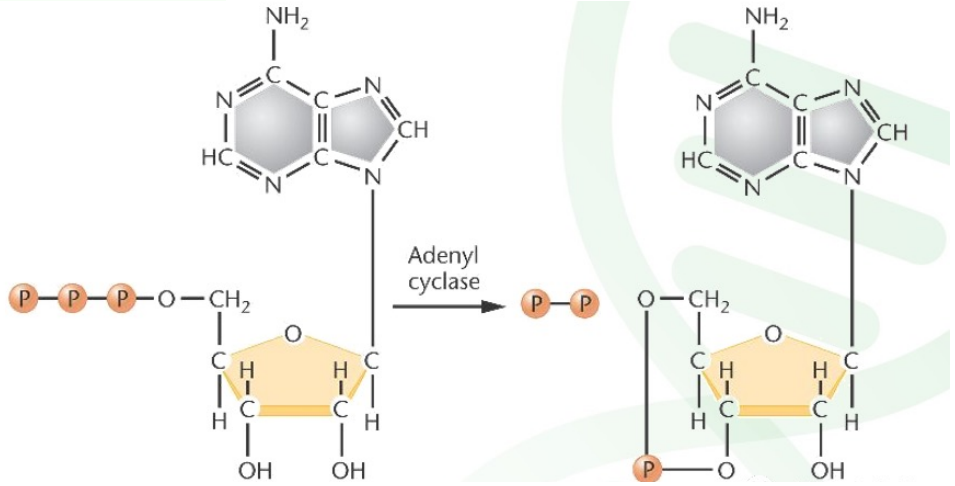
Presenter | Sijie Li

ABSTRACT

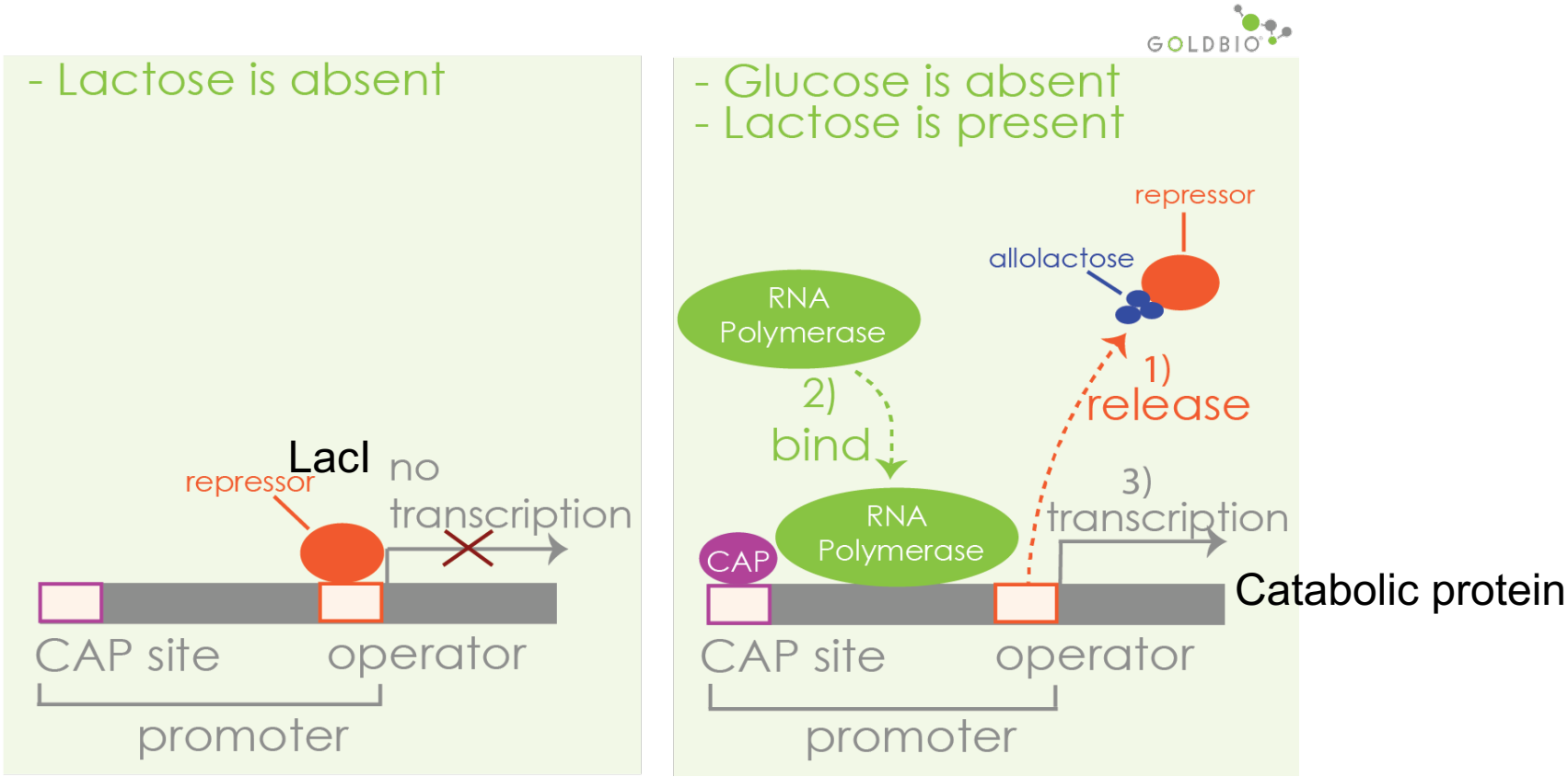
The cyclic AMP (cAMP)-dependent catabolite repression effect in Escherichia coli is among the most intensely studied regulatory processes in biology. However, the physiological function(s) of cAMP signalling and its molecular triggers remain elusive. Here we use a quantitative physiological approach to show that cAMP signalling tightly coordinates the expression of catabolic proteins with biosynthetic and ribosomal proteins, in accordance with the cellular metabolic needs during exponential growth. The expression of carbon catabolic genes increased linearly with decreasing growth rates upon limitation of carbon influx, but decreased linearly with decreasing growth rate upon limitation of nitrogen or sulphur influx. In contrast, the expression of biosynthetic genes showed the opposite linear growth-rate dependence as the catabolic genes. A coarse-grained mathematical model provides a quantitative framework for understanding and predicting gene expression responses to catabolic and anabolic limitations. A scheme of integral feedback control featuring the inhibition of cAMP signalling by metabolic precursors is proposed and validated. These results reveal a key physiological role of cAMP-dependent catabolite repression: to ensure that proteomic resources are spent on distinct metabolic sectors as needed in different nutrient environments. Our findings underscore the power of quantitative physiology in unravelling the underlying functions of complex molecular signalling networks.

cAMP works as a second messenger to transmit extracellular signals.



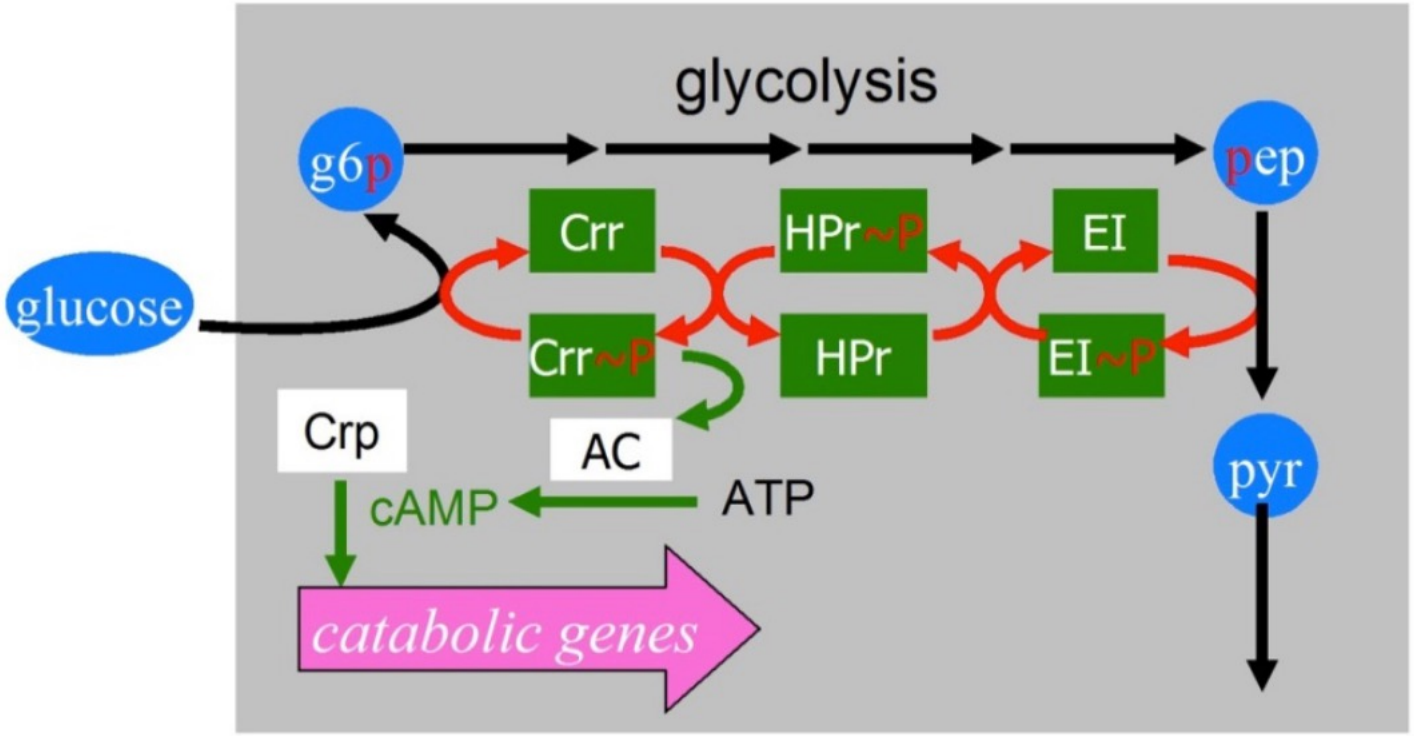


The synthesis of catabolic proteins is inhibited when growing on glucose or other rapidly metabolizable sugars.

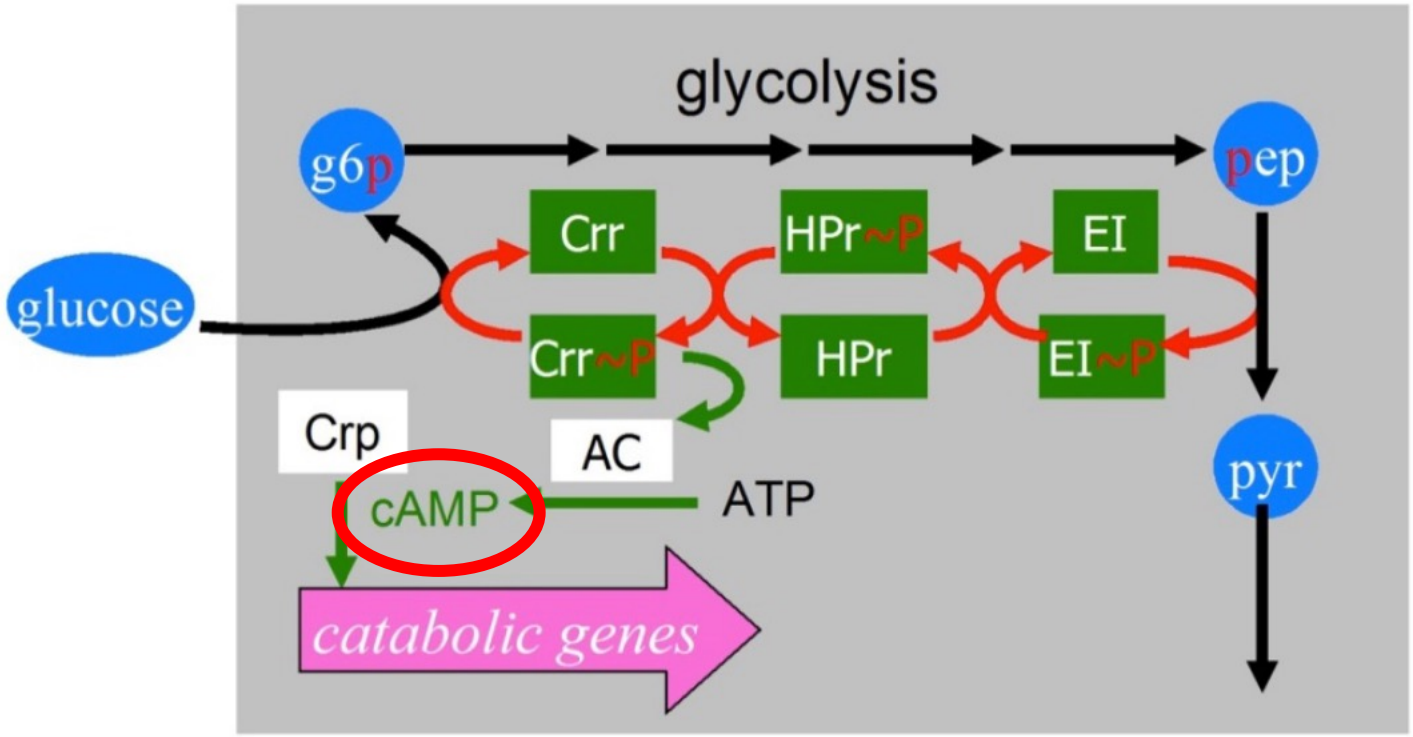


e.g. of CAP Crp-cAMP complex

Phosphotransferase system (PTS) leads to the inhibitory effect of glucose uptake on cAMP synthesis.



This works aim to answer "What is the physiological function (s) and its molecular triggers of cAMP signalling?"



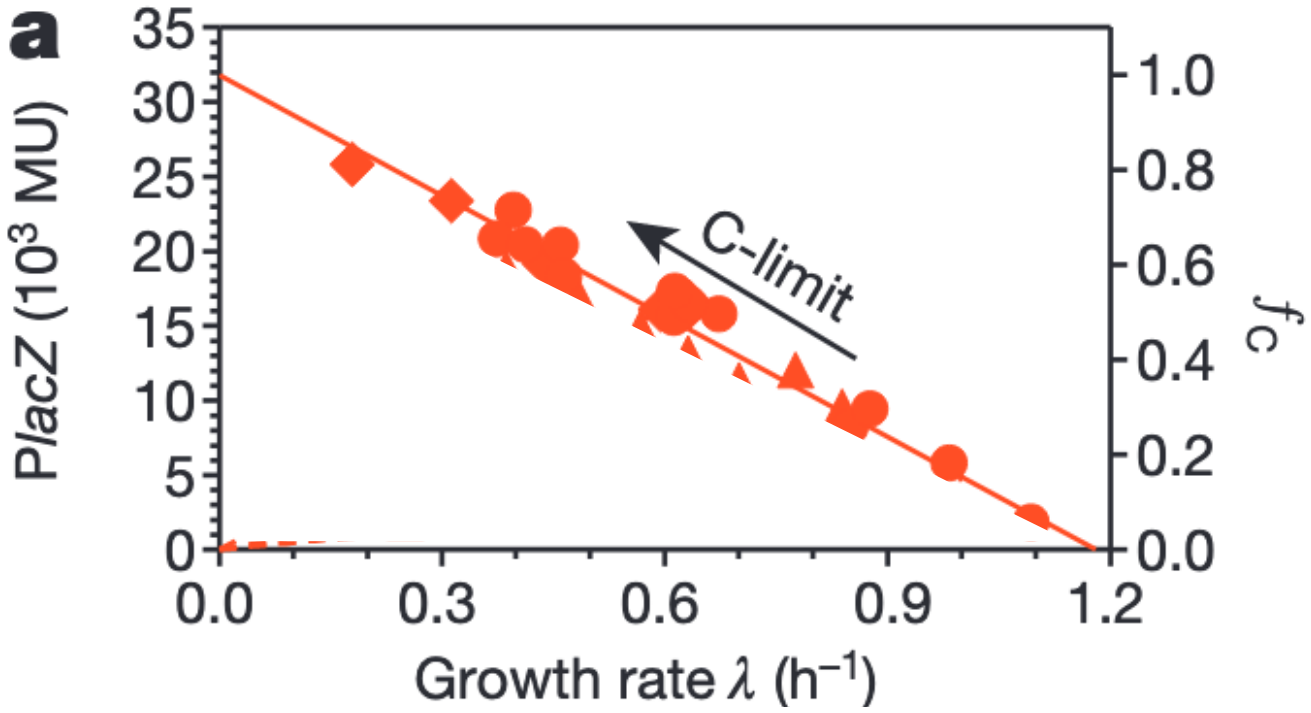
Sensing **glucose signals** and triggered by **PTS**?

To be solved:

1. **PTS-independent** sugars also showed reduced cAMP levels! Why?
2. The **extent** cAMP contribute to the CCR.

Catabolic protein (LacZ) increase linearly w.r.t. the growth rate under C-limit.

MU = M/OD$_{600}$: we always focus on **protein fraction** $L = L_{\max}(1 - \lambda/\lambda_C)$



C-line: common for catabolic genes

C-limit

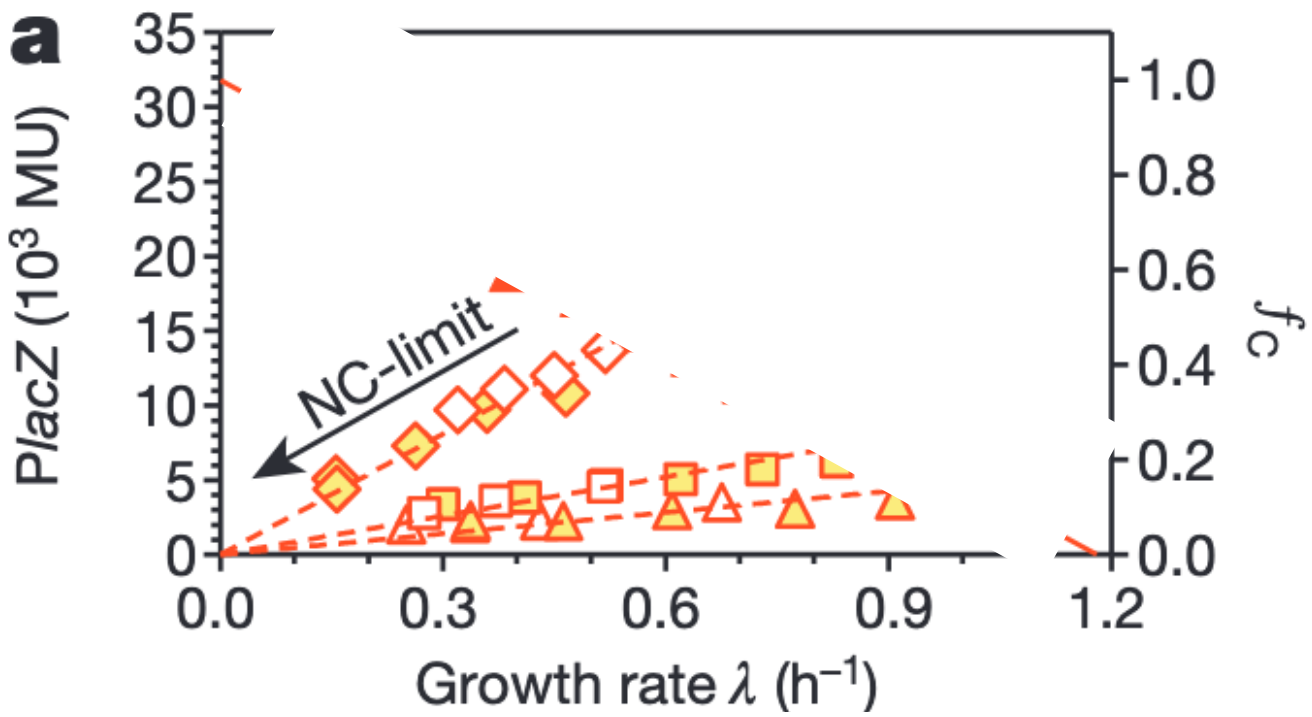
[quality] Carbon sources

[quantity] Carbon influx

Generalize

- Other strains of *E. coli*
- Different catabolic promoter (besides PlacZ)

Catabolic protein (LacZ) decrease linearly w.r.t. the growth rate under NC-limit.



NC-line: **Oppose C-line tendency**

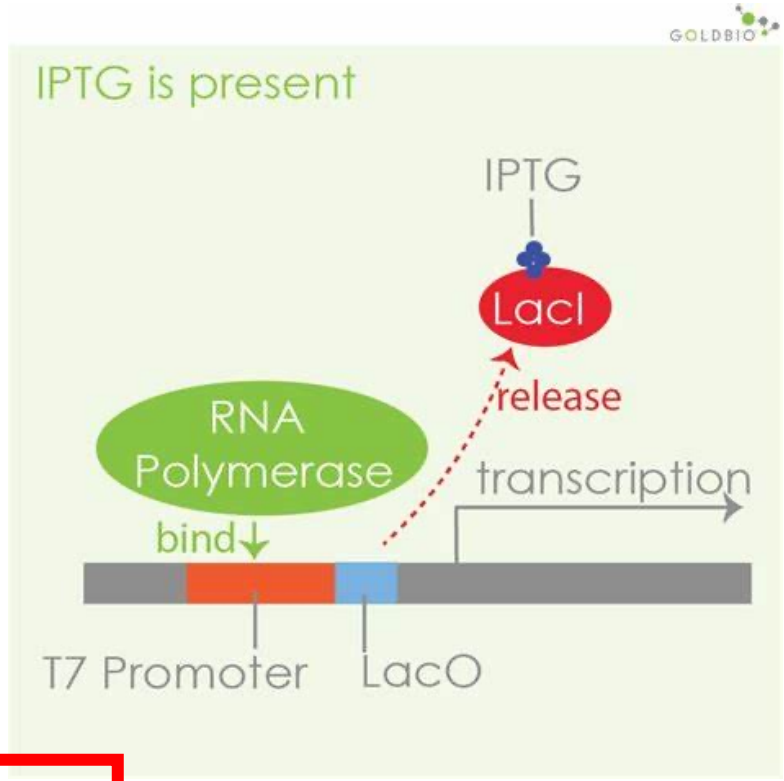
NC-limit [quantity]

- Ammonium / sulphate Imitation
- Titratable nitrogen uptake system (express GDH)

Generalize

- Other catabolic promoters
- Other experimental systems (microfluidic device)

Native LacZ expression indicated the degree of cAMP signalling.



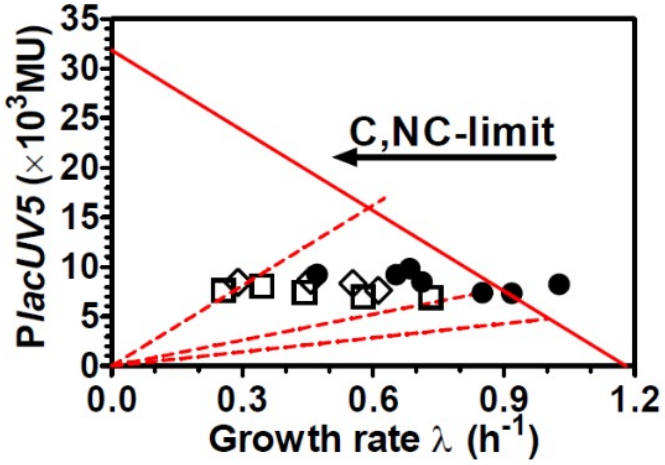
~~Repress: LacI~~
Activate: cAMP

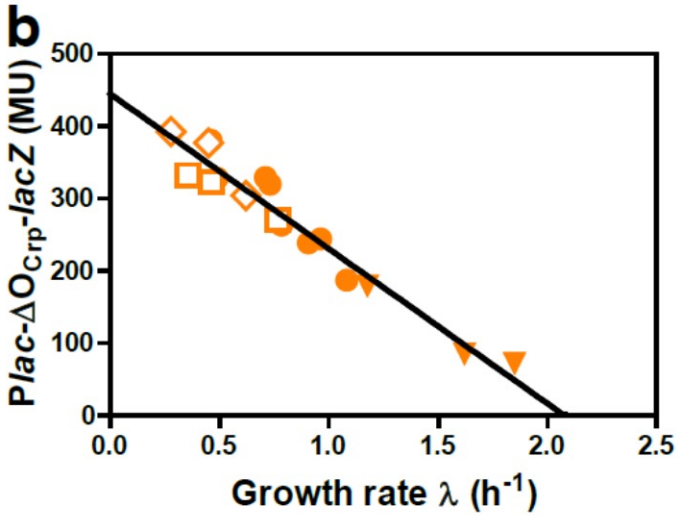
cAMP signalling

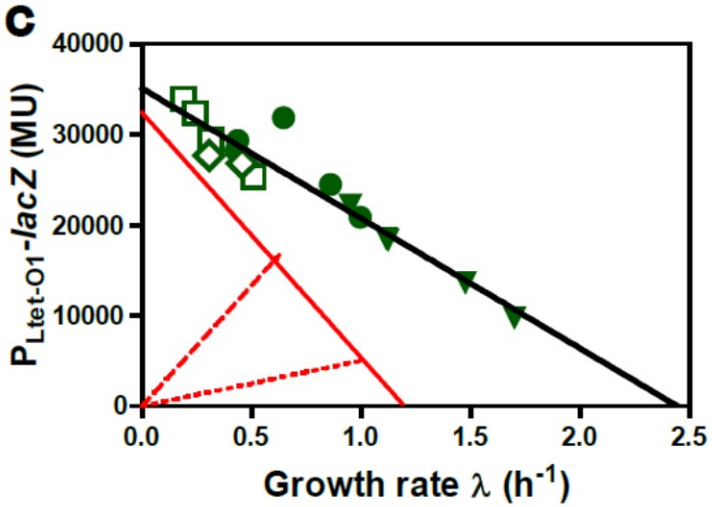
Catabolic protein (LacZ) increase linearly w.r.t. the growth rate under C-limit.

Both the C- and NC-lines rely completely on Crp–cAMP-mediated gene regulation.

Validation1: "**eleminate**" Crp-cAMP ⇒ **no** C- and NC-lines







PlacUV5: independent of Crp-cAMP

Filled: C-limit

Open: NC-limit

Different behavior is beyond research scope

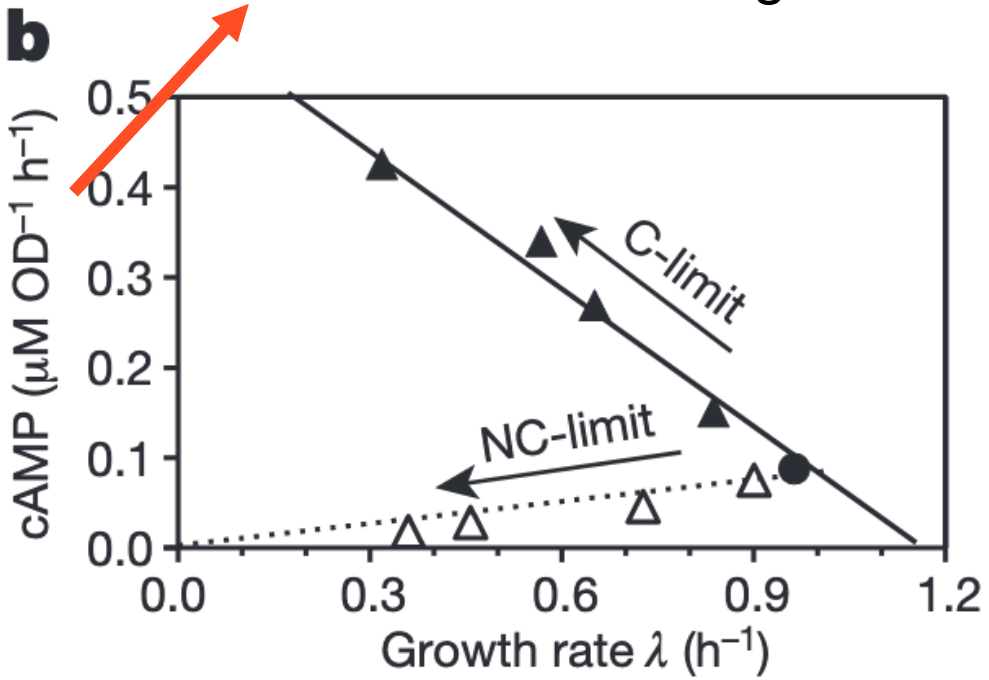
Crp site scrambled

Both the C- and NC-lines rely completely on Crp–cAMP-mediated gene regulation.

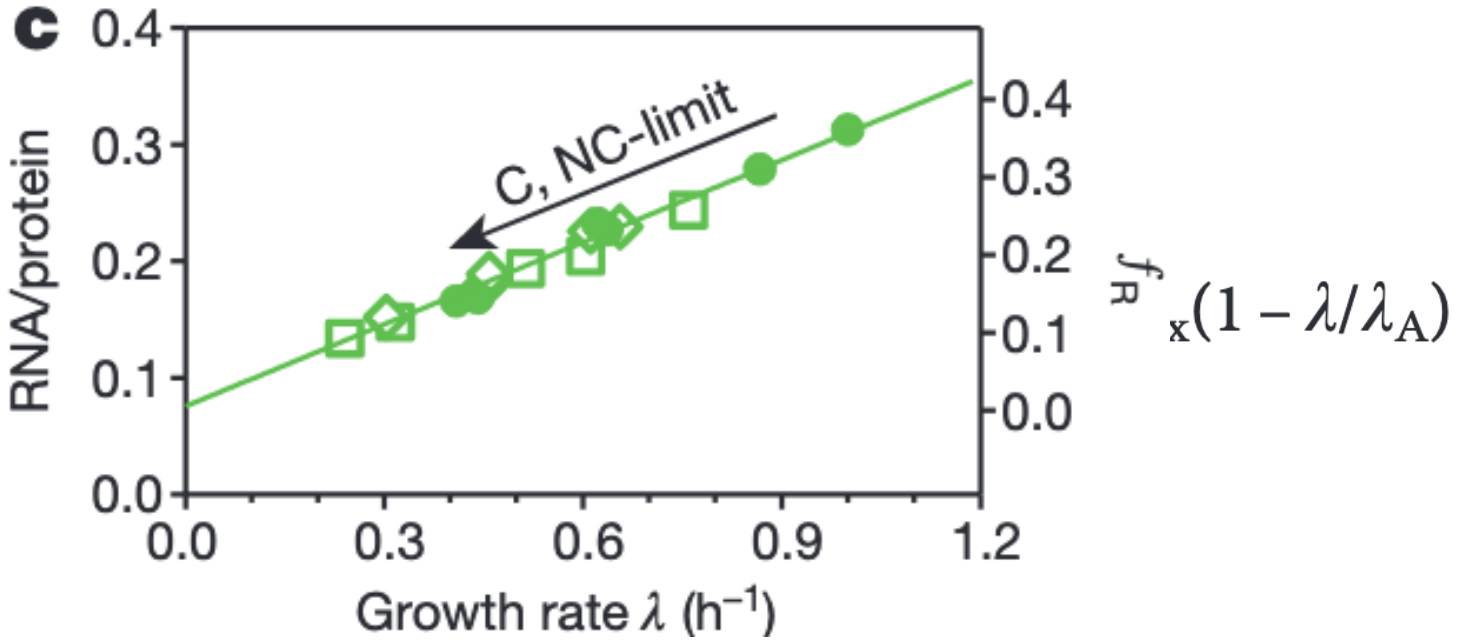
Validation2: cAMP level assemble C- and NC-lines

Internal cAMP level ∝ cAMP excretion rate

cAMP excretion rate = **external cAMP level** × growth rate

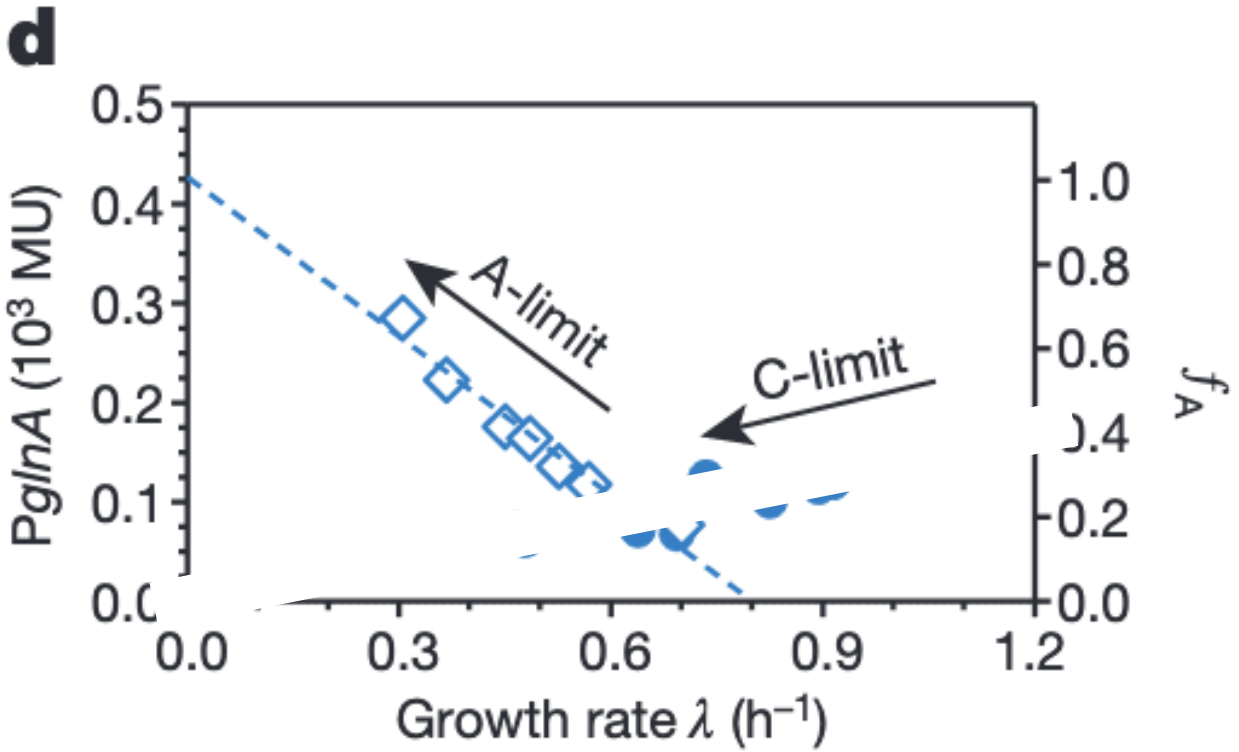


Recall the linear relationship in 2010 science. Similar pattern!



Maybe, the model of **protein allocation** can explain!
If the model works here, since both ribosomal and catabolic **protein fraction decrease** under NC-limit, and the total fraction always remains 1…

The expression of other genes (e.g. anabolic genes) should increase linearly with the growth rate?!!!!



A-line

$$G = G_{\max}(1 - \lambda/\lambda_A)$$

A-limit [quantity] ∈ NC-limit

Titratable GDH system

Generalize

Other genes in amino acid synthesis pathways

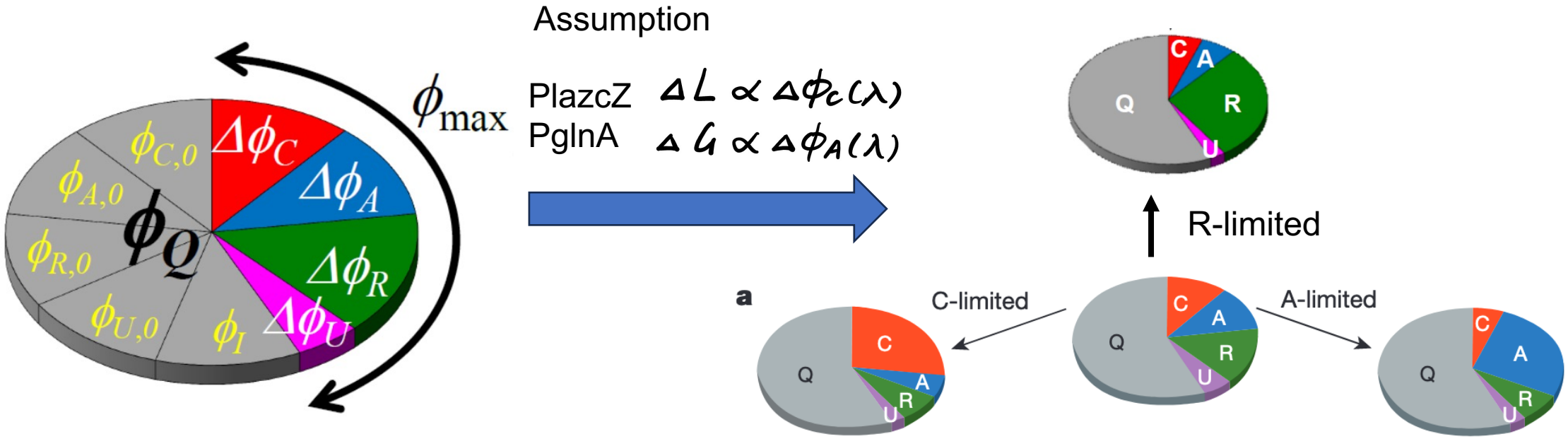
Anabolic gene

glna encodes the major ammonia assimilating protein -- glutamine synthetase

The model of proteome partition (2010 science) is applied and extended.

Definition of sector

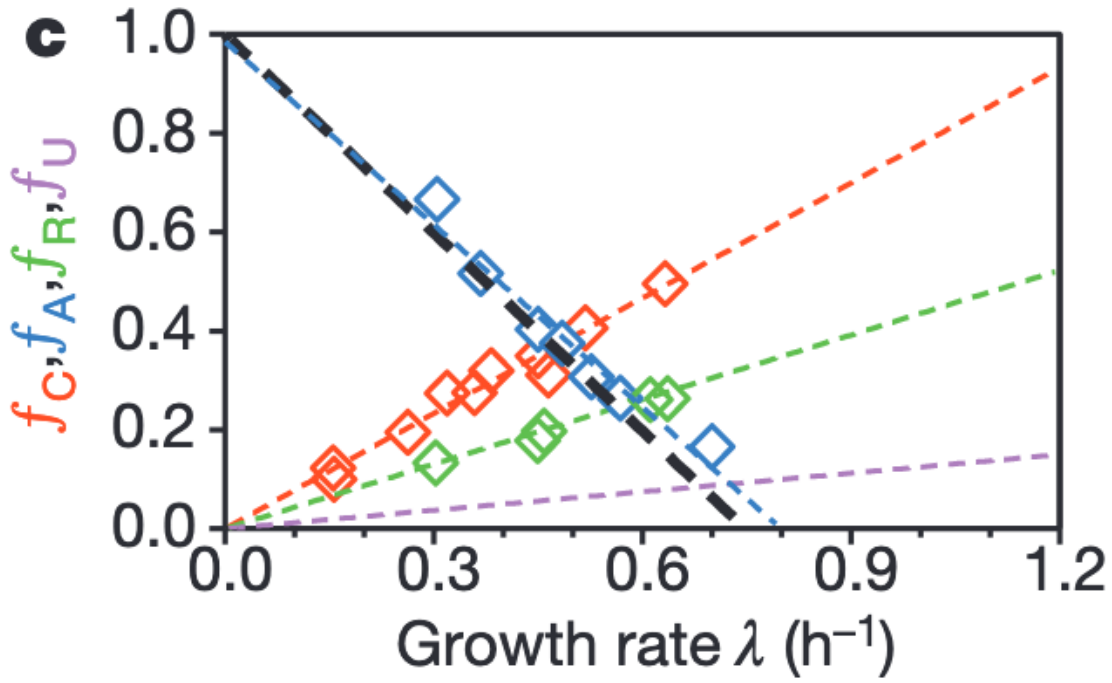
A group of genes whose expression share similar growth-rate dependences upon various modes of growth limitation.



The model of proteome partition (2010 science) explains the above phenomena.

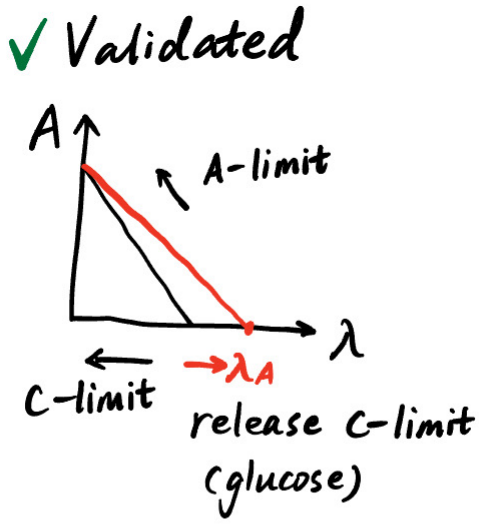
Validation1: Use expression of PlacZ (under A-limit) to predict PglnA (under A-limit)

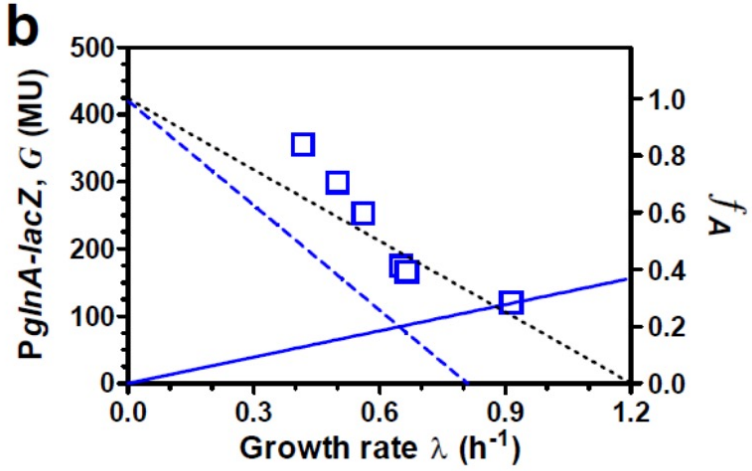
- R-sector follow the growth law
- U-sector is 0.3×R-sector



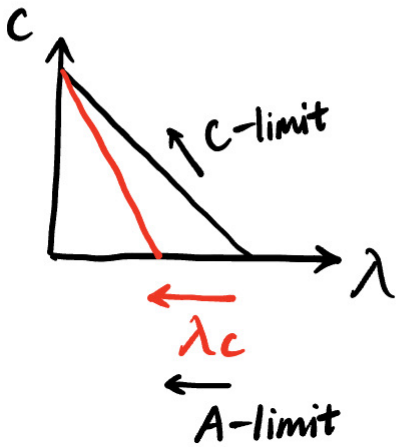
The model of proteome partition (2010 science) explains the above phenomena.

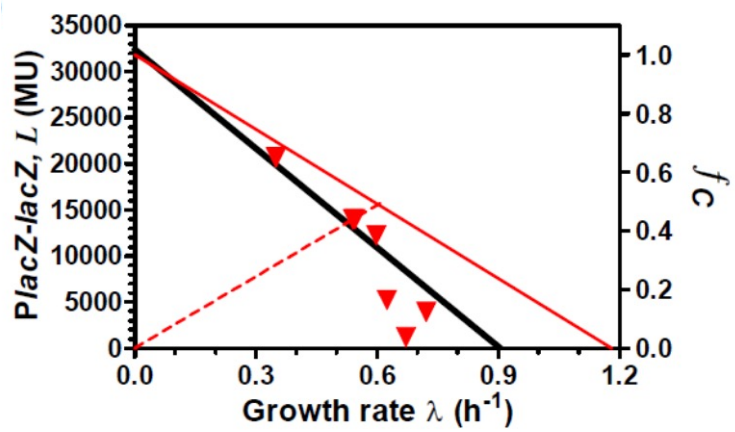
Validation2





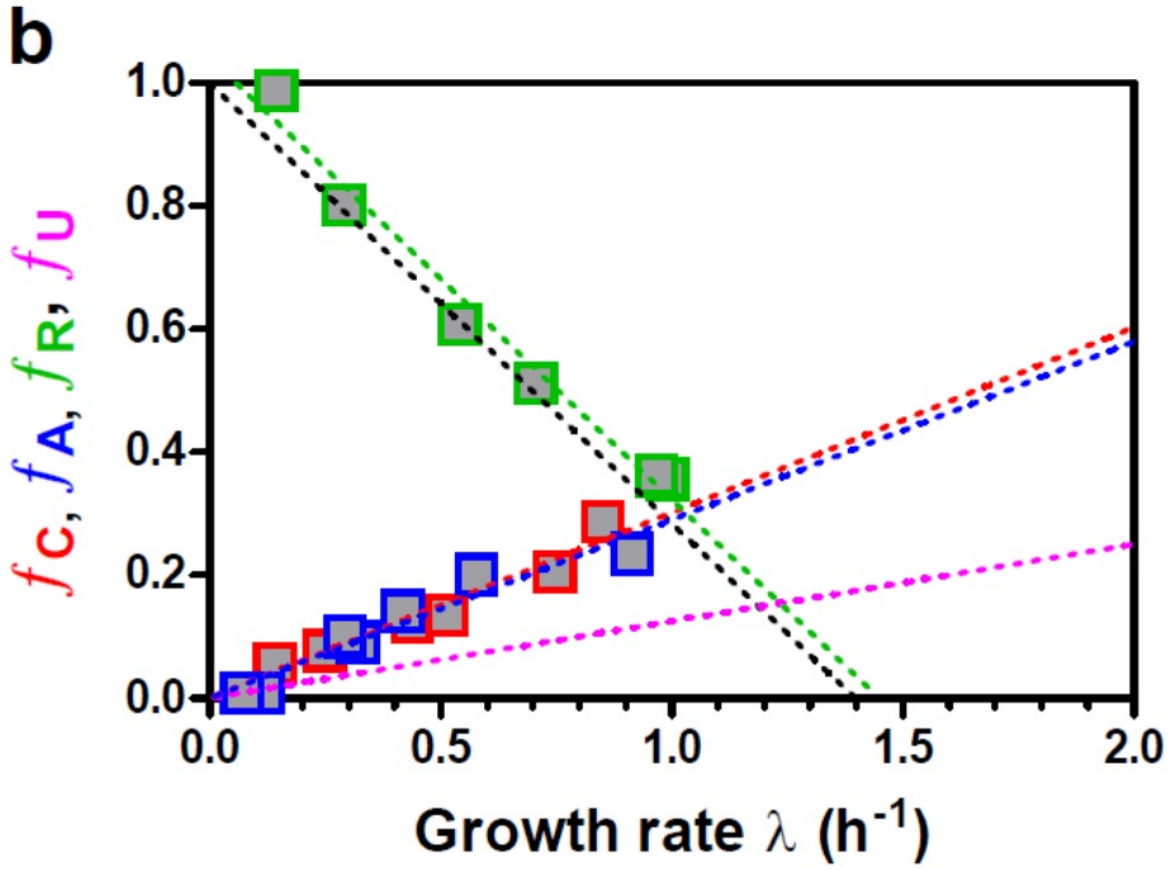
Validation3



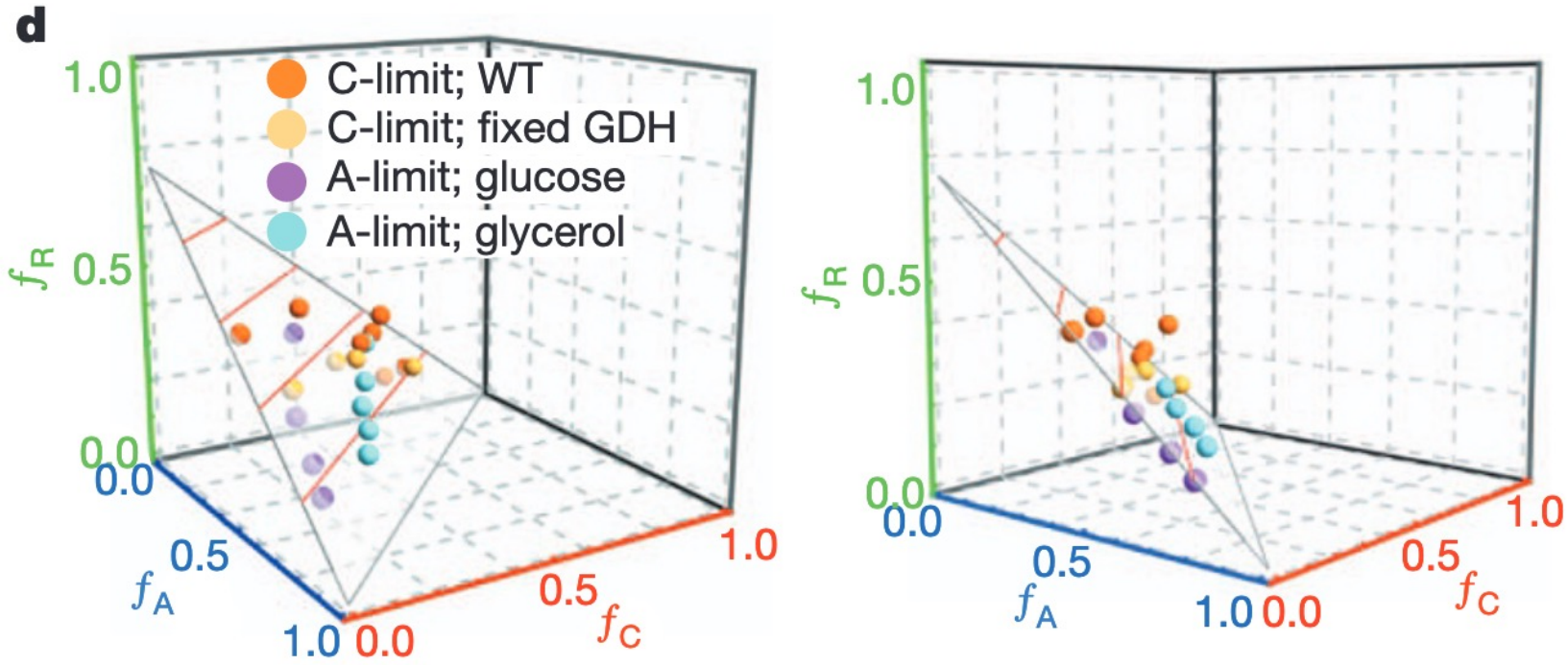


The model of proteome partition (2010 science) explains the above phenomena.

Validation4: under R-limit (antibiotic)—provide a whole picture (C,A,R-limit)



The data lie close to a plane forming a Pareto surface.

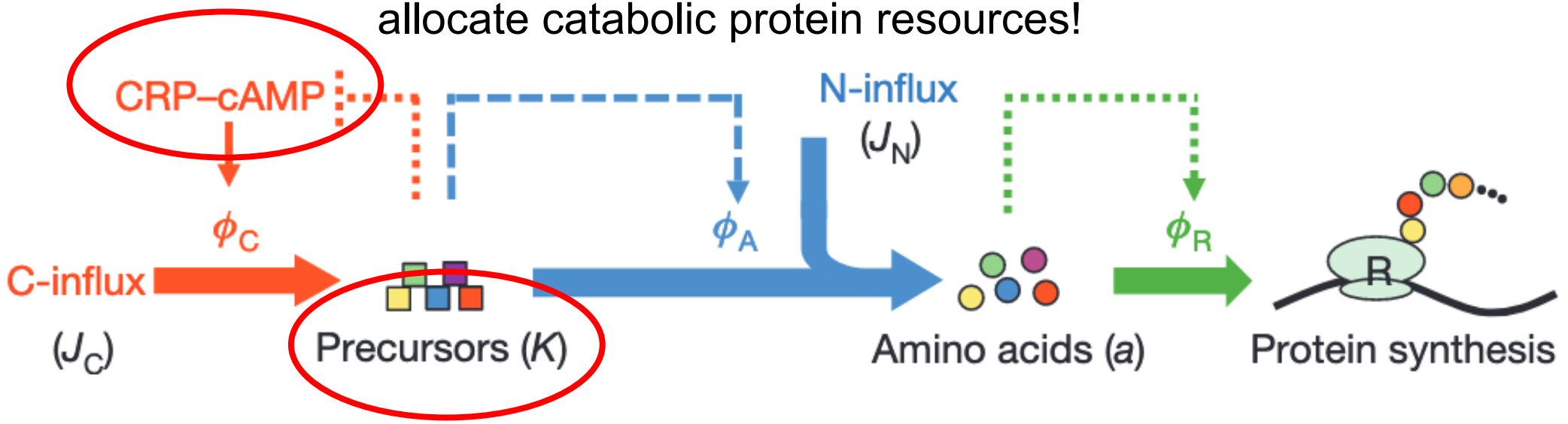


Competing between catabolism, anabolism, translation...

A single signal can sense and eliminate imbalance between metabolic activities on the carbon and nitrogen side.

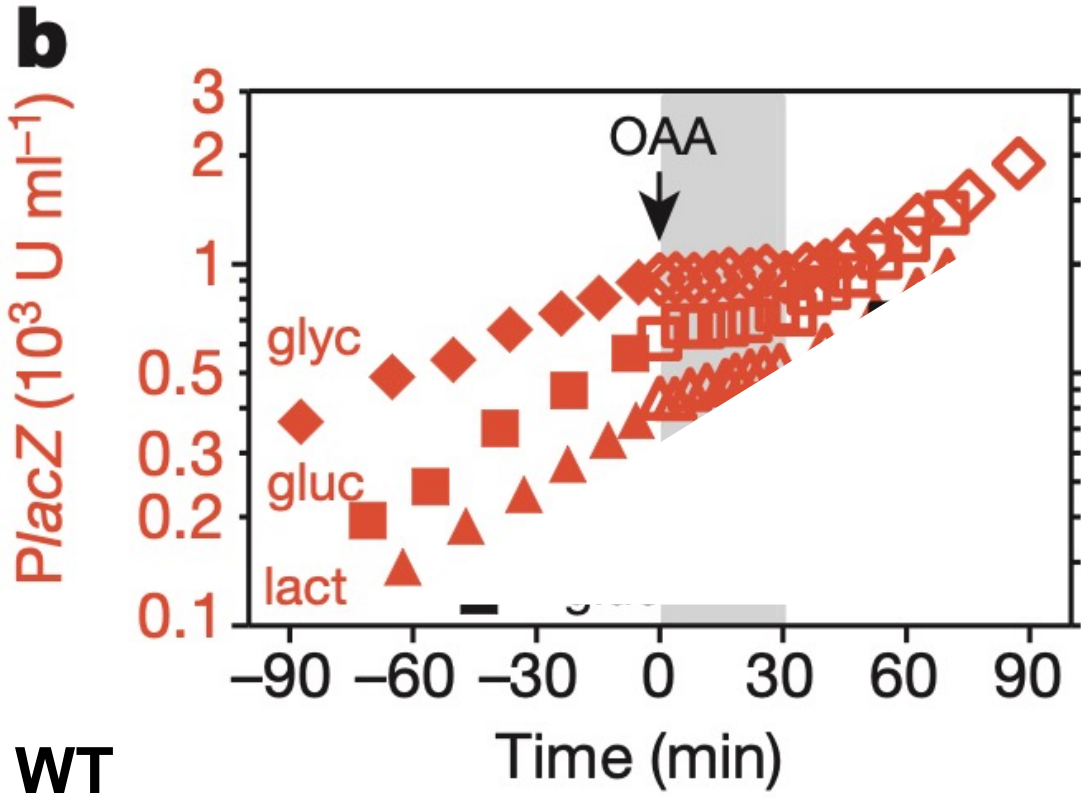
Integral feedback

Physiological function: sence K to allocate catabolic protein resources!

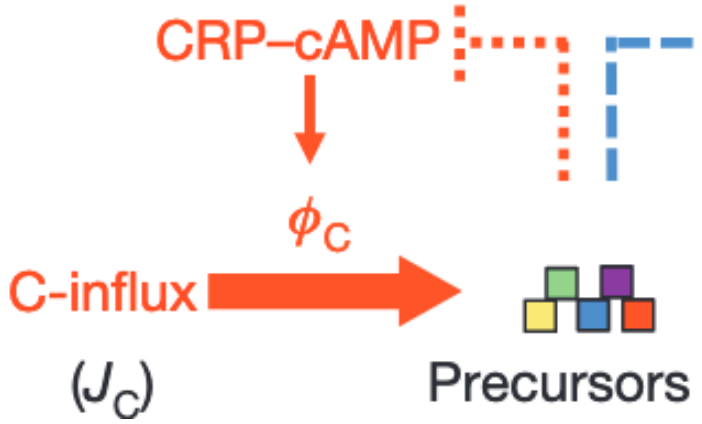


time-integral of the flux difference between J_C and J_N

metabolic precursor K (OAA) represses the expression of metabolic genes (PlacZ).

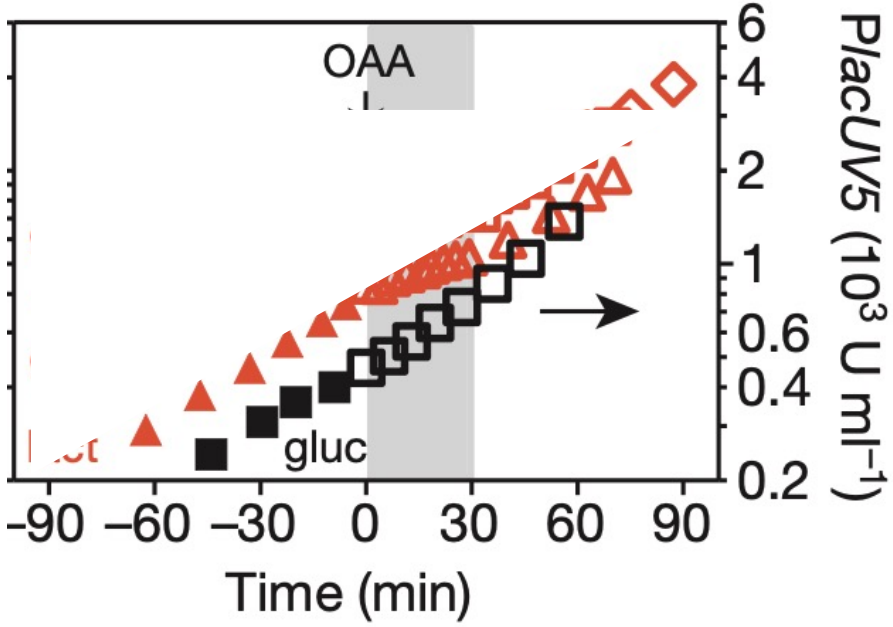


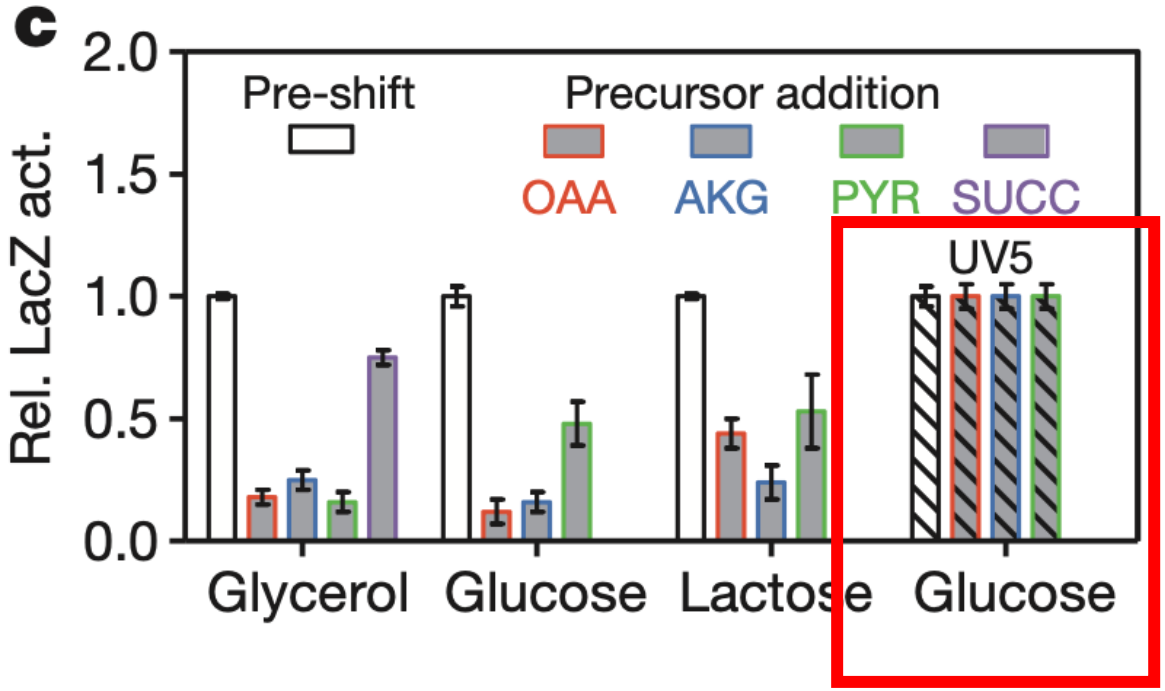
WT



LacI deactivated

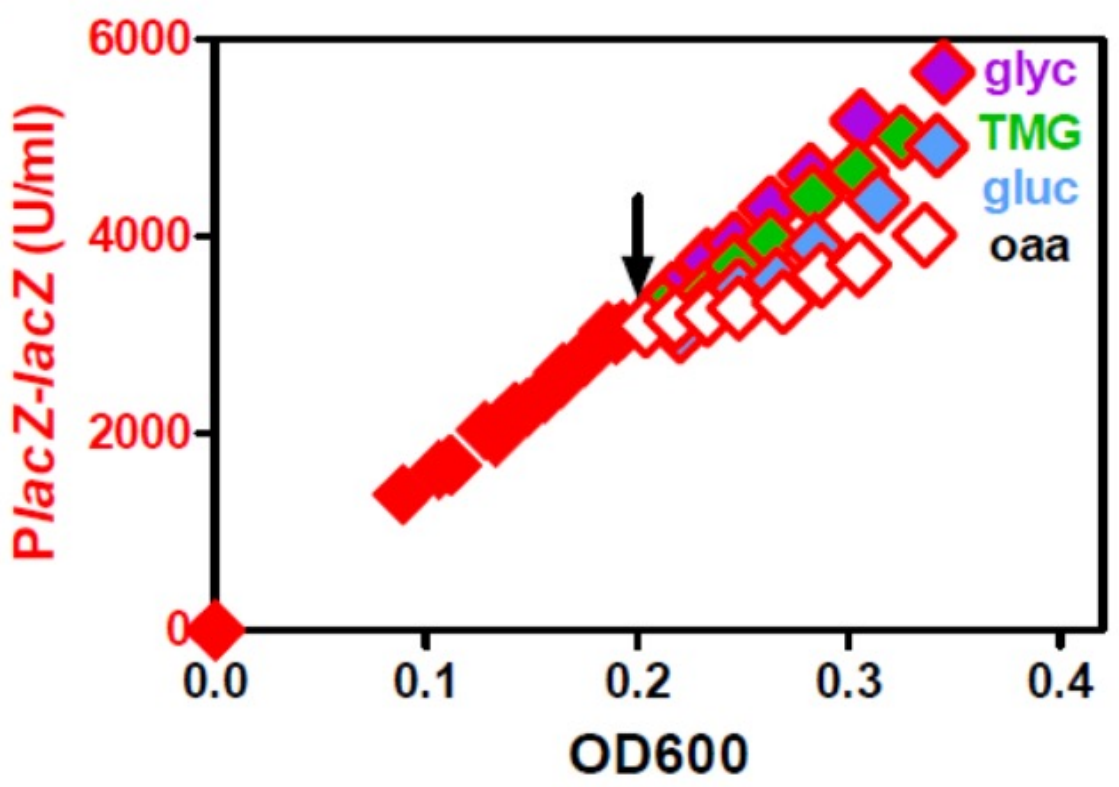
The repression depends on Crp-cAMP.



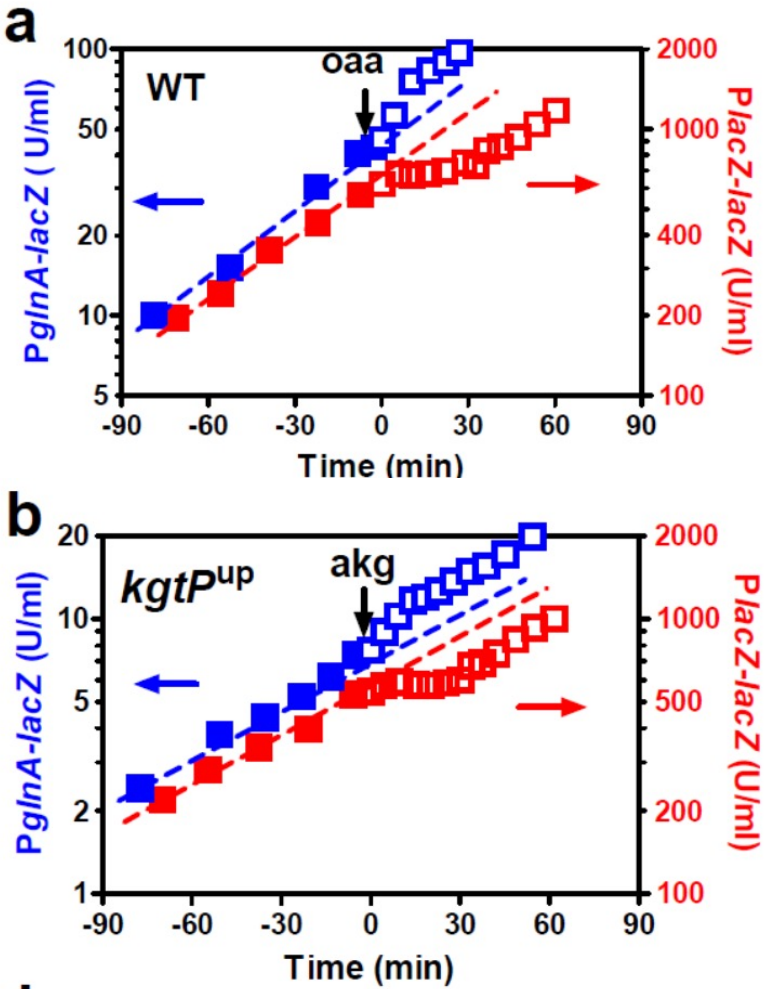


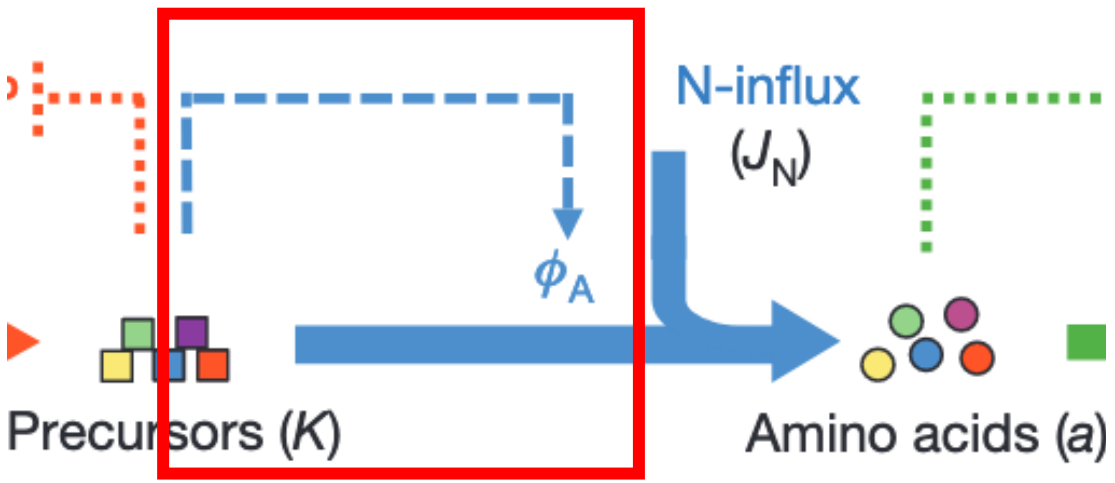
PlacUV5: a Crp-independent promoter

The addition of precursor K is the dominant cause of transient repression.



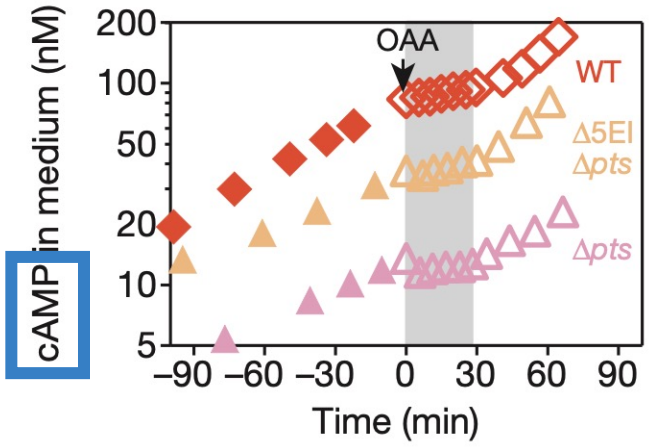
Metabolic precursor K increases the expression of anabolic genes (glnA).

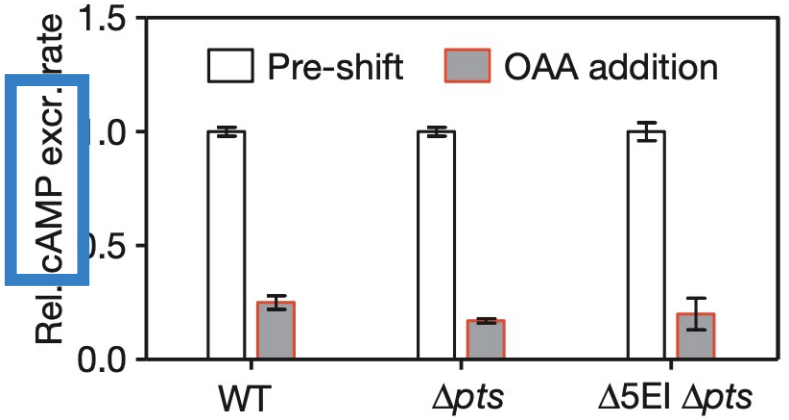


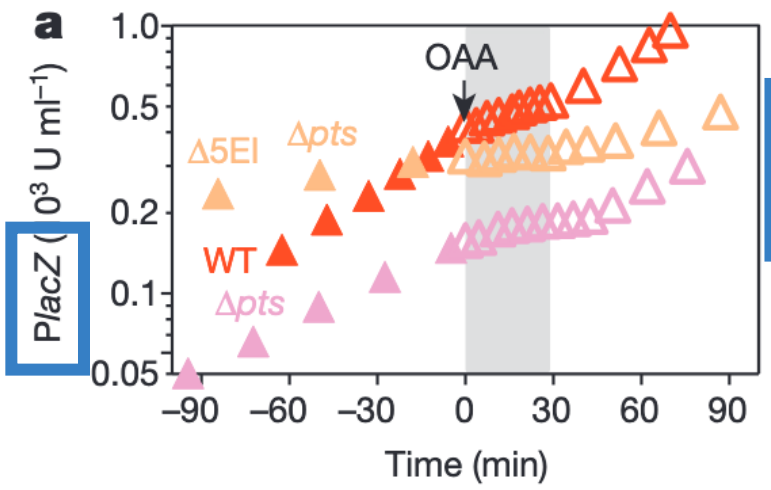


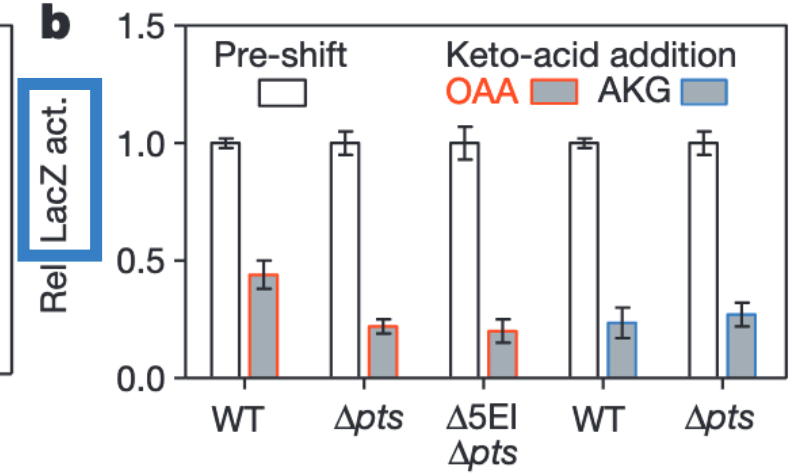
Have been validated: they are cAMP independent

Transient repression was still observed upon the addition of *α*-ketoacids in strains with deletion of PTS protein.









✅ Precursor …→ Crp-cAMP

✅ Precursor → EI

EI ?...→ Crp-cAMP

EI of PTS is **NOT the only candidates** to trigger cAMP signalling!

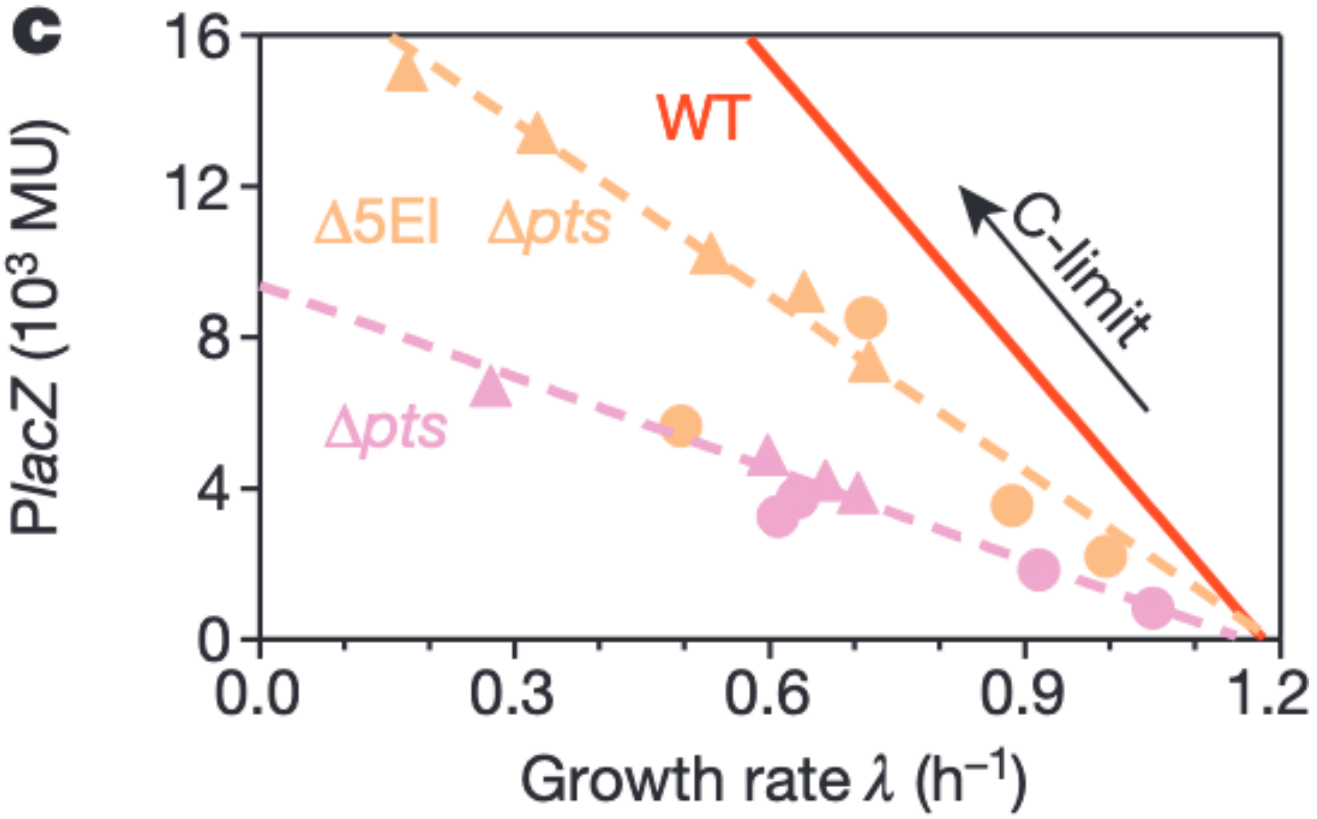
Δpts: delete 3 major PTS protein

Δ5EI *Δpts:* also delete 5EI protein

EI: enzyme I of the PTS protein

/volume in unit

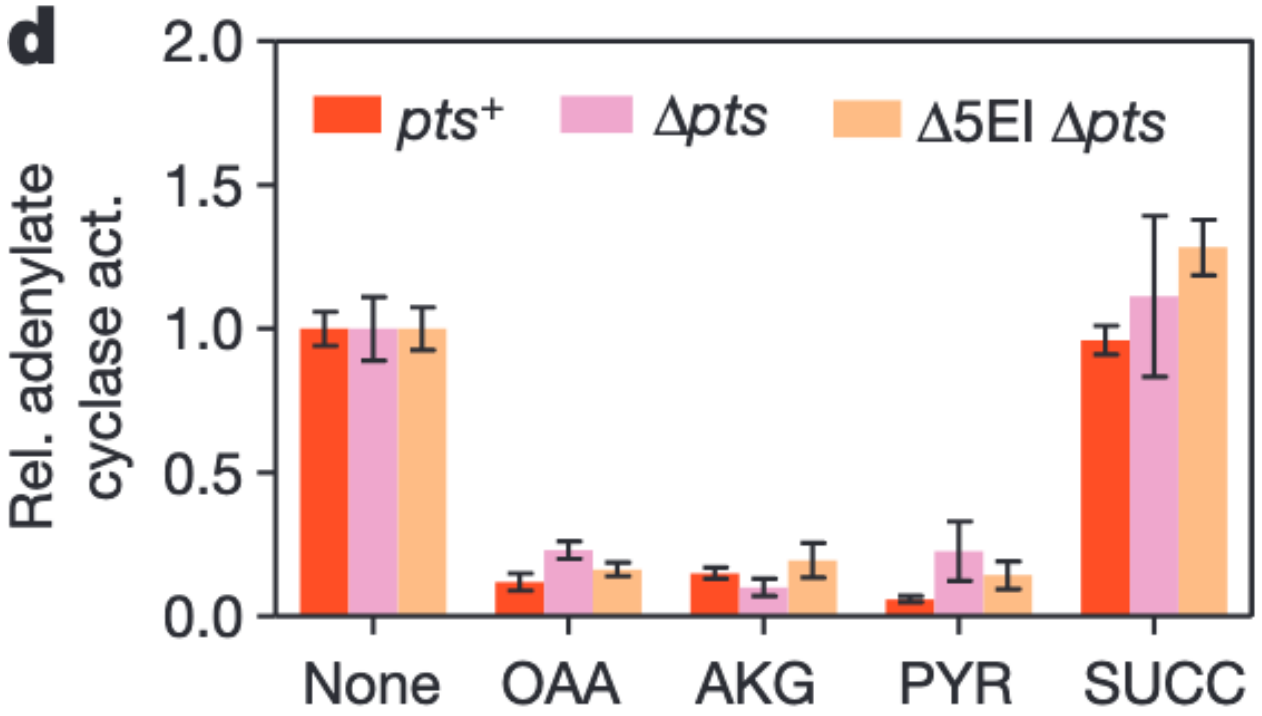
C-line was observed in strains with deletion of PTS protein but effect degree changes.



Precursors inhibit cAMP synthesis by adenylate cyclase (AC)

✅ Precursor (α-ketoacids) → (directly or mediator proteins)→ activity of AC → Crp-cAMP

No need to be PTS proteins



A top-down approach was used to figure out the physiological function of cAMP signalling and its molecular triggers.

Top-down approach

CCR → Linear relationship (C-line, A-line, R-line) → Proteome allocation model → Integral feedback → deduce…

Physiological function

Use most effective sugar ⇒

Allocate proteomic resources according to precursors amount and quality (reflecting nutrient condition: $\int (J_C - J_N) dt$)

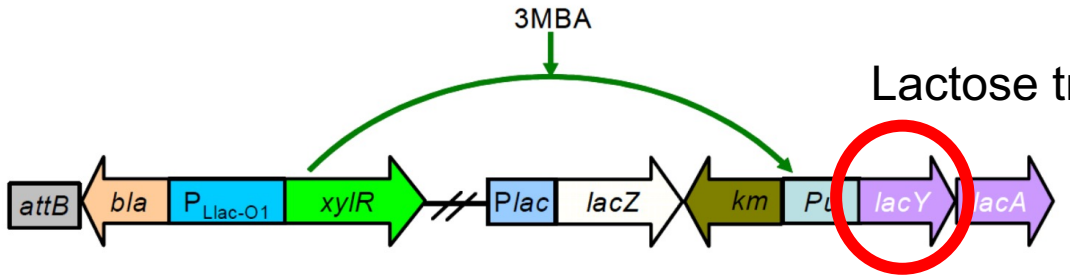
Molecular triggers

PTS → activity of AC → Crp-cAMP ⇒

Precursors → (directly or mediator proteins)→ activity of AC → Crp-cAMP

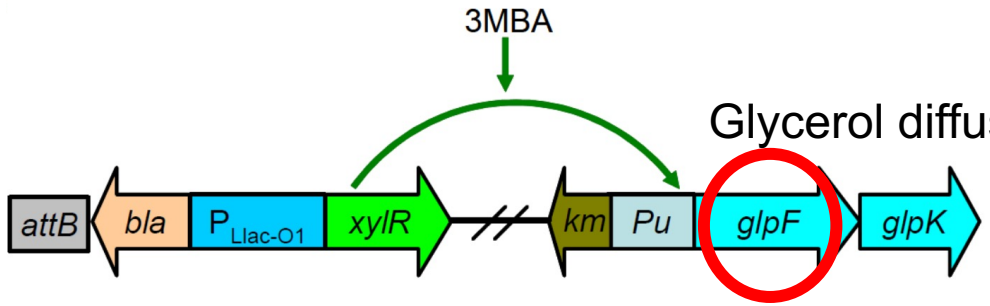
Titratable uptake system in Figure 1.

NQ381
Insert promoter *Pu*
- 3MBA induce *xylR*
- *xylR* activate *Pu*



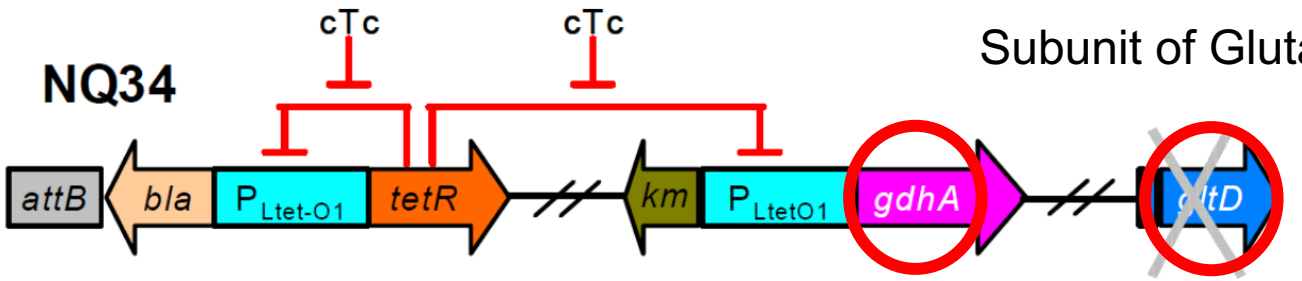
Lactose transporter (only one)

NQ399
Replace glpFK operon promoter with *Pu*



Glycerol diffusion facilitator

Delete gltD
Replace gdhA promoter with $P_{ltet-O1}$ Promoter
- TetR repress $P_{ltet-O1}$ Promoter and itself
- inducer chloro-tetracycline (cTc) release the repression



Subunit of Glutamine synthase

Glutamine Synthetase