Design and Evolution of Carbon Conservation and Fixation Pathways

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- Non-Oxidative Glycolysis (NOG)

  Bogorad Nature 2013; Lin PNAS 2018

6"H"

$$C_6H_{12}O_6 \rightarrow 3\ CH_3COOH \rightarrow 3\ C_2H_5OH$$

- Malyl-CoA Glycerate (MCG) pathway for CO_2 fixation Yu Nat Comm 2018

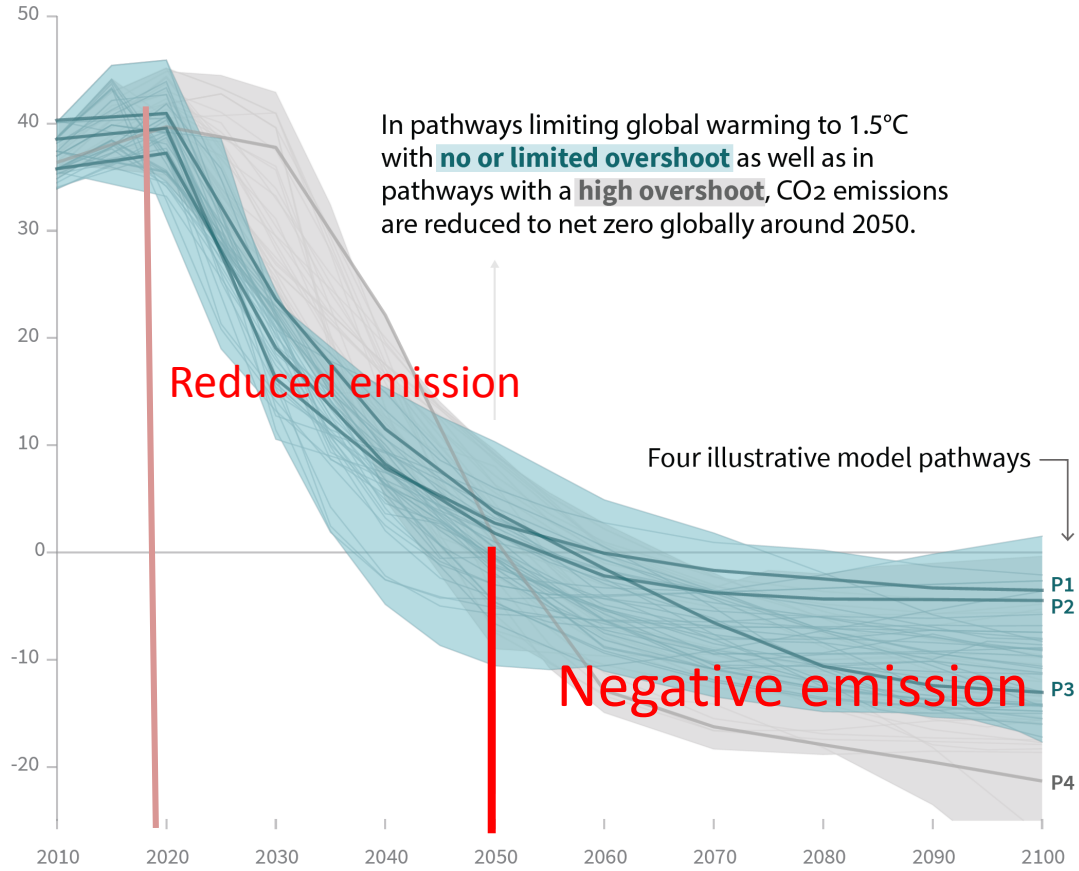
4"H" 8"H"

$$C_6H_{12}O_6 + 2\ CO_2 \rightarrow 4\ CH_3COOH \rightarrow 4\ C_2H_5OH$$

Global net CO_2 emission pathway (1.5 °C limit)

Global total net CO_2 emissions

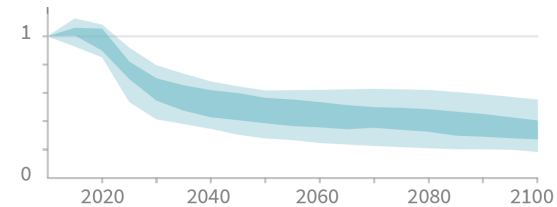
Billion tonnes of CO_2/yr



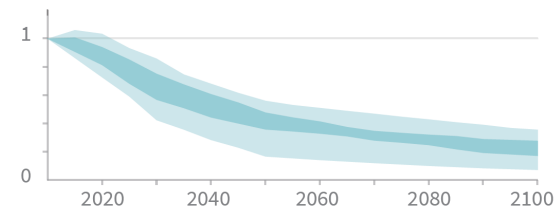
Non-CO_2 emissions relative to 2010

Emissions of non-CO_2 forcers are also reduced or limited in pathways limiting global warming to 1.5°C with **no or limited overshoot**, but they do not reach zero globally.

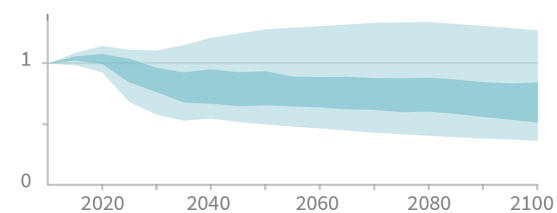
Methane emissions



Black carbon emissions

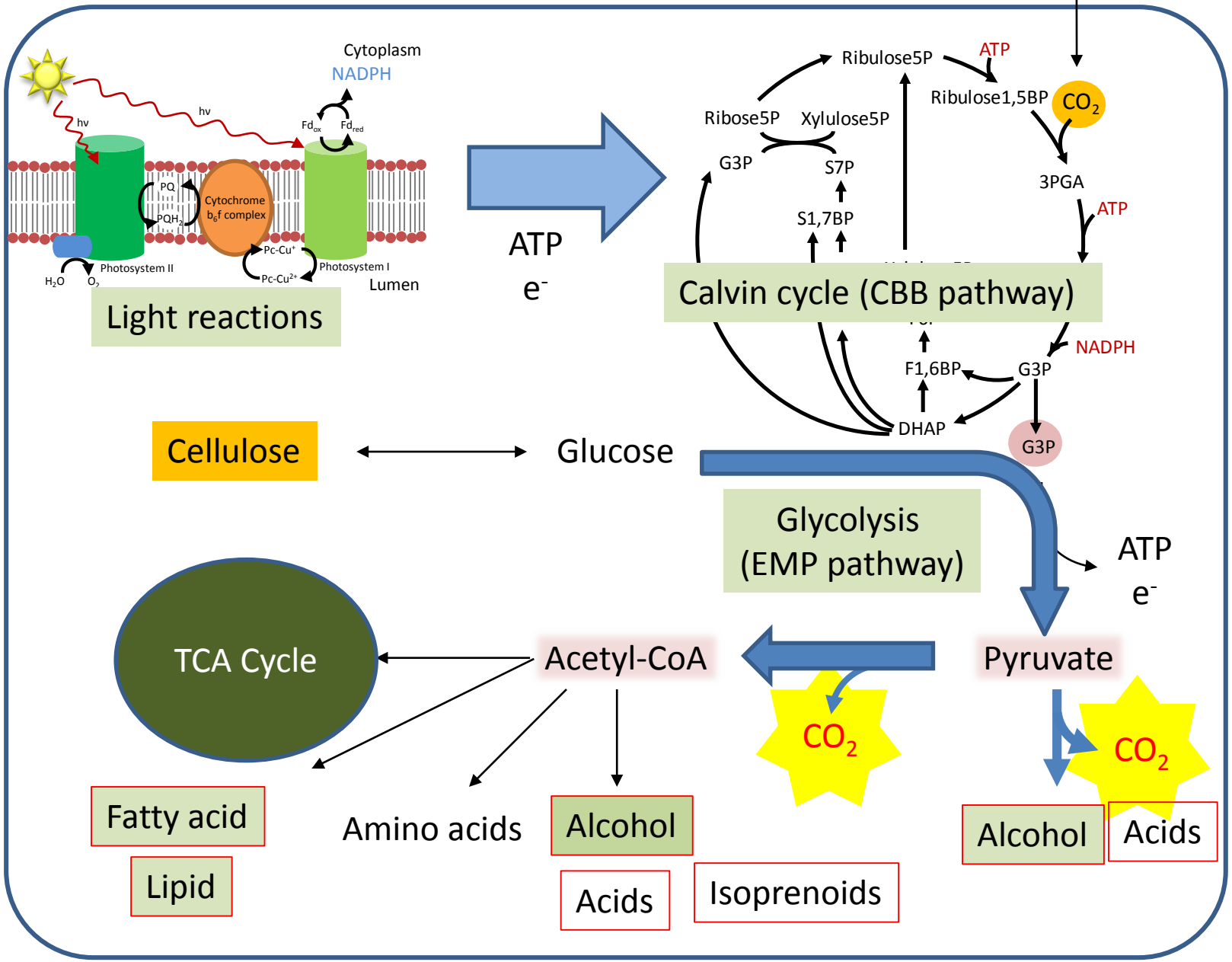


Nitrous oxide emissions

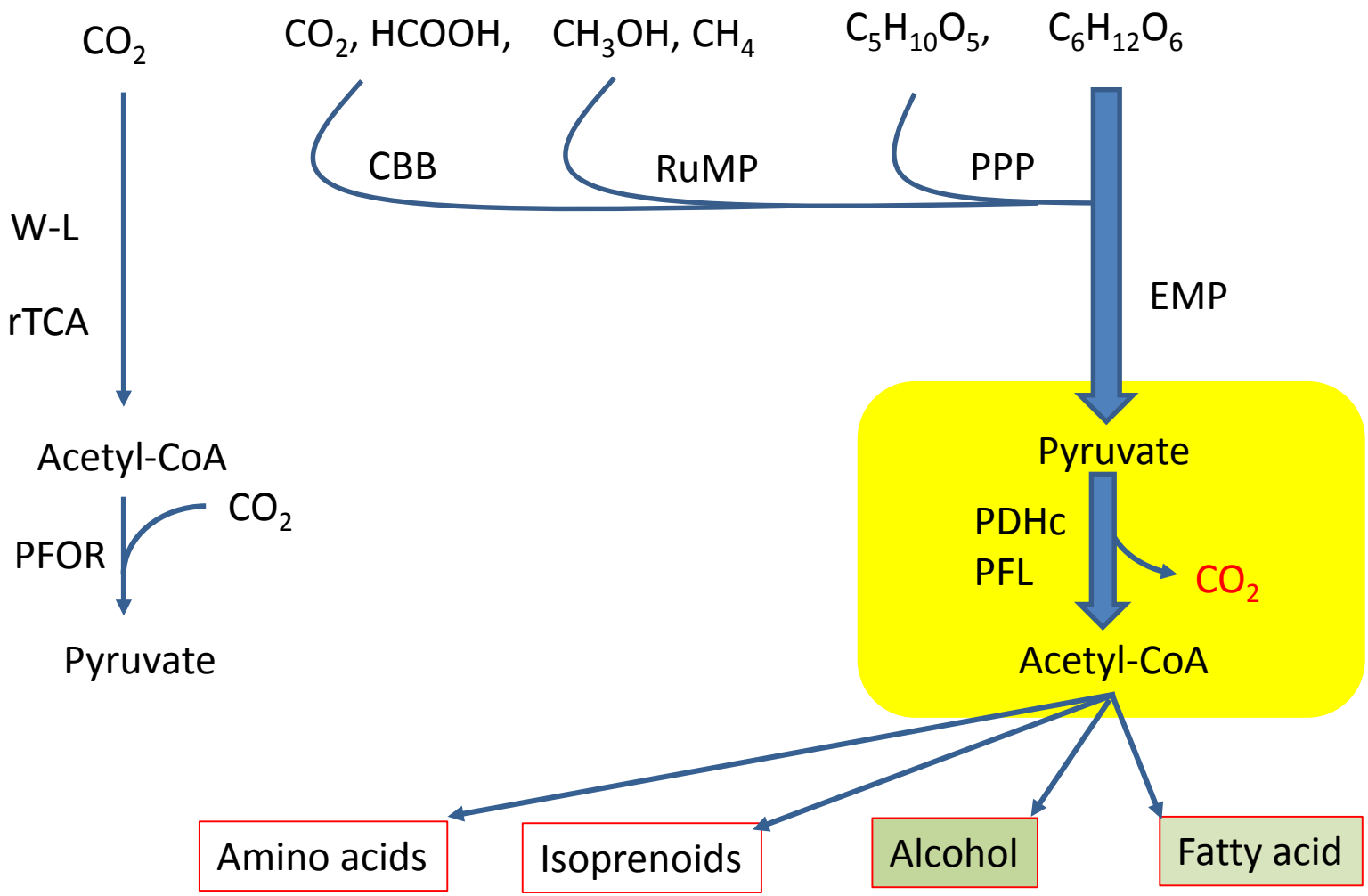


IPCC "Global Warming of 1.5°C" Oct 2018

Pathways to Life



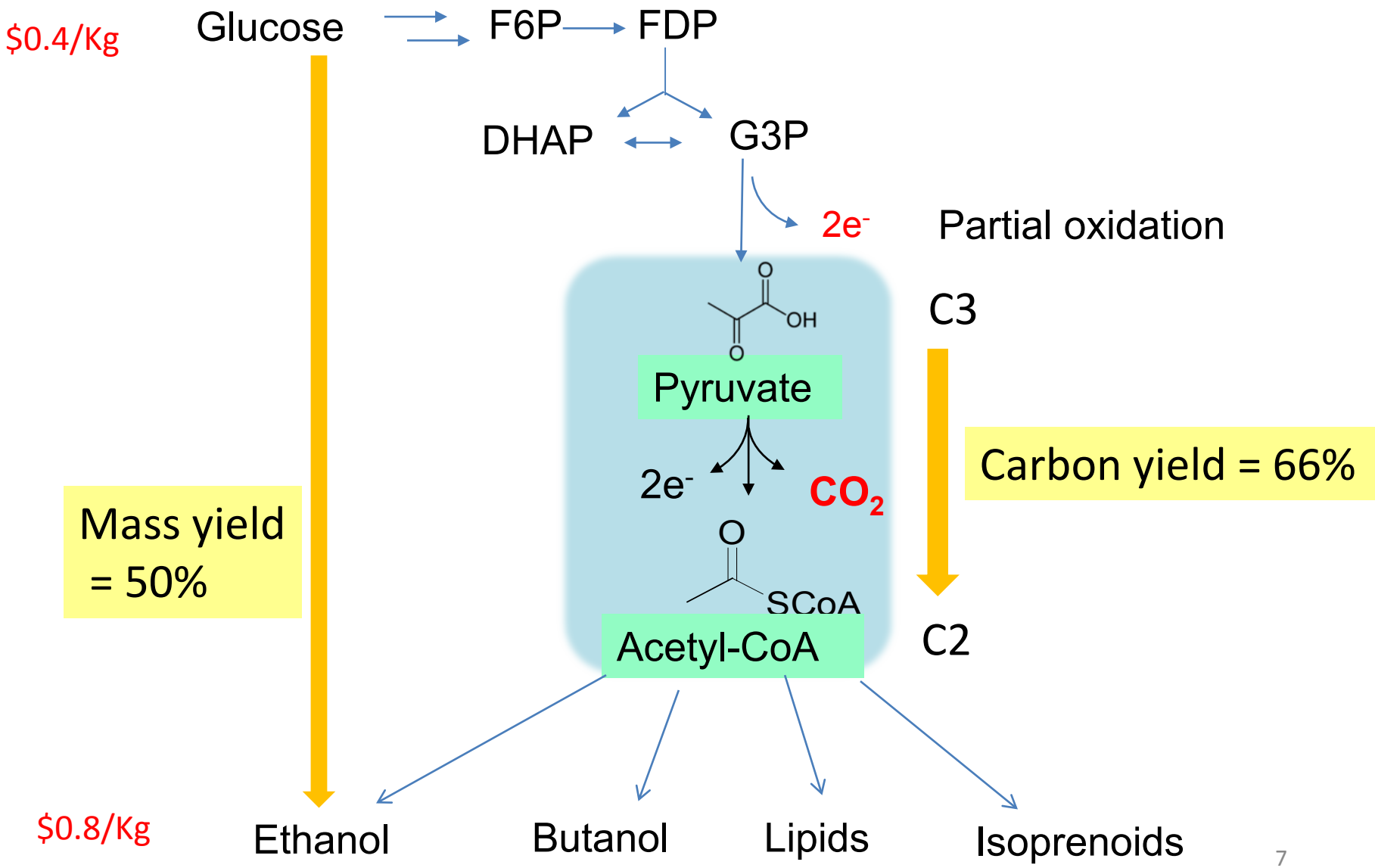
CO_2 loss is a common problem in cellular metabolism



Non-Oxidative Glycolysis (NOG)

$$C_6H_{12}O_6 \longrightarrow 3\ CH_3COOH$$

Oxidative Glycolysis
Embden-Meyerhof-Parnas (EMP) pathway

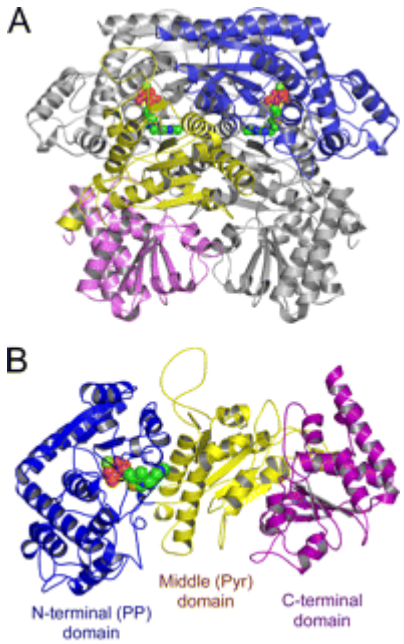


Theoretical Energy and Mass Yields

			energy %	mass %
$C_6H_{12}O_6$ →	$2C_2H_6O$ +	$2\ CO_2$		
2540	2 x 1235 kJ/mol		97.24%	51%
$C_6H_{12}O_6$ →	$C_4H_{10}O$ +	$2\ CO_2$		
2540	2455 kJ/mol		96.6%	41%
$2\ C_6H_{12}O_6$ →	C_7H_{16} +	$4CO_2$ + CO		
2x 2540	4543 kJ/mol		89.4%	27%

Jet fuel

Non-oxidative Glycolysis (NOG)



Bifidobacterium breve.

Glucose → F6P

3 F6P (C6)

3 AcP (C2) + 3 E4P (C4) → Carbon Rearrangement → 2 F6P

3 AcCoA → Ethanol, Butanol, Acetate

Carbon yield = 100%

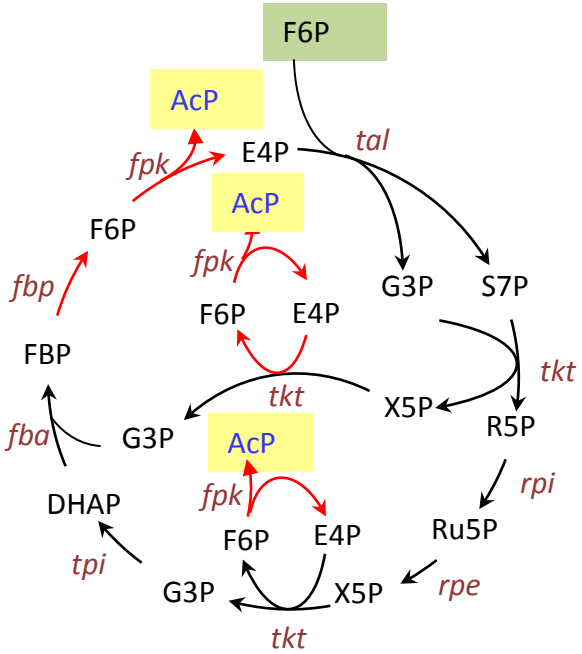
Phosphoketolase

F6P → AcP + E4P

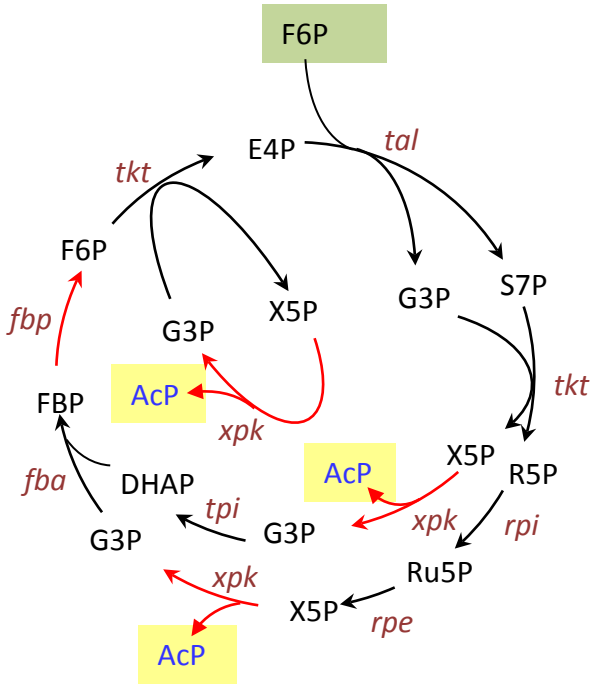
X5P → AcP + G3P

Two Modes of Non-Oxidative Glycolysis (NOG)

F6P phosphoketolase
F6P → AcP + E4P



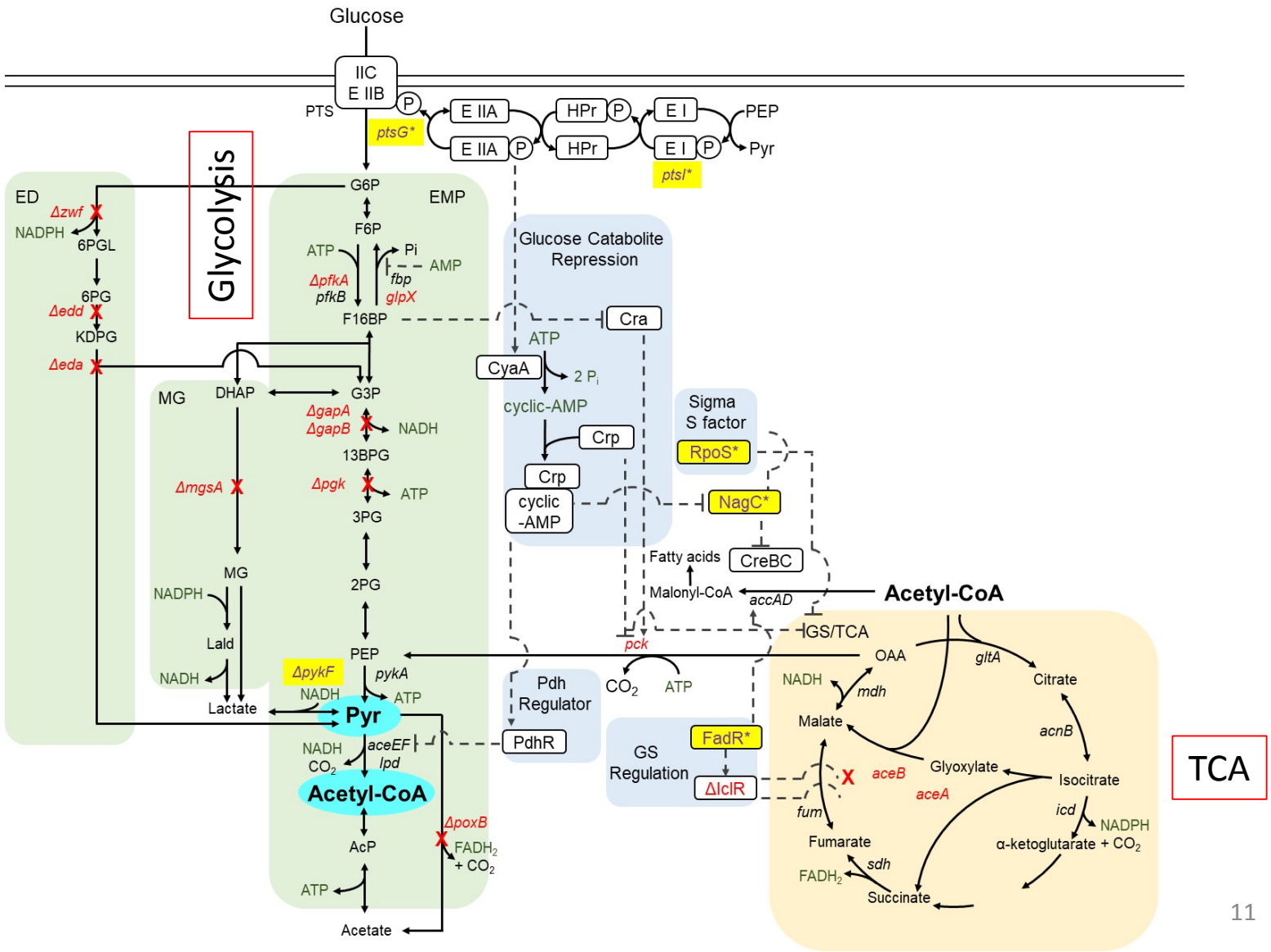
X5P phosphoketolase
X5P → AcP + G3P



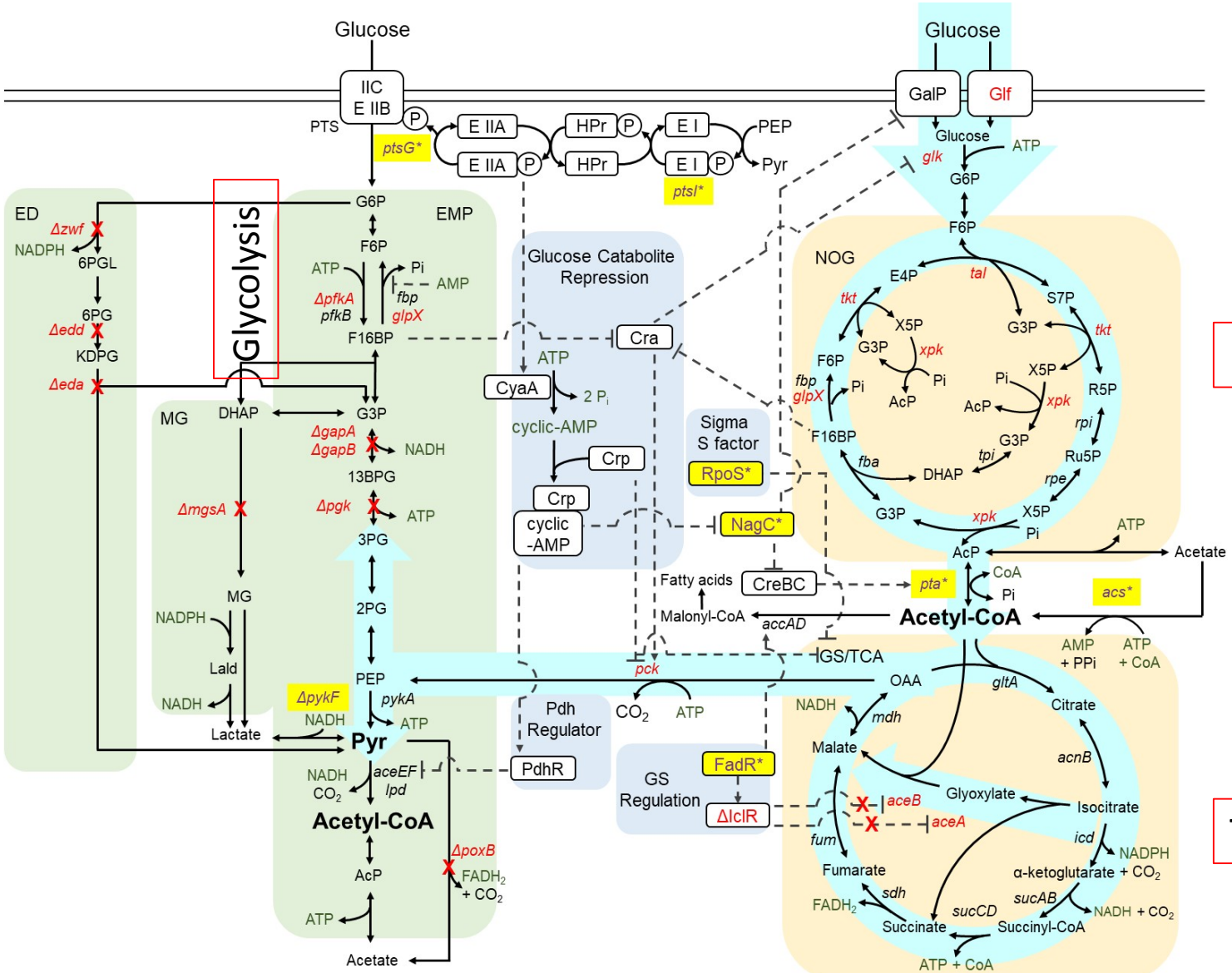
Net reaction: F6P → 3 AcP

Bogorad et al. Nature 2013

Construction and Evolution of an NOG *E. coli* strain

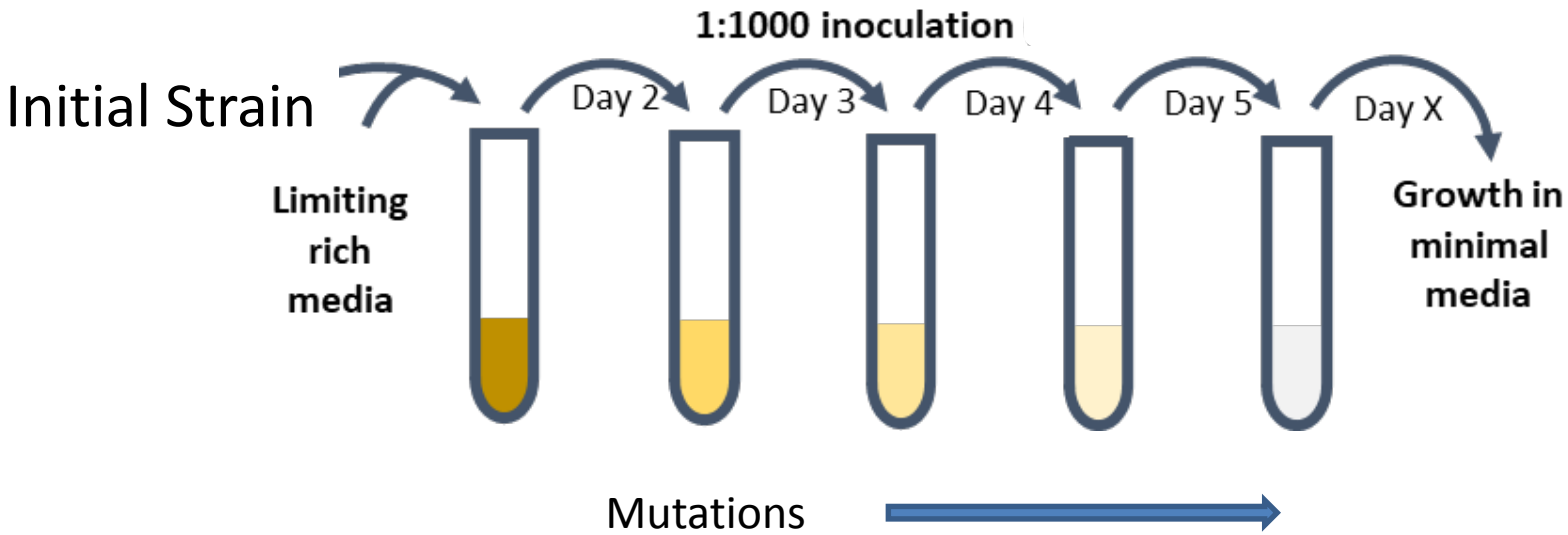


Construction and Evolution of an NOG E. coli strain



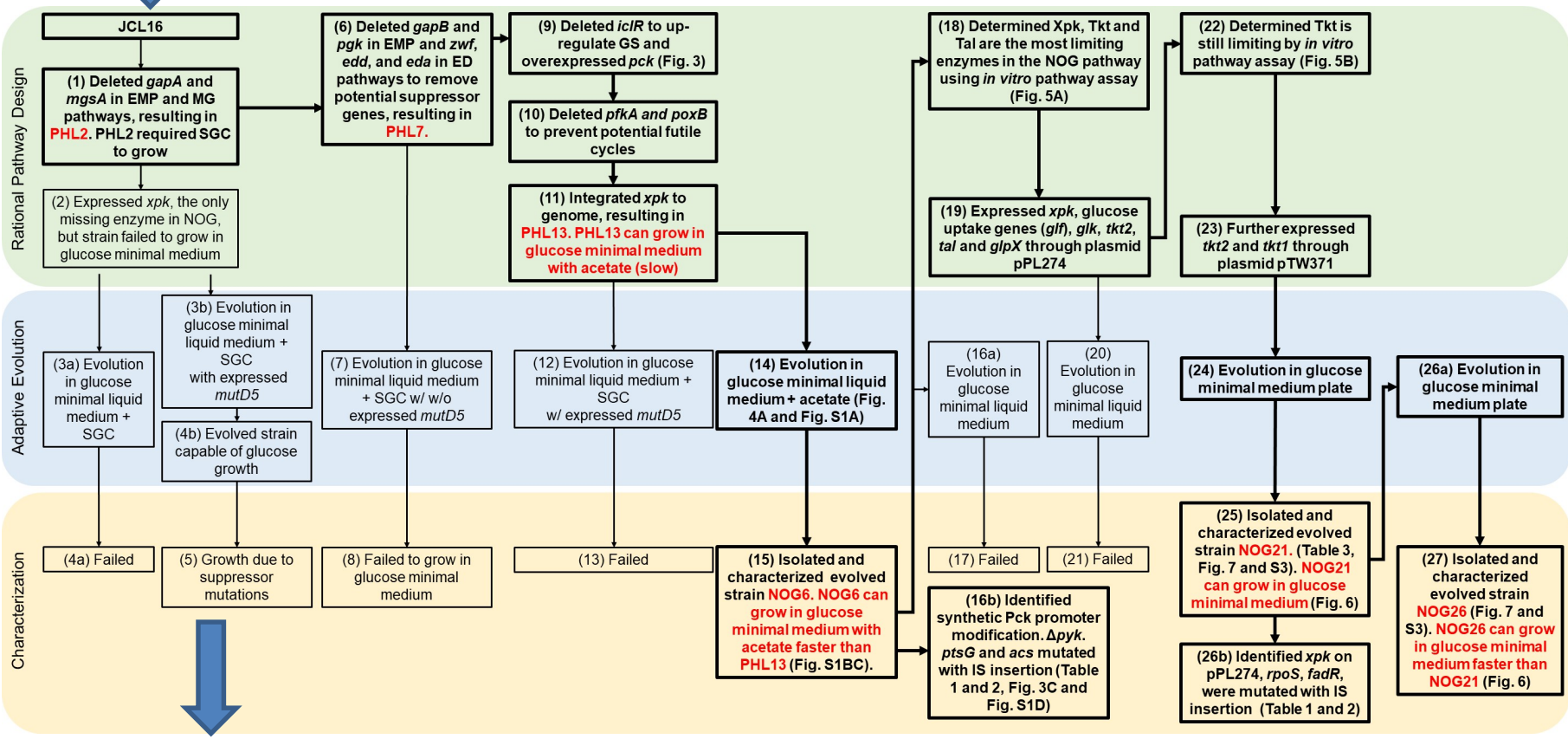
Lin et al. PNAS 2018

Laboratory evolution



Deleting Regular Glycolysis

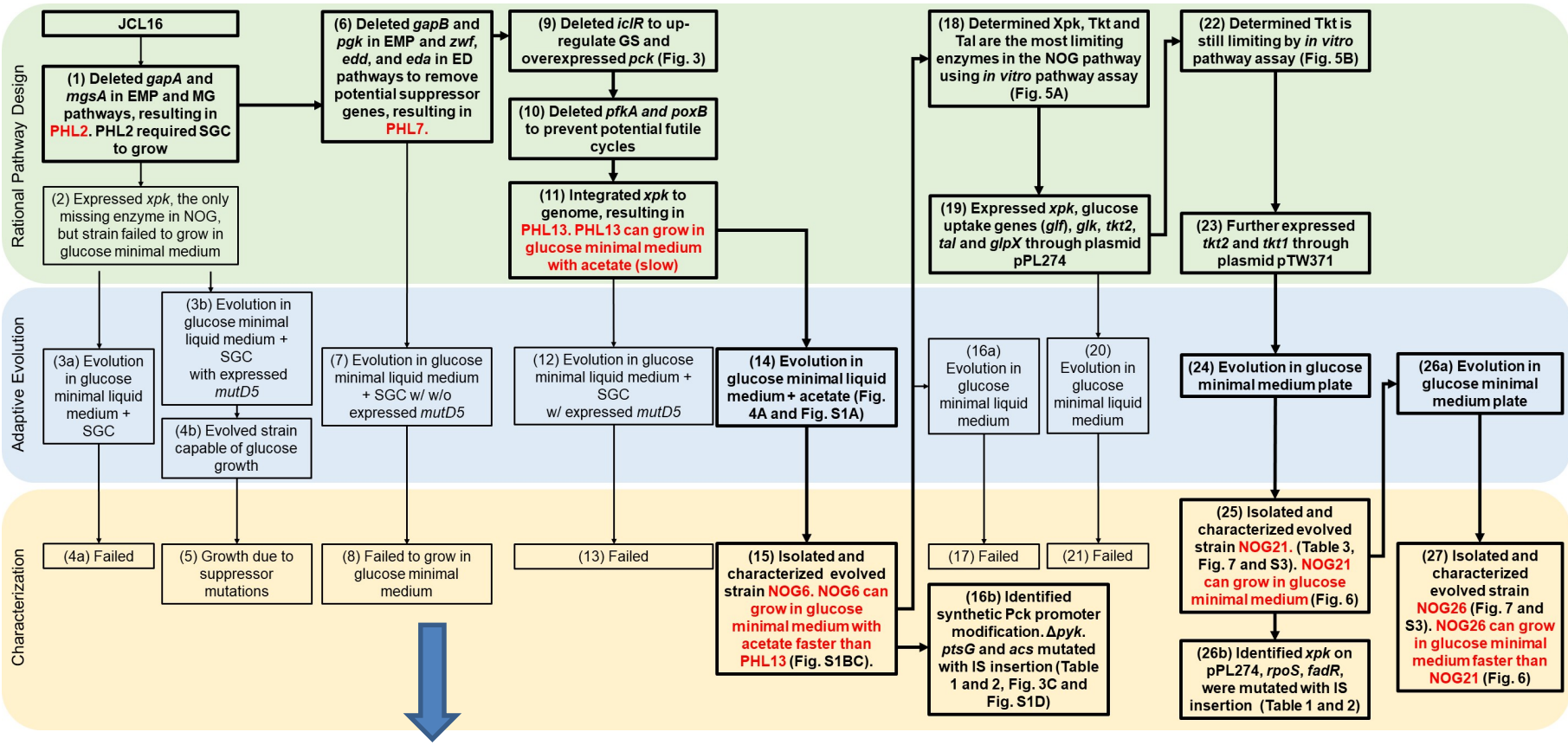
Use directed strain evolution to force the cell



"Loophole" mutations

Lin et al. PNAS 2018

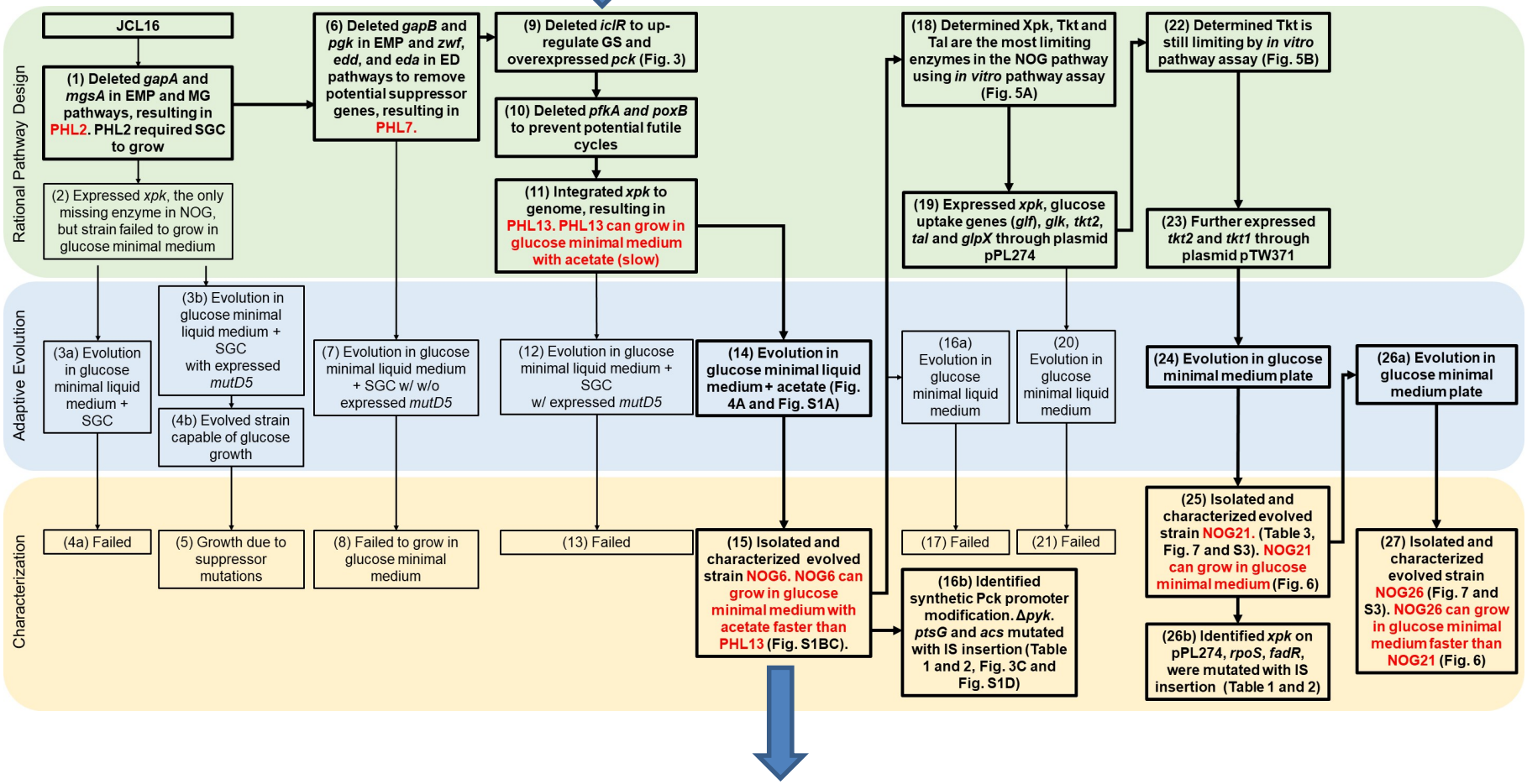
Deleting Potential Loopholes



No loopholes
But no growth

Lin et al. PNAS 2018

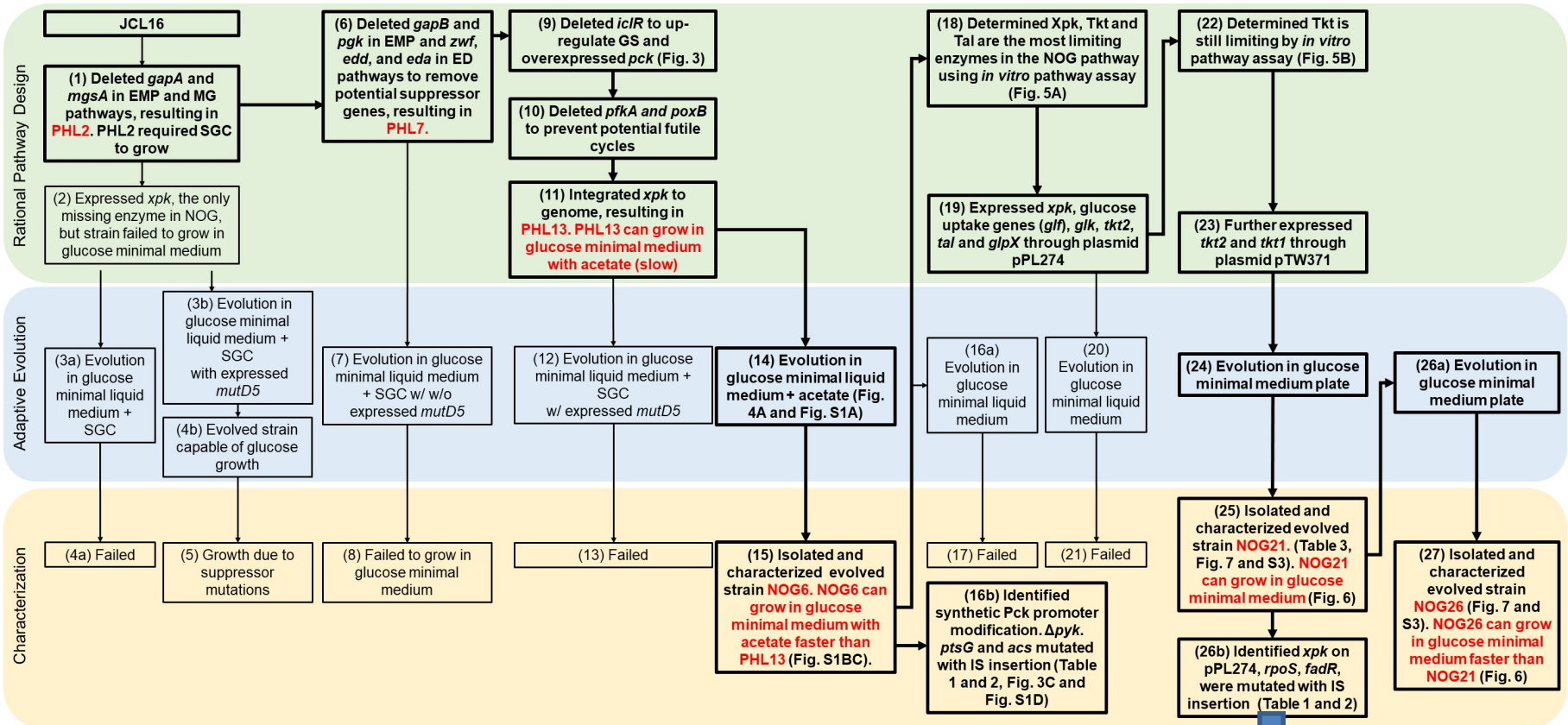
Tune-up desired pathways,
Delete potential futile cycles



Growth in
Glucose + Acetate

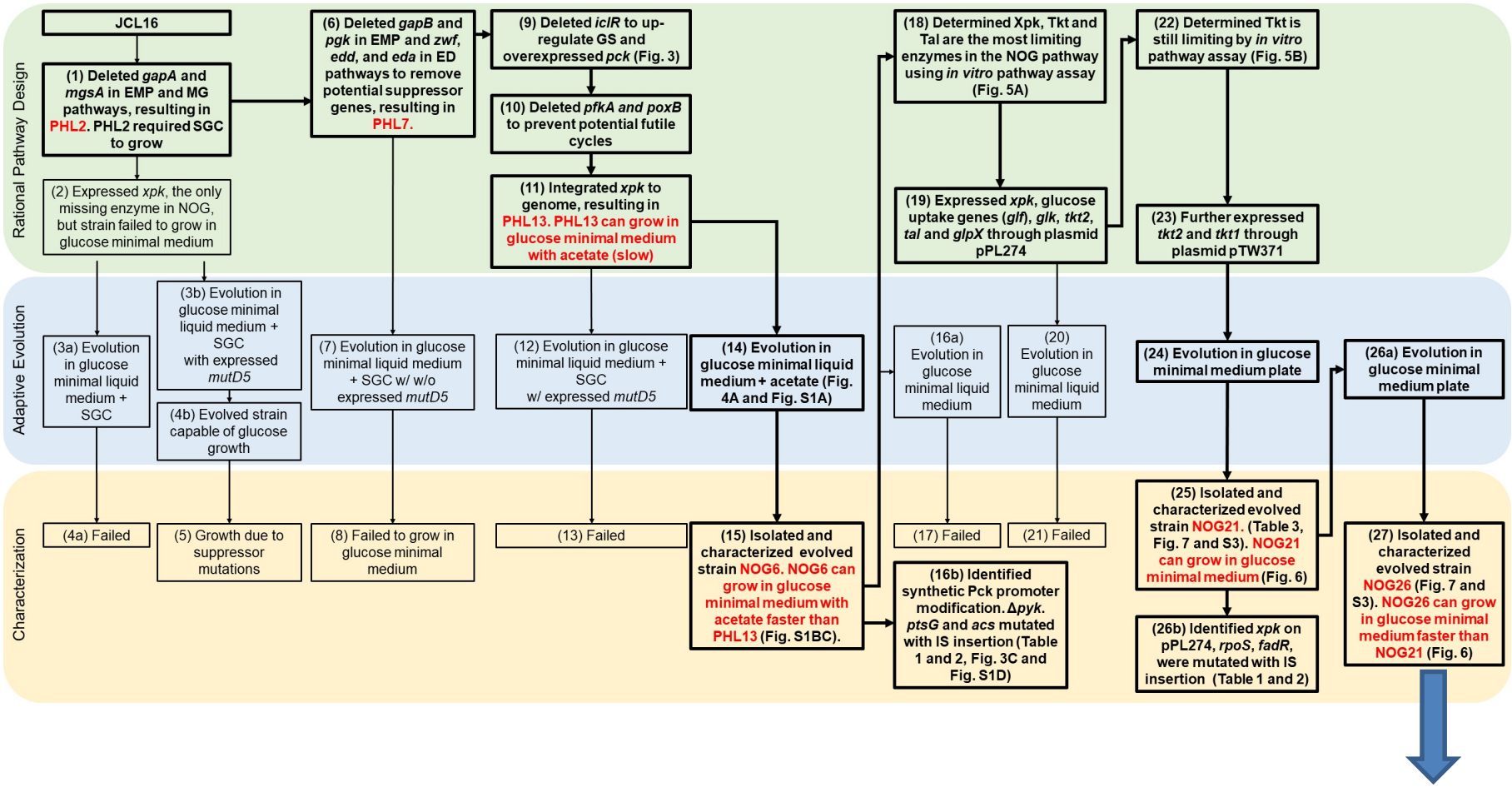
Lin et al. PNAS 2018

Use pathway assays to identify limiting enzymes
De-bottlenecking



Growth in Glucose

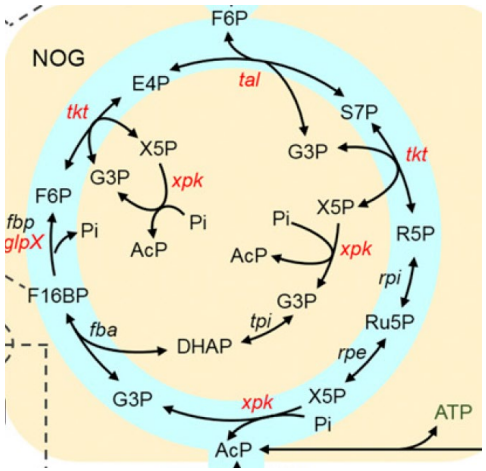
Lin et al. PNAS 2018

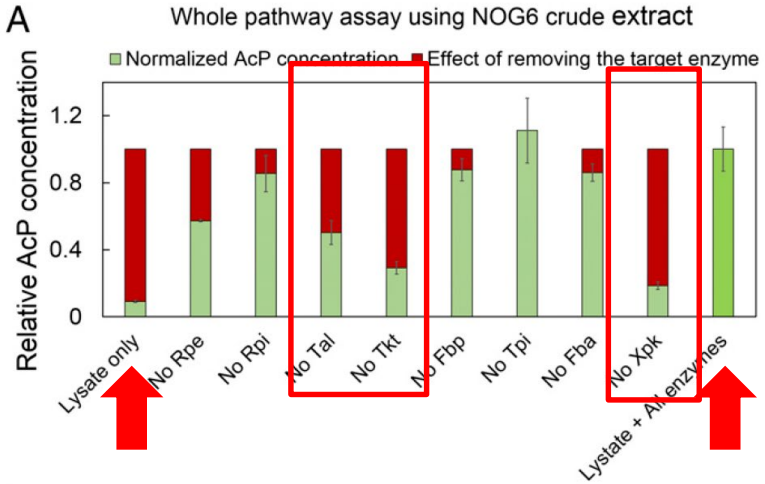


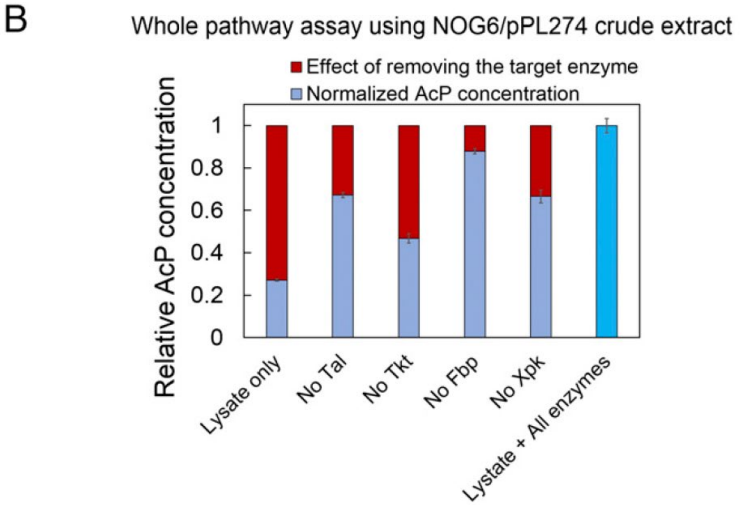
Improved growth

Lin et al. PNAS 2018

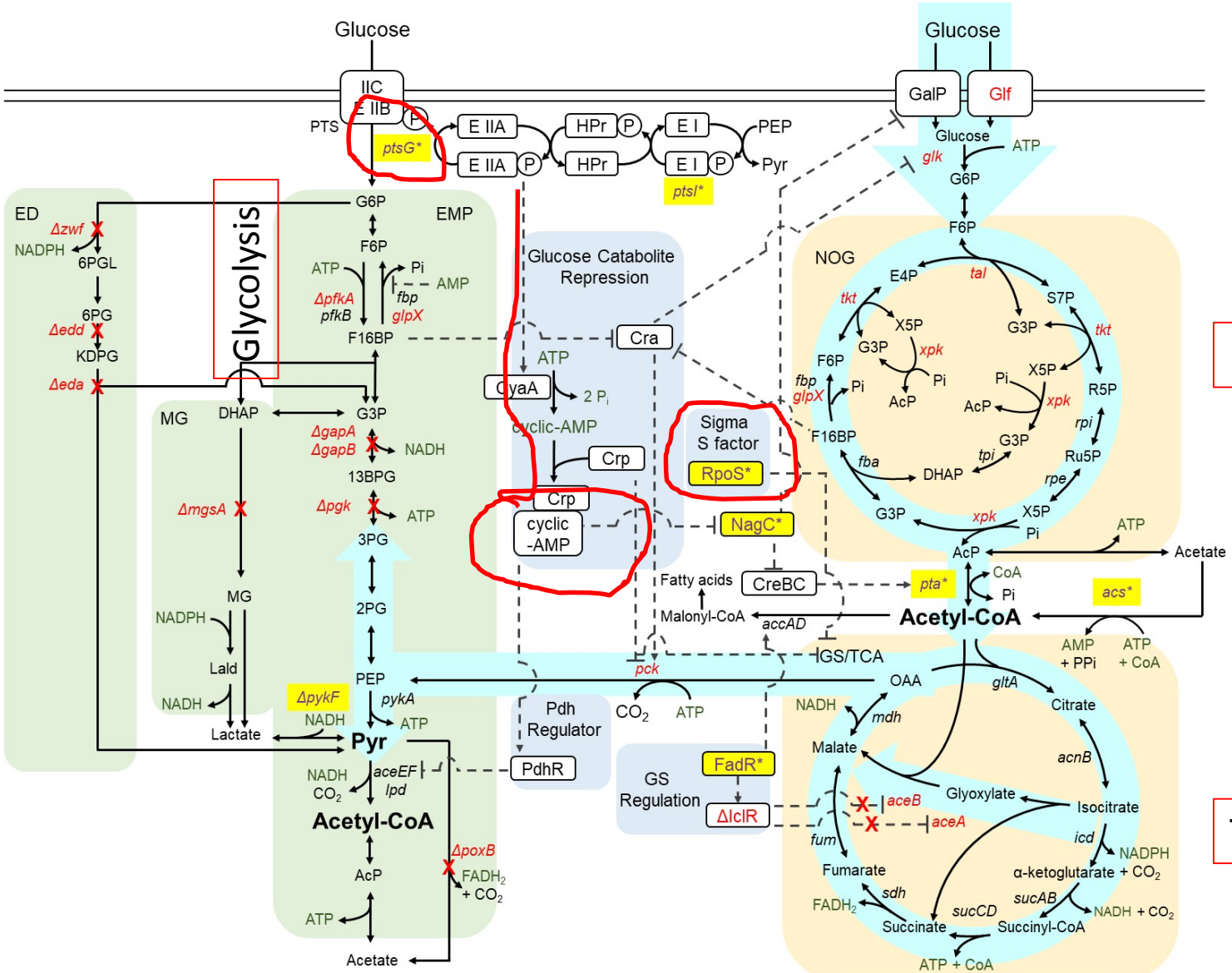
Pathway assay to identify limiting enzymes







Construction and Evolution of an NOG E. coli strain



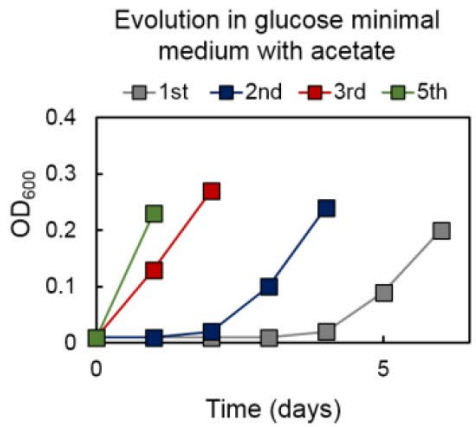
NOG

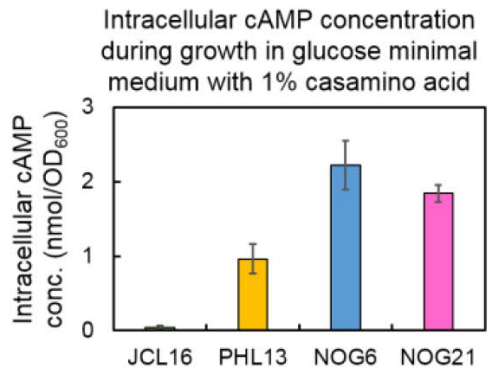
TCA/GS

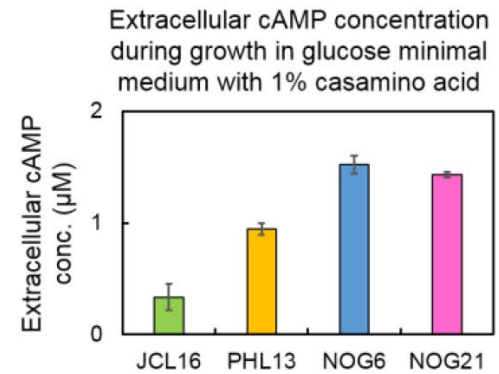
Lin et al. PNAS 2018

Important mutation occurring during evolution

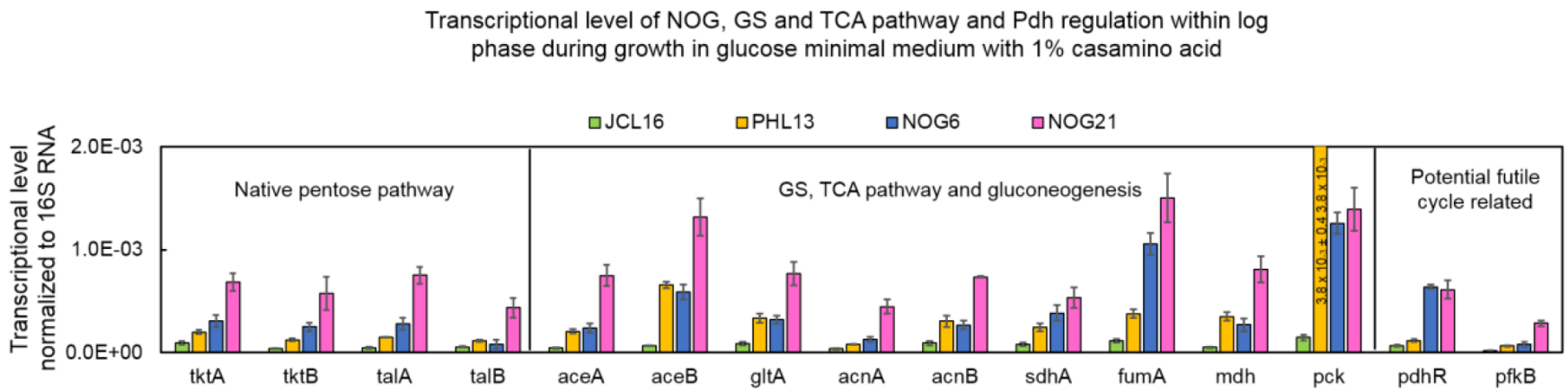
ΔptsG → EII-P ↑ → CyaA ↑ → cAMP ↑



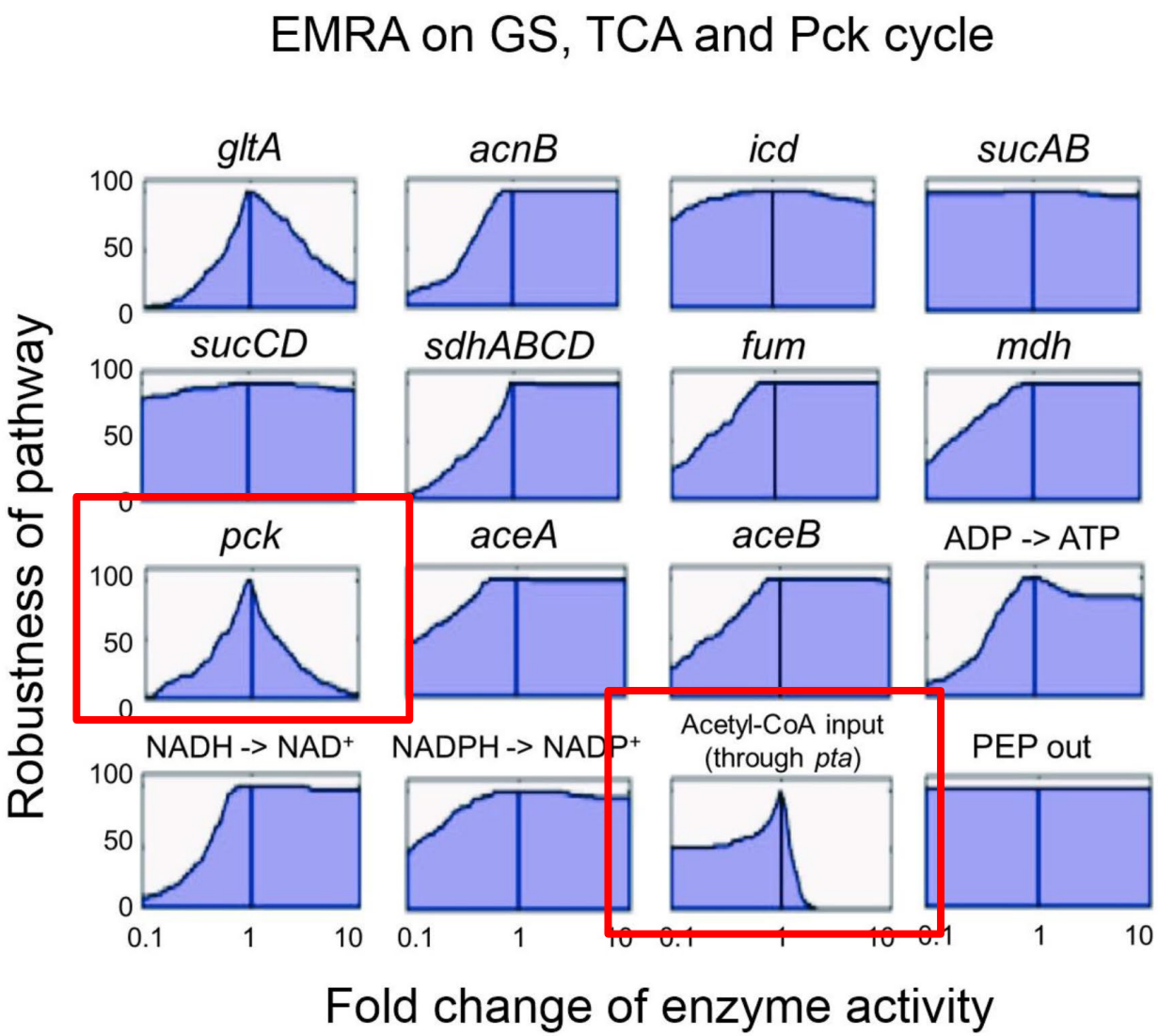




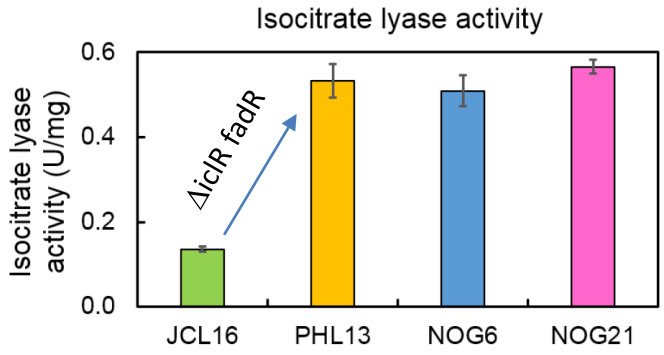
rpoS::transposon

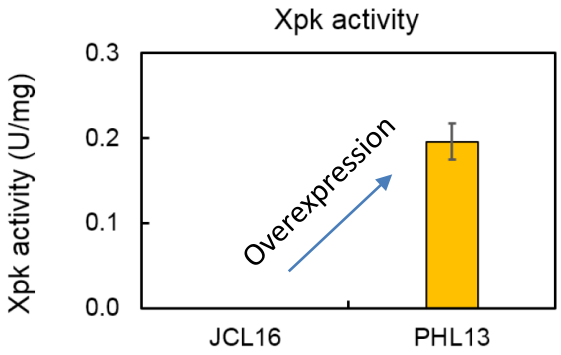


Fine-tuning of enzyme activity
Ensemble Modeling for Rubustness Analysis

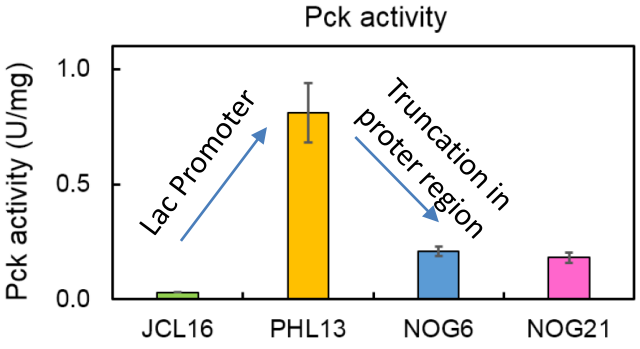


Fine-tuning of enzyme activity

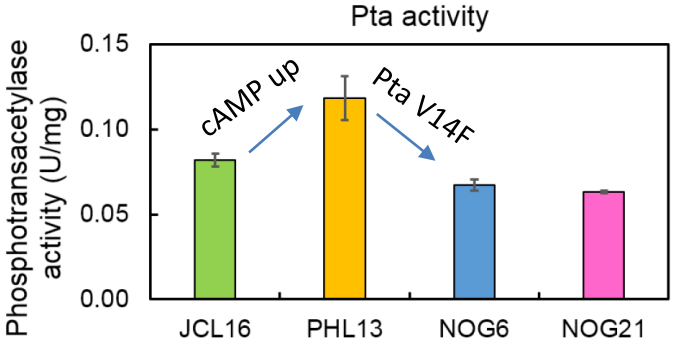




C



D

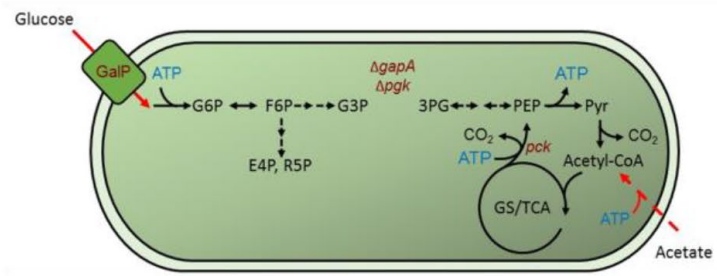


Installed Stronger promoter

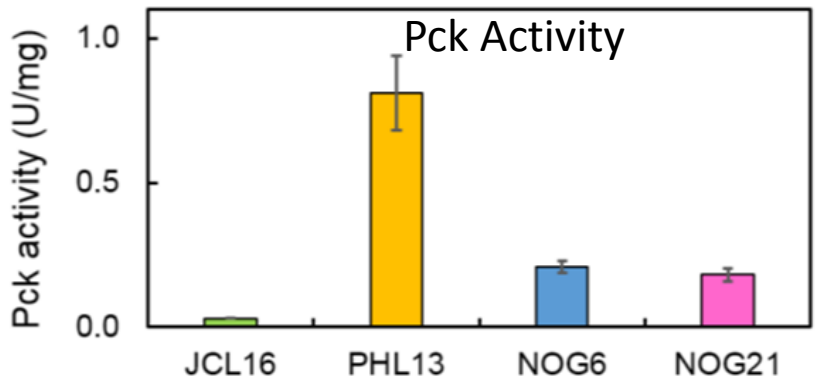
Wt

Evolved away

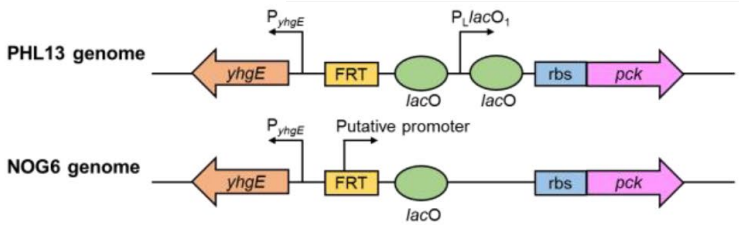
A



B



D



PHL13 genome

Putative promoter

-35 -10 FRT

P_L*lacO*$_1$ promoter

lacO -35 *lacO* -10

GTGTAGGCTGGAGCTGCTTCGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGGAATAGGAACTAAGGAGGATATTCATATGGAAATTGTGAGCGGATAACAATTGACATTGTGAGCGGATAACAAGATACTGAGCACATCAGCAGGACGCACTGACC

NOG6 genome

Putative promoter

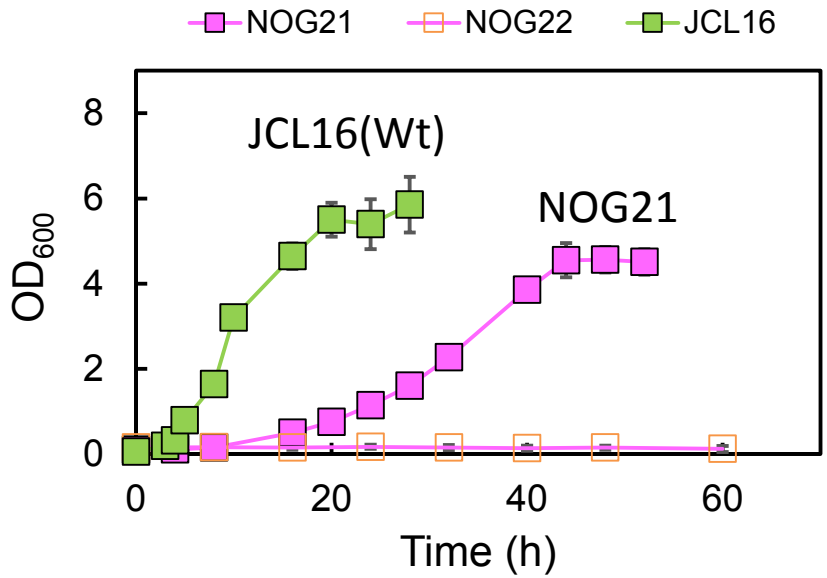
-35 -10 FRT

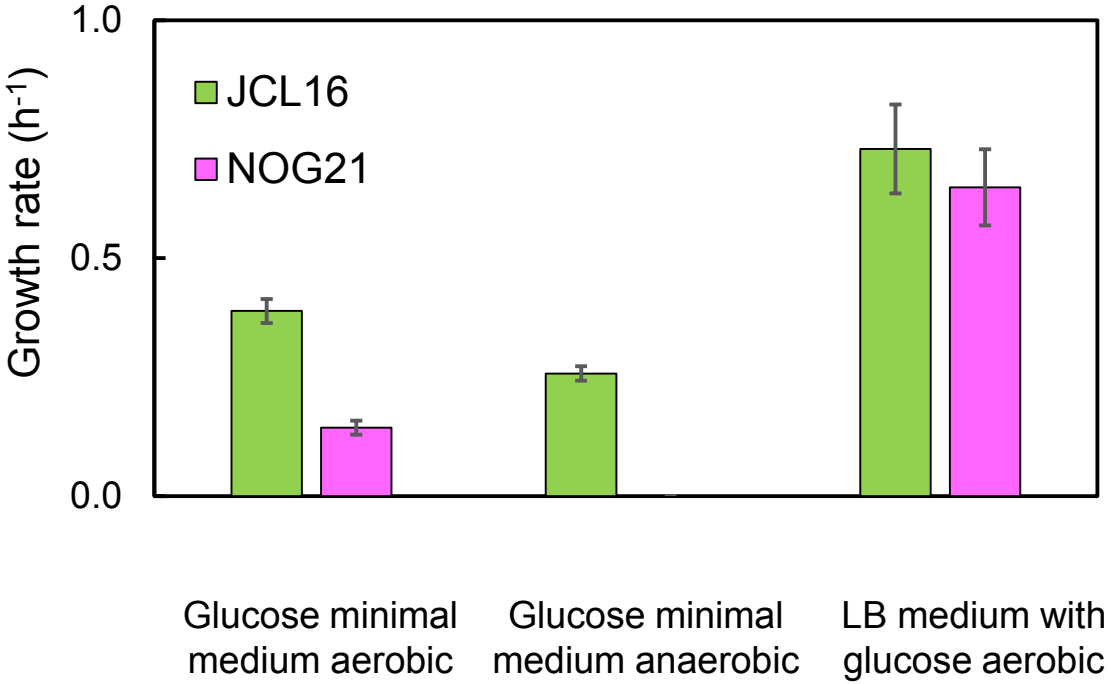
lacO

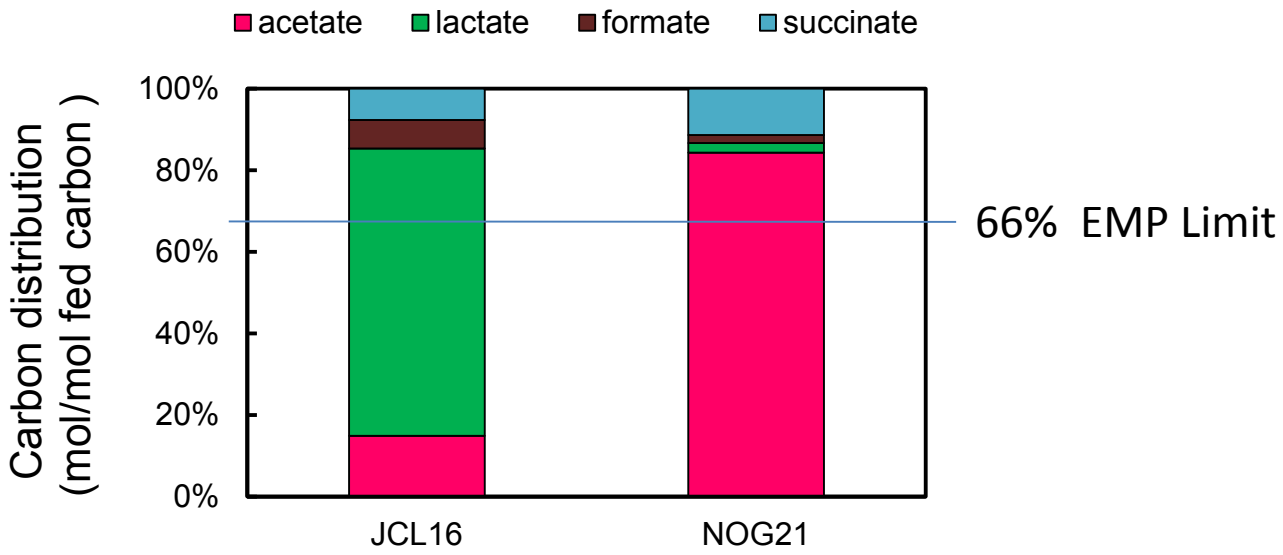
GTGTAGGCTGGAGCTGCTTCGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGGAATAGGAACTAAGGAGGATATTCATATGGAAATTGTGAGCGGATAACAA GATACTGAGCACATCAGCAGGACGCACTGACC

truncated

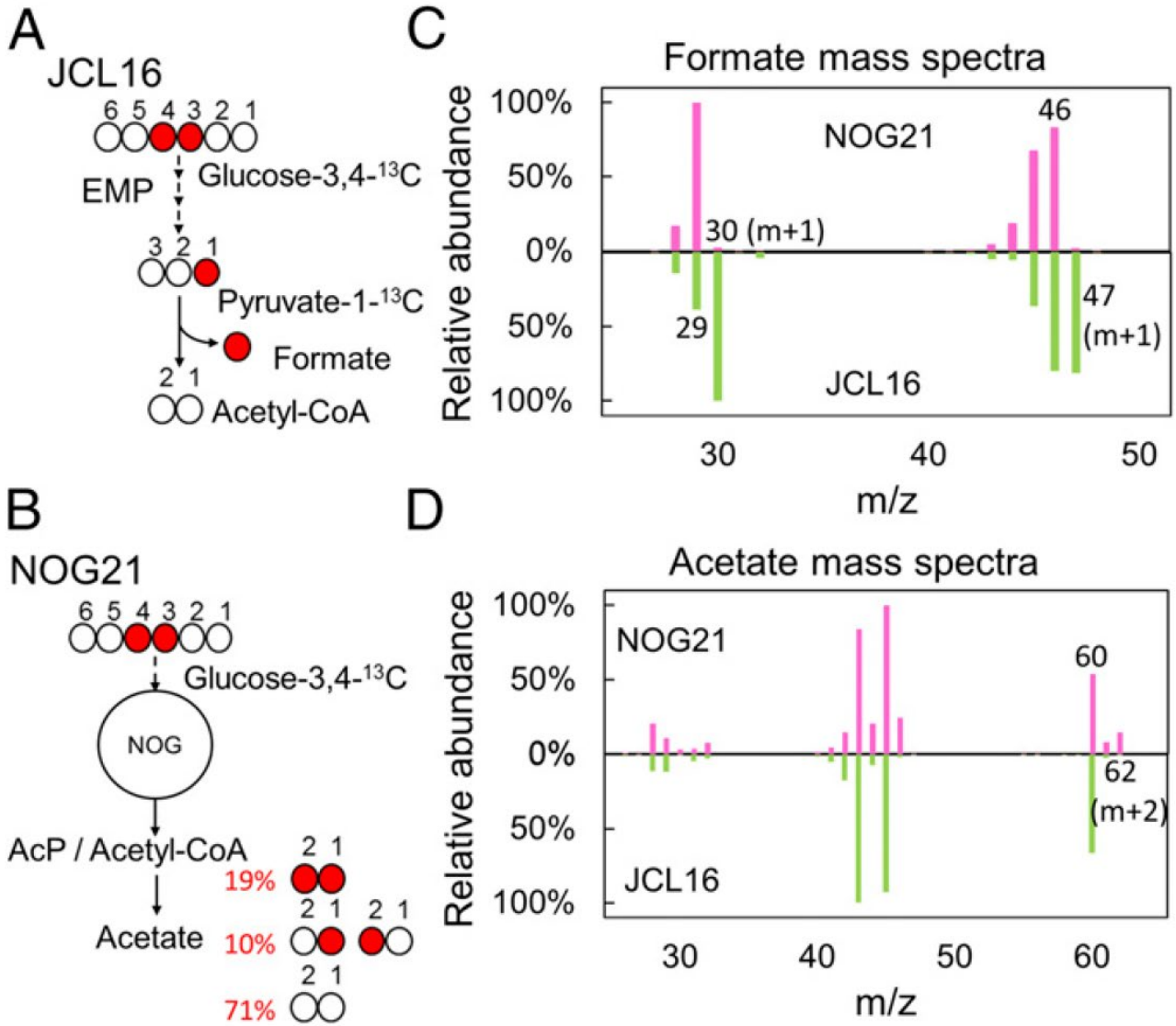
Lin et al. PNAS 2018


NOG21
NOG22
JCL16
JCL16(Wt)
NOG21
OD600
8
6
4
2
0
0
20
40
60
Time (h)


1.0
0.5
0.0
Growth rate (h-1)
JCL16
NOG21
Glucose minimal medium aerobic
Glucose minimal medium anaerobic
LB medium with glucose aerobic


acetate
lactate
formate
succinate
Carbon distribution
(mol/mol fed carbon)
100%
80%
60%
40%
20%
0%
66% EMP Limit
JCL16
NOG21

An E. coli strain that uses solely NOG for sugar catabolism



Economy of oxidative and non-oxidative glycolysis for making ethanol

Traditional fermentation

$$1.5\ C_6H_{12}O_6 \rightarrow 3C_2H_6O + 3\ CO_2$$

sugar price = $0.4/Kg

ethanol price = $0.8/Kg

http://www.nasdaq.com/markets/sugar.aspx

NOG

$$C_6H_{12}O_6 + 6\ H_2 \rightarrow 3\ C_2H_6O + 3\ H_2O$$

When

$$\frac{\text{sugar price}}{\text{H2 price}} > \frac{12}{90} \quad (= 0.133)$$

Then it makes sense to use NOG + H2.

H2 price < $3/Kg

Reductive Fermentation (H_2-assisted CO_2 fixation)

Traditional fermentation

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2\,CO_2$$

$$+ \quad 6\,H_2 + 2CO_2 \rightarrow C_2H_5OH + 3\,H_2O$$

NOG: Reductive fermentation

$$C_6H_{12}O_6 + 6\,H_2 \rightarrow 3\,C_2H_5OH + 3\,H_2O$$

CO_2 Saving

Non-oxidative (NOG) $C_6H_{12}O_6 + 6\ H_2 \rightarrow 3\ C_2H_5OH + 3\ H_2O$

Methane steam reforming $1.5\ CH_4 + 3\ H_2O \rightarrow 1.5CO_2 + 6\ H_2$

$C_6H_{12}O_6 + 1.5\ CH_4 \rightarrow 3\ C_2H_5OH + 1.5\ CO_2$

Traditional Fermentation $1.5\ C_6H_{12}O_6 \rightarrow 3\ C_2H_5OH + 3\ CO_2$

Methane upgrading

Traditional Fermentation

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$$

\+ Methane upgrading

$$\boxed{0.5\ CO_2 + 1.5\ CH_4 \rightarrow C_2H_5OH}$$

NOG + Steam Reforming

$$C_6H_{12}O_6 + 1.5\ CH_4 \rightarrow 3\ C_2H_5OH + 1.5\ CO_2$$

Methane upgrading

Reactions	ΔG^o (kJ/mole)	Energy* efficiency	Carbon# yield	Comment
1) $C_6H_{12}O_6$ --> 2 C_2H_6O + 2 CO_2	-228.4	0.97	66.70%	Current fermentation
2) 0.5 CO_2 + 1.5 CH_4 --> C_2H_6O	100.05	1.03	133%	Proposed Methane upgrading (ΔG^o >0)
3) = 1)+2) $C_6H_{12}O_6$ + 1.5 CH_4 --> 3 C_2H_6O + 1.5 CO_2	-128.35	0.99	80%	Proposed reductive fermentation
4) 2CH_4 + O_2--> C_2H_6O+ H_2O	-309.1	0.77	100%	REMOTE

*Energy efficiency is calculated from the lower heating value (LHV) of combustion of products divided by the LHV of the reactants.

! ΔG_f^o and ΔH^o_{LHV} values were taken from Table 2

Carbon yield calculation does not include CO_2.

Malyl-CoA Glycerate (MCG) pathway for CO_2 fixation

$C_6H_{12}O_6 + 2\ CO_2 + 4$"H" $\longrightarrow$ $4\ CH_3COOH$

Pyruvate + CO_2 $\longrightarrow$ 2 Acetyl-CoA

Glyoxylate $\longrightarrow$ Acetyl-CoA

Comparison of different carboxylases

Carboxylase abbrev.	Carbon species	Cofactor	Function in natural CO_2 fixation pathway	Oxygen sensitivity	Specific Activity (µmol/min/mg)
RuBisco	CO_2	No	Yes (CBB)	No, but has oxygenase activity	3.5[b]
ACC	HCO_3^-	ATP, biotion	Yes (HP bi, HP/HB)	No	18[b]
PCC	HCO_3^-	ATP, biotion	Yes (HP bi, HP/HB)	No	29.6[b]
PYC	HCO_3^-	ATP, biotion	No (mainly in anaplerosis)	No	32.4[b]
PPC	HCO_3^-	No	Yes (DC/HB)	No	35.2[b]
PFOR	CO_2	ferredoxin, TPP	Yes (WL, rTCA, DC/HB)	Yes	<1
KOR	CO_2	ferredoxin, TPP	Yes (rTCA)	Yes	<1
CCR	CO_2	NADPH	No (found in ethylmalonyl-CoA pathway)	No	130[b]

Using Ppc for CO_2 fixation

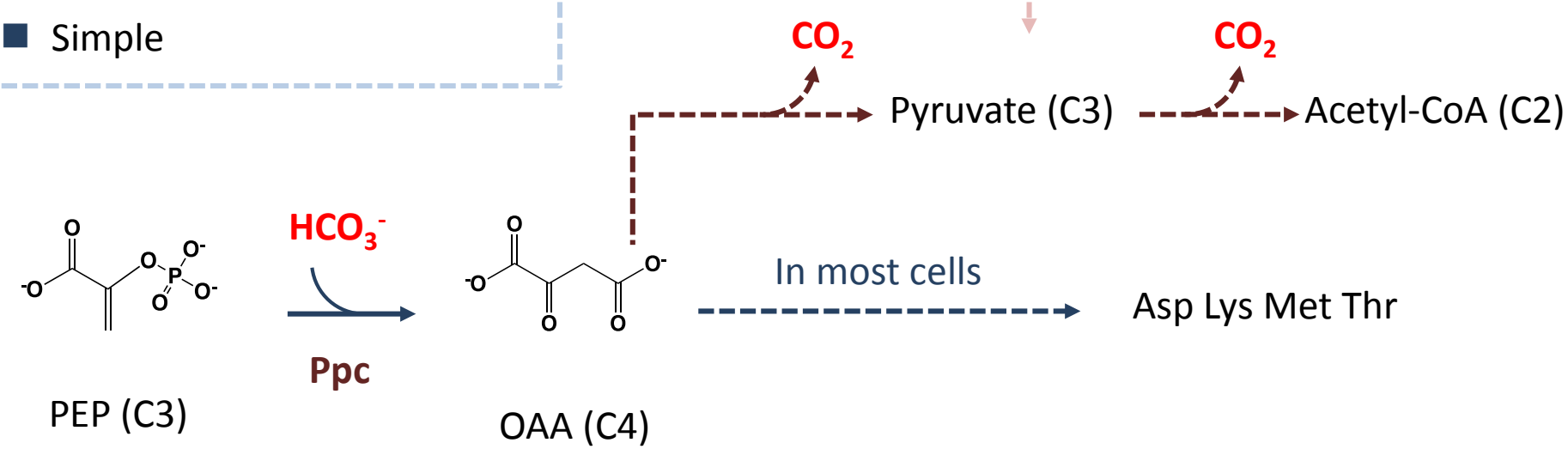
Ppc:

Phosphoenolpyruvate carboxylase

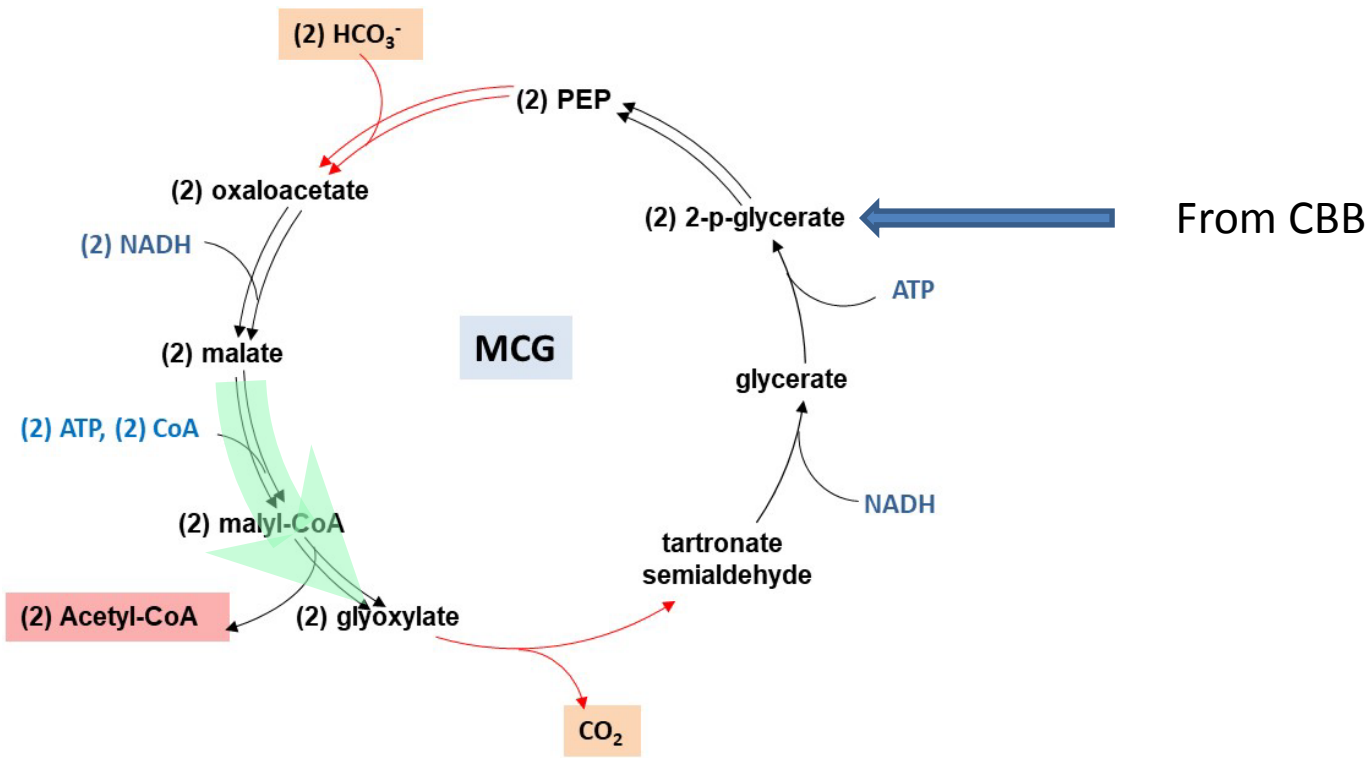
- Efficient
- Favor carboxylation direction
- No oxygen sensitivity
- Simple

Problem:

- OAA can not be converted to general building blocks without carbon loss

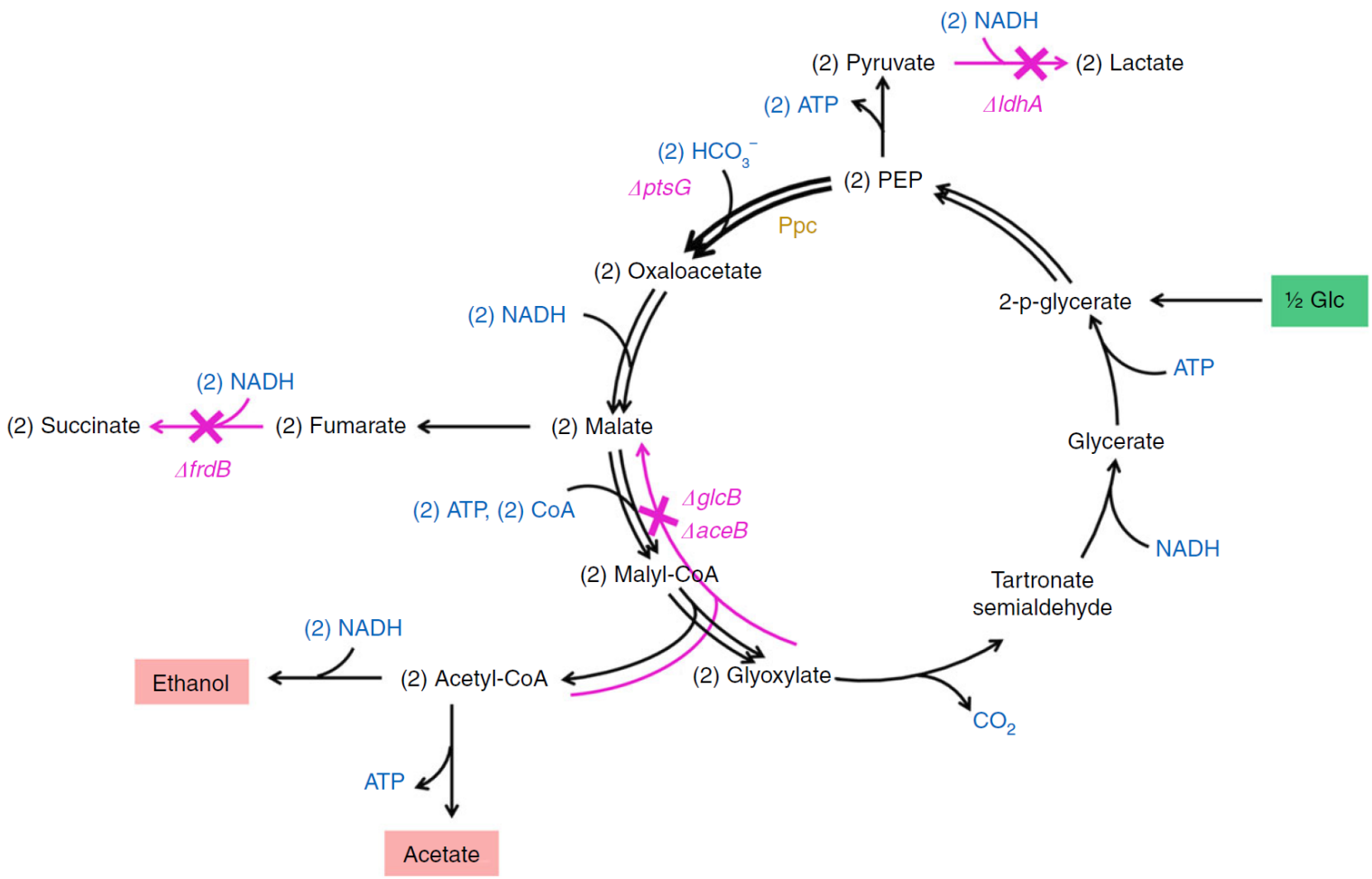


Reversal of glycoxylate shunt allows fixation of CO2 using Ppc

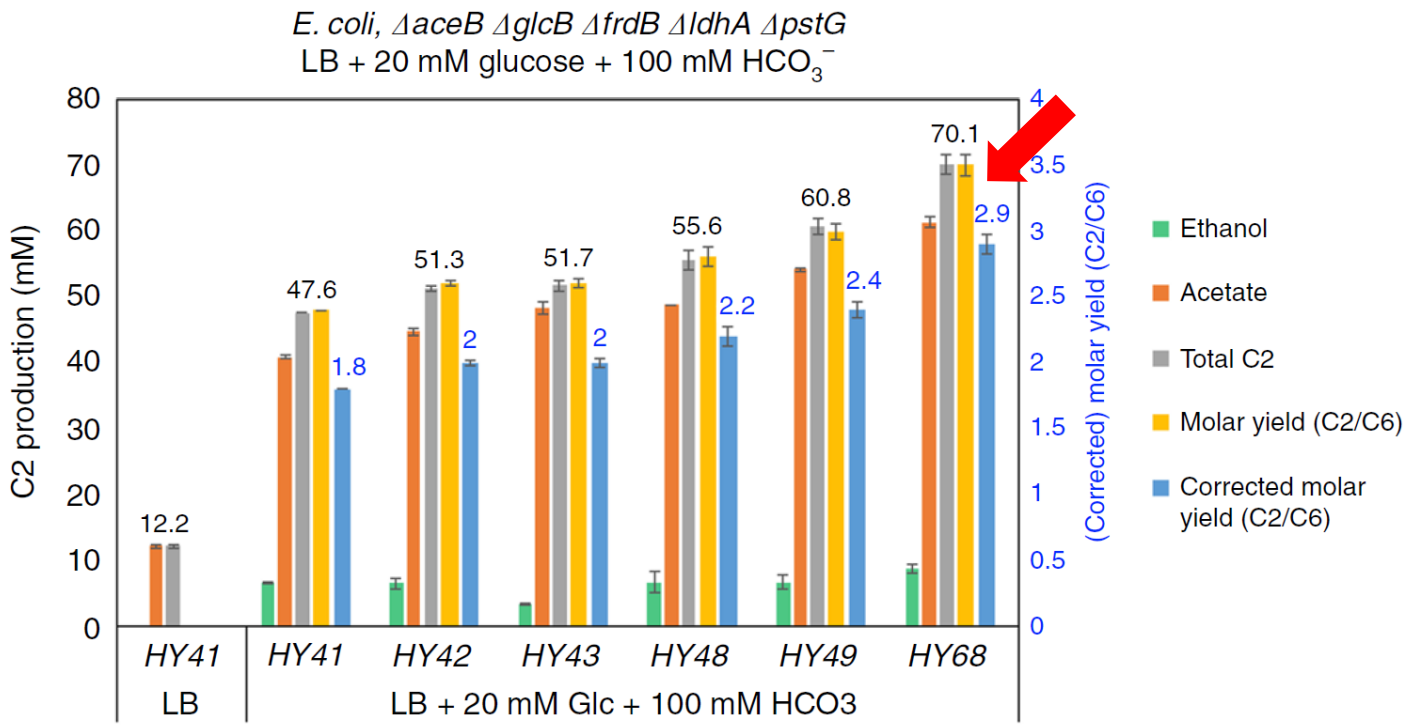


$C_6H_{12}O_6 + 2\ CO_2 + 4$"H" $\longrightarrow$ $4\ CH_3COOH$

Constructing MCG in E. coli

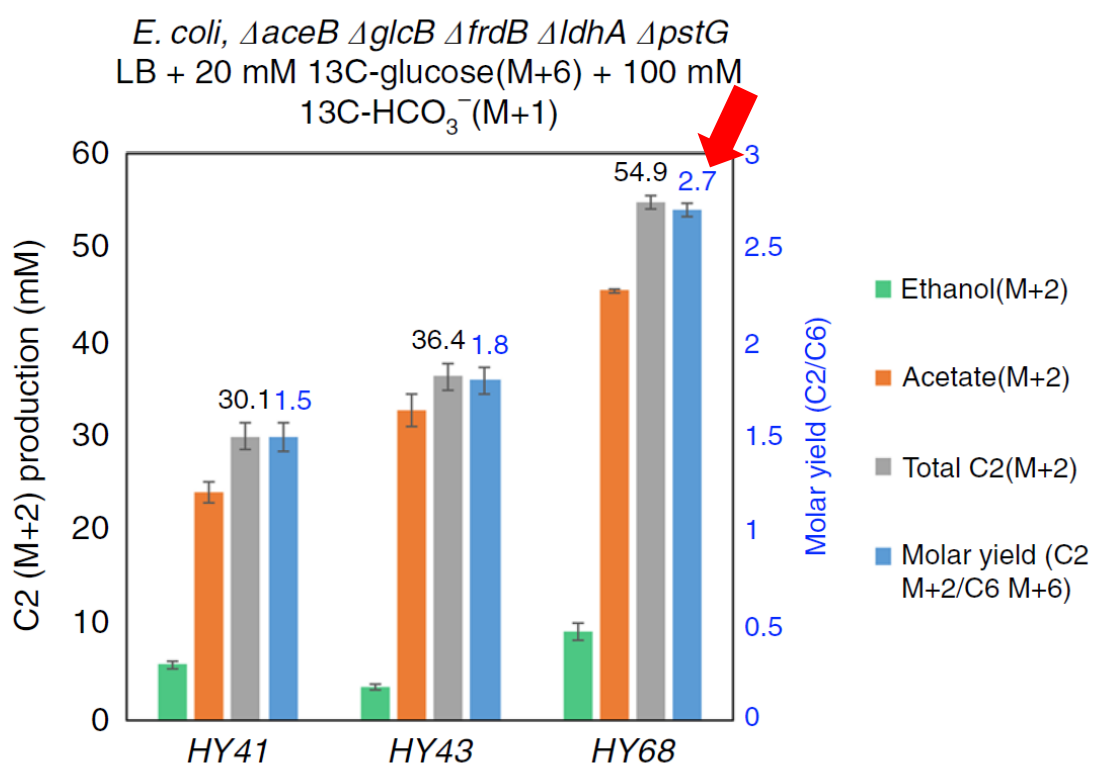


MCG in *E. coli*



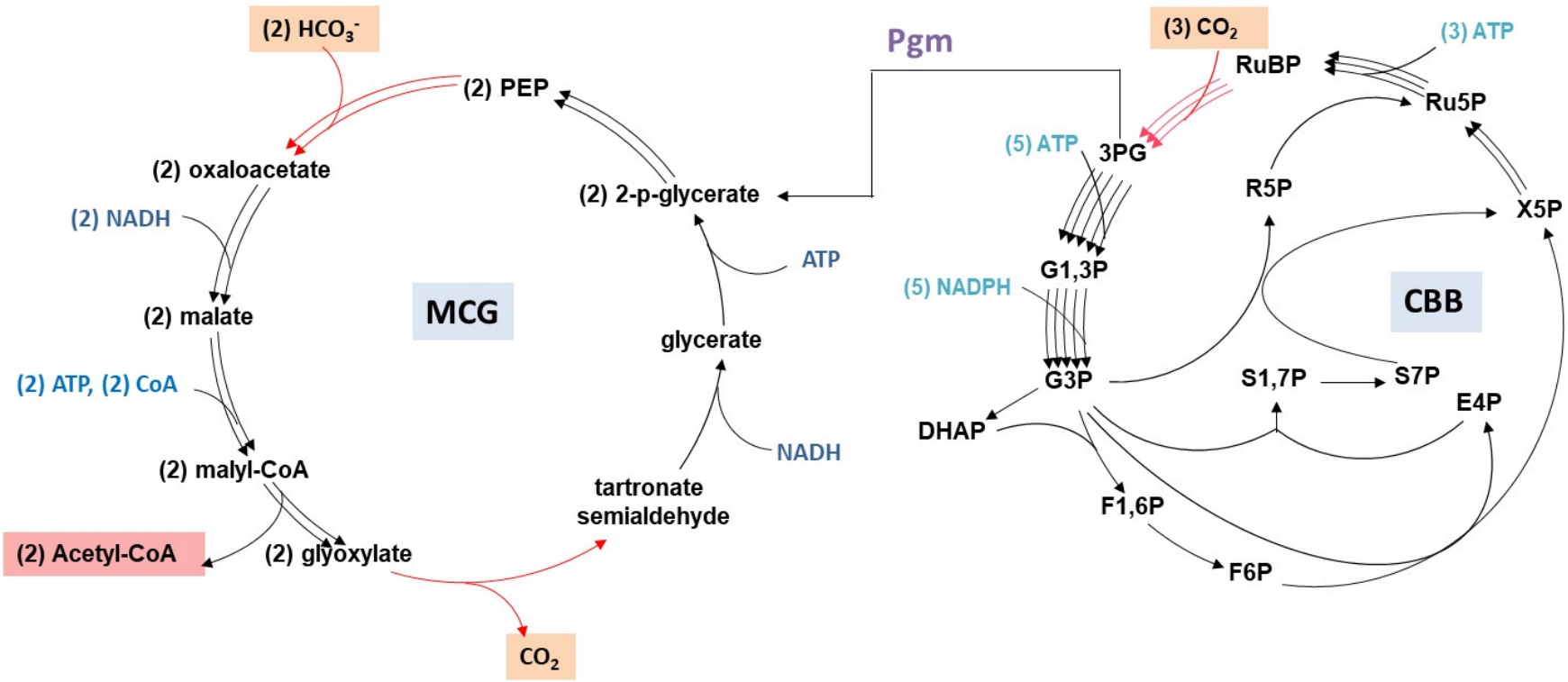
	HY41	HY41	HY42	HY43	HY48	HY49	HY68
	LB	LB + 20 mM Glc + 100 mM HCO3					
Mtk/Mcl	–	–	+	+	+	+	+
Gcl/Hyi	–	–	–	+	+	+	+
GarK	–	–	–	–	+	+	+
Mdh	–	–	–	–	–	+	+
Ppc	–	–	–	–	–	–	+

c



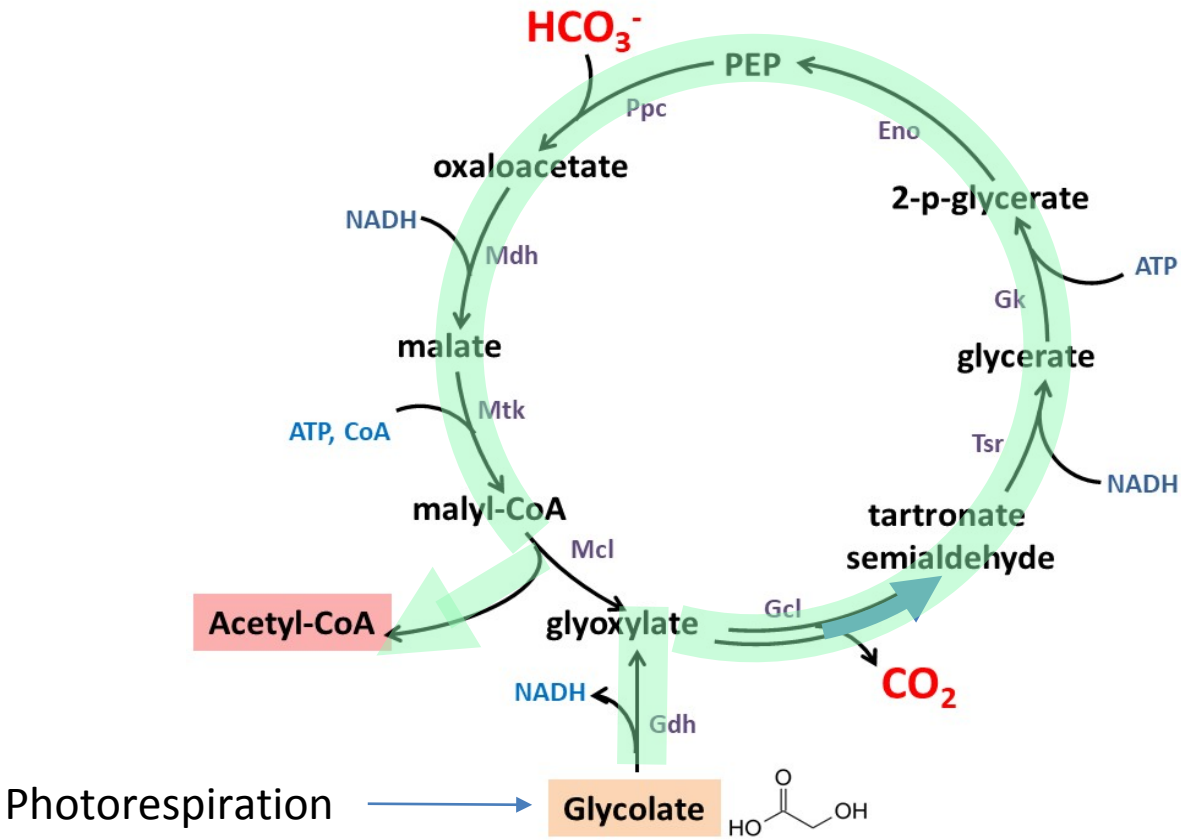
Mtk/Mcl	–	+	+
Gcl/Hyi	–	+	+
GarK/Mdh/Ppc	–	–	+

MCG augments CBB pathway



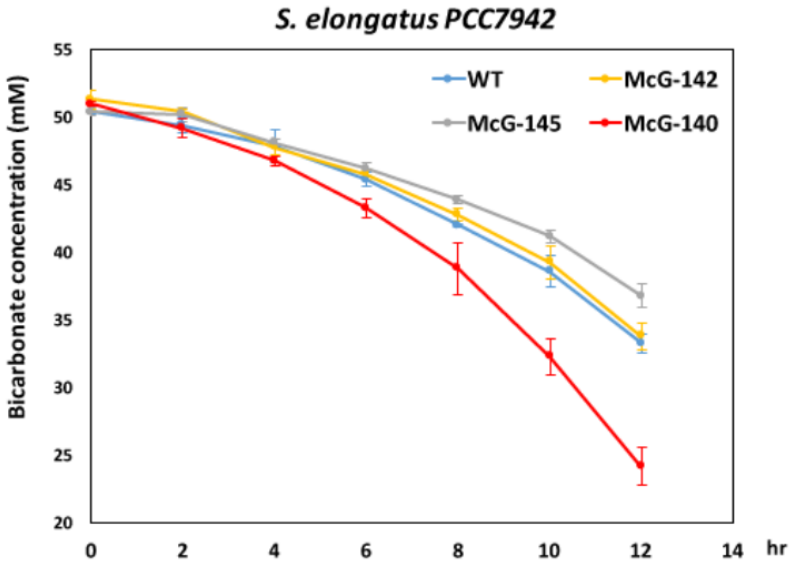
Per Ac-CoA Synthesis from CO_2	NAD(P)H consumption	ATP consumption	Rubisco turnover	The theoretical carbon yield
CBB+ PDH	4	7	3	66% (Ac-CoA/C3)
CBB+ NOG	4	6	2	100% (1.5 Ac-CoA/C3)
CBB+ MCG	4	5.5	1.5	100% (2 Ac-CoA/C3+C1)

MCG pathway recycles photorespiration product



Per Ac-CoA Synthesis from glycolate	NAD(P)H consumption	ATP consumption	The theoretical carbon yield
Native photorespiration pathway[17]	0	0	50% (1 Ac-CoA/2 glycolate)
The bacterial glycolate assimilation route[17]	-2	0	50% (1 Ac-CoA/2 glycolate)
The MCG pathway	1	2	100% (1 Ac-CoA/1 glycolate)

MCG in S. elongates increased CO_2 fixation



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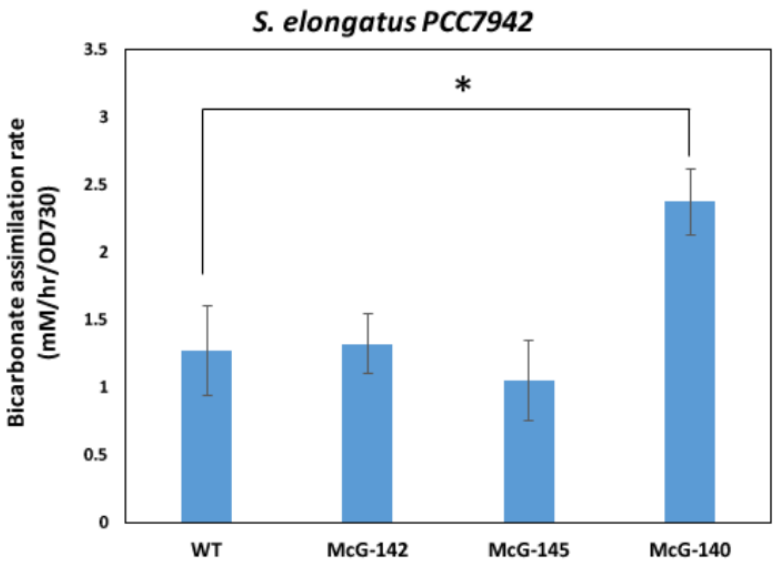

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