Modelling Biochemical Reaction Networks

Lecture 11: Metabolic control analysis of glycerol metabolism

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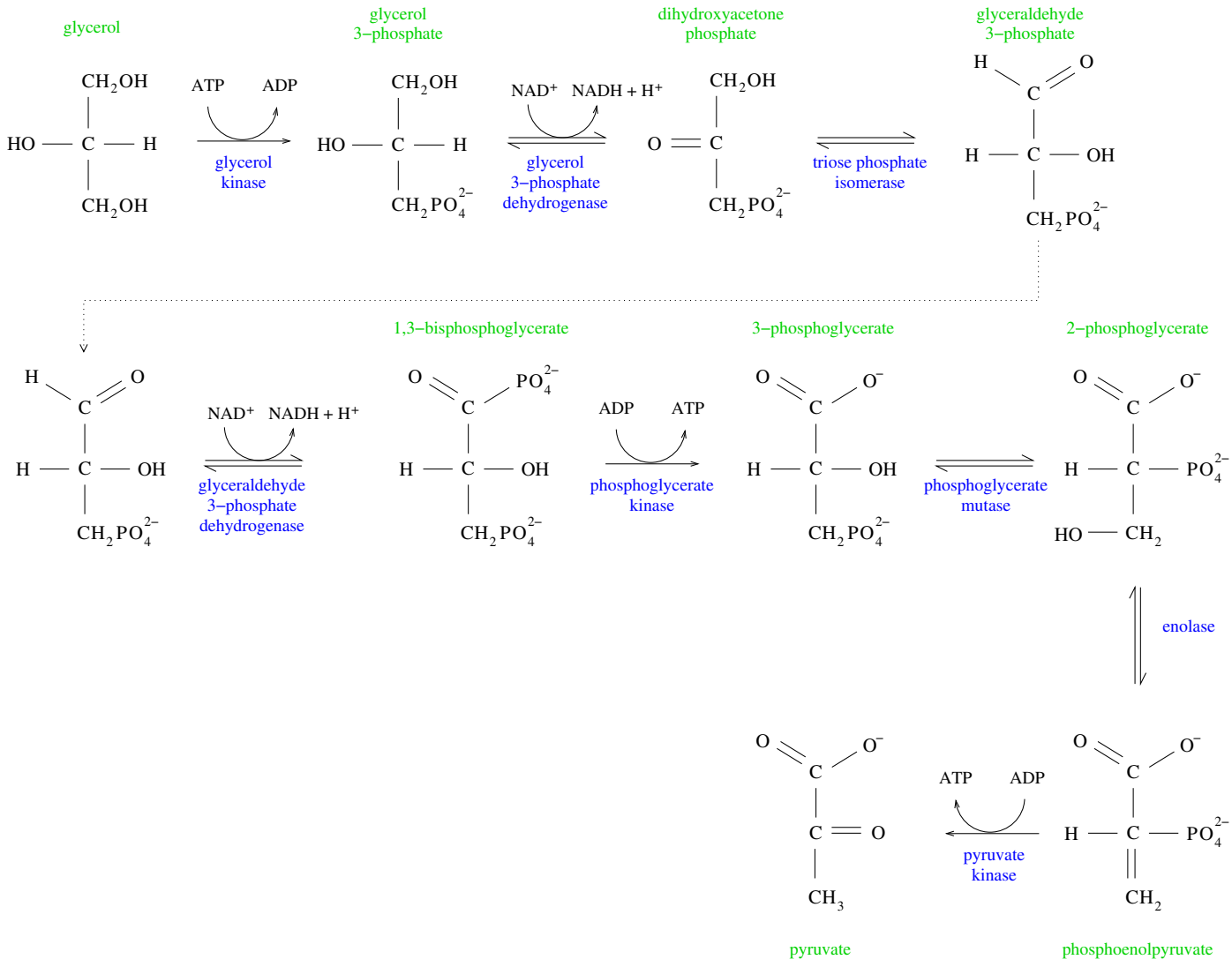
Model


glycerol
glycerol 3–phosphate
dihydroxyacetone phosphate
glyceraldehyde 3–phosphate
ATP
ADP
glycerol kinase
NAD+
NADH + H+
glycerol 3–phosphate dehydrogenase
triose phosphate isomerase
1,3–bisphosphoglycerate
3–phosphoglycerate
2–phosphoglycerate
glyceraldehyde 3–phosphate dehydrogenase
phosphoglycerate kinase
phosphoglycerate mutase
enolase
pyruvate kinase
pyruvate
phosphoenolpyruvate

Questions, revisited

- Flux through pathway: rate of formation of pyruvate
- When we started developing our glycerol metabolism model, we had two questions:
  1. What factor(s) limit the flux through this pathway?
  2. Can we engineer a strain of *Saccharomyces cerevisiae* that is capable of a higher flux through this pathway?
- Easier to answer 2 if you know the answer to 1
- 1 can be addressed using Metabolic Control Analysis (MCA)

Metabolic Control Analysis

- Imagine an experiment in which we change a parameter (p) and measure the resulting change in the flux (J).
- Rate of change of flux with respect to changes in $p = \frac{\partial J}{\partial p} \approx \frac{\Delta J}{\Delta p}$

Problem: size of rate of change is difficult to interpret because a change of (e.g.) $1\,\mu$M/min in J can be a small change if $J \sim 1000\,\mu$M/min or a very large change if $J \sim 1\,\mu$M/min.

Solution: Use relative changes $\Delta J/J$ and $\Delta p/p$.

Control coefficient:

$$C_p^J = \frac{\partial J/J}{\partial p/p} = \frac{\partial \ln J}{\partial \ln p} \approx \frac{\Delta J/J}{\Delta p/p} \approx \frac{\Delta \ln J}{\Delta \ln p}$$

Metabolic Control Analysis

Control coefficients

$$C_p^J = \frac{\partial \ln J}{\partial \ln p}$$

- Control coefficients can be positive or negative.
- A very small control coefficient would imply that a particular parameter has little effect on the flux.
- Special case: If $p = E$ is the concentration of an enzyme (or transporter), then C_E^J is called a flux control coefficient.
- The classical idea of a rate-limiting step would correspond to $C_E^J \sim 1$, i.e. doubling the enzyme concentration doubles the flux.
- Because of the logarithms, any quantity proportional to E (e.g. $v_{\max}$) will give the same value for the flux control coefficient.

Metabolic control analysis

Measurement of flux control coefficients

- For irreversible steps, increase $v_{\max}$ (or equivalent parameter) by a small amount (say, 5%).
  Then decrease it by the same amount (to check for consistency).
  Calculate

$$C_E^J \approx \frac{\Delta \ln J}{\Delta \ln v_{\max}} = \frac{\ln J(v_{\max}+\delta) - \ln J(v_{\max}-\delta)}{\ln(v_{\max}+\delta) - \ln(v_{\max}-\delta)} = \frac{\ln\left(\frac{J(v_{\max}+\delta)}{J(v_{\max}-\delta)}\right)}{\ln\left(\frac{v_{\max}+\delta}{v_{\max}-\delta}\right)}$$

Metabolic control analysis

Measurement of flux control coefficients

- For reversible steps, the rate for both directions is proportional to E.
  Introduce a "dummy" parameter that scales both the forward and reverse $v_{\max}$ in proportion, e.g.

$$v = e\frac{v^+_{\max} S/K_S - v^-_{\max} P/K_P}{1 + \frac{S}{K_S} + \frac{P}{K_P}}$$

  $e = 1$: original enzyme concentration
  $e = 2$: doubling of enzyme concentration
- Calculate C^J_E using (e.g.) $J(e = 1.05)$ and $J(e = 0.95)$.

Steady-state flux

- We need to get steady-state fluxes.
- If we run our model, we find that it does not reach a steady state: The glycerol 3-phosphate concentration just keeps rising.
- This is a common problem when we extract a set of reactions from a metabolic system.
  What we're leaving out might be important for homeostasis.
- In our case, the model includes an arbitrary external glycerol concentration. We can adjust this downward to avoid overwhelming glycerol 3-phosphate dehydrogenase.
  A steady-state is reached if $[\mathrm{Glyc}_{(\mathrm{ext})}] = 5 \times 10^{-5}$ mM.
- $[\mathrm{Glyc}_{(\mathrm{ext})}]$ is really tiny: Probably should reconsider model instead.
- Use the corresponding steady-state concentrations to accelerate simulations.

Metabolic control analysis of glycerol metabolism

Example: Control coefficient with respect to glycerol diffusion

- We need to consider all steps from source (external glycerol) to sink (pyruvate), including transport.
- The model contains a rate law for diffusive transport of glycerol (Glyc) through the cell membrane:

$$v_{\text{diff,Glyc}} = \frac{k_{16}}{Y_{\text{vol}}} \left([\text{Glyc}] - [\text{Glyc}_{(\text{ext})}] \right)$$

- Here, the diffusive rate constant k_{16} acts as the equivalent of an enzyme concentration.

Metabolic control analysis of glycerol metabolism

Example: Control coefficient with respect to glycerol diffusion

- Data collected from simulations:

k_{16}/s^{-1}	$J/\mathrm{mM\,s}^{-1}$	
1.8	8.9677×10^{-5}	
1.9	9.4640×10^{-5}	(default)
2.0	9.9602×10^{-5}	

- Control coefficient:

$$C^{J}_{\mathrm{diff,Glyc}} = \frac{\ln\left(\frac{9.9602\times10^{-5}}{8.9677\times10^{-5}}\right)}{\ln\left(\frac{2.0}{1.8}\right)} = 0.9963$$

Metabolic control analysis of glycerol metabolism

Flux control coefficients

Enzyme/process	Parameter	C_E^J
Glycerol diffusion	k_{16}	0.9963
Glycerol kinase	$v_{\mathrm{max,gk}}$	0.0038
Glycerol 3-phosphate dehydrogenase	e_{g3pd}	0
Triose phosphate isomerase	e_{tpi}	0
Glyceraldehyde 3-phosphate dehydrogenase	e_{GAPDP}	0
PEP synthesis	e_{PEPsynth}	0
Pyruvate kinase	V_{10m}	0
		1.0001

Conclusions

- Under the conditions considered here, the flux through the glycerol to pyruvate pathway is mostly controlled by transport into the cell.
- Overexpressing a transporter alone is not sufficient because the glycerol 3-phosphate dehydrogenase becomes saturated at higher concentrations of glycerol 3-phosphate resulting from higher levels of glycerol.
- Might be worth investigating the addition of a gene for a more-efficient glycerol 3-phosphate dehydrogenase (perhaps from another organism)