Systems Biology 551-1174-00L

Metabolic Regulation

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Content:

- **Metabolic regulation mechanisms & mechanistic interpretation of metabolite data**
- Can engineers understand glycolytic control?
- Can a biologist improve control of glycolysis?

Learning Goals: Metabolic Regulation

- **Know basic mechanisms how cells regulate metabolic flux & explain biological reasons why cells need them.**
- **Describe the relationship between metabolite [c] and flux.**
- **Critically assess how different types of feedback affect the dynamic regulation of metabolism.**

Exercise 5

Transfer knowledge to new problems: formulate and implement a model of *E. coli* glycolysis.

The bonus exercise will enable you to:

- **analyze a real system, understand its steady state and dynamic behavior using your model implementation.**
- **estimate kinetic parameters and thus improve model quality, by comparing simulation and experimental results.**

OMICS Data

- General term for measurements of the entire complement of all metabolites, mRNAs or proteins in a cell
- Typically these methods provide concentration data on hundreds to thousands of cellular species in one sample
- The speed of the cellular response to a stimulus is typically metabolites -> mRNAs -> protein
- Once the proteins start to change, it typically leads to new changes in the metabolites ....
- A major difficulty in data interpretation is to delineate direct and causal from indirect consequences ⋯..

Metabolite Data: Metabolomics

- Measurement of all metabolite concentrations in a cell, body fluid etc

What's in a metabolome:

- **Microbes** > 1000 species
- **Single plant species** ≈ 5000 in Arabidopsis
- **All plant species** together ≈ 90,000-200,000
- **Humans** 41,514 metabolite entries (water- and lipid soluble metabolite in human metabolome database! http://www.hmdb.ca/

- Main techniques are mass spectrometry & NMR with a current maximum of a few hundred compounds for a given method

Dynamic Data are Information Rich – But How to Infer Causality & Mechanisms ?

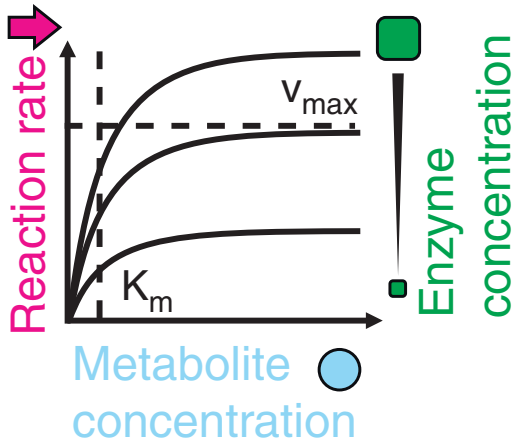
Which physico-(bio)chemical processes contribute to the emergence of a new metabolic flux pattern ?

Which of these are under the control of the cell?
Which regulation process does the cell employ to modulate them?

Framework for Mechanistic Understanding

$$v = K_{cat} E \frac{S}{S + K_m}$$

ODE-based kinetic models:
e.g. Michaelis-Menten

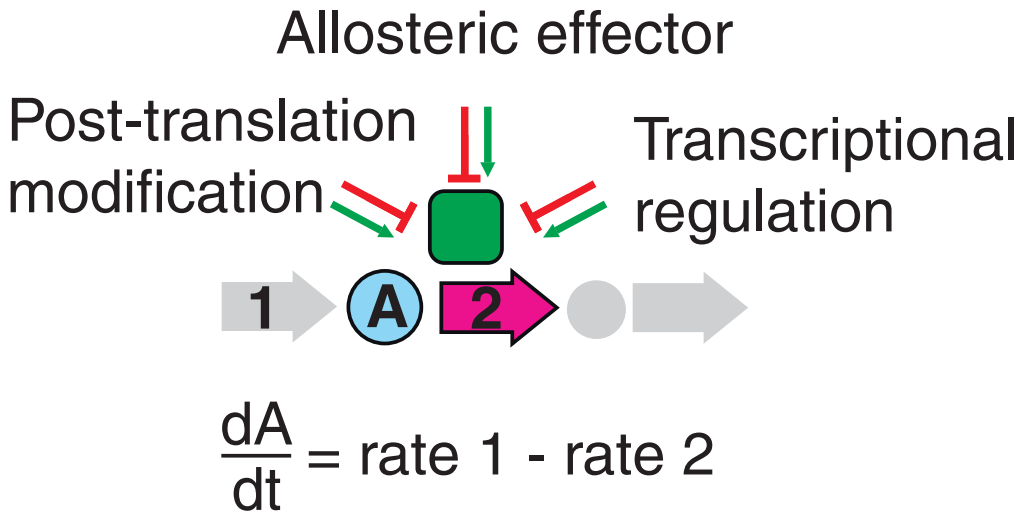


Virtually all models of enzyme kinetics decompose flux into contributions of **enzyme** and **metabolites** (*i.e.* substrates, effectors ….)

Reaction kinetics and their parameters determine how concentrations of metabolites and enzymes influence the reaction rate. Mass action kinetics are summed up in ordinary differential equations that describe the mass balances around a metabolite.

By which mechanisms can cells control their reaction rates ?

Through Which Mechanisms Can Cells Control Reaction Rates ?



Processes that alter enzyme activity and/ or abundance

- Transcriptional – gene expression/translation
- Post-translation modification such as:
  - .....
  - .....
  - .....
- Allosteric regulation by metabolite-protein interactions

How Can Cells Coordinate Metabolic Fluxes?

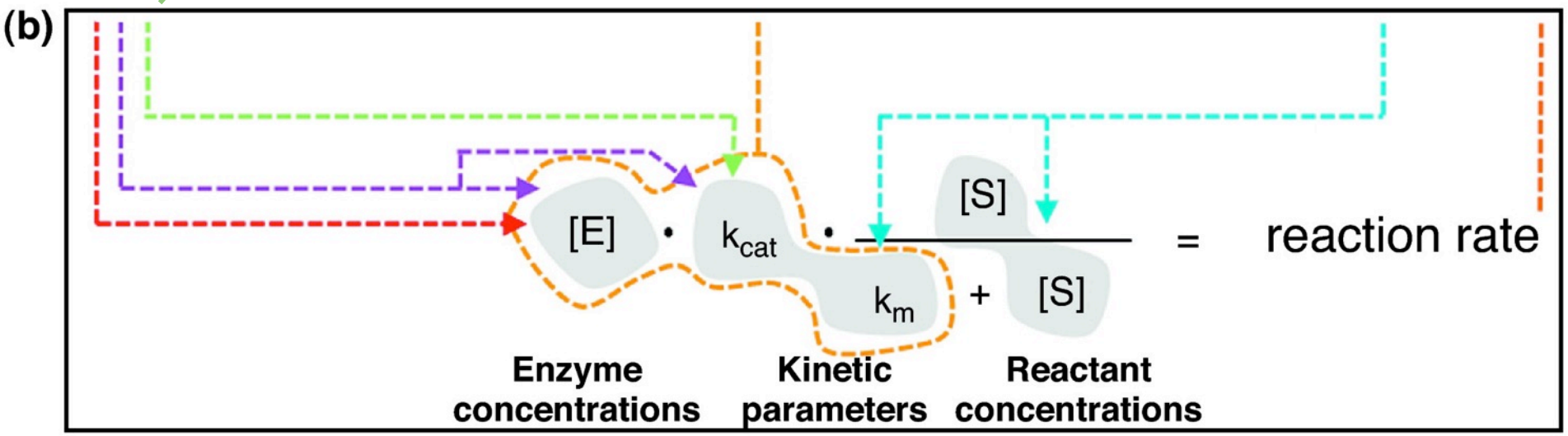
Gene expression

Post-translational modification

Allosteric regulation

Enzyme capacity

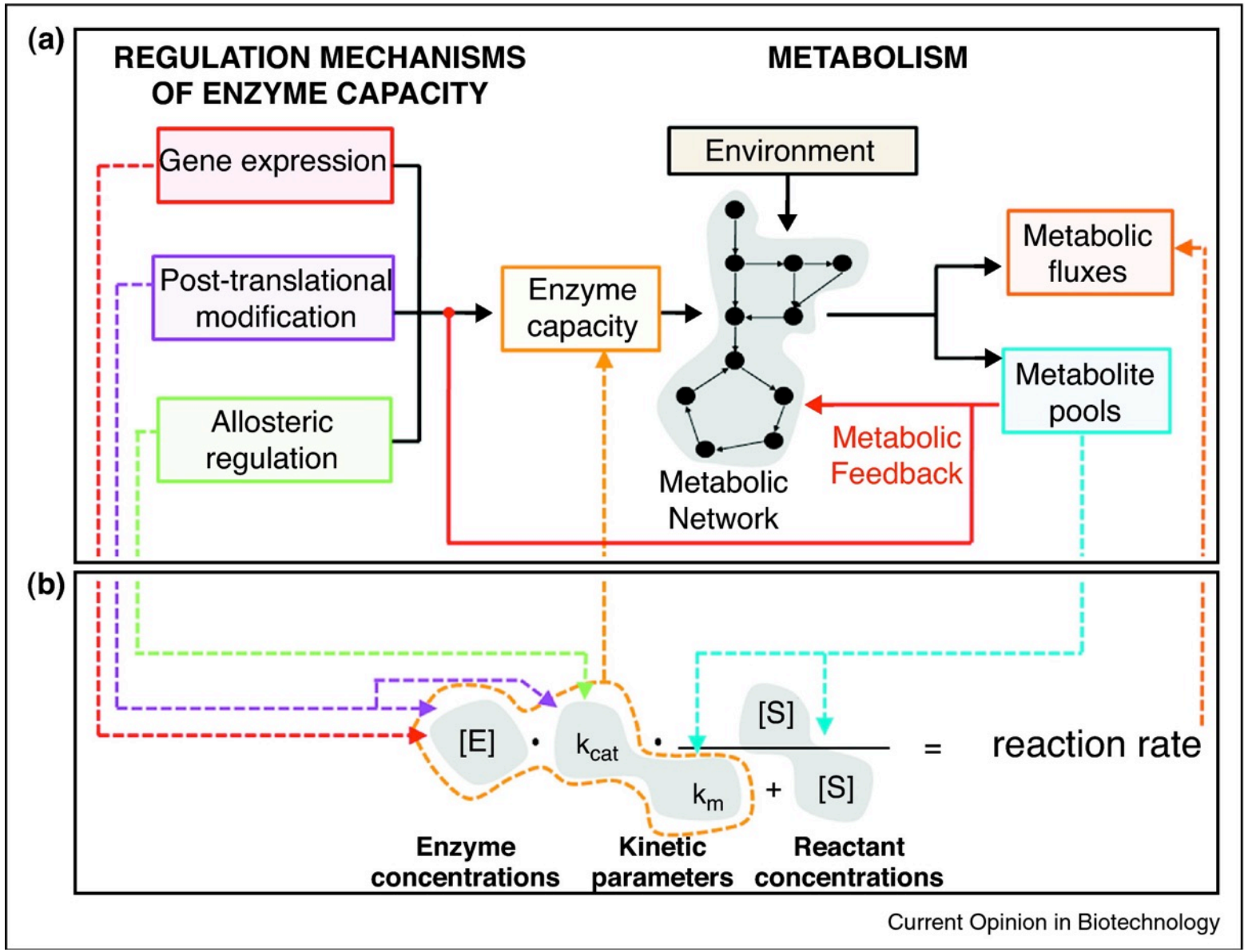
Metabolite level



Current Opinion in Biotechnology

How Can Cells Coordinate Metabolic Fluxes?

Gerosa & Sauer 2011 Curr Opin Biotechnol



How can we then interpret cellular metabolite data ??

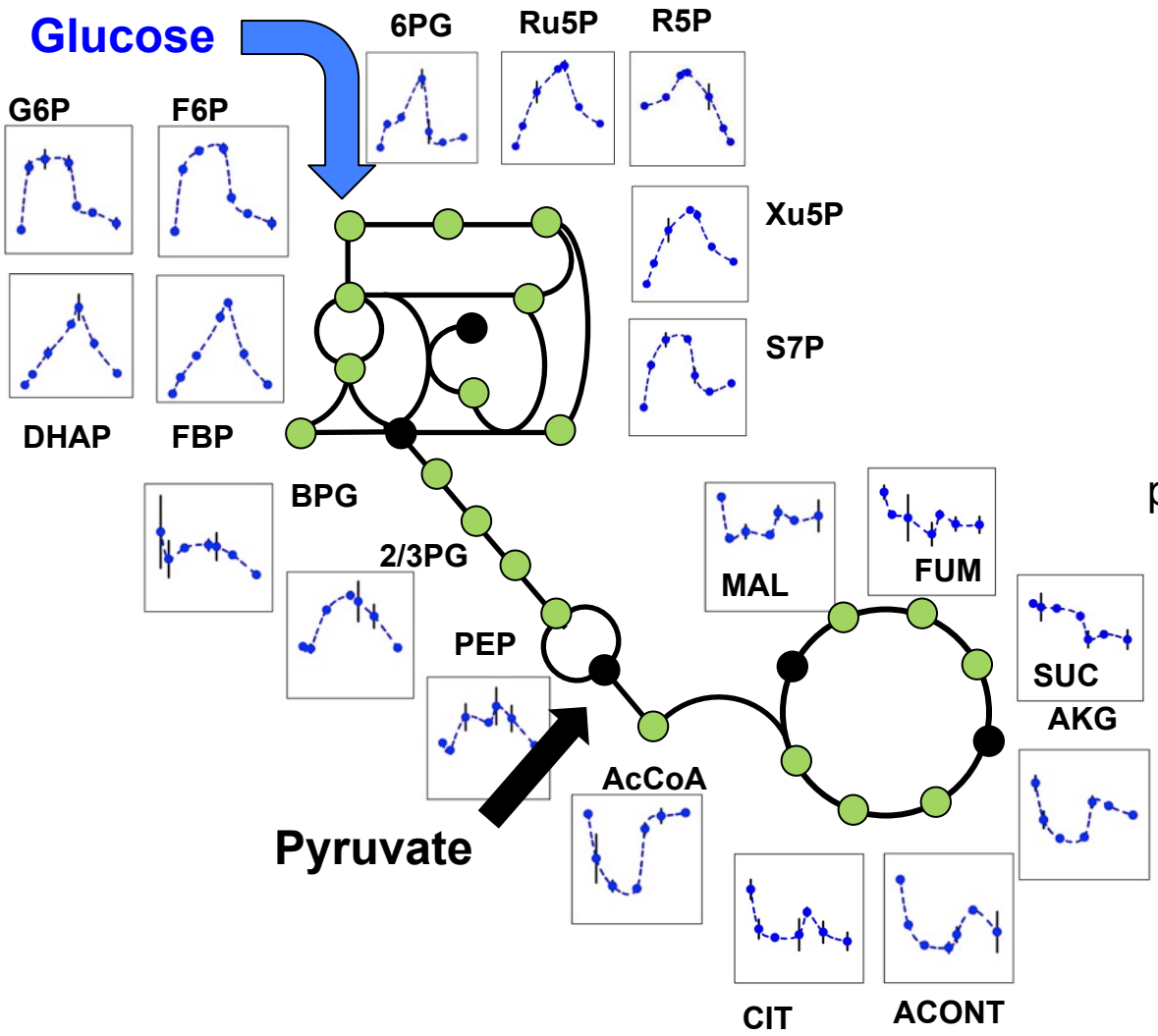
(for example by focusing on conditions/time-scales where only one or few of the regulation processes matter)

How?

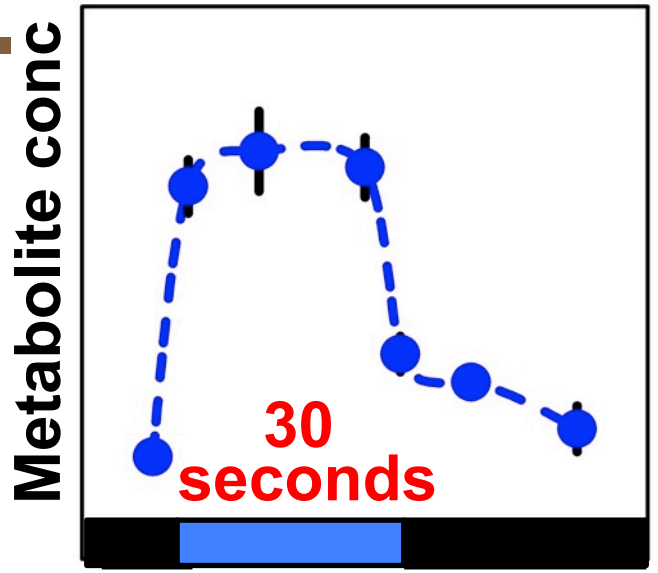
In the following (and the exercise) we attempt a mechanistic interpretation. In the second half of the course we use statistics

Metabolite Dynamics

Very short time scales; *i.e.* only enzyme kinetics rule!



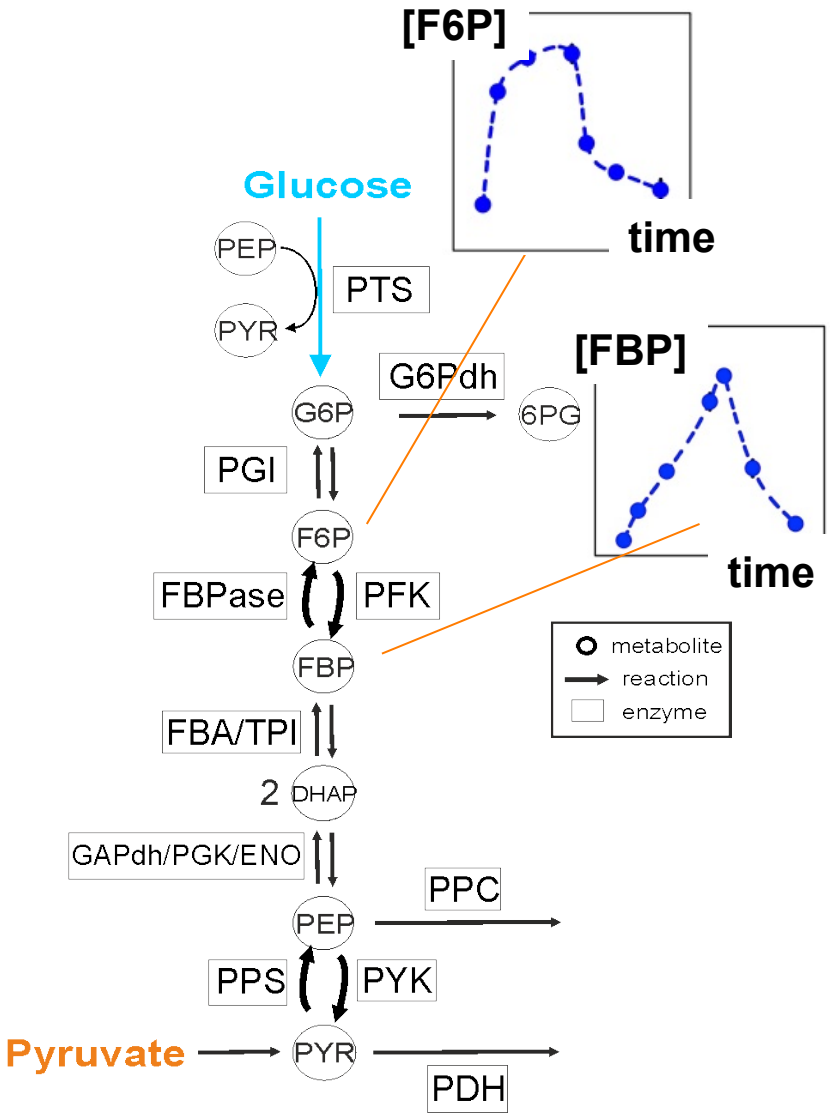
Perturbation of steady state
Pyruvate -> Glucose -> Pyruvate



pyruvate steady state → **glucose pulse** → return to pyruvate steady state

What is the relationship between [metabolite] and fluxes ?

Mass Balances and Kinetic Equations



Fluxes determine metabolite dynamics

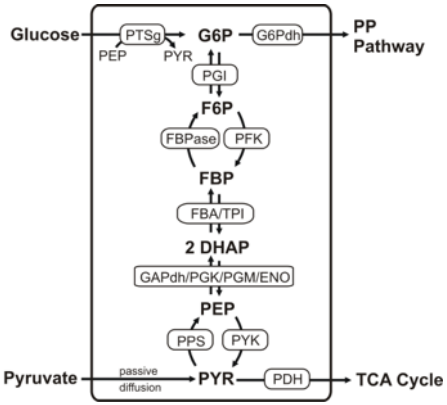
$$\frac{d[FBP]}{dt} = v_{PFK} - v_{FBA} - v_{FBPase} \neq 0$$

Metabolites determine fluxes

$$v_{PFK} = v_{\max}^{PFK} \frac{[F6P]}{K_m + [F6P]}$$

- concentrations and fluxes are intertwined
- hard to understand intuitively
- dynamic ODE modelling
- **w/o kinetics – no statements on metabolite concentrations!**
- ***Which metabolic modeling framework works w/o kinetics ?***

Glycolysis Simulations in Your Exercises



Set of Michaelis-Menten equations

$$v_{PFK} = v_{\max}^{PFK} \frac{[F6P]}{K_m + [F6P]}$$

System of ODEs on mass balances (in matrix form)

$$\frac{d[G6P]}{dt} = \ldots.$$

$$\frac{d[FBP]}{dt} = v_{PFK} - v_{FBA} - v_{FBPase}$$

...

Metabolites and fluxes are mechanistically connected here through Michaelis-Menten kinetics

Perturbation: eg higher influx to G6P

X_o *Steady state metabolite [conc]*

Kinetics

X → **v(X)** → **v** → **Mass balances dX/dt** → ∫ → X_1

Numerical integration
solving for short intervals t

[conc] at time point 1

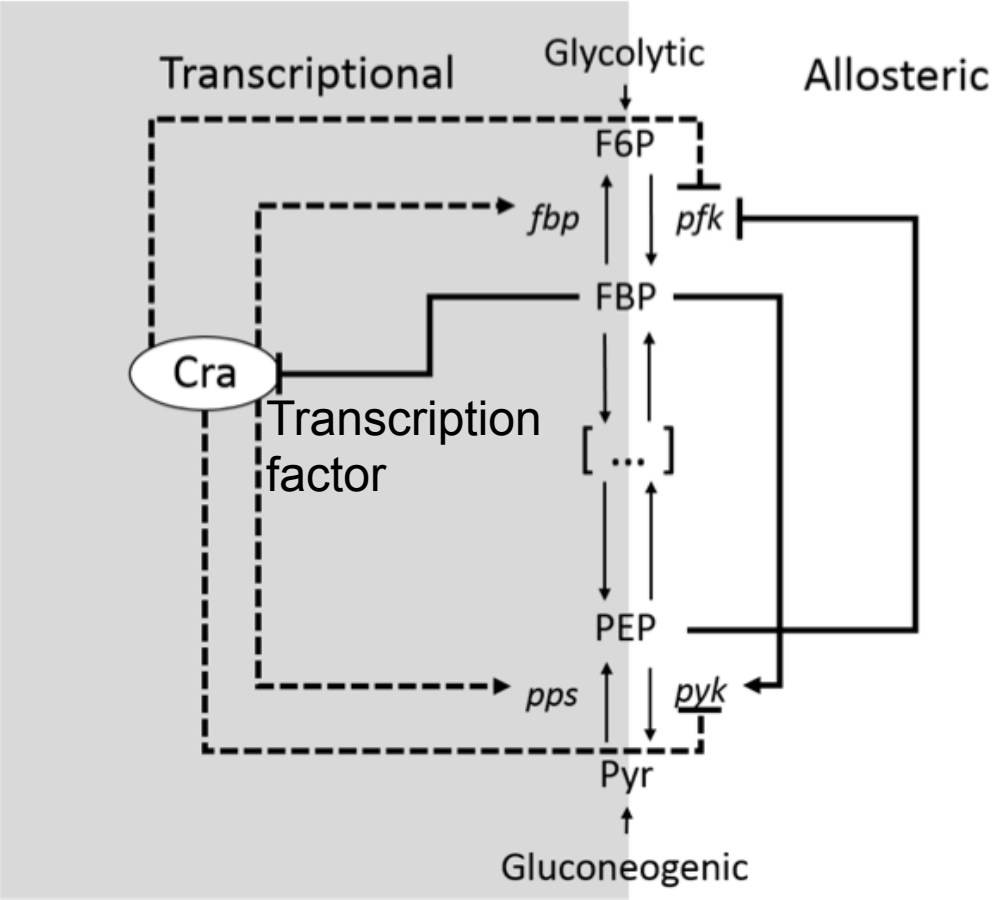
Flux Coordination at 2 Temporal Levels

Simplified Regulation Model of Glycolysis/Gluconeogenesis in *E. coli*

Some key allosteric (solid lines) and transcriptional (dotted lines) regulatory interactions are indicated.

fbp is fructose 1,6-bisphosphatase
pfk is phosphofructokinase
pps is phosphoenolpyruvate synthase
pyk is pyruvate kinase.
Cra is the DNA-binding transcriptional dual regulator *Cra* formerly known as *fruR*.

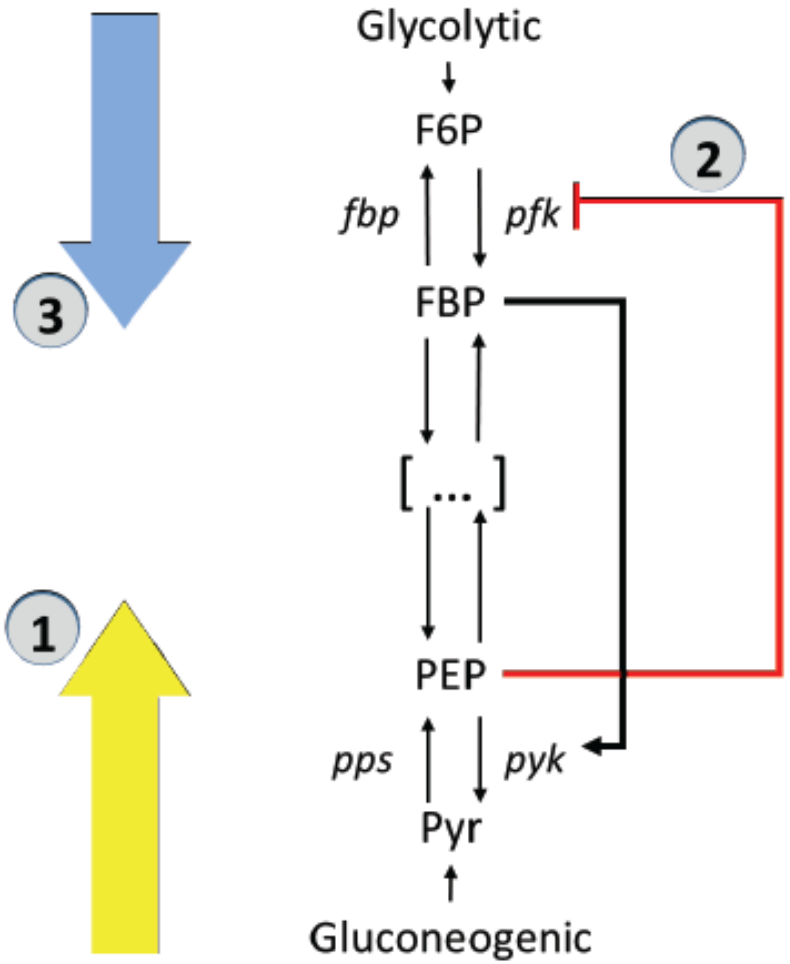
Can you postulate glycolytic roles of allosteric regulation by FBP and PEP ?



Gedankenexperiment with Simplified Glycolysis Model

Imagine *E. coli* growing on a gluconeogenic substrate (1, yellow), e.g. pyruvate. At steady-state, high PEP pool cause inhibition of *pfk* (2). This interaction helps the cell to switch from usage of a glycolytic substrate to usage of a gluconeogenic substrate. Now let us assume perfect, 100% effective allosteric inhibition of *pfk* activity by PEP for the purpose of this thought experiment. The gluconeogenic carbon source is maintained (1) and a glycolytic substrate (3, blue), e.g. lactose, is introduced in the medium. In this case, the cells could not switch to the preferential utilization of the glycolytic carbon source enabling faster growth, if *pfk* is completely shut off by PEP. This is a theoretical example of a regulatory interaction that is essential in one condition but could be detrimental in another.

Thus, also the quantity of the regulatory influence matters !

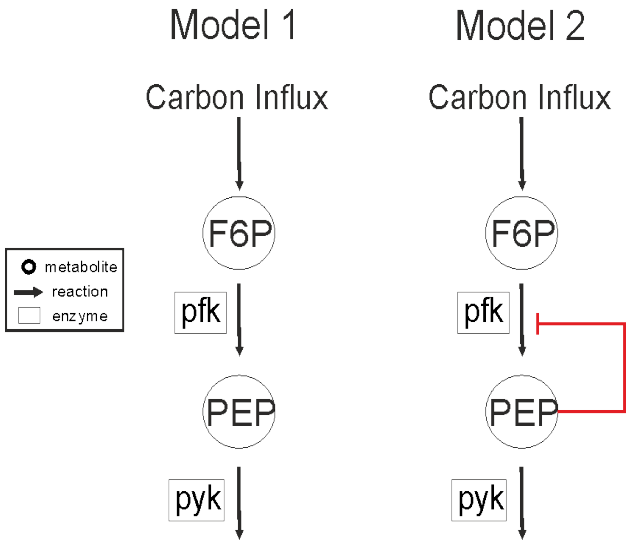


Dynamic Analysis with Kinetic Models:
Regulatory Interactions

Why did cells have to evolve regulatory interactions ?

How can we study and understand them ?

What experiments would you design?



How could cells mechanistically achieve the feedback from lower to upper glycolysis?
the example from the exercise

Recap

Be able to:

- explain basic mechanisms of metabolic regulation.
- explain their biological need (ie why they evolved).
- describe the relationship between metabolite [c] and flux.
- explain how metabolite data can be interpreted mechanistically
- apply this reasoning to other pathways (Glycolysis was only the consistent example).

Exercise 5: Glycolysis Model

Goal

- Formulate and implement an abstract representation of *E. coli* glycolysis, given its topology, parameters and initial conditions.
- Estimate model parameters using available experimental data.