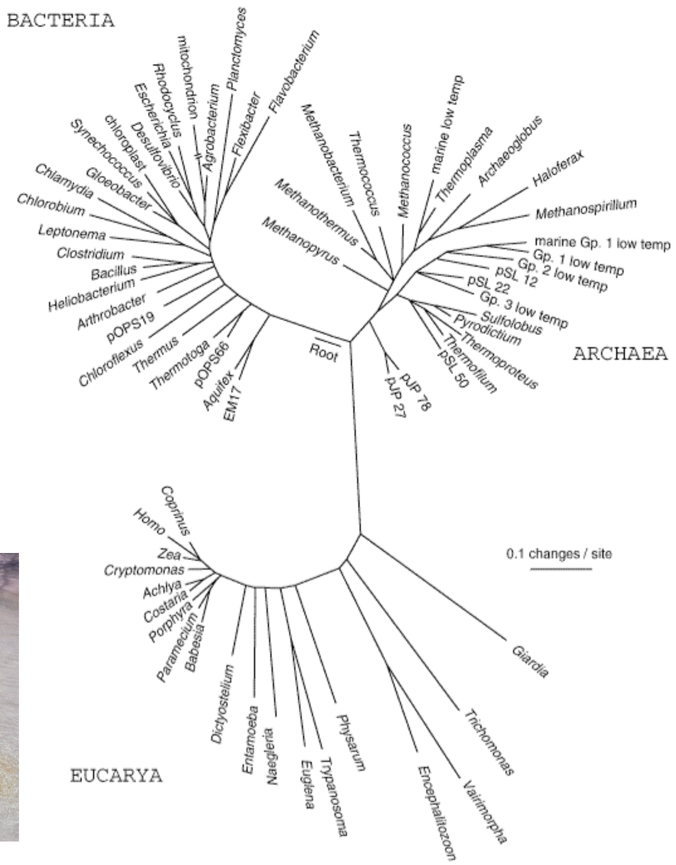
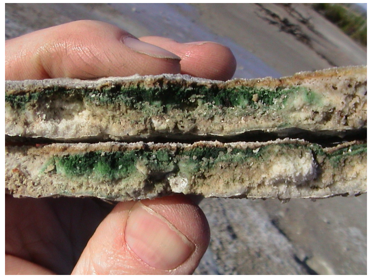
Metabolic enzymes and networks: Function and evolution

Shelley Copley

University of Colorado Boulder

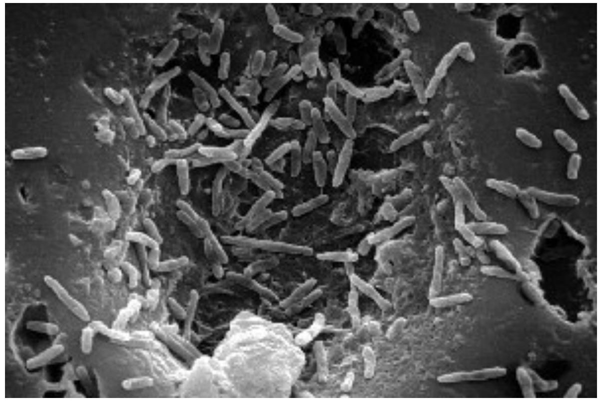
Why metabolism is interesting: Evolution of lifestyles



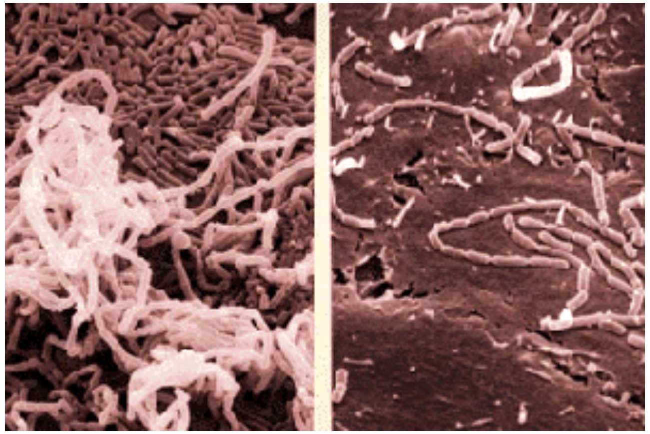


Why metabolism is interesting: Potential drug targets

Pseudomonas aeruginosa



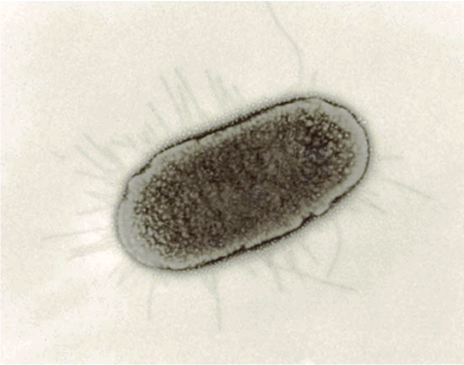
Mycobacterium tuberculosis



Haemophilus influenzae

Why metabolism is interesting: Metabolic engineering

food



Biofuels

Biodegradable polymers

Pharmaceuticals

Commodity chemicals

Sources of energy

Oxidation of organic or inorganic compounds

Light

↓

ATP
NAD(P)H → biosynthesis

Redox reactions

Oxidation – loss of electrons

(oxidant – the species that receives the electrons)

Reduction – gain of electrons

(reductant – the species that gives up the electrons)

LEO the lion says GER

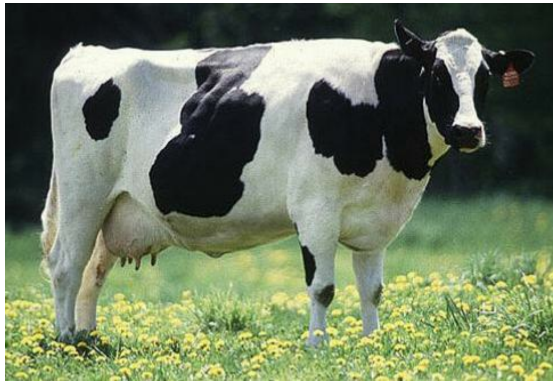
OIL RIG

Oxidation reactions release energy

$$H_2 + 1/2\ O_2 \longrightarrow H_2O + \text{energy}$$

$$C_6H_{12}O_6 + O_2 \longrightarrow 6\ CO_2 + 6\ H_2O + \text{energy}$$

(carbohydrate)

$$C_6H_{12}O_6 + O_2 \longrightarrow 6\ CO_2 + 6\ H_2O + \text{energy}$$

(carbohydrate)

There are more things in heaven and earth, Horatio, than are dreamt of in your philosophy.

Shakespeare (Hamlet)

Many different compounds can serve as electron donors (reductants)

E.g. sulfur oxidation

Sulfolobus acidocaldarius

Electron donor – H_2S
Electron acceptor – O_2

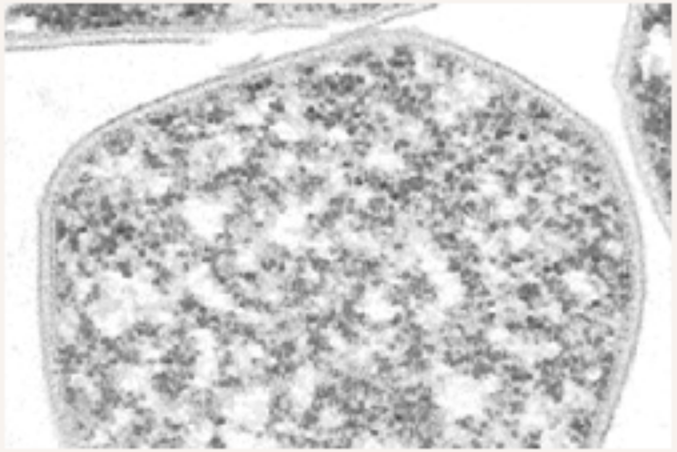


Photo: D.Janckovik and W.Zillig


US National Park Service

(Lithotroph – an organism that derived energy from redox reactions involving inorganic molecules)

Iron-oxidizing bacteria

$$4\ Fe(OH)_2 + O_2 \rightarrow 4\ H_2O + 2\ Fe_2O_3$$

Many different compounds can serve as electron acceptors (oxidants)

Table 6.3 Compounds that can serve as electron acceptors in anaerobic respiration, replacing oxygen

Organic compounds	Inorganic compounds
Fumarate	Nitrate (NO_3^-)
Dimethylsulfoxide (DMSO)	Nitrite (NO_2^-)
Trimethylamine *N*-oxide (TMAO)	Nitrous oxide (N_2O)
	Chlorate (ClO_3^-)
	Perchlorate (ClO_4^-)
	Manganic ion (Mn^{4+})
	Ferric ion (Fe^{3+})
	Gold (Au^{3+})
	Selenate (SeO_4^{2-})
	Arsenate (AsO_4^{3-})
	Sulfate (SO_4^{2-})
	Sulfur (S^0)

Sulfur oxidation

Thiobacillus denitrificans

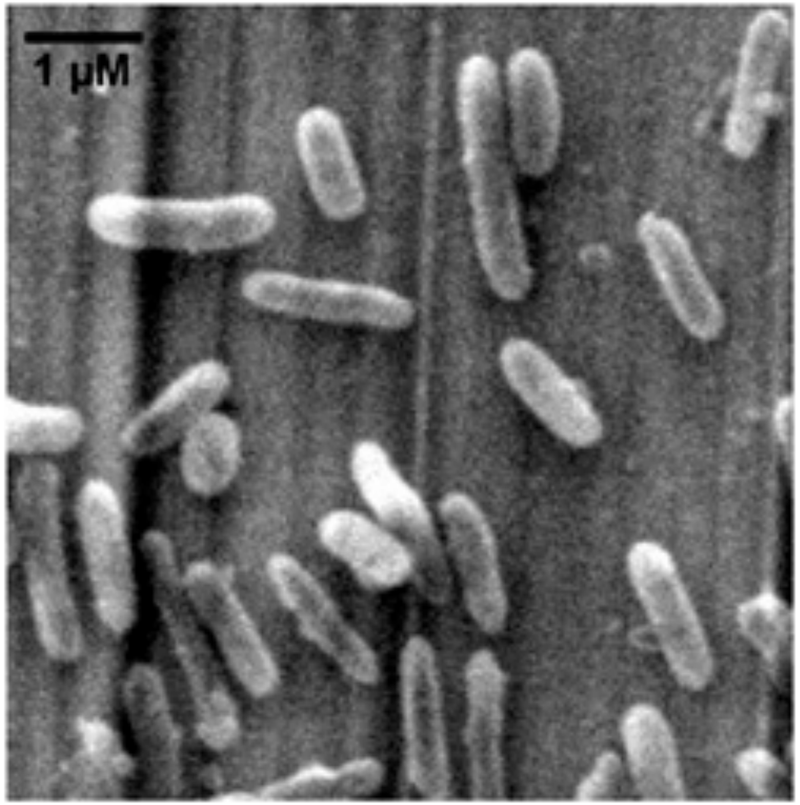


Photo credit: T.E. Letain, S.I. Martin, H.R. Beller

Electron donor – H_2S

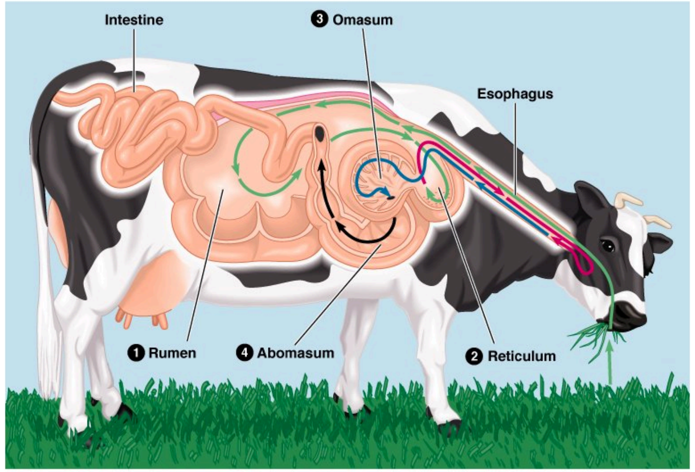
Electron acceptor – NO_3^- (nitrate)

Methanogens

$$4\ H_2 + CO_2 \rightarrow 2\ H_2O + CH_4$$

Anoxic guts

Figure 1 | Home for an abundant microbiological flora. The human gut and (inset) a scanning electron micrograph of part of the small intestine, with some bacterial inhabitants picked out in green.



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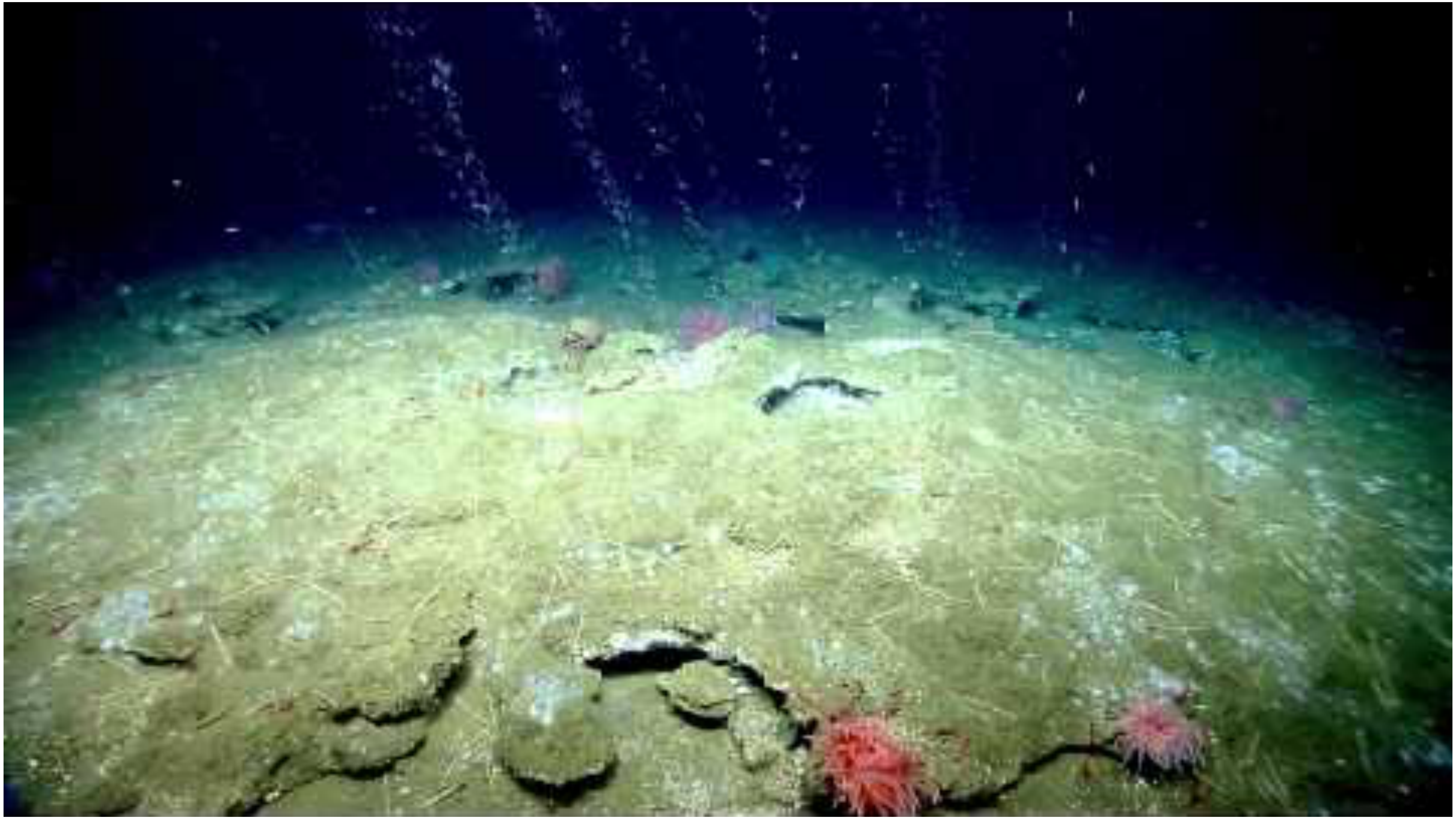


The point:

There are many different reductants and many different oxidants that can be used to supply energy.

Sources of energy

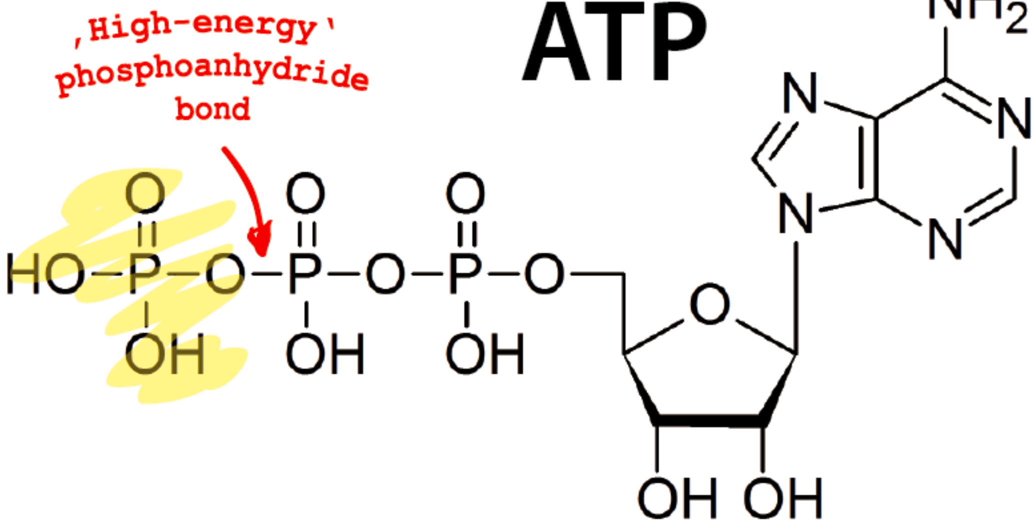
Oxidation of organic or inorganic compounds

Light

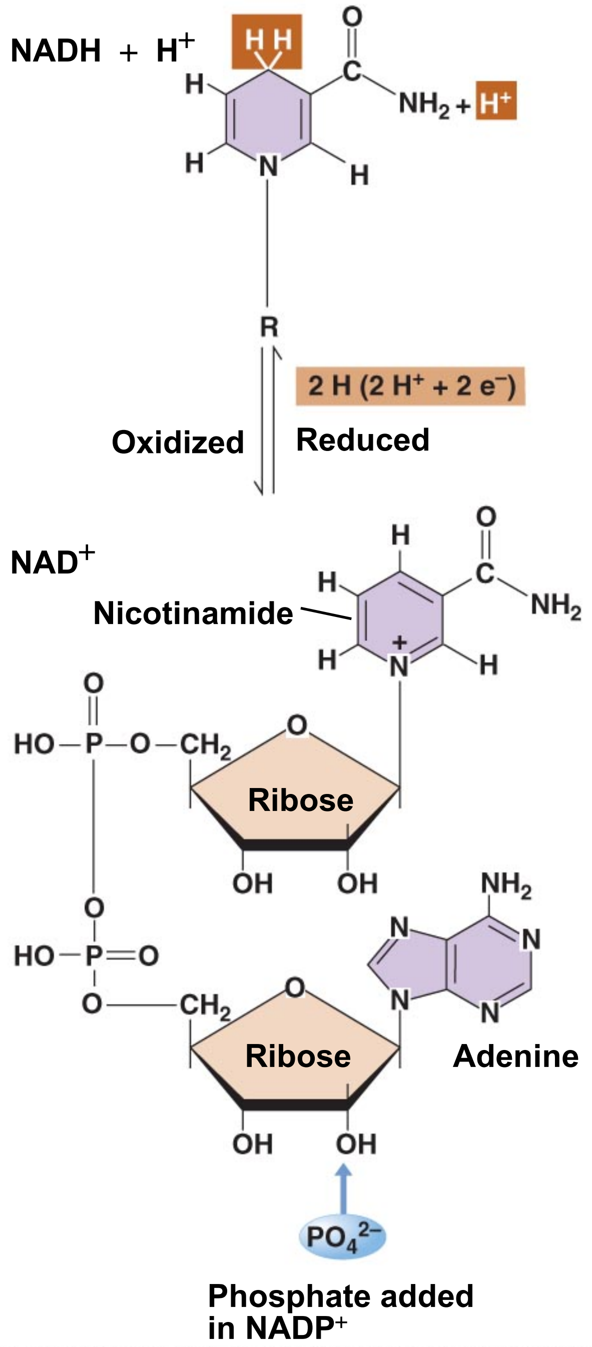
↓

ATP
NAD(P)H → biosynthesis

ATP



Adenosine triphosphate



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NAD = Nicotinamide adenine dinucleotide

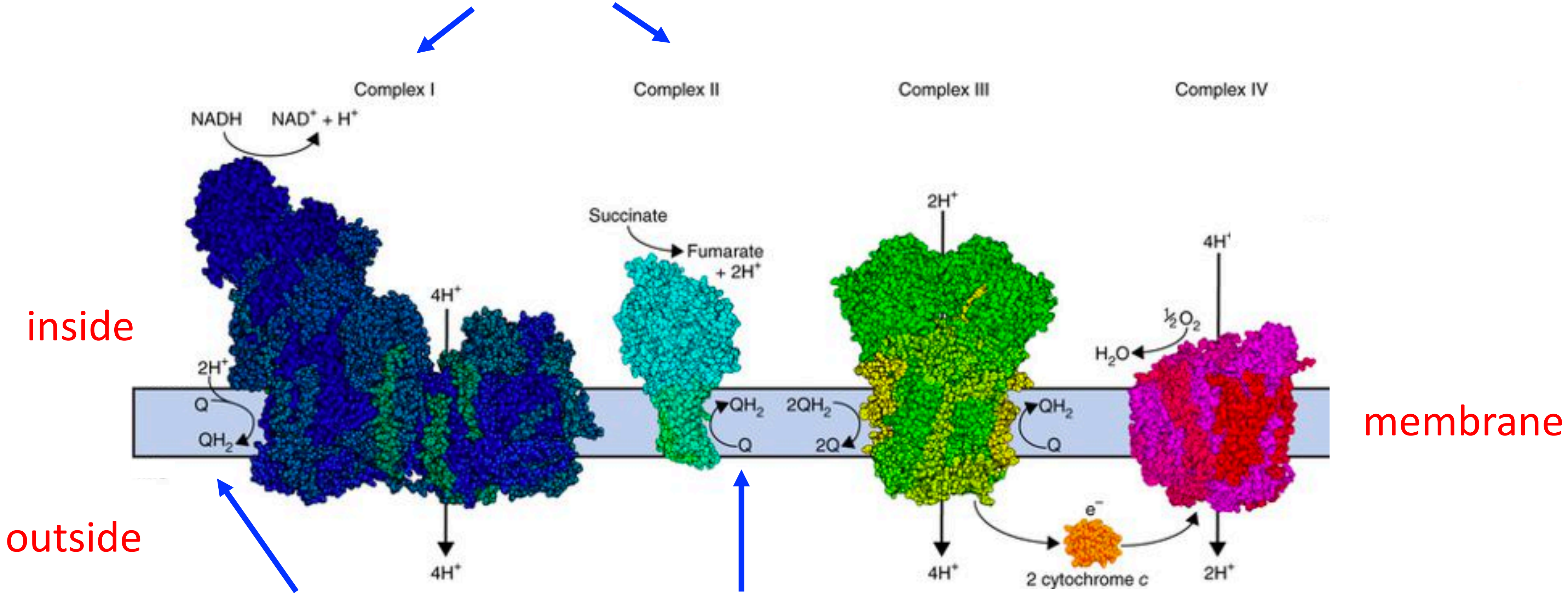
NADH used for energy generation

NADPH used for biosynthesis

How energy from redox reactions is captured

Redox energy is captured in a proton gradient

Two entry points for electrons



QH_2 generated by Complex I or Complex II reduces Complex III

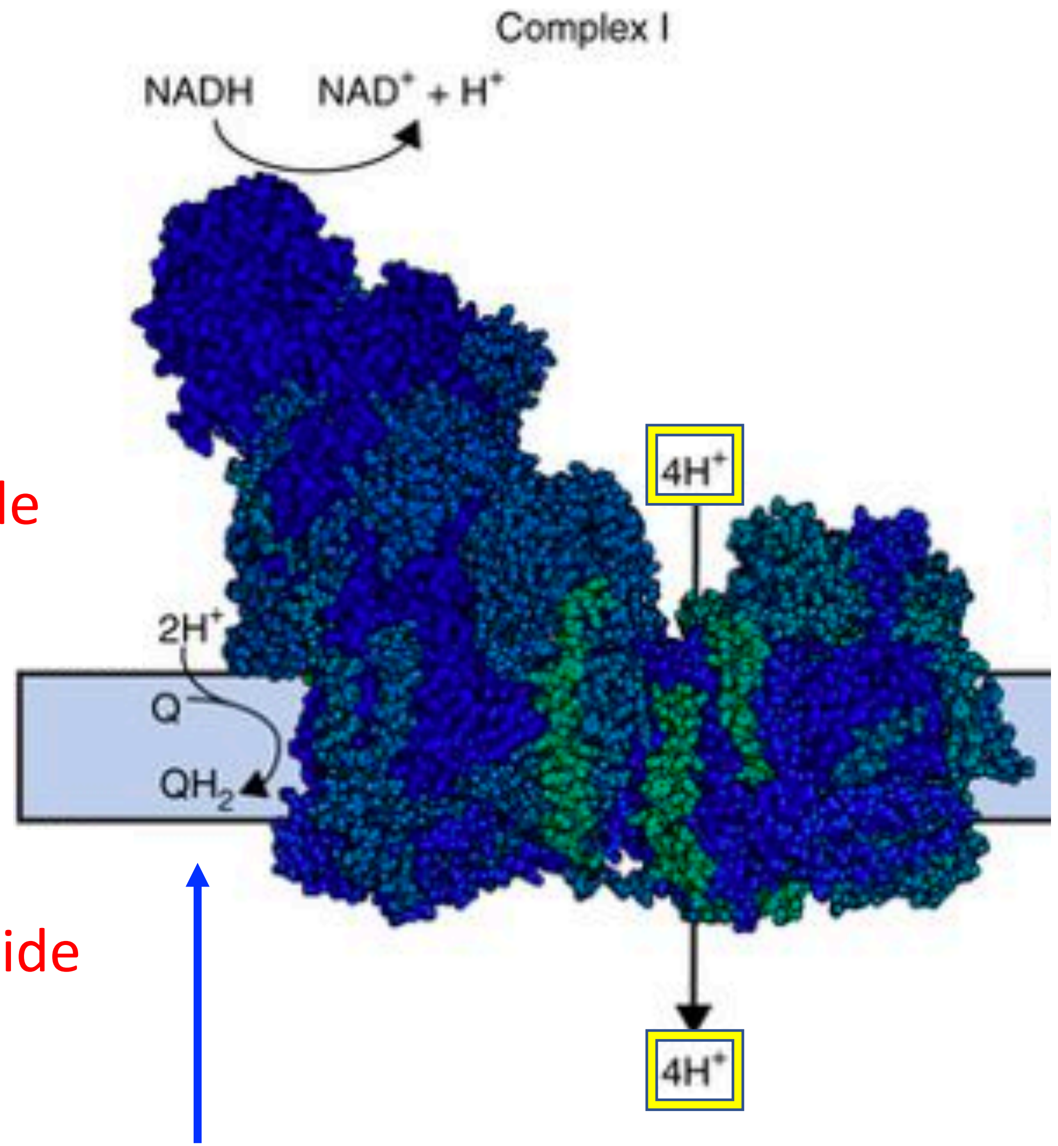
Electrons from Complex III are carried to Complex IV by cytochrome c

Nature Structural & Molecular Biology **volume24**, pages800–808 (2017)

inside

Q = quinone

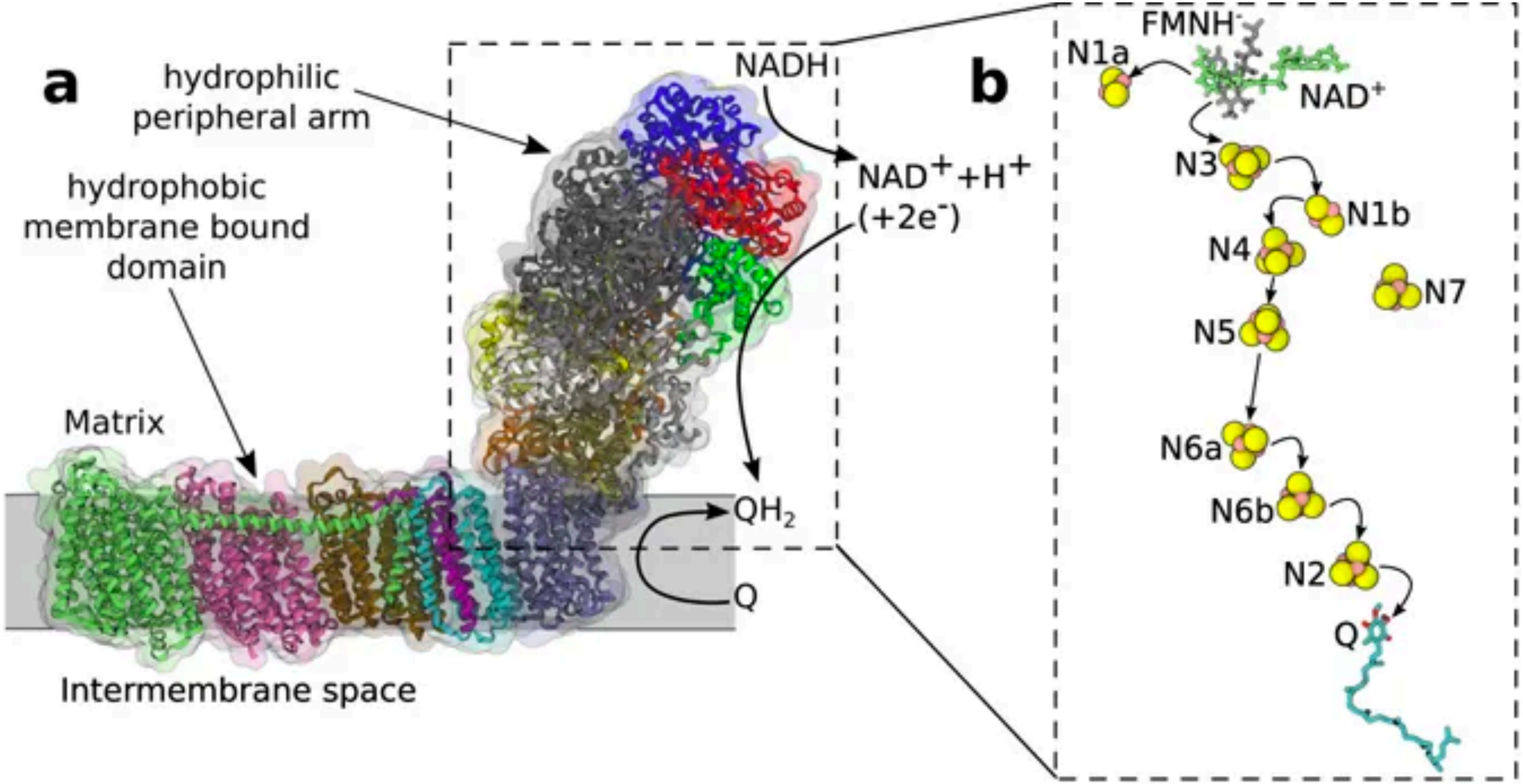
QH_2 = reduced quinone



membrane

outside

Nature Structural & Molecular Biology **volume24**, pages800–808 (2017)

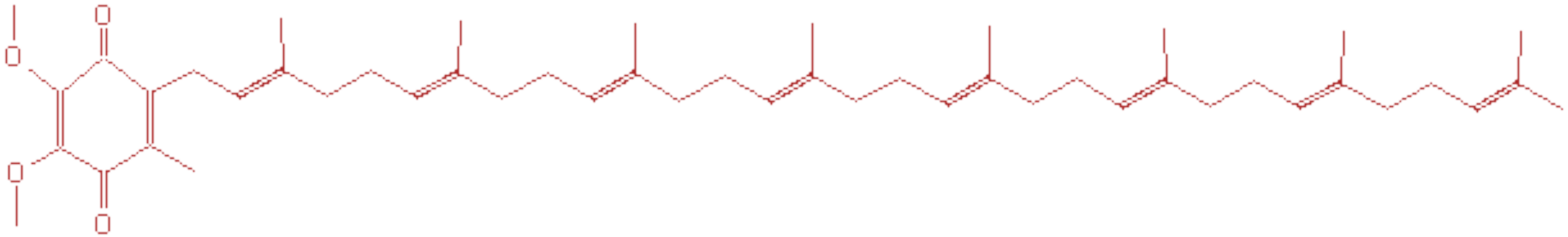


2 Fe_2S_2

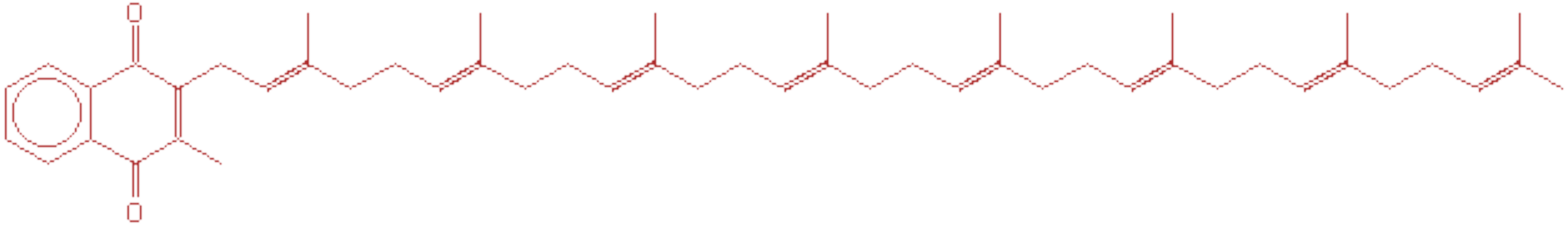
7 Fe_4S_2

Scientific Reports **volume 7**, Article number: 5495 (2017)

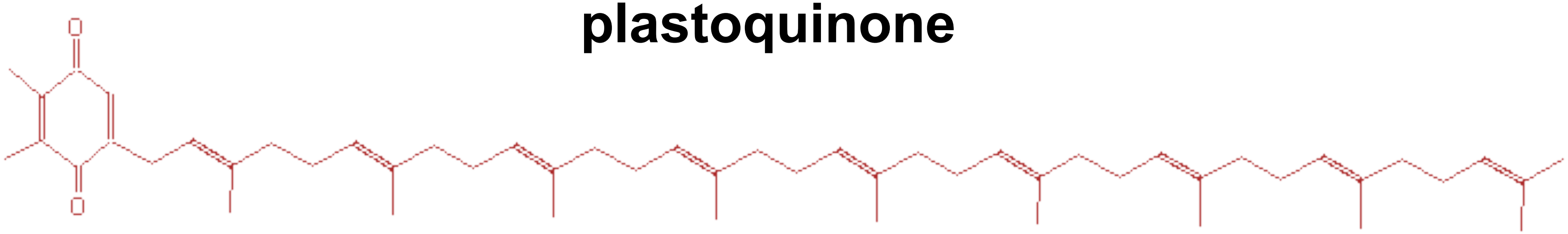
ubiquinone



menaquinone

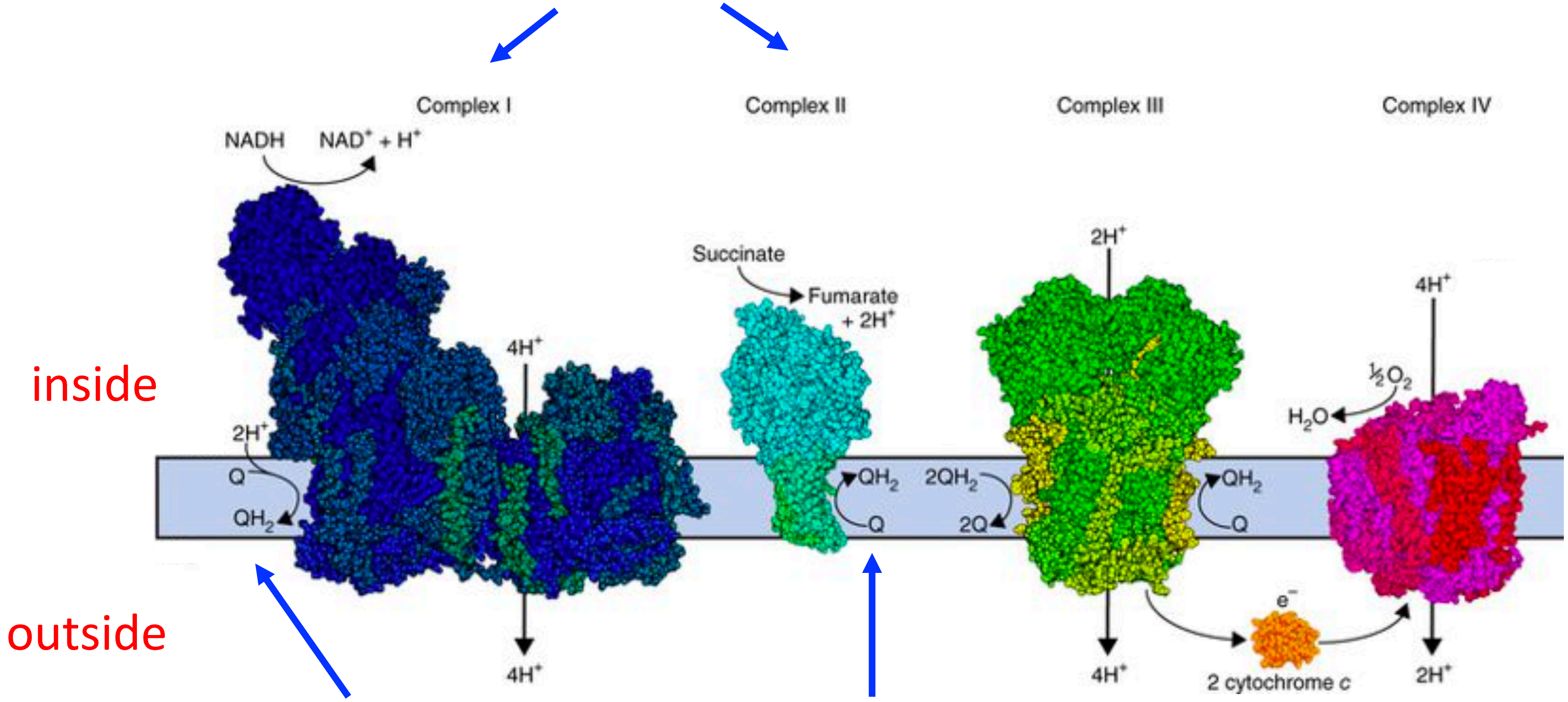


plastoquinone



Redox energy is captured in a proton gradient

Two entry points for electrons



QH_2 generated by Complex I or Complex II reduces Complex III

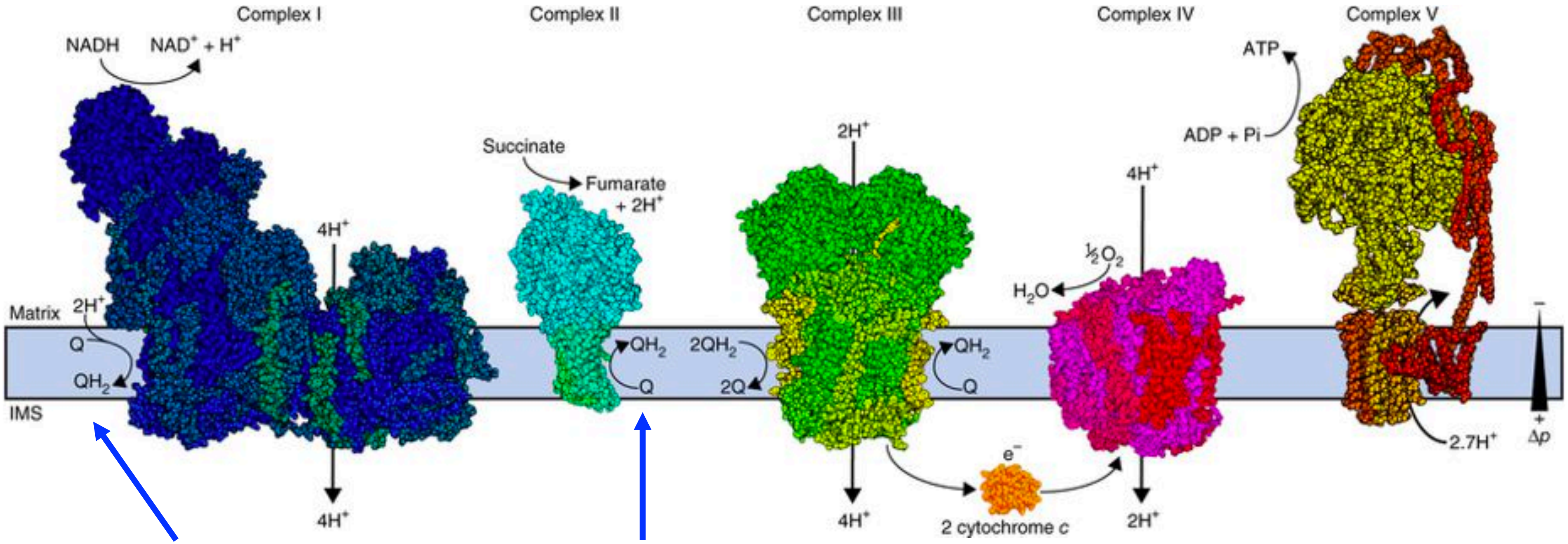
Electrons from Complex III are carried to Complex IV by cytochrome c

Nature Structural & Molecular Biology **volume24**, pages800–808 (2017)

Oxidative phosphorylation

Two entry points for electrons

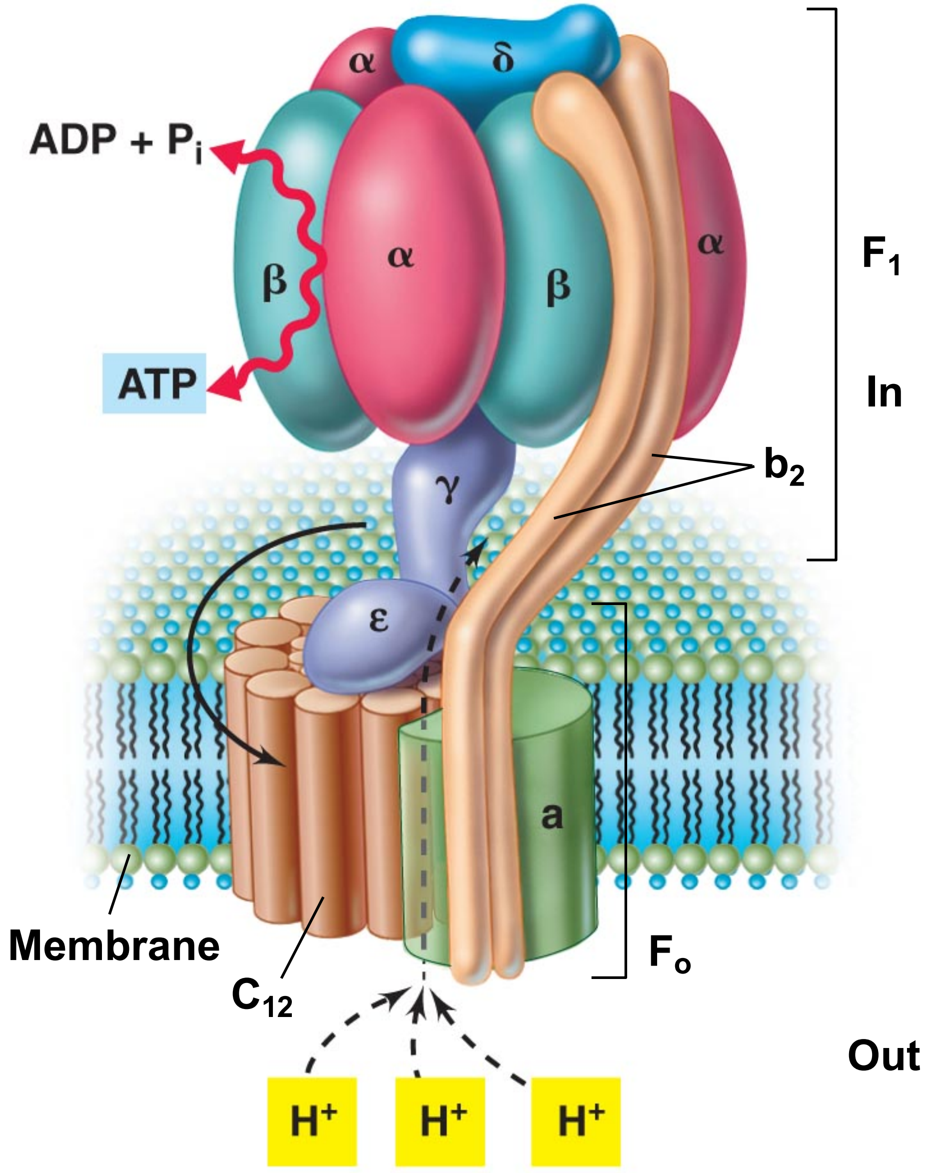
ATP synthase



QH_2 generated by Complex I or Complex II reduces Complex III

Electrons from Complex III are carried to Complex IV by cytochrome c

Nature Structural & Molecular Biology **volume24**, pages800–808 (2017)



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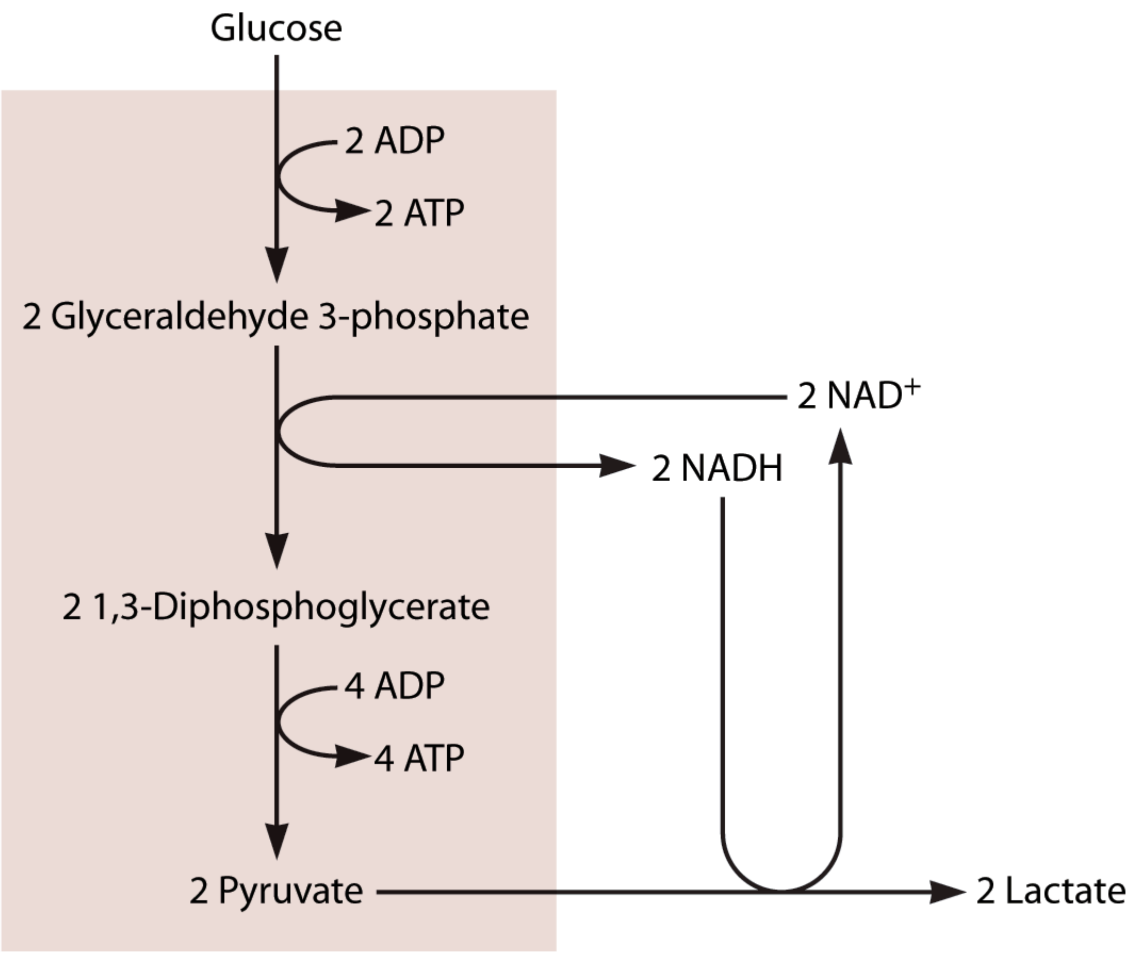
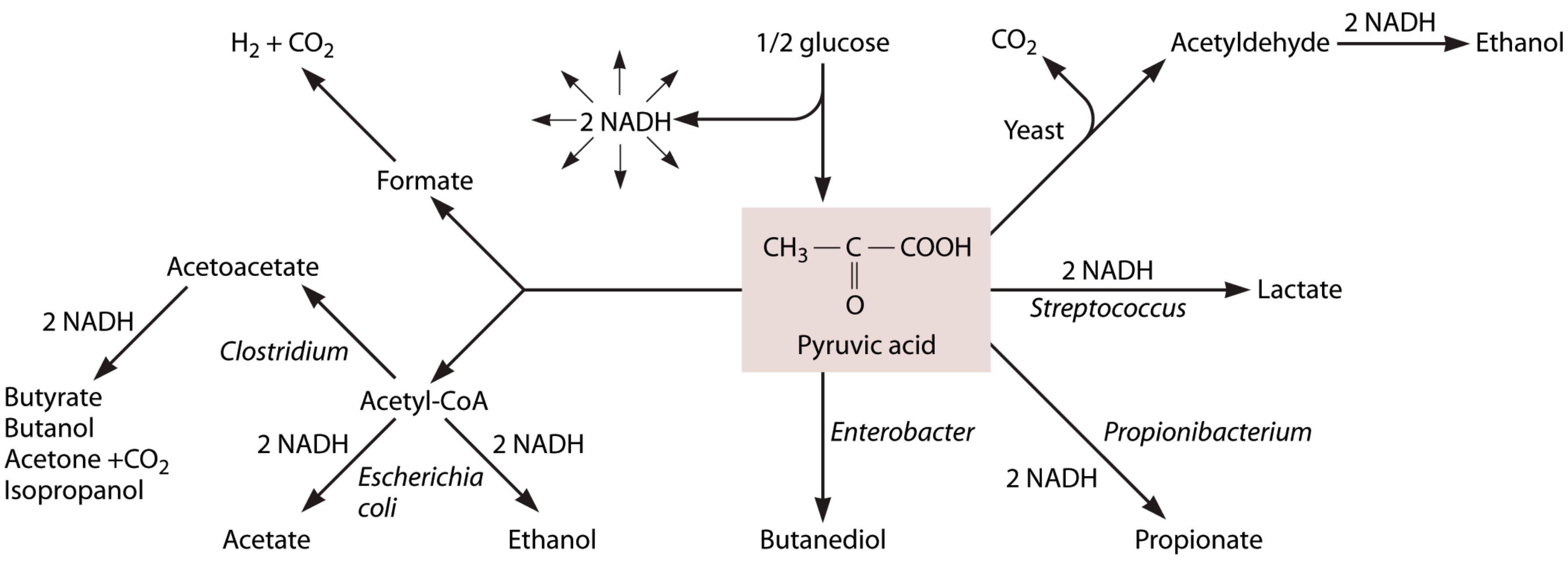
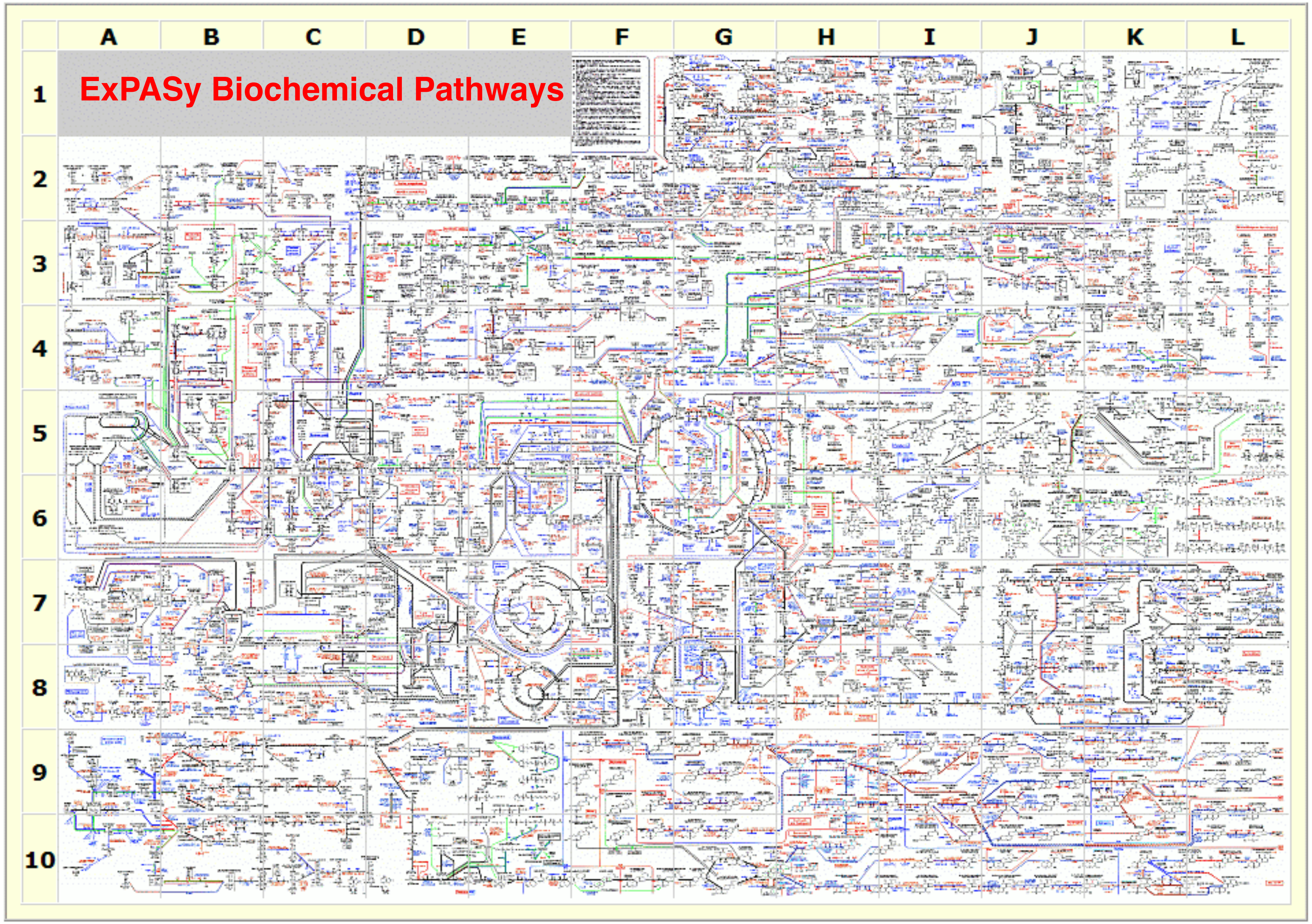
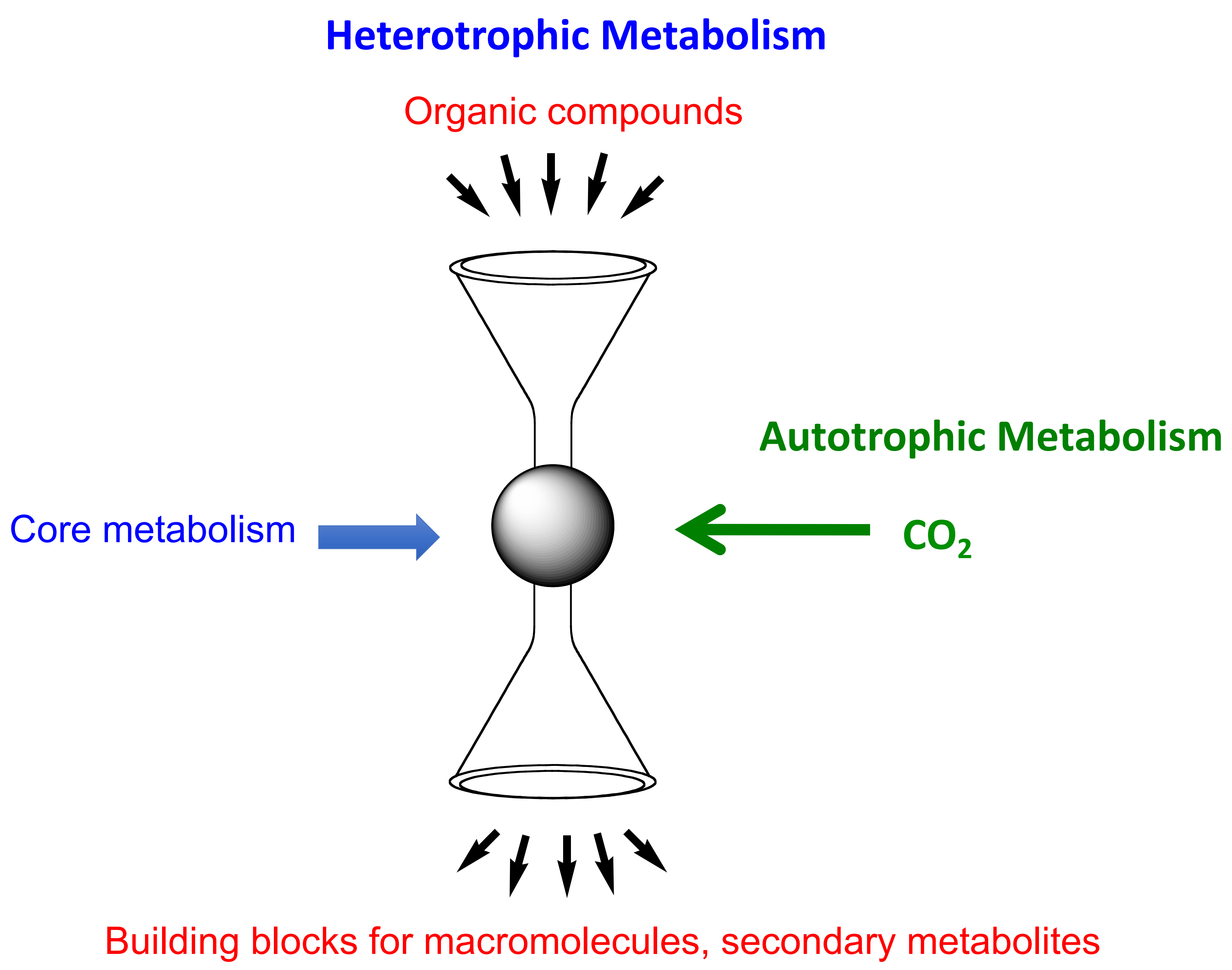
Fermentation:
A way to skip the electron transport chain



Various organic electron acceptors




ExPASy Biochemical Pathways
A
B
C
D
E
F
G
H
I
J
K
L
1
2
3
4
5
6
7
8
9
10


Heterotrophic Metabolism
Organic compounds
Autotrophic Metabolism
Core metabolism
CO_2
Building blocks for macromolecules, secondary metabolites

Features in the metabolic network

1) cycles
2) pathways
3) local networks

Precursors for biosynthesis

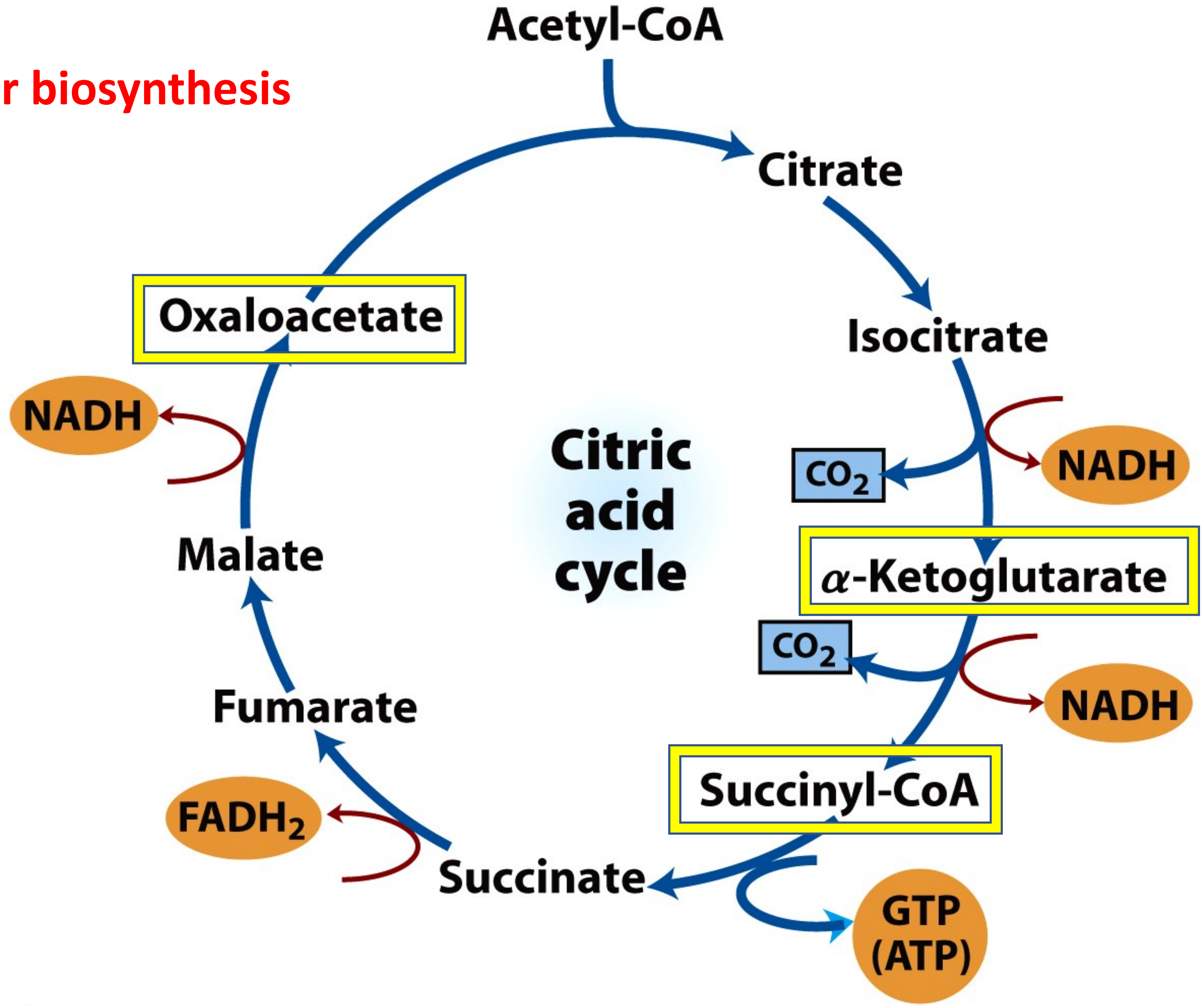


Figure 16-13
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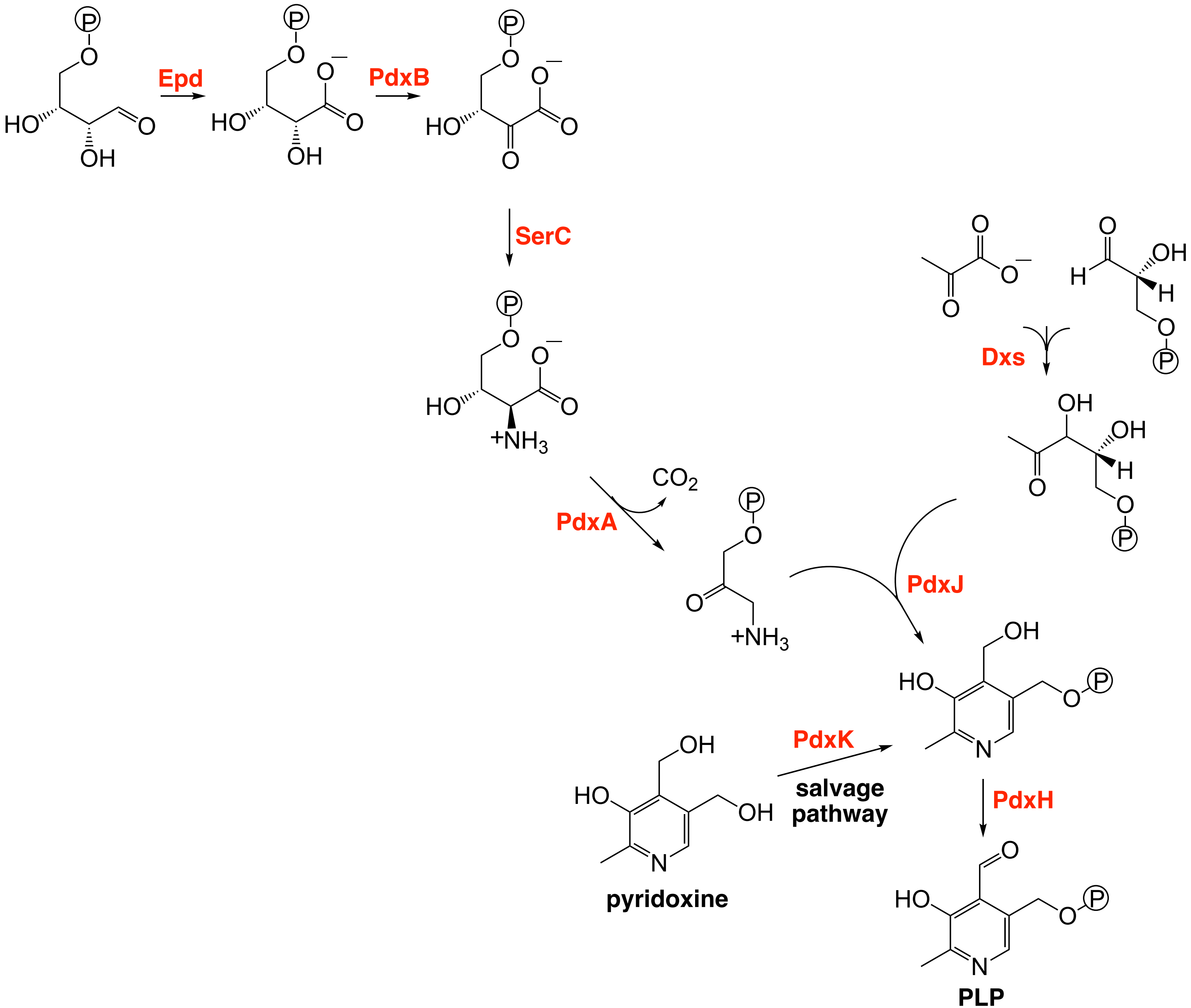
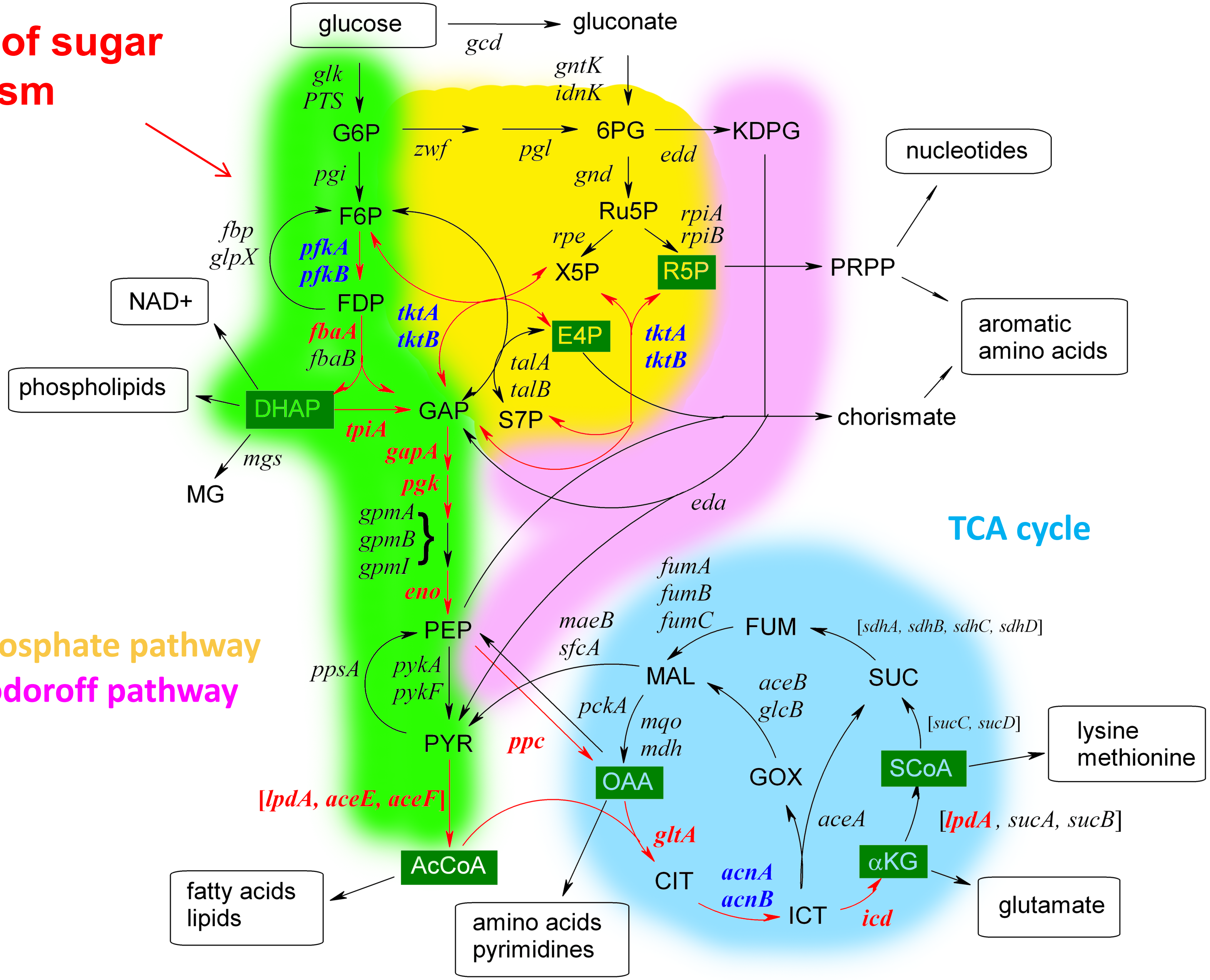
A pathway (for synthesis of pyridoxal phosphate)

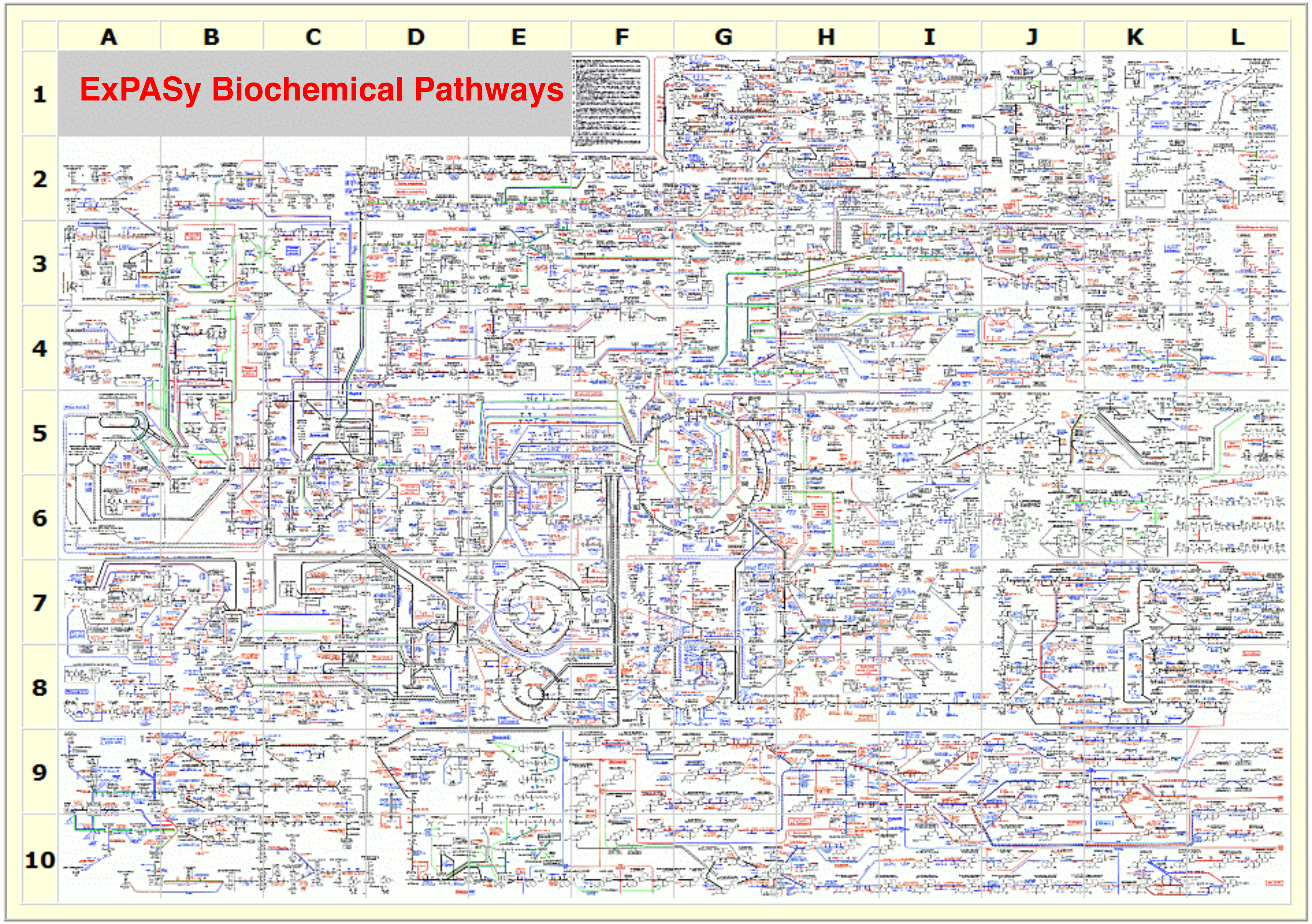


Network of sugar metabolism



Glycolyisis
Pentose phosphate pathway
Entner-Duodoroff pathway

Almost every reaction in this network is catalyzed by an enzyme



Why catalysis is needed

reaction	$t_{1/2}$
triose isomerization	2 days
ester hydrolysis	4 years
phosphomonoester hydrolysis	>500,000 years
fumarate hydration	700,000 years
phosphodiester hydrolysis	>13 million years
OMP decarboxylation	1.1 billion years

Wolfenden, Acc. Chem. Res. 34, 938, 2001

Catalysts are involved in

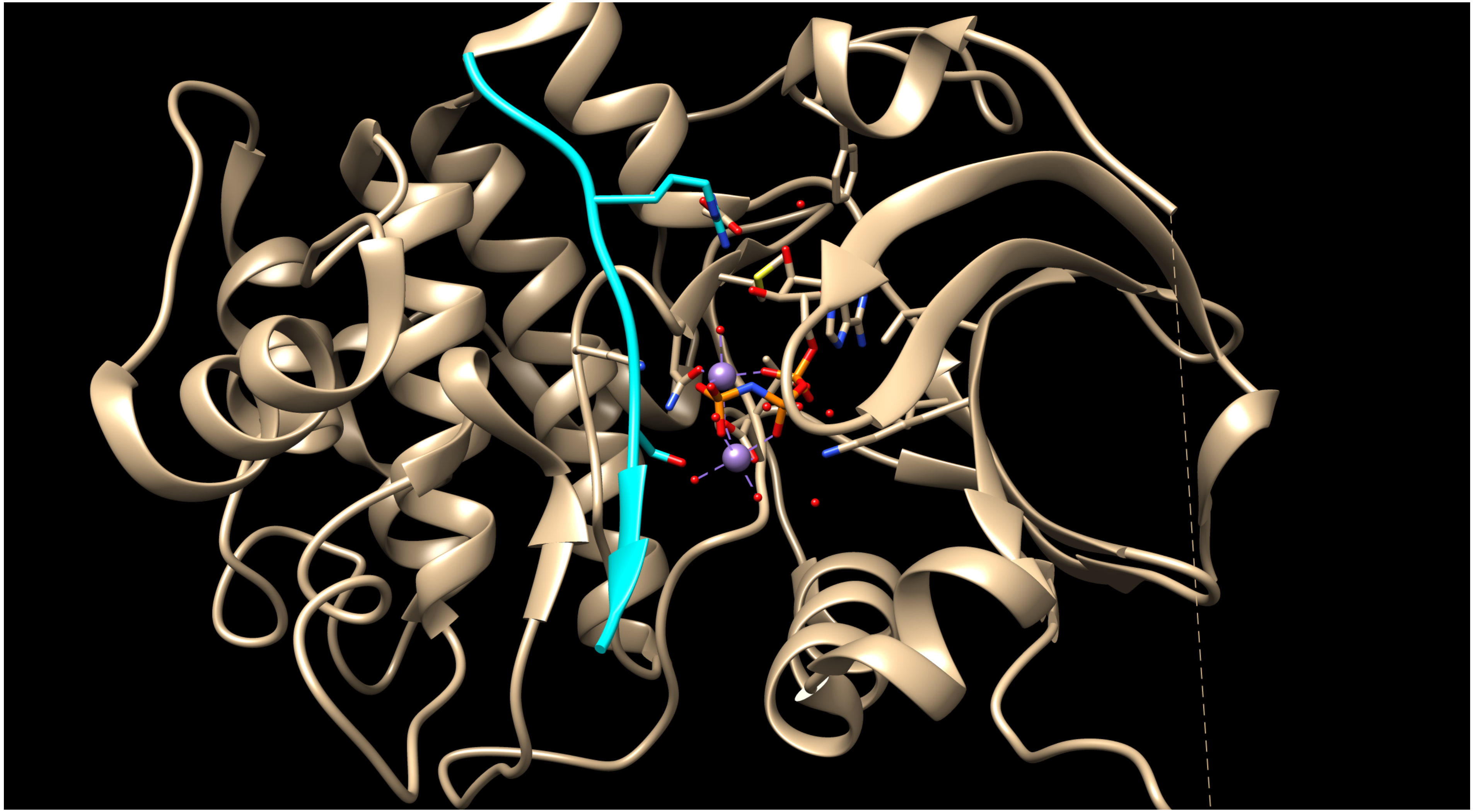
- genome replication
- transcription
- translation
- regulation
  - histone modification
  - phosphorylation
  - acetylation
  - etc etc etc
- metabolism
- transport
- movement

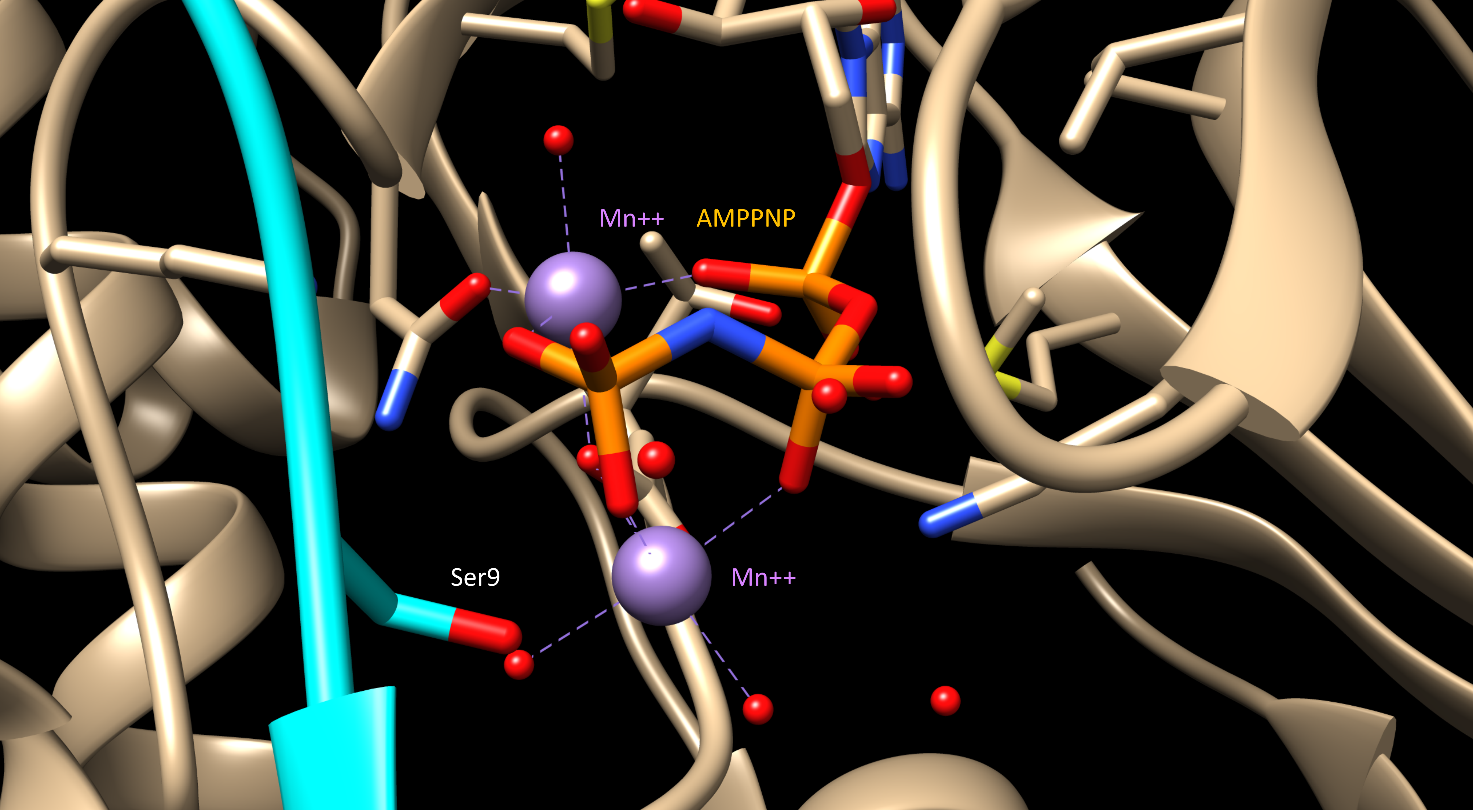
How enzymes catalyze reactions

approximation
transition state stabilization
acid-base catalysis
metal ion catalysis

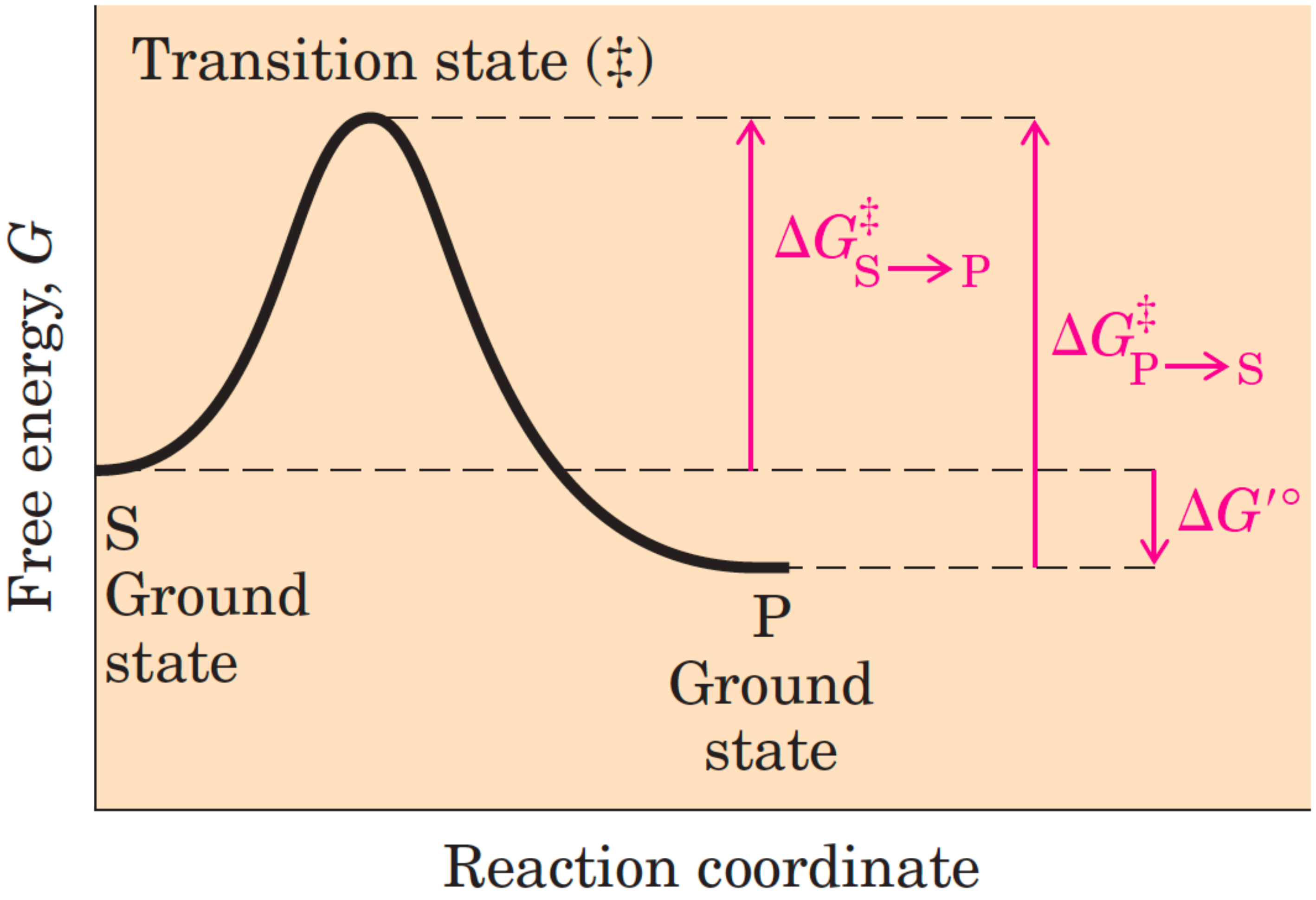
Approximation

PDB 1OK6 – Akt/protein kinase B in complex with GSK-3 peptide and AMPPNP




Mn++
AMPPNP
Ser9
Mn++

Transition state stabilization



$\Delta G'^{0}$ = standard free energy change when reactants and products are at 1 M pH 7

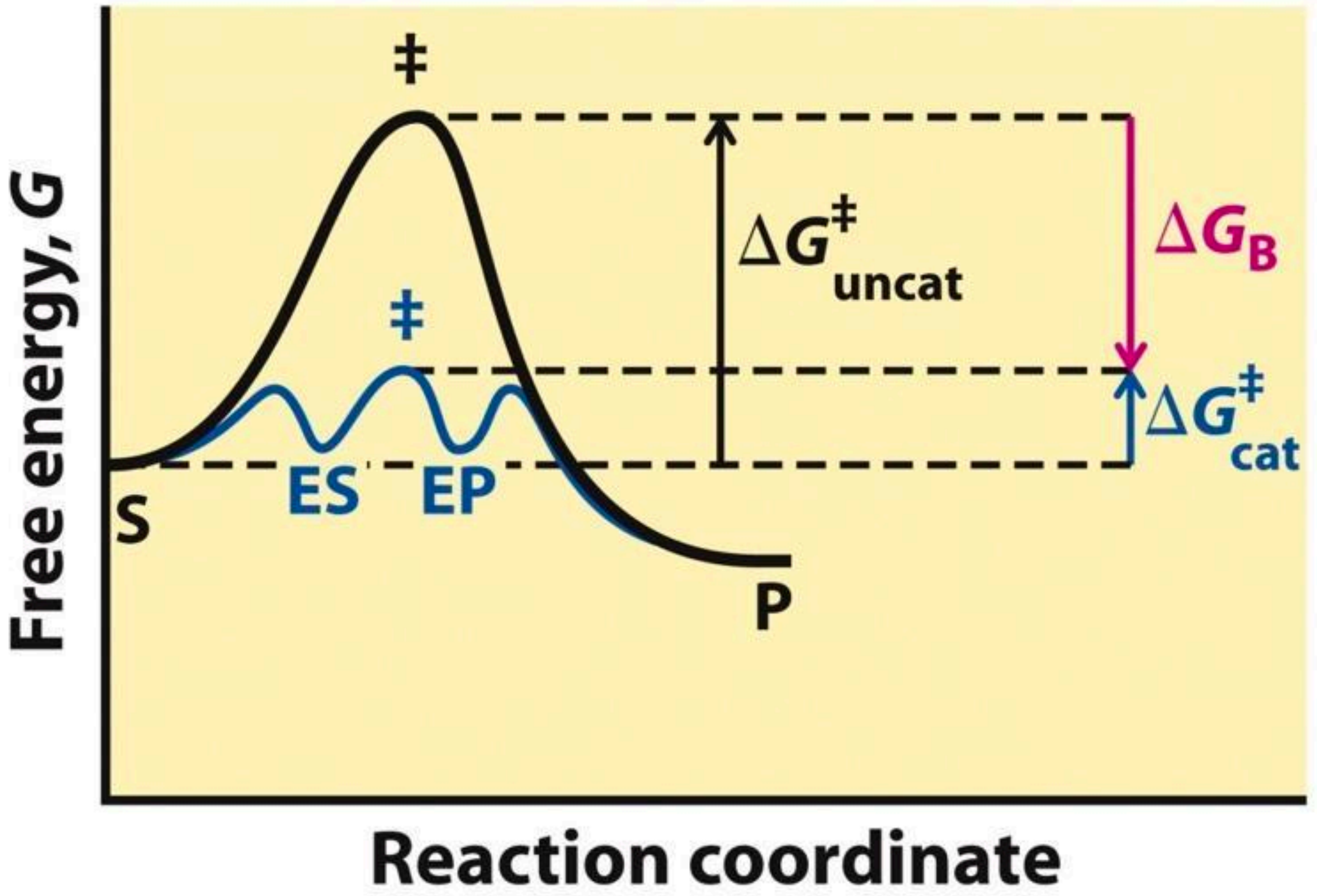


Figure 6-6
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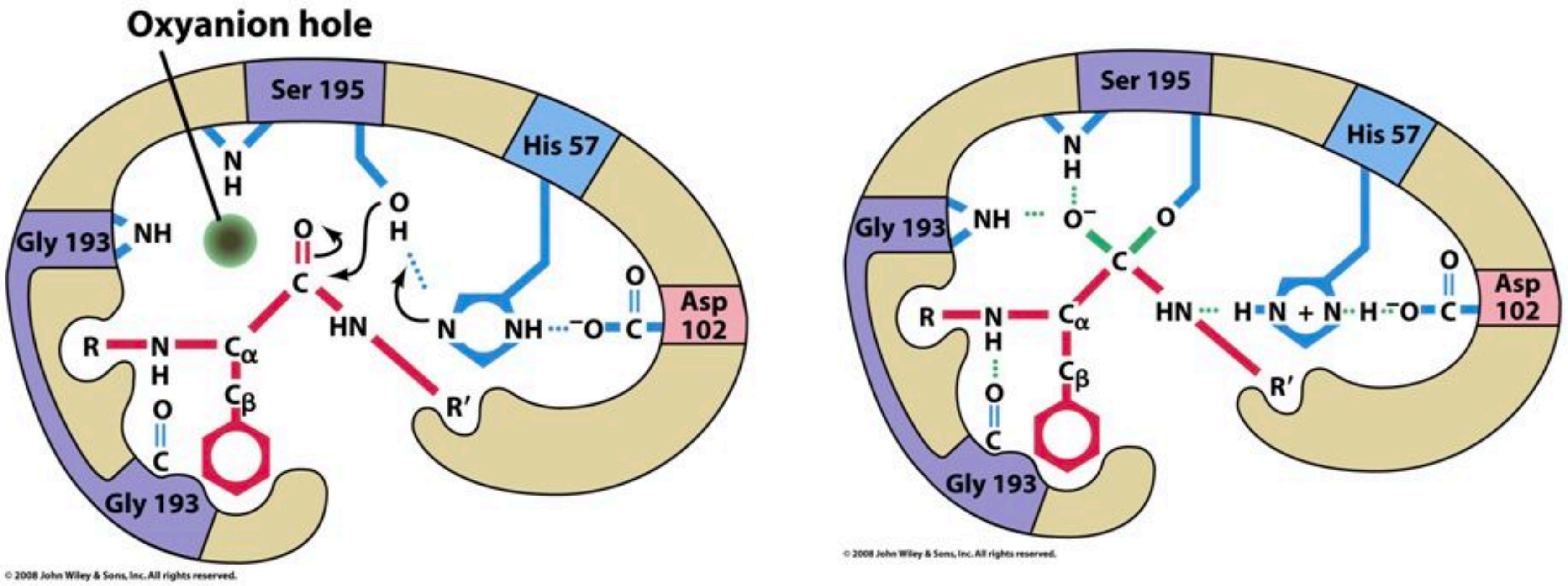
Serine proteases: features for transition state stabilization



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This is actually an intermediate, not a transition state, but the interactions shown on the right are developing in the transitions state

- ‘oxyanion hole’ to stabilize negative charge on tetrahedral intermediate
- H-bonds to stabilize distorted protein backbone

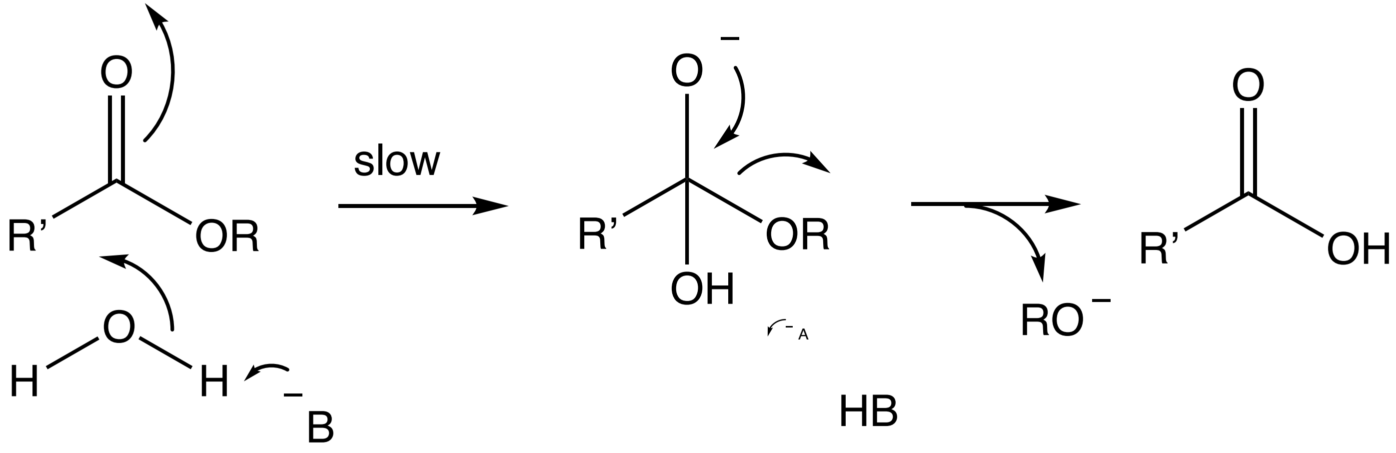
Figure 11-30a

General acid and base catalysis

(General means not H^+ and OH^-)

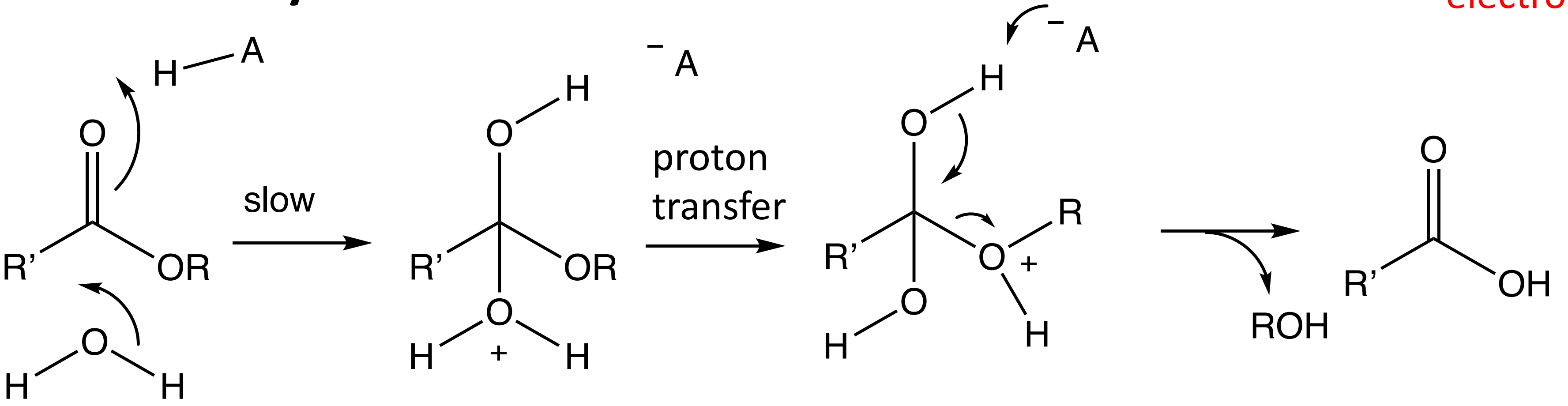
Example: ester hydrolysis – catalysis needed because water is a poor nucleophile

General base catalysis

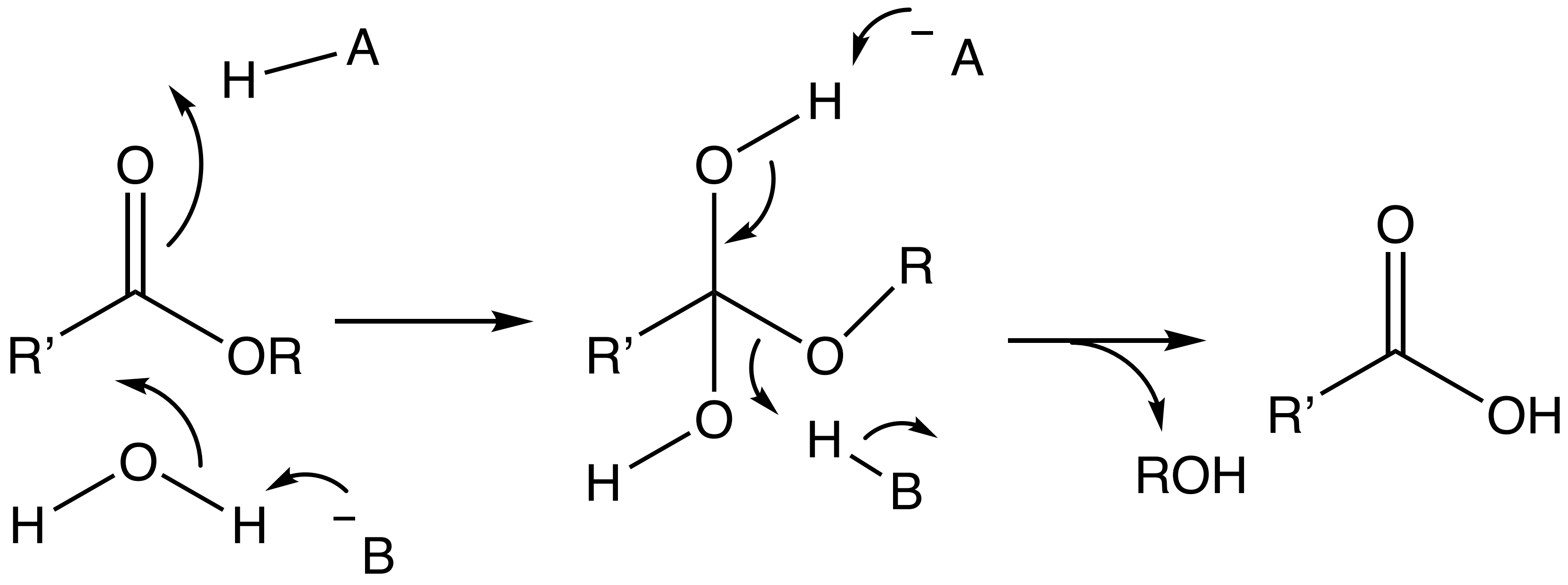


(note: arrows always denote movement of electrons, not protons!!!)

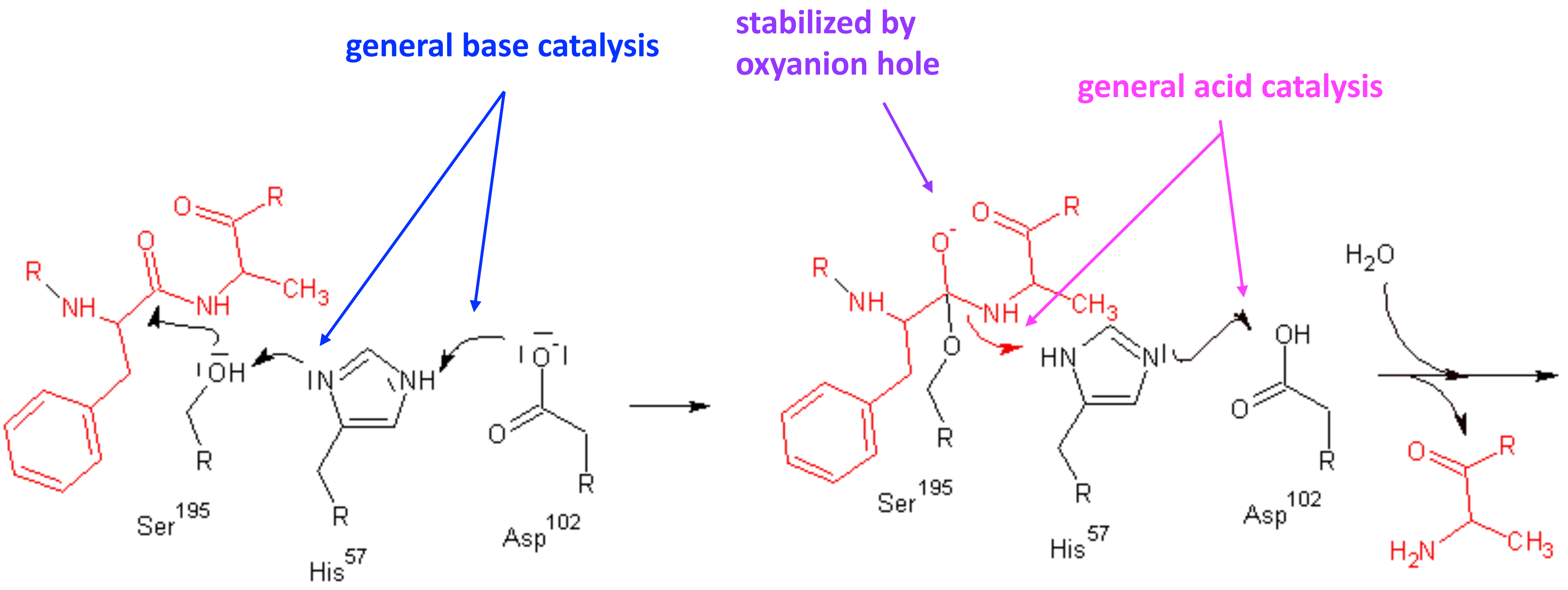
General acid catalysis



The best of both worlds – only possible for enzymes



Multple modes of catalysis: Chymotrypsin (a serine protease)



Metalloenzymes

Fe, Ni, Zn, Mn, W, Co, Cu, Mo

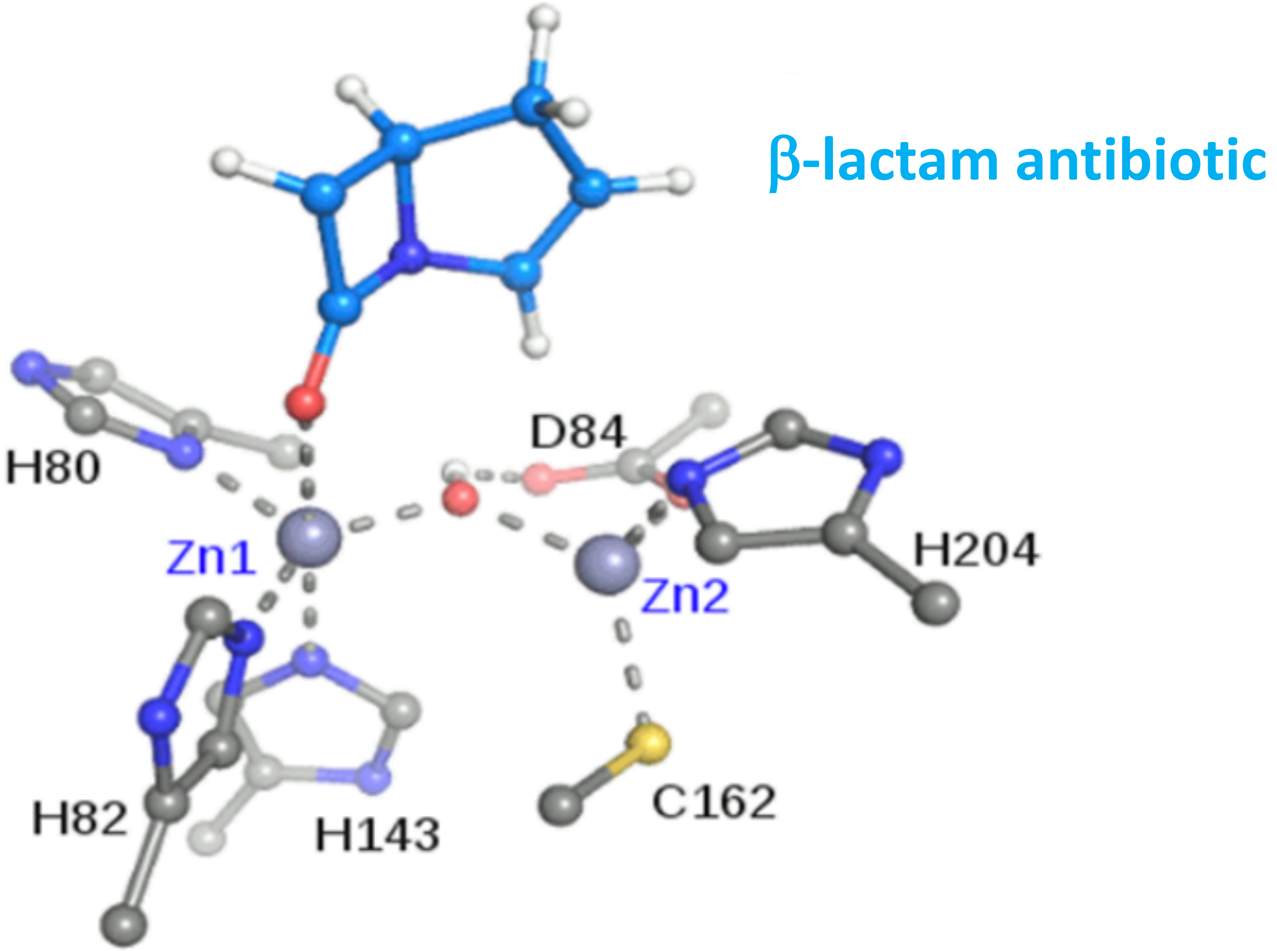
metals can act as Lewis acids

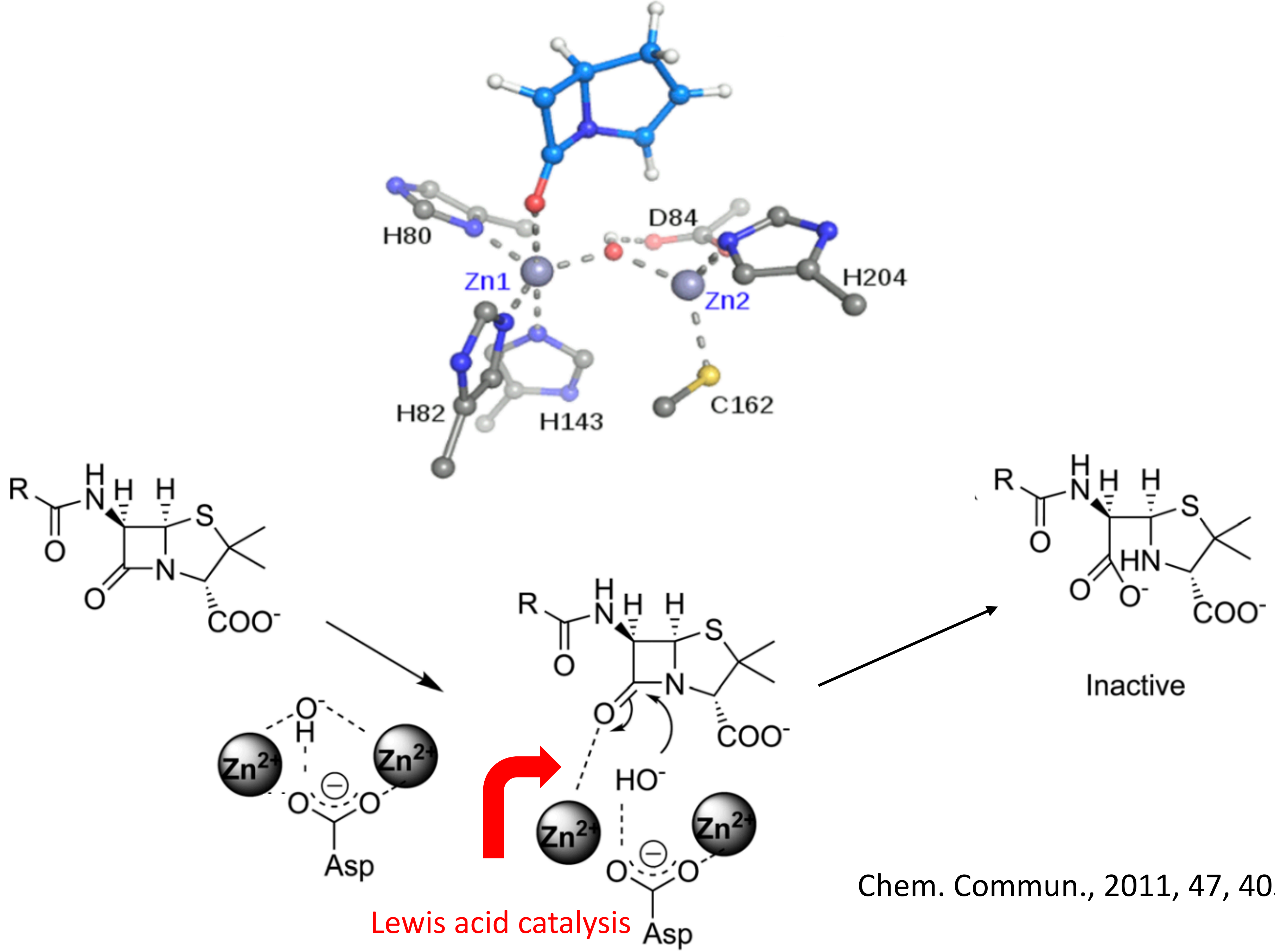
Lewis acid has an empty orbital that can interact with a lone pair

(i.e. Lewis acids do not donate protons)

some metals can do redox chemistry

An enzyme with two metal ions – a metallo-β-lactamase





Chem. Commun., 2011, 47, 4055–4061

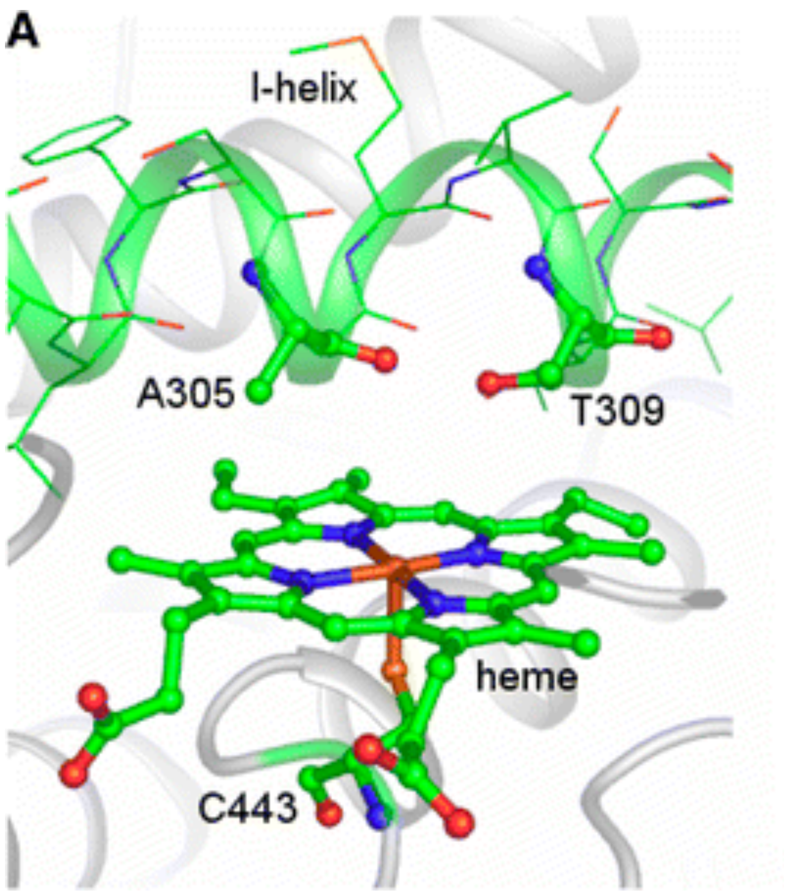
Redox metalloenzymes

Can contain one or multiple metal ions

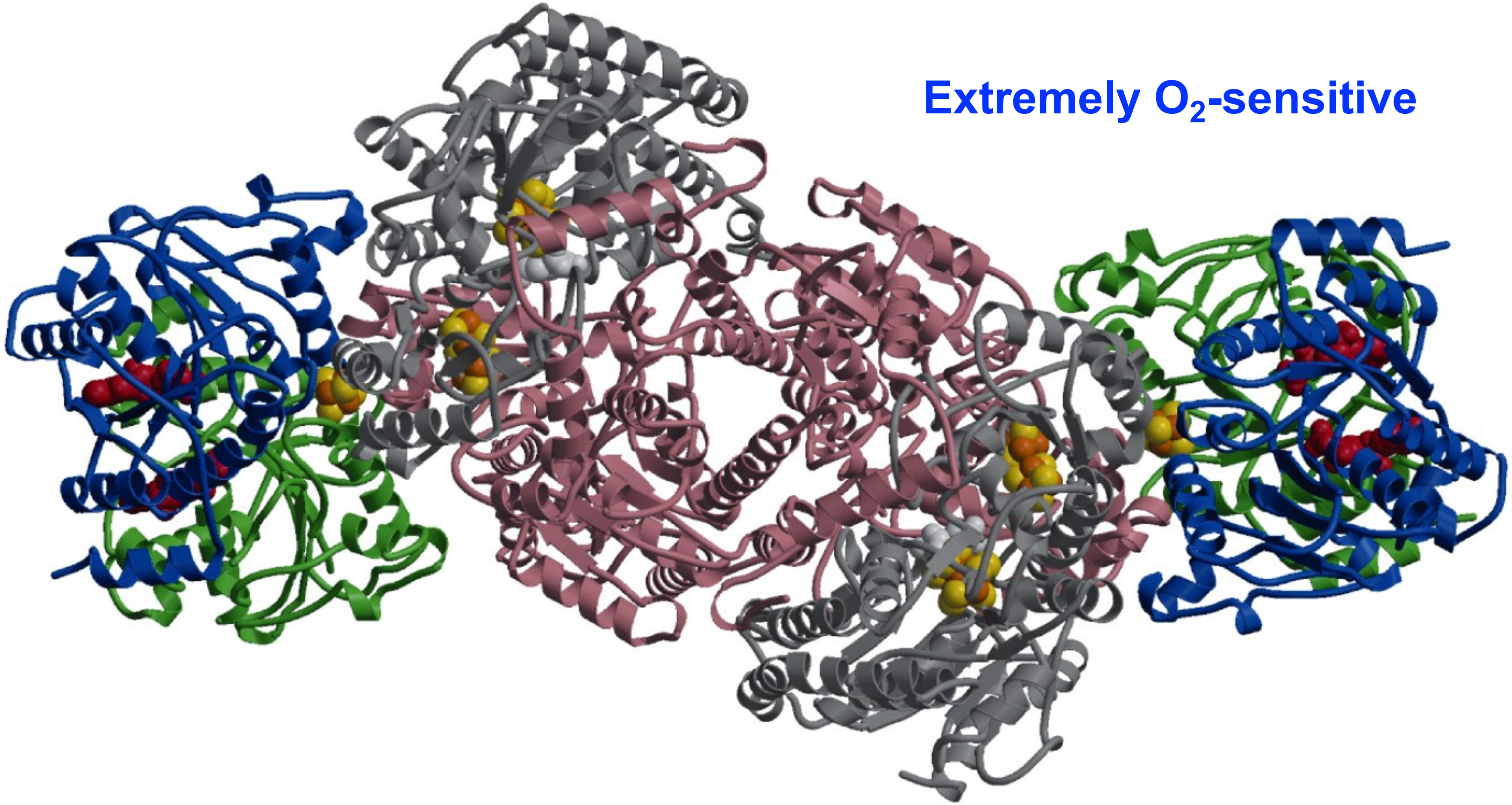
Metal ions must be able to undergo changes in oxidation state

Metal ions can be found in organic cofactors
e.g. Fe in heme in cytochrome P450s

Oxidize endogenous and exogenous substrates, including steroids and drugs
Found in all organisms



Even more elaborate metal ion cofactors: nitrogenase – responsible for fixing N_2



Extremely O_2-sensitive

Figure 22-3a
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10^{11} kg per year!

$$N_2 + 8\ e^- + 10\ H^+ + 16\ ATP \rightarrow 2\ NH_4^+ + 16\ ADP + 16\ P_i + H_2$$

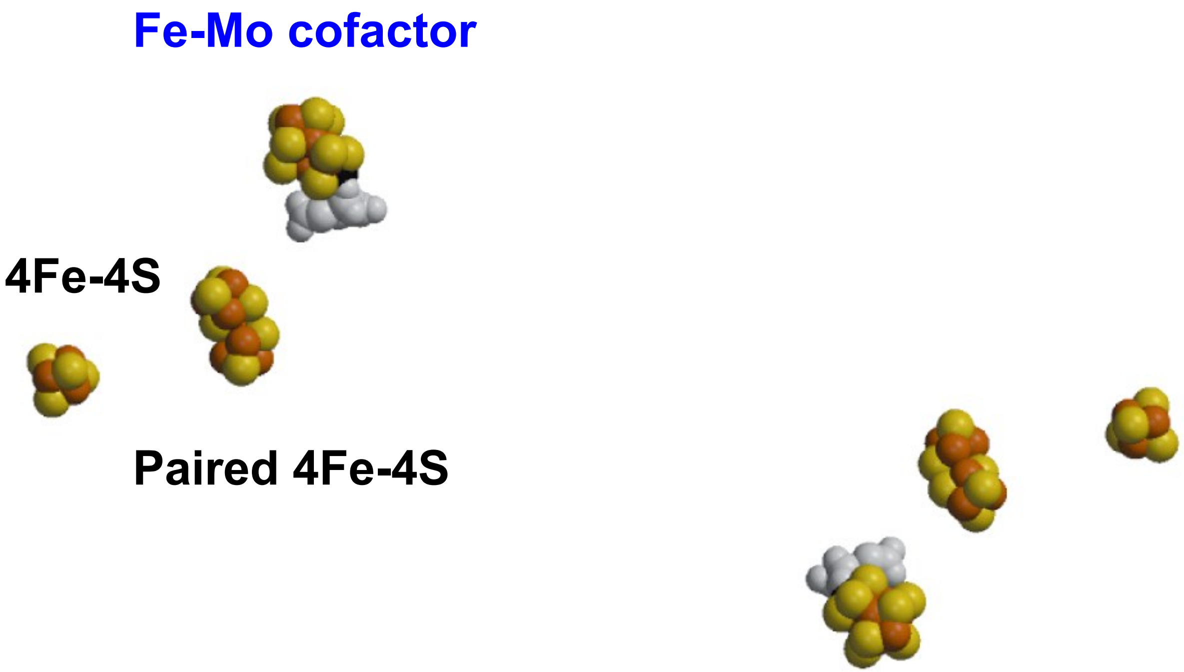

Figure 22-3b
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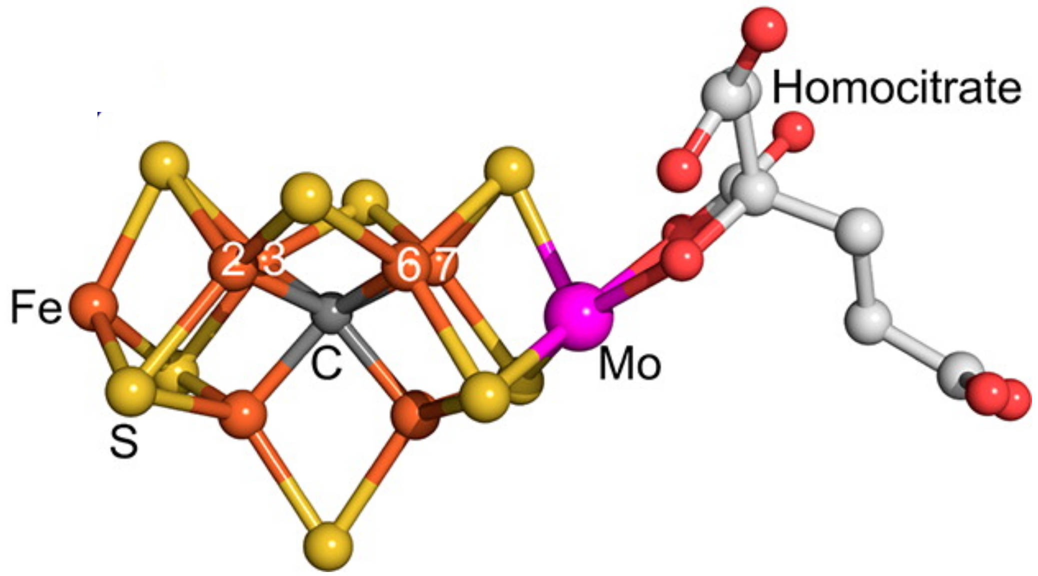

Published in: Brian M. Hoffman; Dmitriy Lukoyanov; Dennis R. Dean; Lance C. Seefeldt; *Acc. Chem. Res.* Article ASAP
DOI: 10.1021/ar300267m
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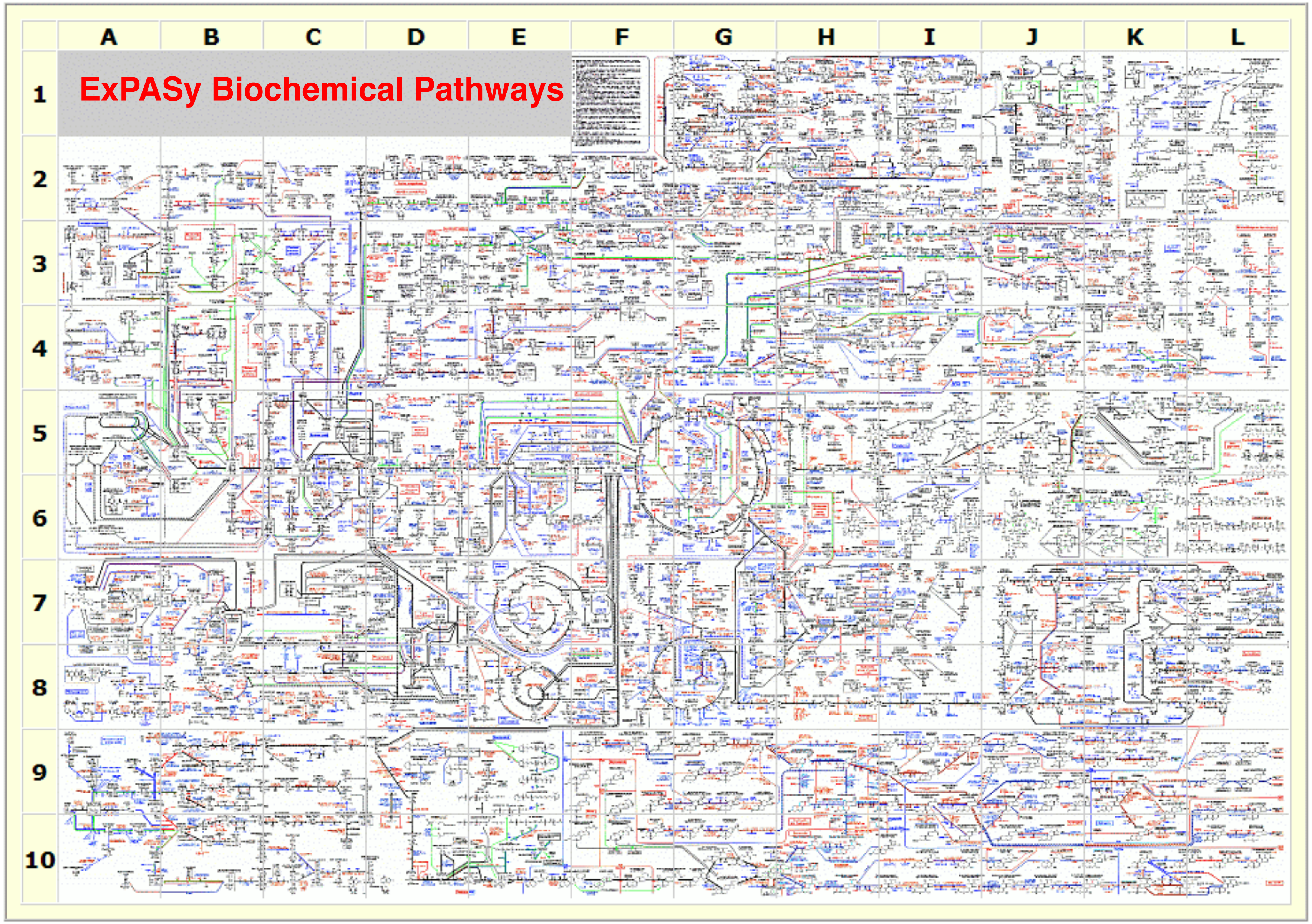
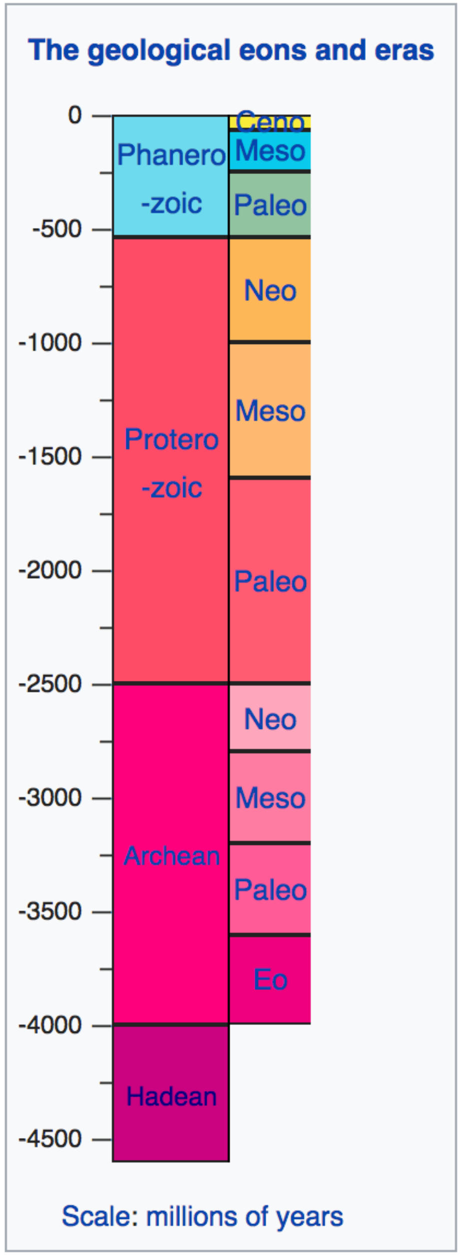
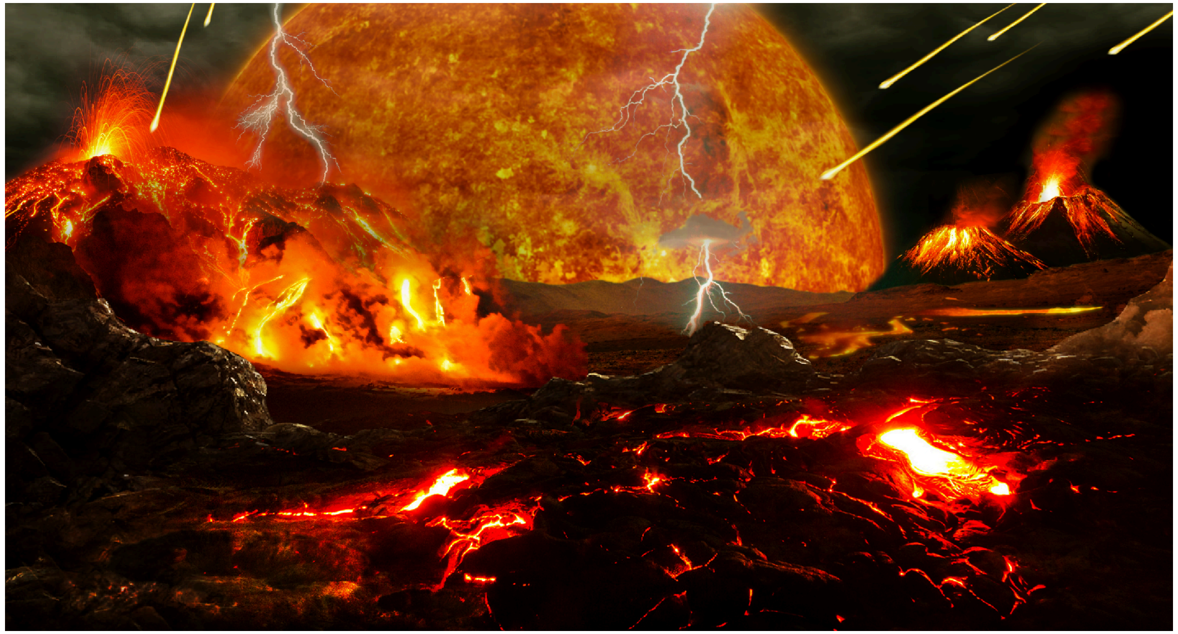
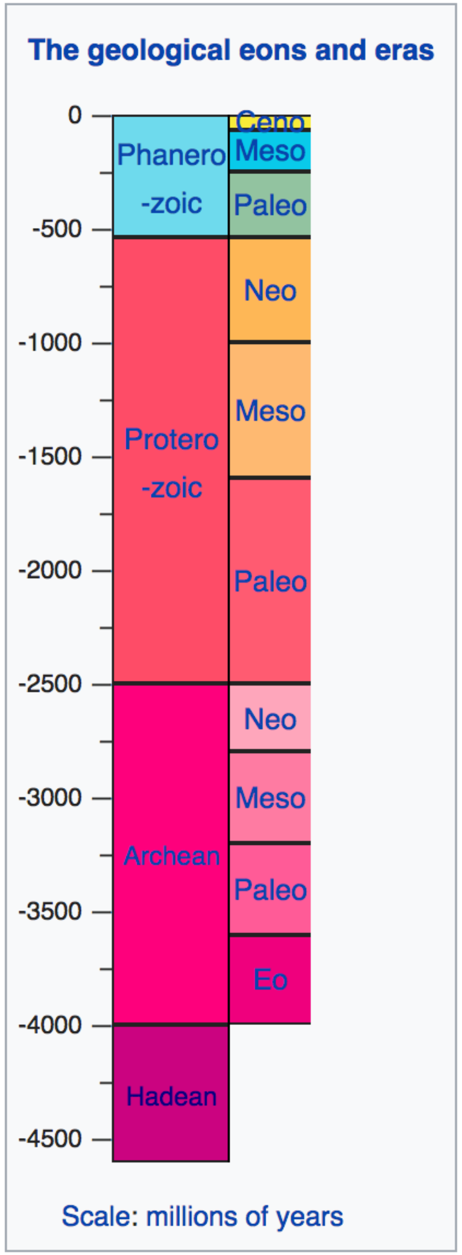
Where did all this come from?





The Hadean eon





Archaean era

Mostly oceans – small islands but no continents yet
Hydrothermal vents and spreading zones at the bottom

The LUCA (last universal common ancestor)

?

What we know:

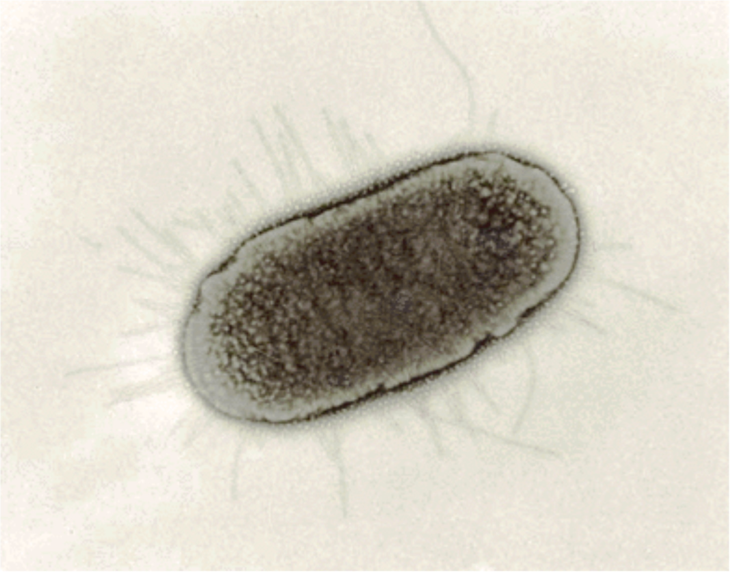
It was microbial

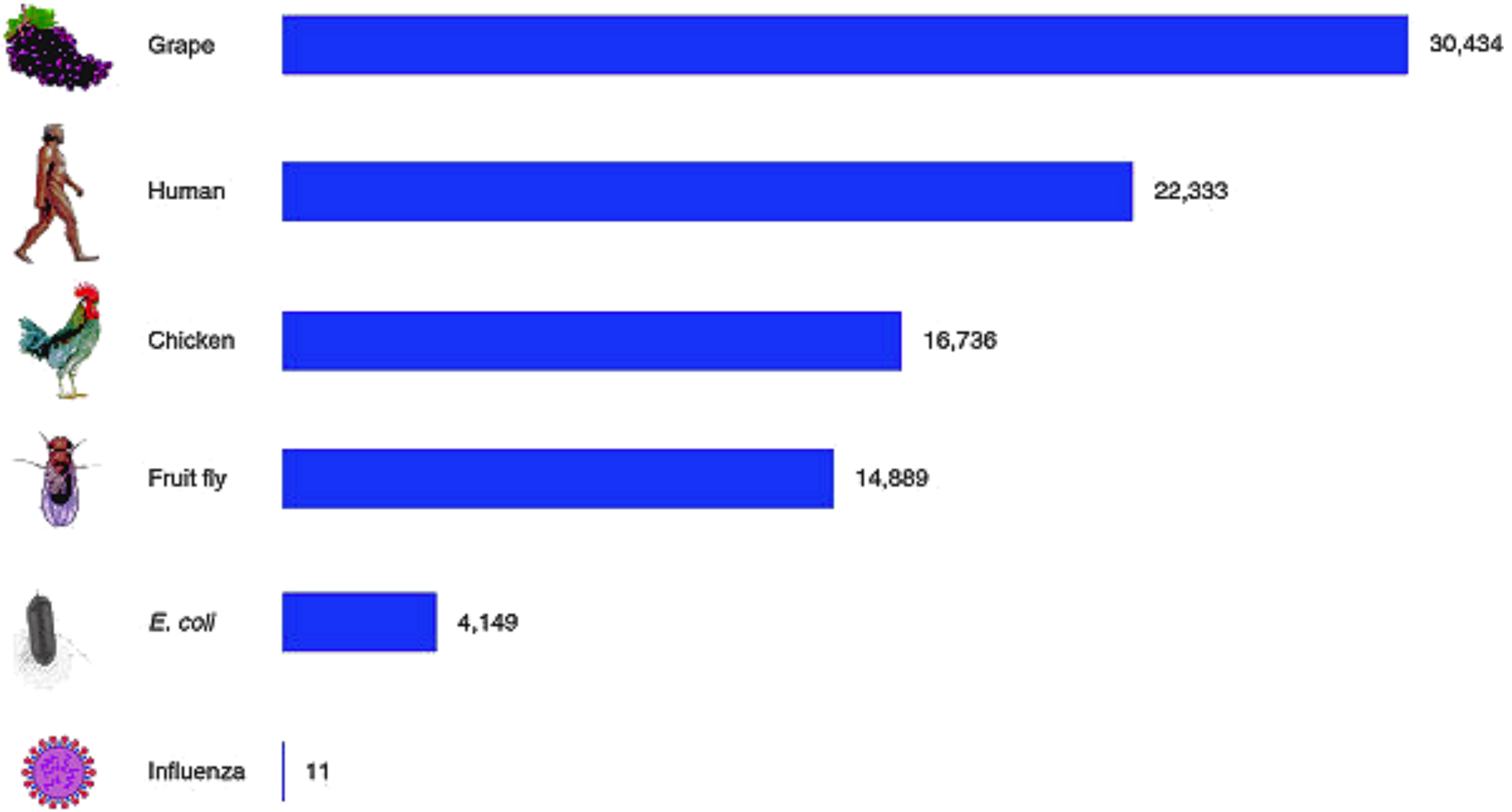
It had ribosomes
proteins
a cell wall
core biosynthetic pathways

The Proteome of the LUCA

669 genes (*Res. Microbiol. 157*, 57-68, 2006)

Including enzymes for synthesis of
- amino acids
- nucleotides
- sugars
- fatty acids
- cofactors



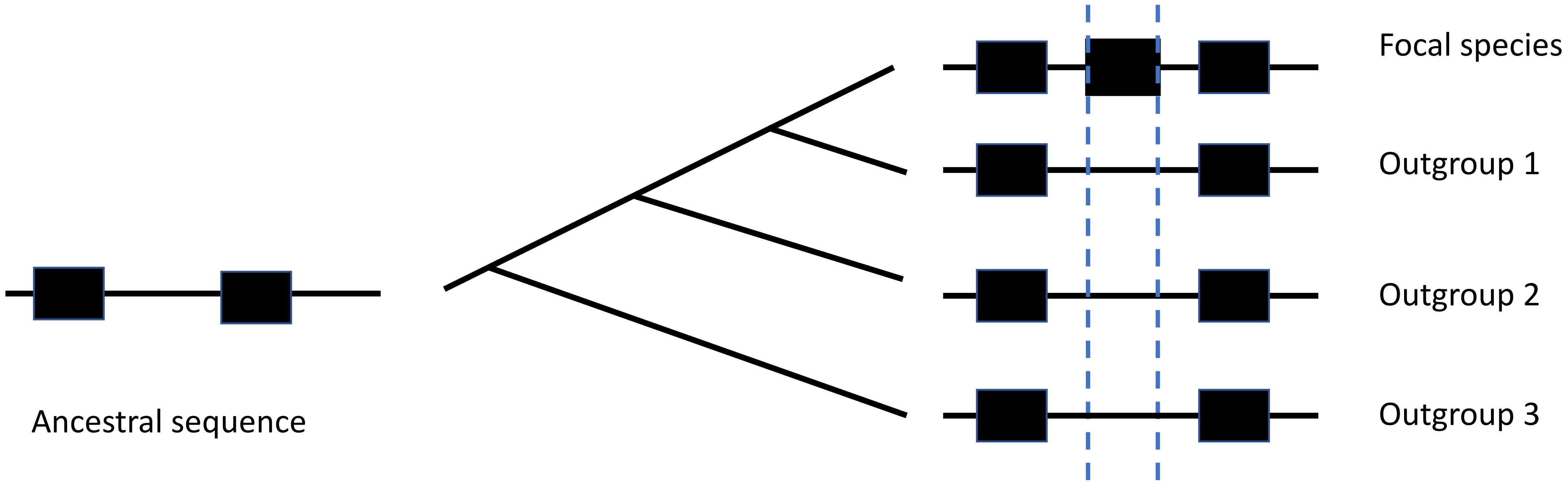


http://universe-review.ca/F11-monocell13.htm

Evolution continues today

Origin of new genes?

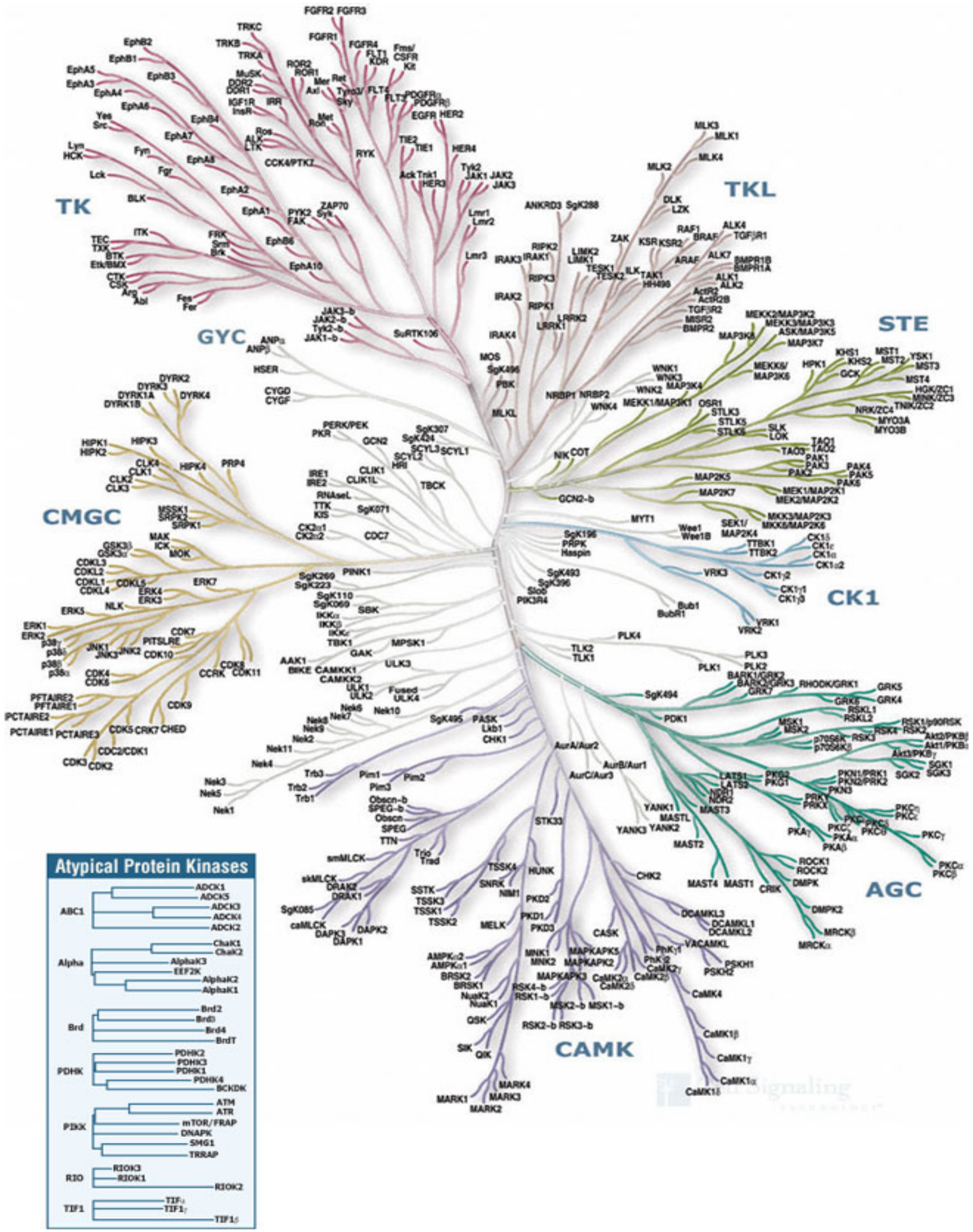
De novo genes: genes that originate from a previously non-coding sequence



Gene duplication and divergence into superfamilies

Superfamily = a group of genes that has evolved from a common ancestor by gene duplication and divergence

Members of a superfamily generally retain some structural and mechanistic features of the ancestor



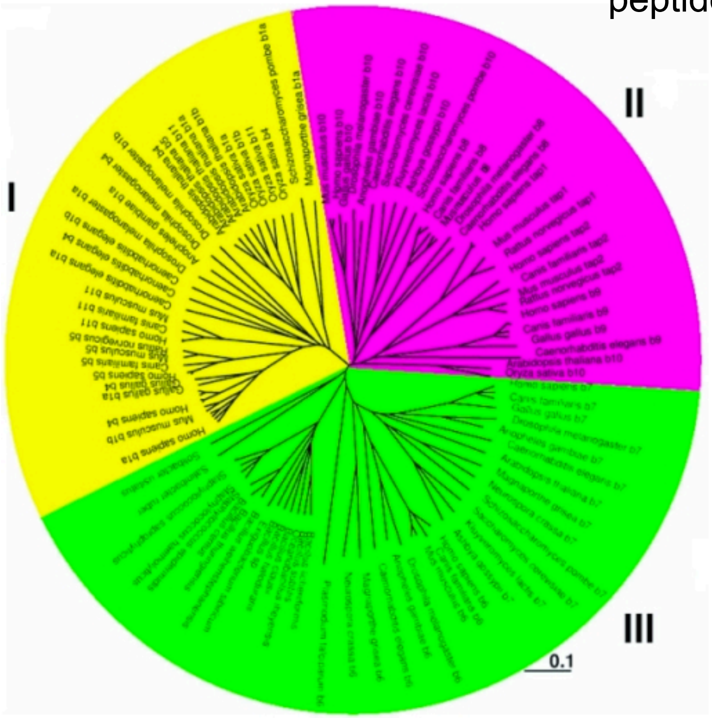
The human kinome

ABC (ATP-binding cassette) transporters

Sub-family B

peptide

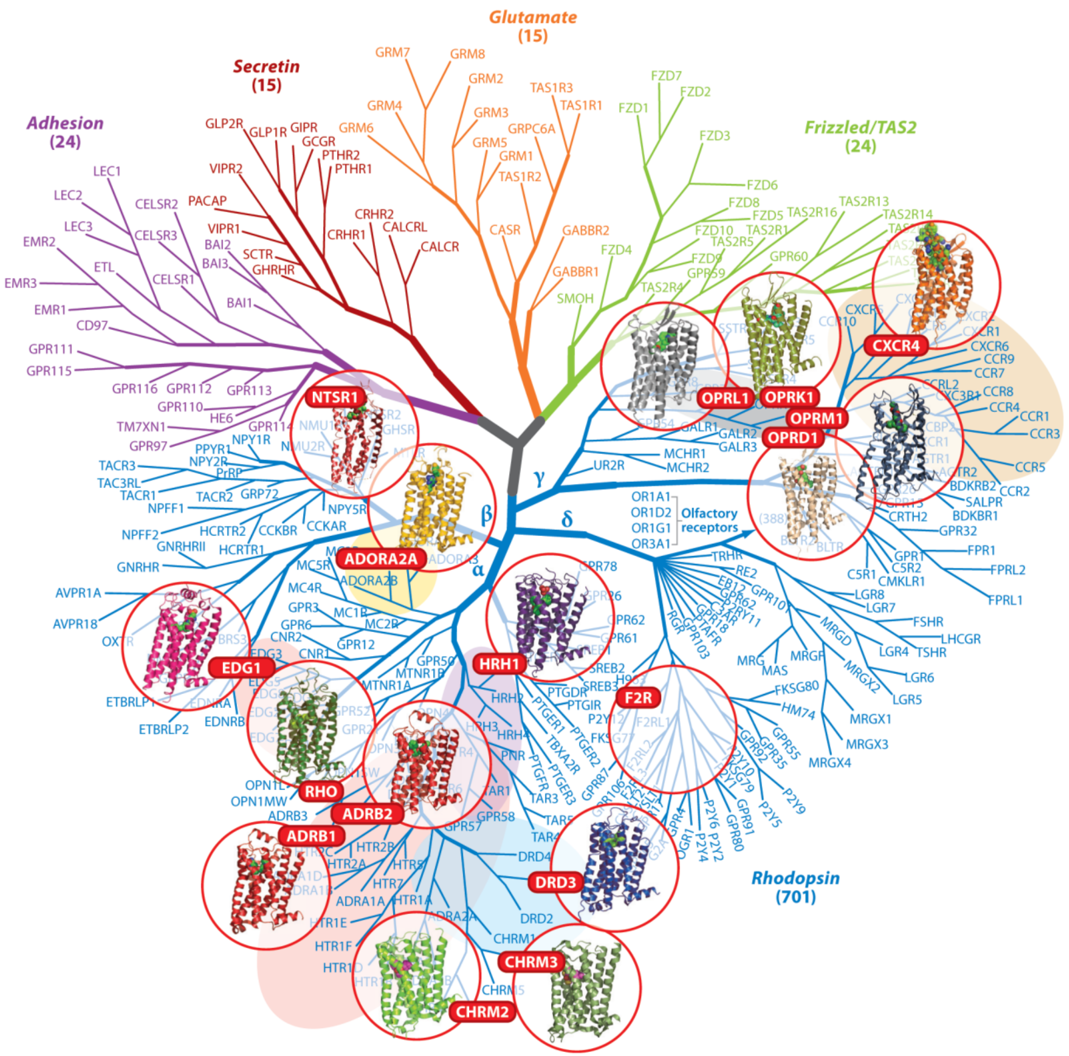
Drug/lipid/b
ile salt



iron

Flanagan JU, Huber T - *Evol. Bioinform.* Online (2007)

G-Protein Coupled Receptors



Katritch V, et al. 2013.
Annu. Rev. Pharmacol. Toxicol. 53:531–56

The importance of superfamilies

Supfam.org

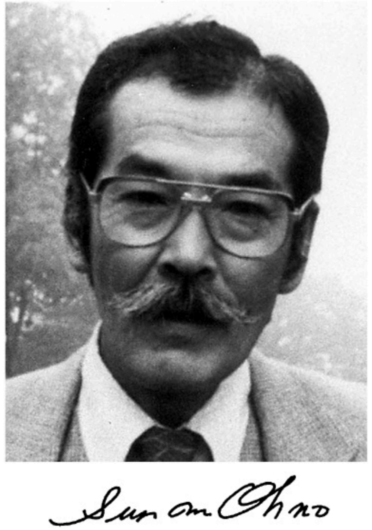
2478 genomes

# of superfamilies per genome 278-1175

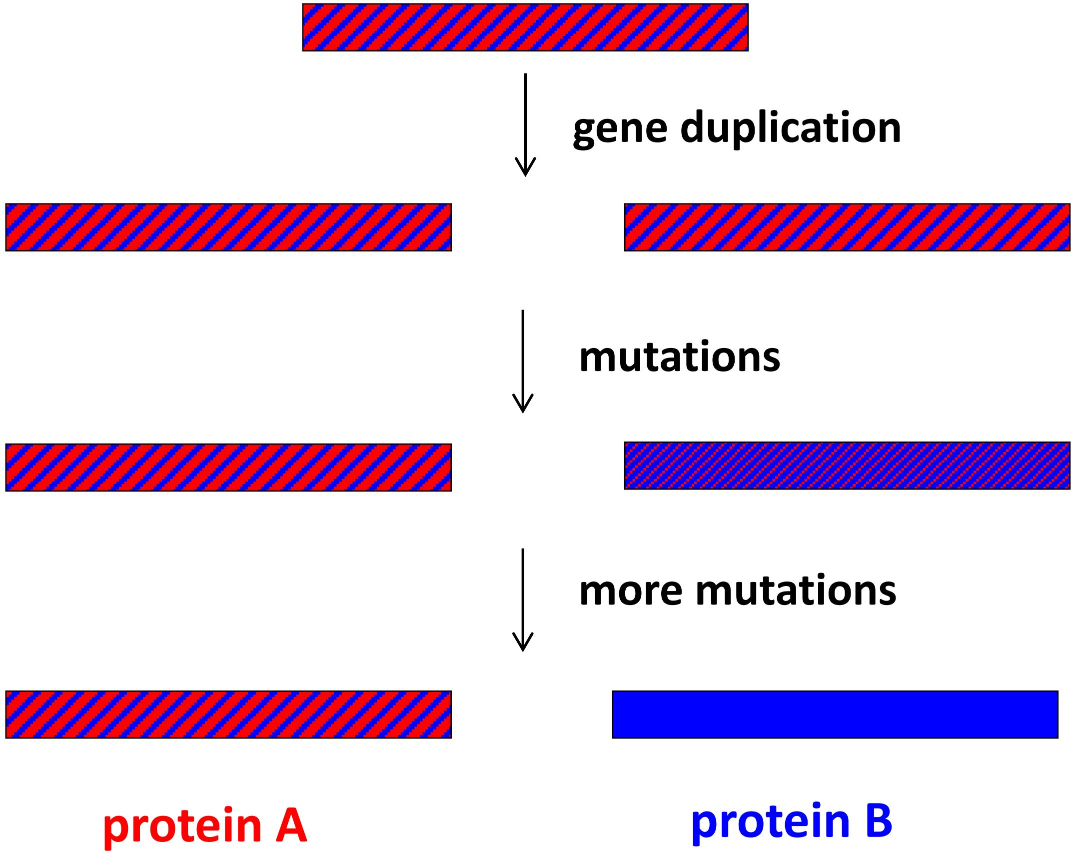
Humans – 1113 superfamilies, average of 25 members/superfamily

E. coli – 839 superfamilies, average of 5.3 members/superfamily

How have superfamilies evolved?

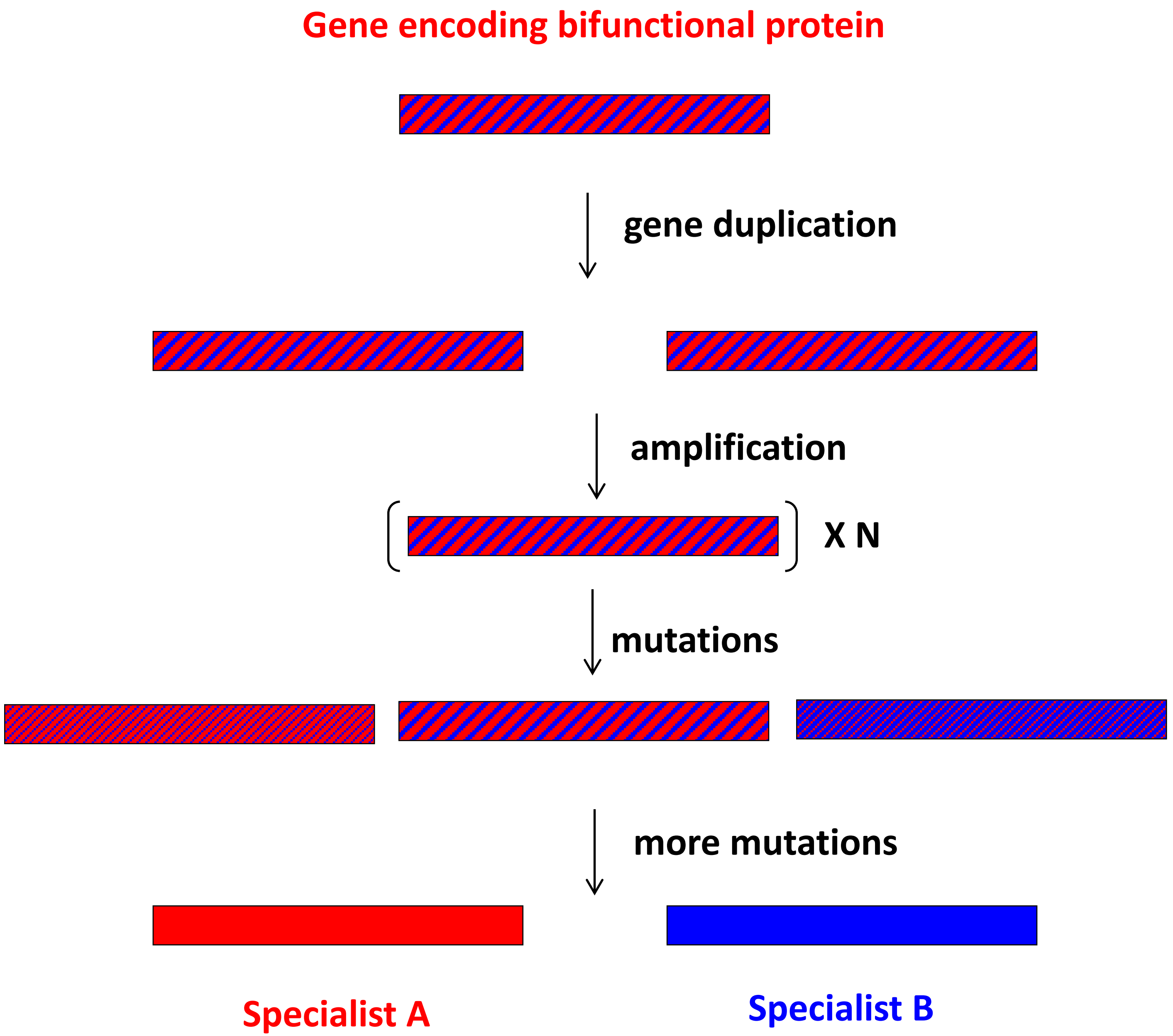


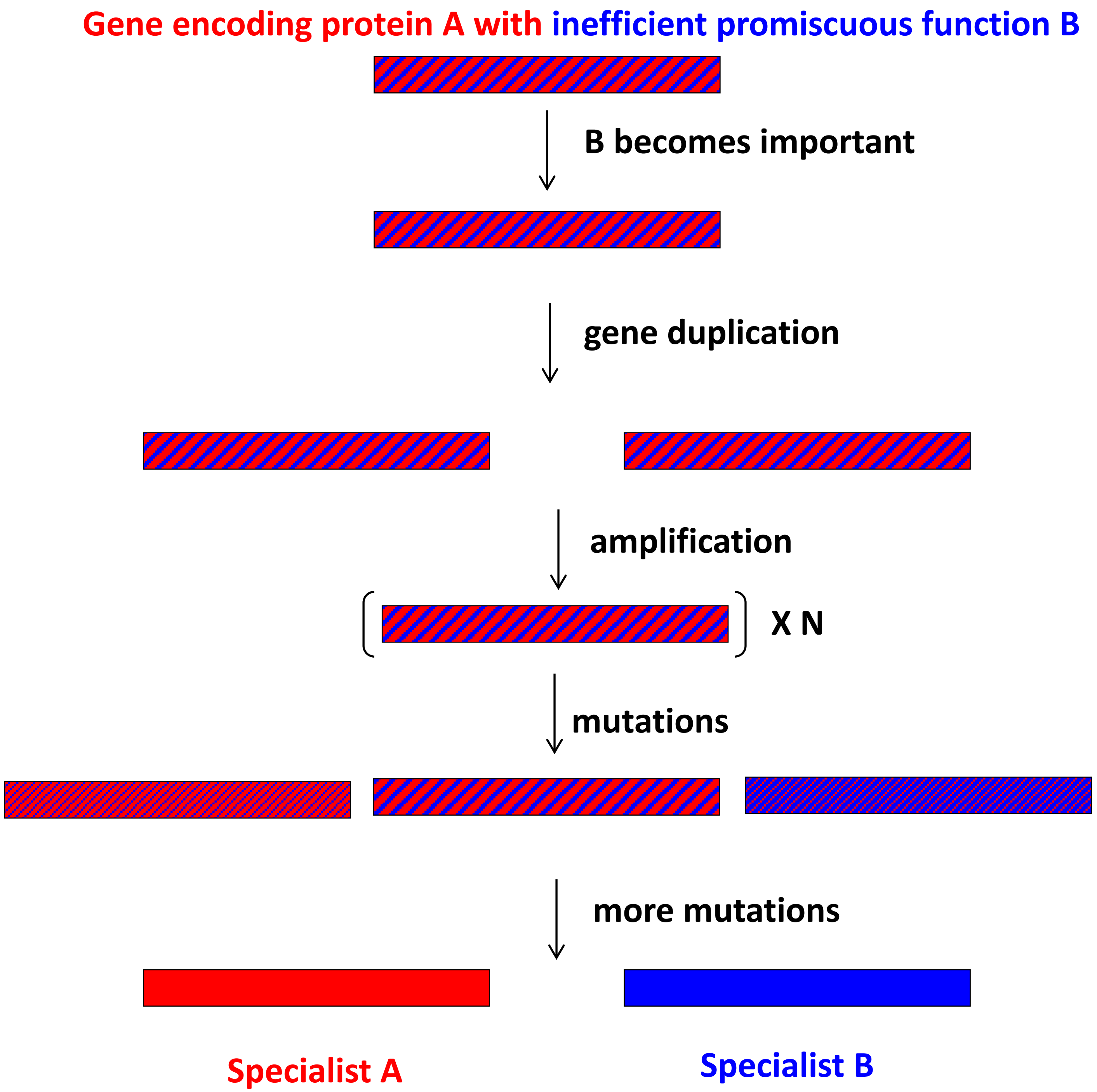
The Ohno Model - 1970



Innovation-Amplification-Divergence Model

Austin Hughes
John Roth
Dan Andersson





Innovation-
Amplification-
Divergence
Model

Austin Hughes
John Roth
Dan Andersson

Enzyme promiscuity
(according to Shelley)

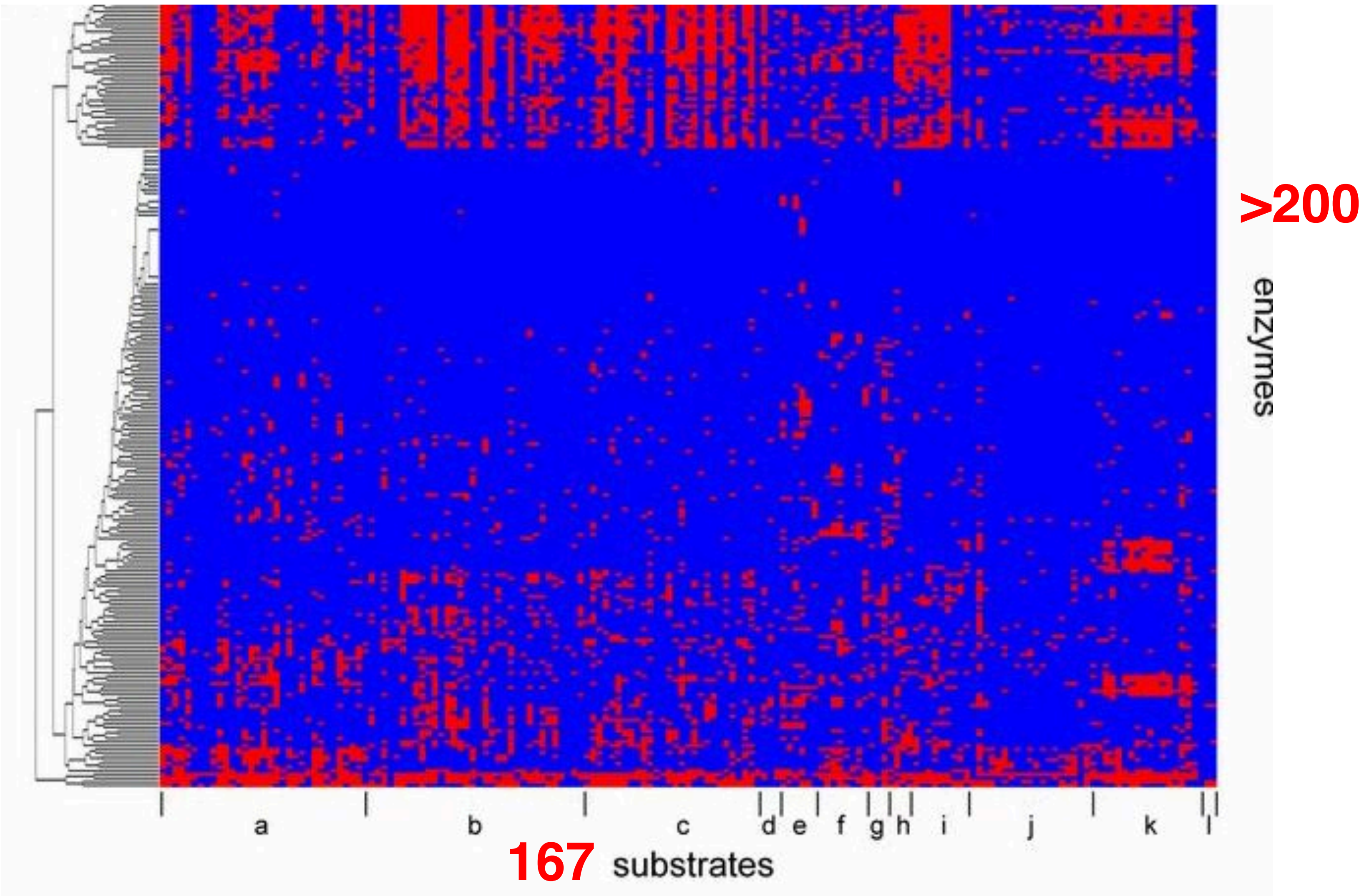
catalysis of adventitious secondary reactions that are physiologically irrelevant

Promiscuity according to Evan

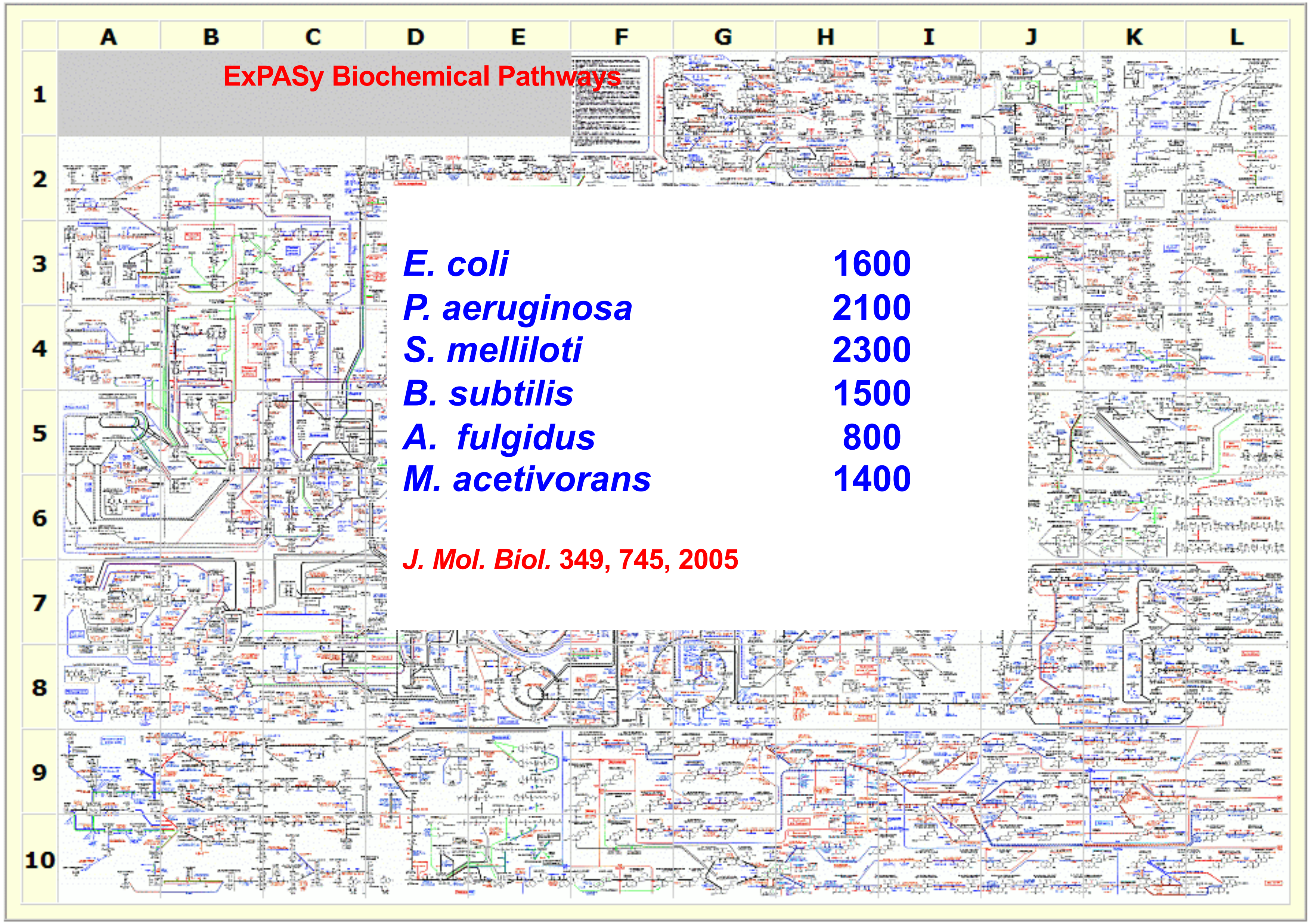
"Oh, I get it – it's when enzymes cheat on their substrates."

Promiscuity is common

Panoramic view of a superfamily of phosphatases through substrate profiling, Huang, et al, *PNAS* **112**, E1974 – E1983, 2015



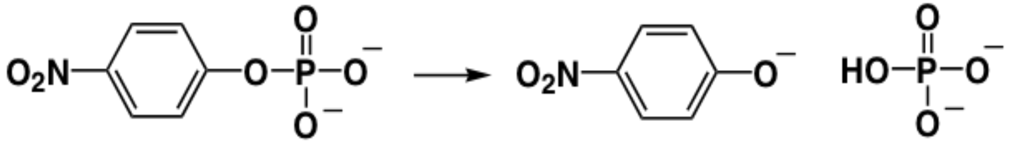
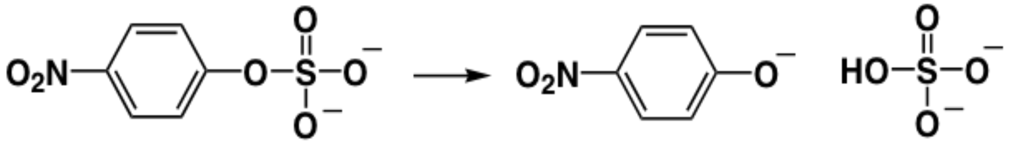
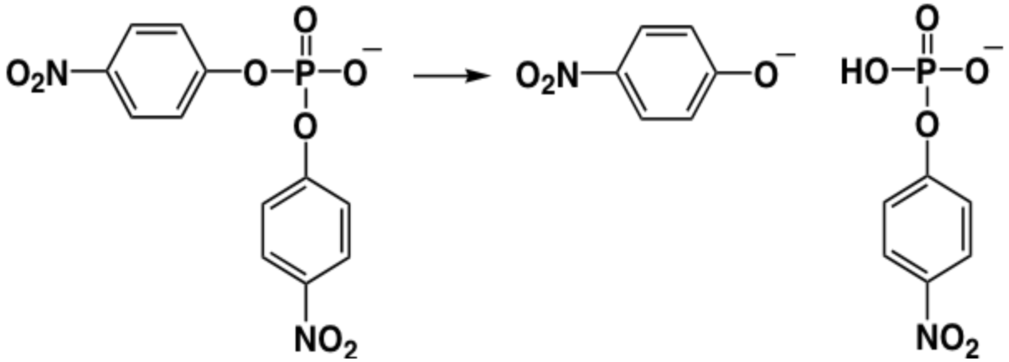
The number of promiscuous activities is unknown but undoubtedly huge



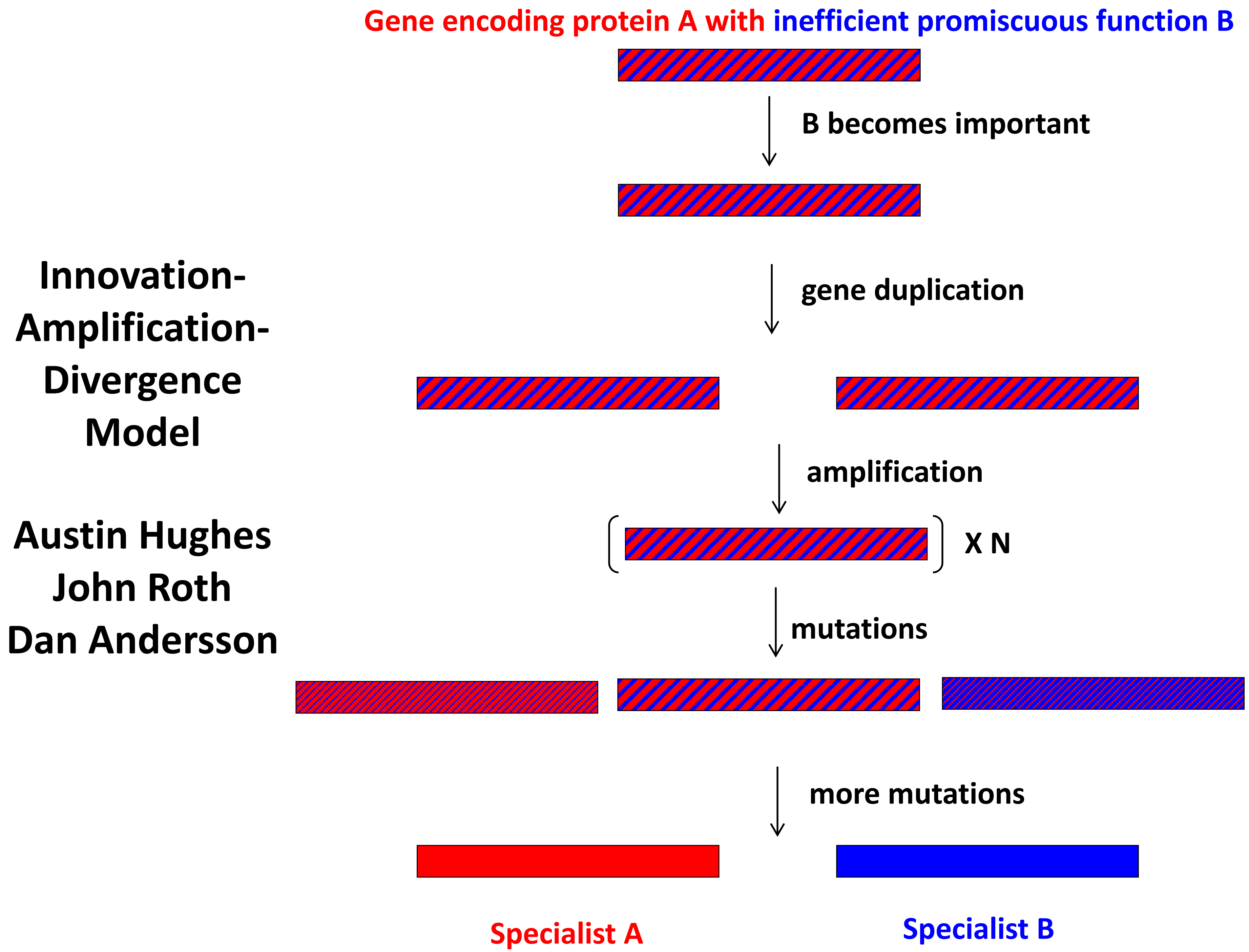
E. coli	1600
P. aeruginosa	2100
S. melliloti	2300
B. subtilis	1500
A. fulgidus	800
M. acetivorans	1400

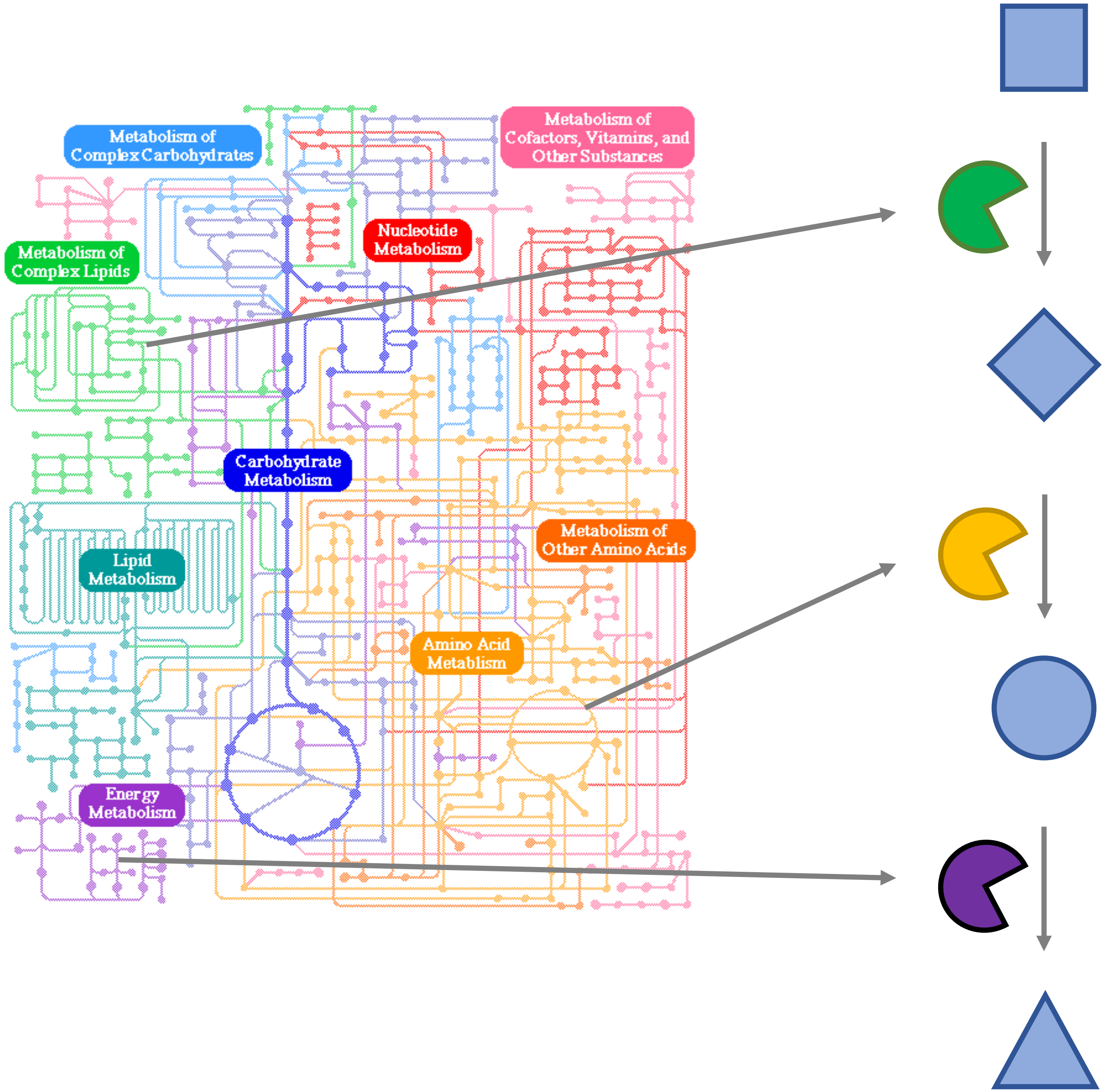
J. Mol. Biol. 349, 745, 2005

Even inefficient promiscuous activities can accelerate reactions by orders of magnitude

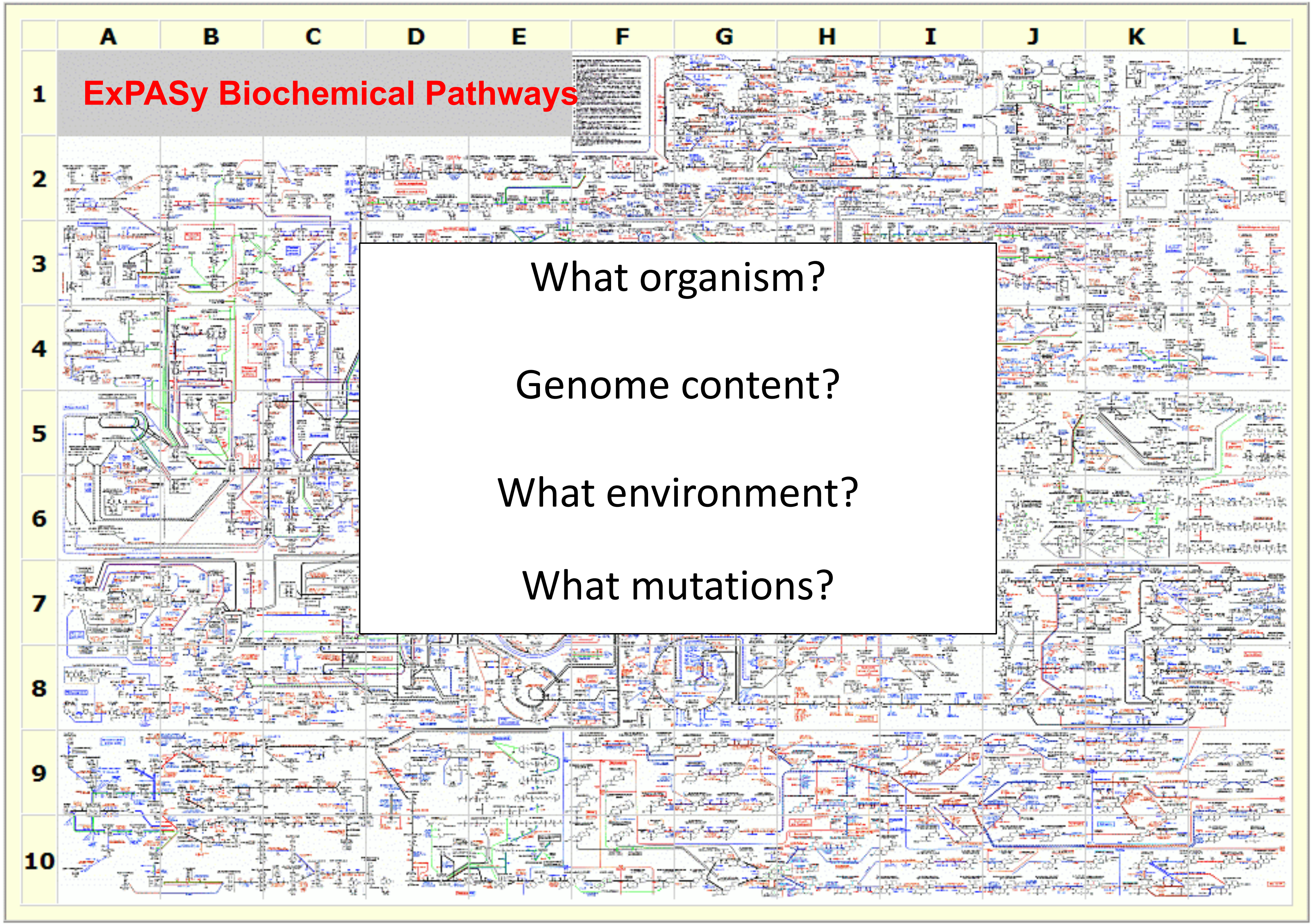
e.g. *E. coli* alkaline phosphatase	k_{cat} / K_M ($M^{-1}s^{-1}$)	"proficiency"
	3×10^{7}	10^{17}
	1×10^{-2}	10^{9}
	1×10^{2}	10^{11}

O'Brien and Herschlag, *J. Am. Chem. Soc.* 1998, 120-12369 and *Biochemistry* 2001, 40, 5691


Gene encoding protein A with inefficient promiscuous function B
B becomes important
Innovation-
Amplification-
Divergence
Model
gene duplication
amplification
X N
Austin Hughes
John Roth
Dan Andersson
mutations
more mutations
Specialist A
Specialist B


Metabolism of Complex Carbohydrates
Metabolism of Cofactors, Vitamins, and Other Substances
Nucleotide Metabolism
Metabolism of Complex Lipids
Carbohydrate Metabolism
Metabolism of Other Amino Acids
Lipid Metabolism
Amino Acid Metablism
Energy Metabolism

Lost in time…….



What organism?

Genome content?

What environment?

What mutations?

A story about the evolutionary potential of promiscuous enzymes

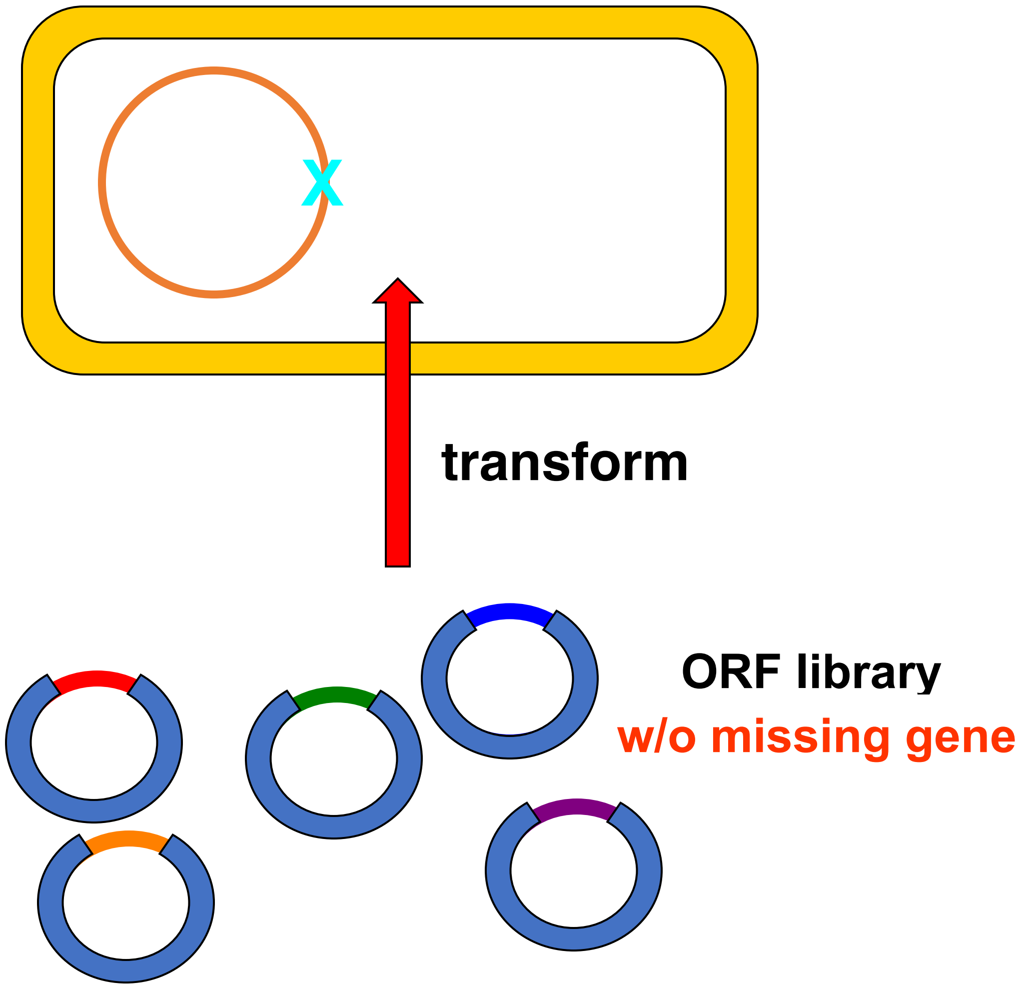
Hidden resources in the *Escherichia coli* genome restore PLP synthesis and robust growth after deletion of the essential gene *pdxB*

Juhan Kim[a,b,1], Jake J. Flood[a,b,1], Michael R. Kristofich[a,b], Cyrus Gidfar[a,b], Andrew B. Morgenthaler[a,b], Tobias Fuhrer[c], Uwe Sauer[c], Daniel Snyder[d], Vaughn S. Cooper[d], Christopher C. Ebmeier[a], William M. Old[a], and Shelley D. Copley[a,b,2]

[a]Department of Molecular, Cellular and Developmental Biology, University of Colorado Boulder, Boulder, CO 80309; [b]Cooperative Institute for Research in Environmental Sciences, University of Colorado Boulder, Boulder, CO 80309; [c]Institute of Molecular Systems Biology, ETH Zurich, 8093 Zurich, Switzerland; and [d]Center for Evolutionary Biology and Medicine, University of Pittsburgh, Pittsburgh, PA 15260

Edited by Michael Lynch, Arizona State University, Tempe, AZ, and approved October 11, 2019 (received for review September 7, 2019)

Multicopy suppression

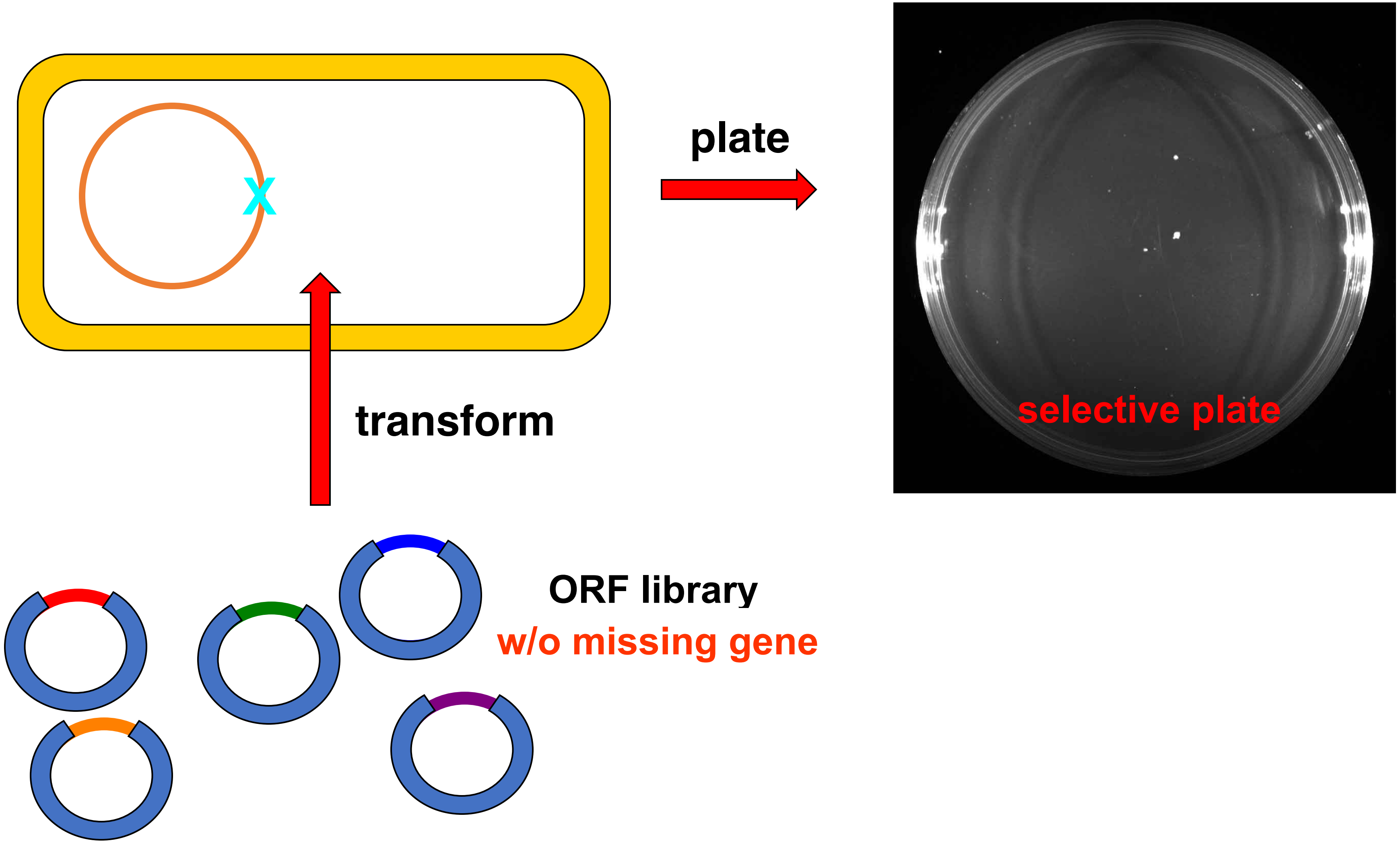


ASKA library

(A complete set of E. coli K-12 ORF Archive)

DNA Res. 2005;12(5):291-9

Multicopy suppression



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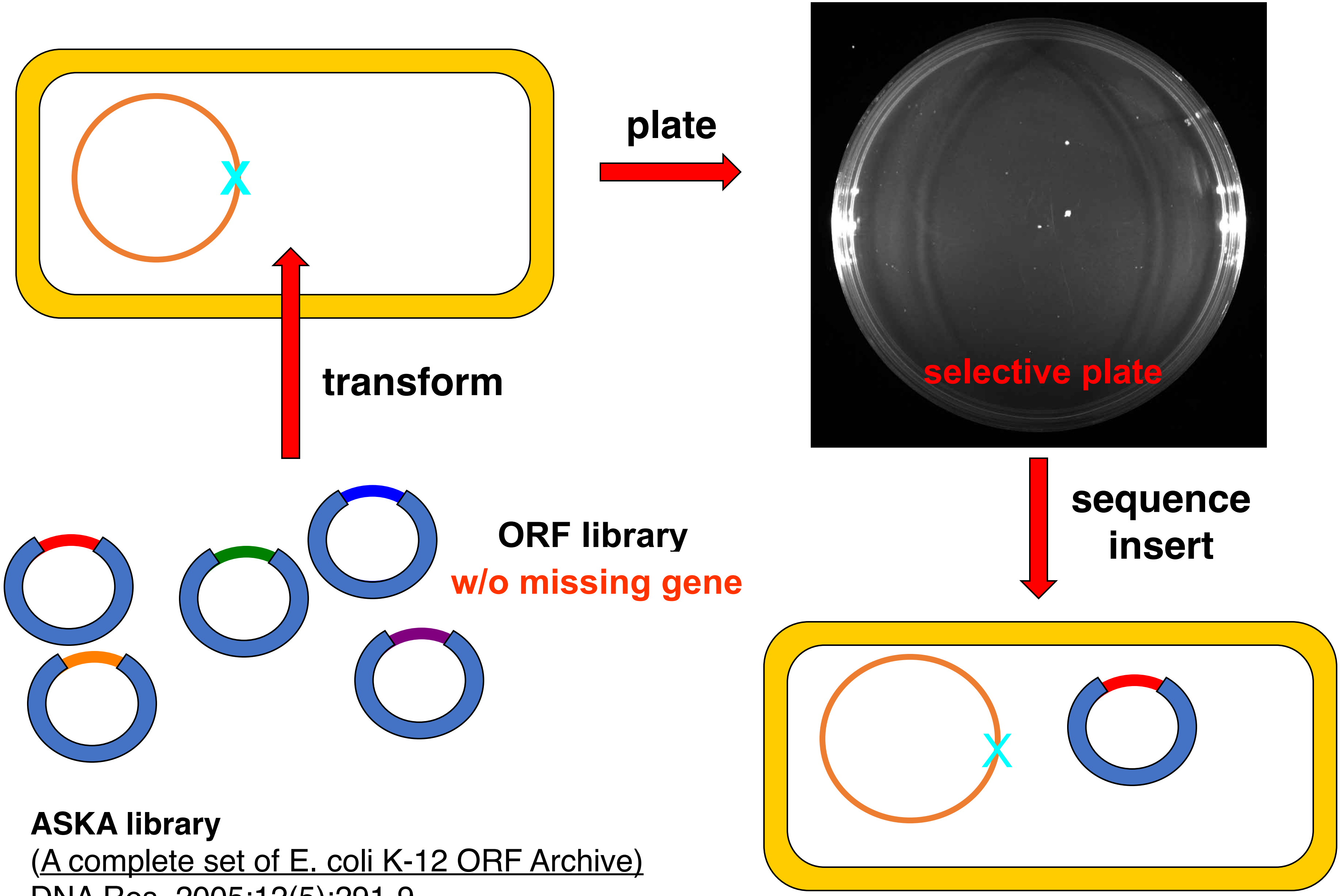
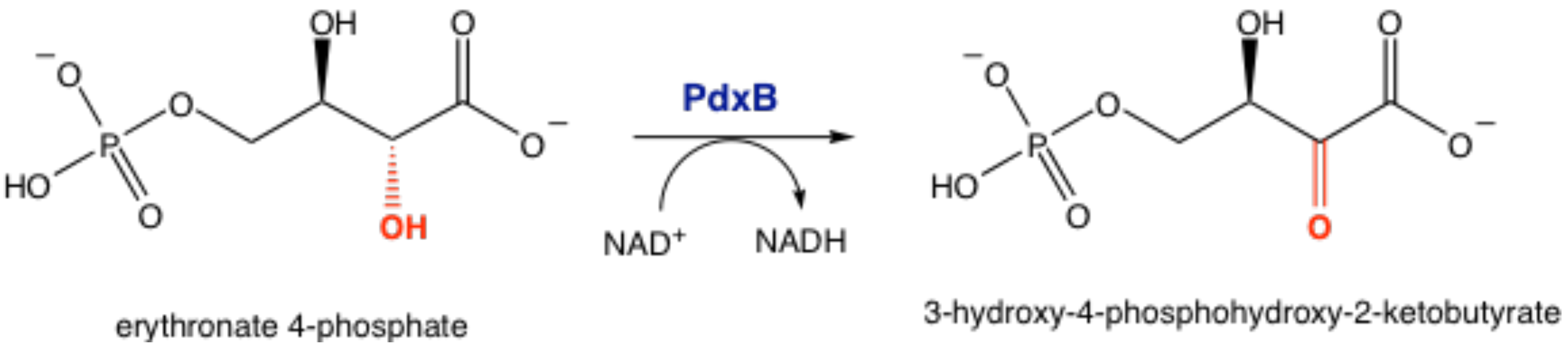
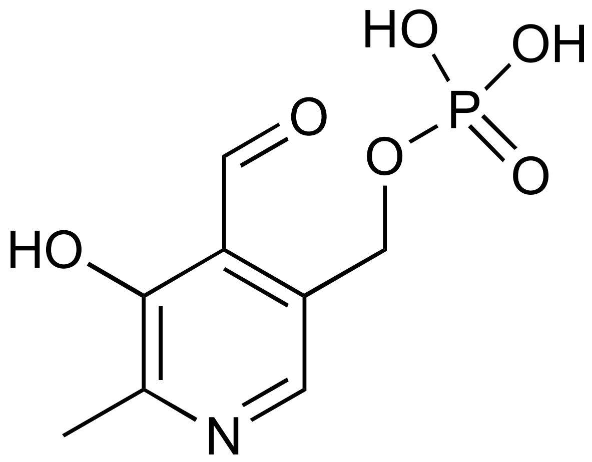


ASKA library
(A complete set of E. coli K-12 ORF Archive)
DNA Res. 2005;12(5):291-9

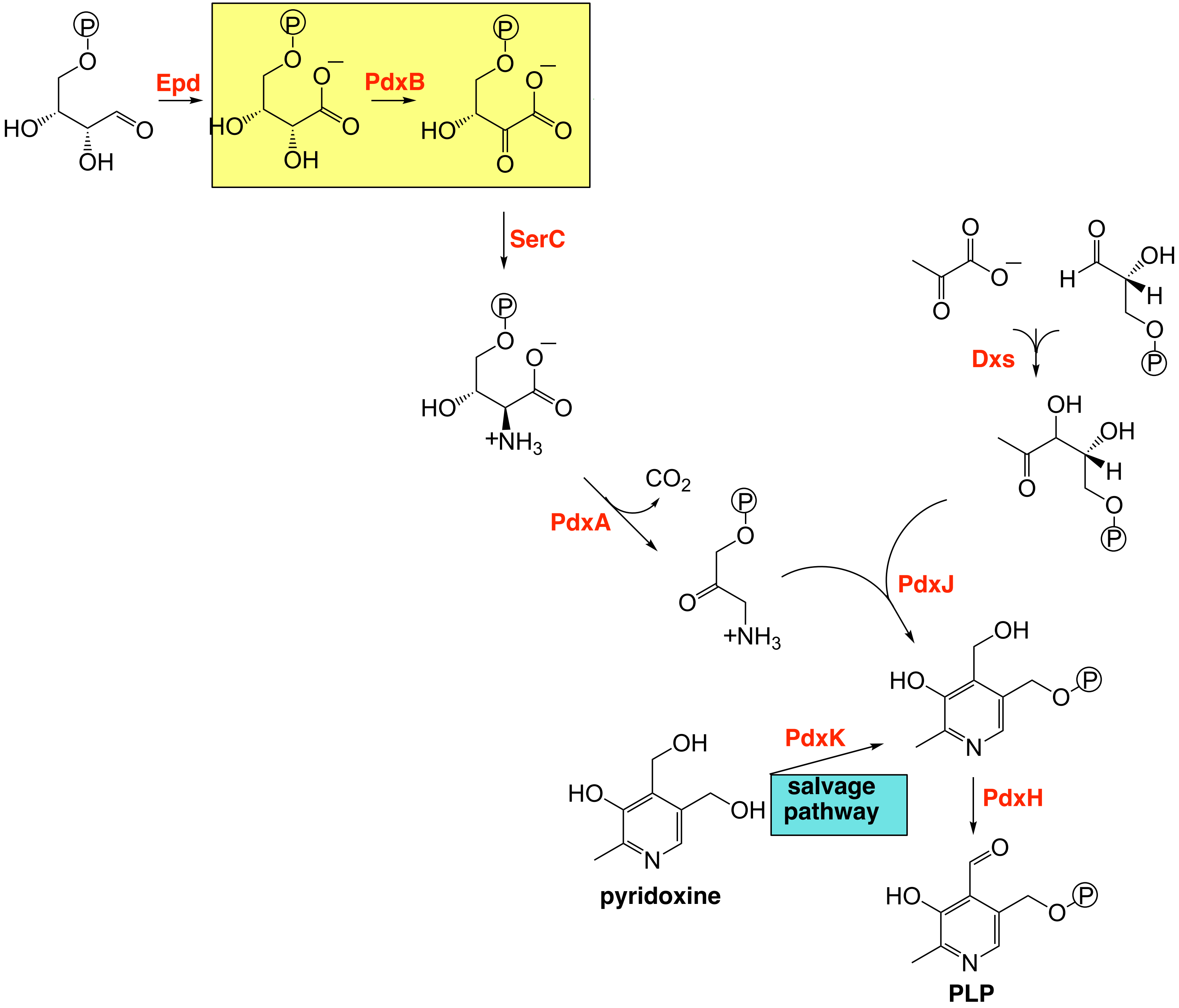
PdxB: Erythronate-4-phosphate dehydrogenase



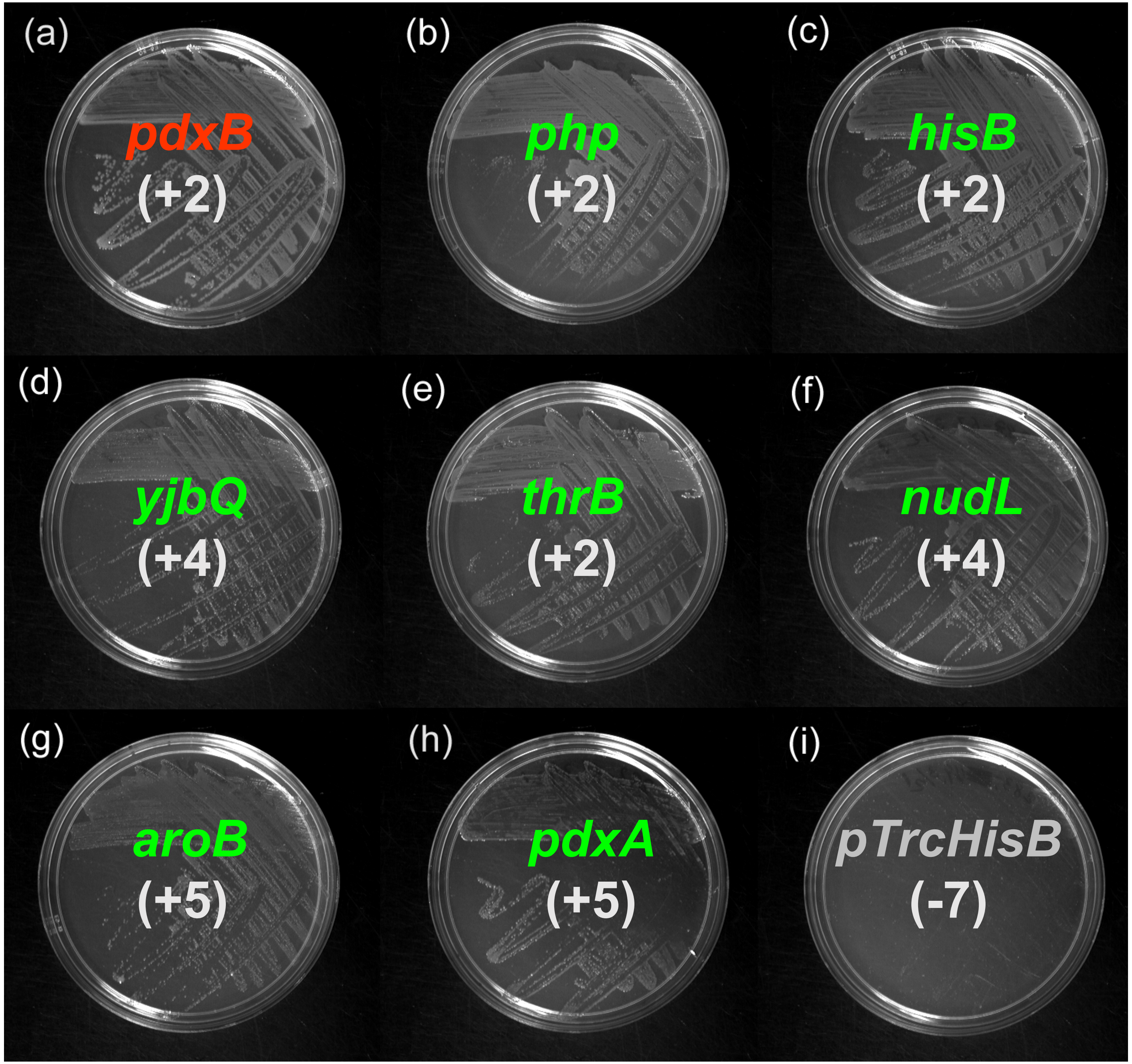
Pyridoxal 5-phosphate
(PLP)



Transamination
Racemization
β-elimination
Retro aldol cleavage
Radical reactions


Epd
PdxB
SerC
PdxA
CO2
Dxs
PdxJ
PdxK
salvage pathway
PdxH
pyridoxine
PLP

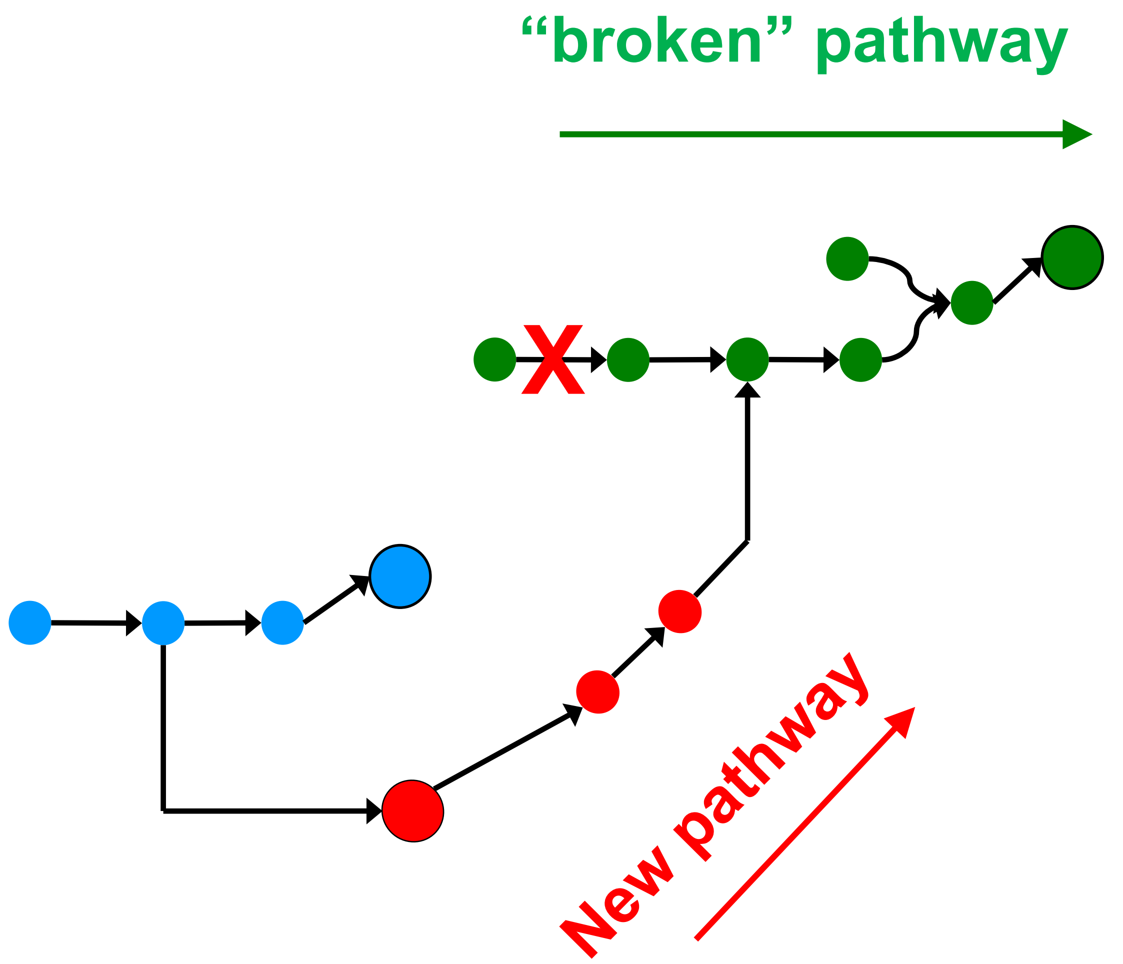
ΔpdxB E. coli
M9/glucose plates

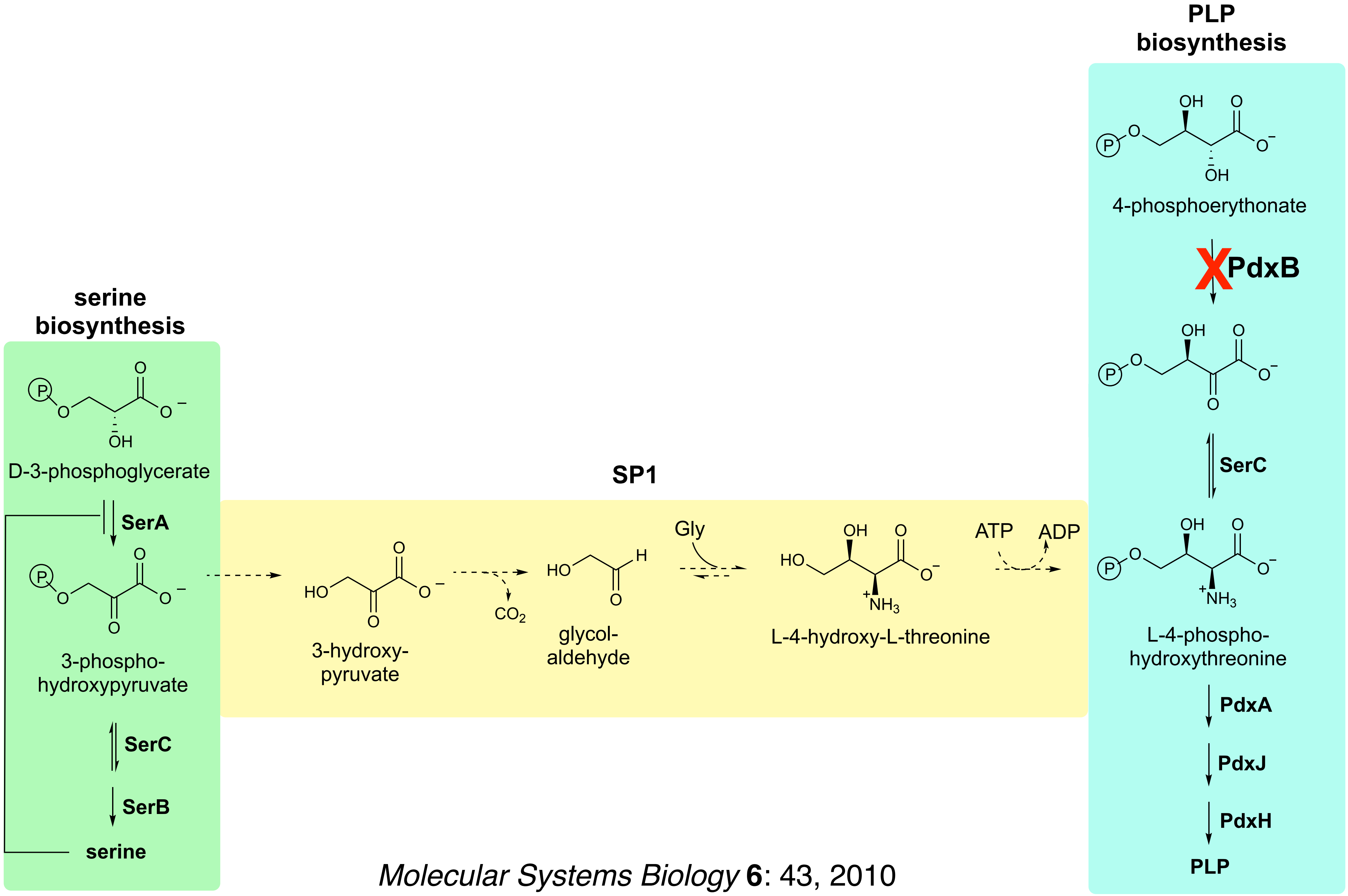


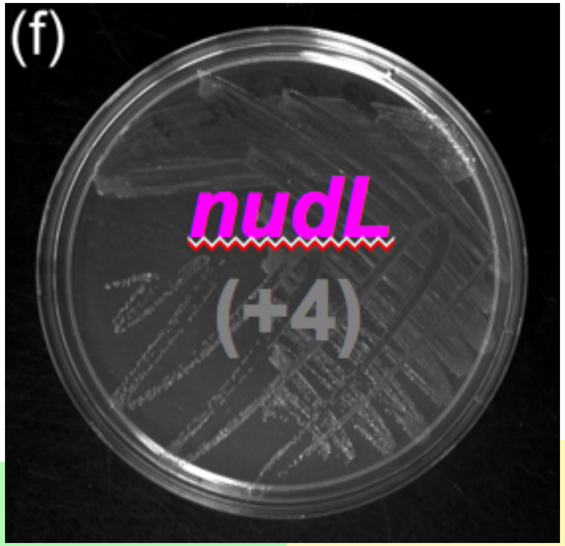
enzyme	activity
PdxA	dehydrogenase
AroB	synthase
ThrB	kinase
HisB	dehydratase
Php	predicted hydrolase
NudL	hydrolase
YjbQ	conserved protein of unknown function

enzyme	activity
PdxA	dehydrogenase
AroB	synthase
ThrB	kinase
HisB	dehydratase
Php	predicted hydrolase
NudL	hydrolase
YjbQ	conserved protein of unknown function

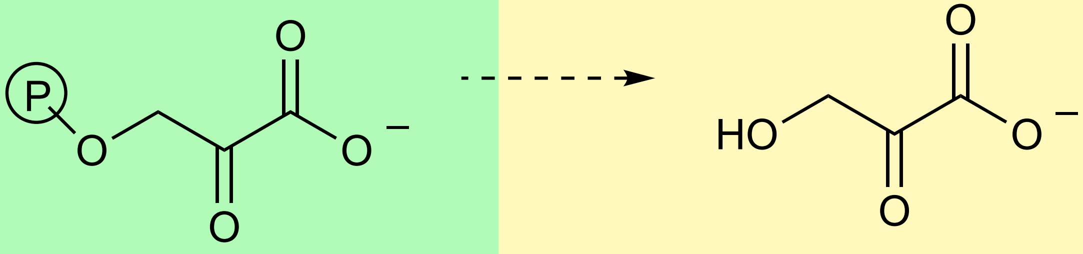
Serendipitous pathway




PLP
biosynthesis
4-phosphoerythonate
PdxB
SerC
L-4-phospho-
hydroxythreonine
PdxA
PdxJ
PdxH
PLP
serine
biosynthesis
D-3-phosphoglycerate
SerA
3-phospho-
hydroxypyruvate
SerC
SerB
serine
SP1
3-hydroxy-
pyruvate
CO_2
glycol-
aldehyde
Gly
L-4-hydroxy-L-threonine
ATP
ADP



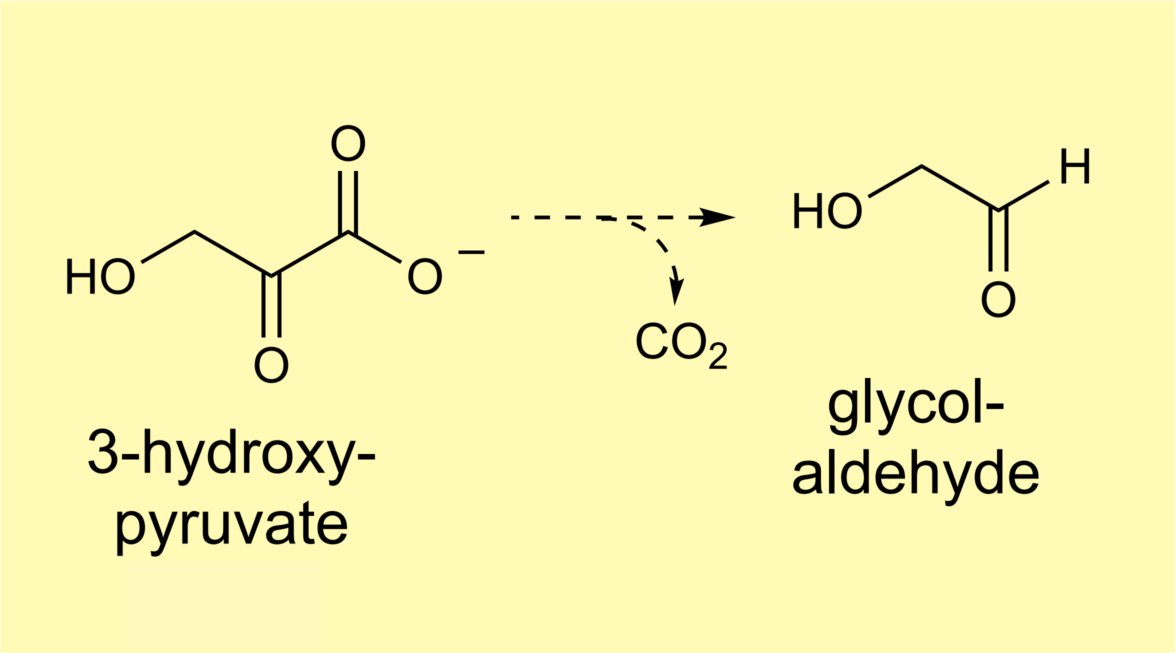
predicted CoA pyrophosphorylase



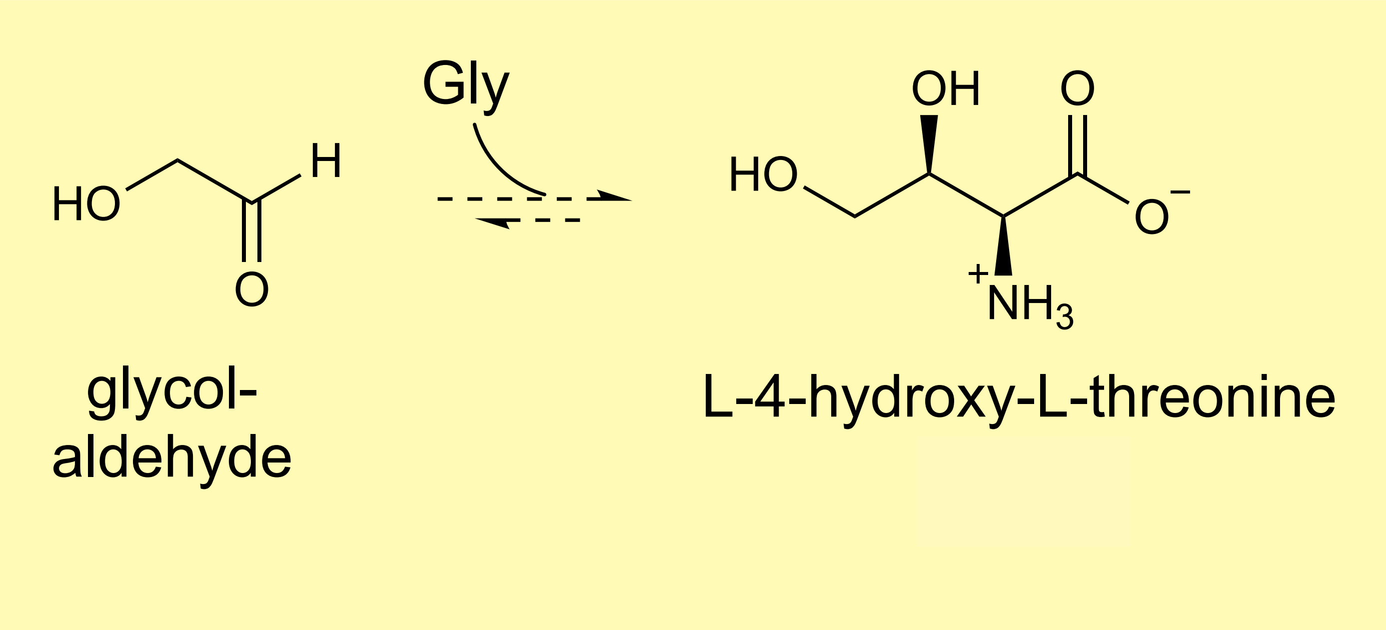
3-phospho-hydroxypyruvate

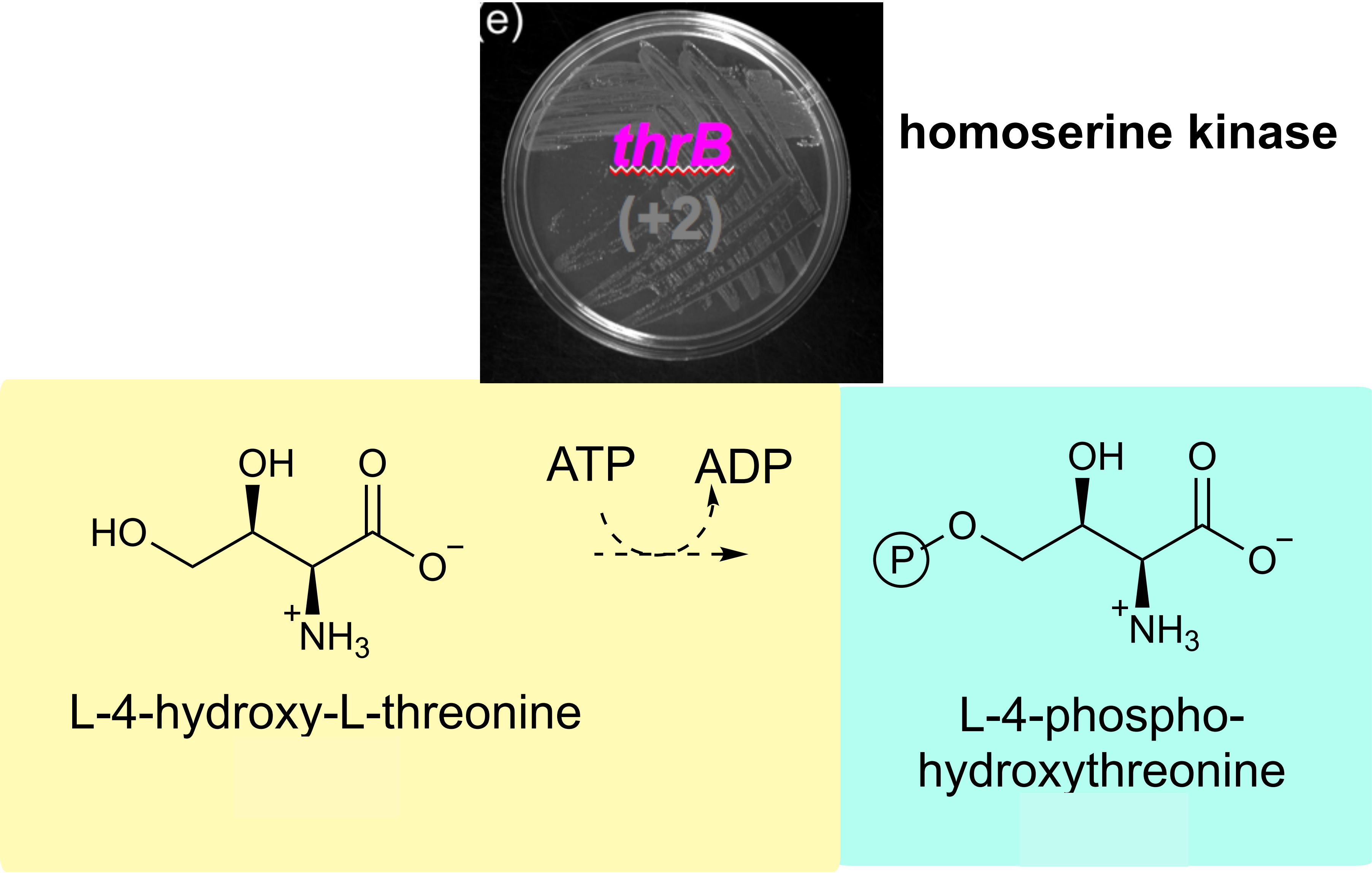
3-hydroxy-pyruvate

non-enzymatic

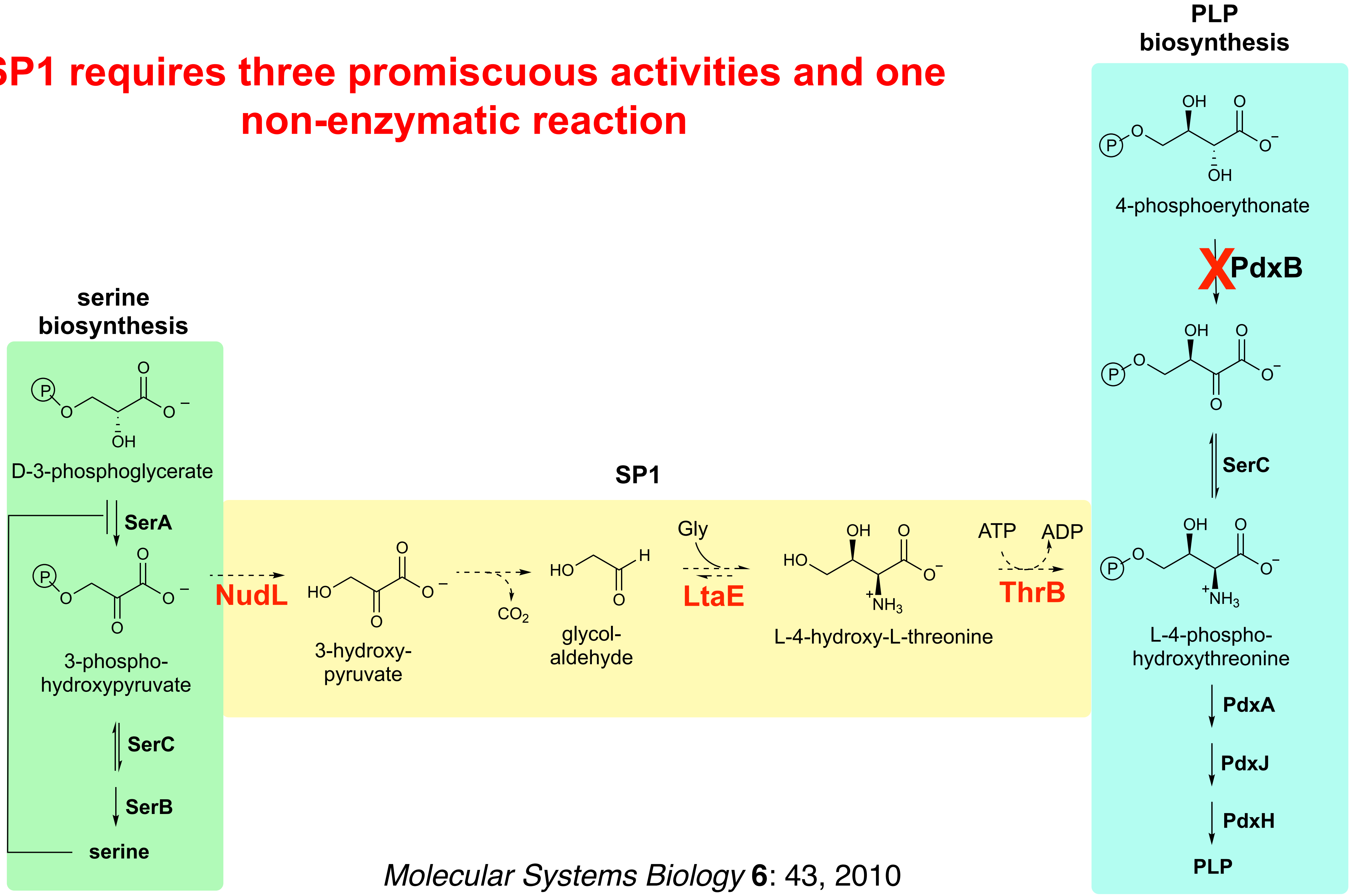


low-specificity threonine aldolase
LtaE



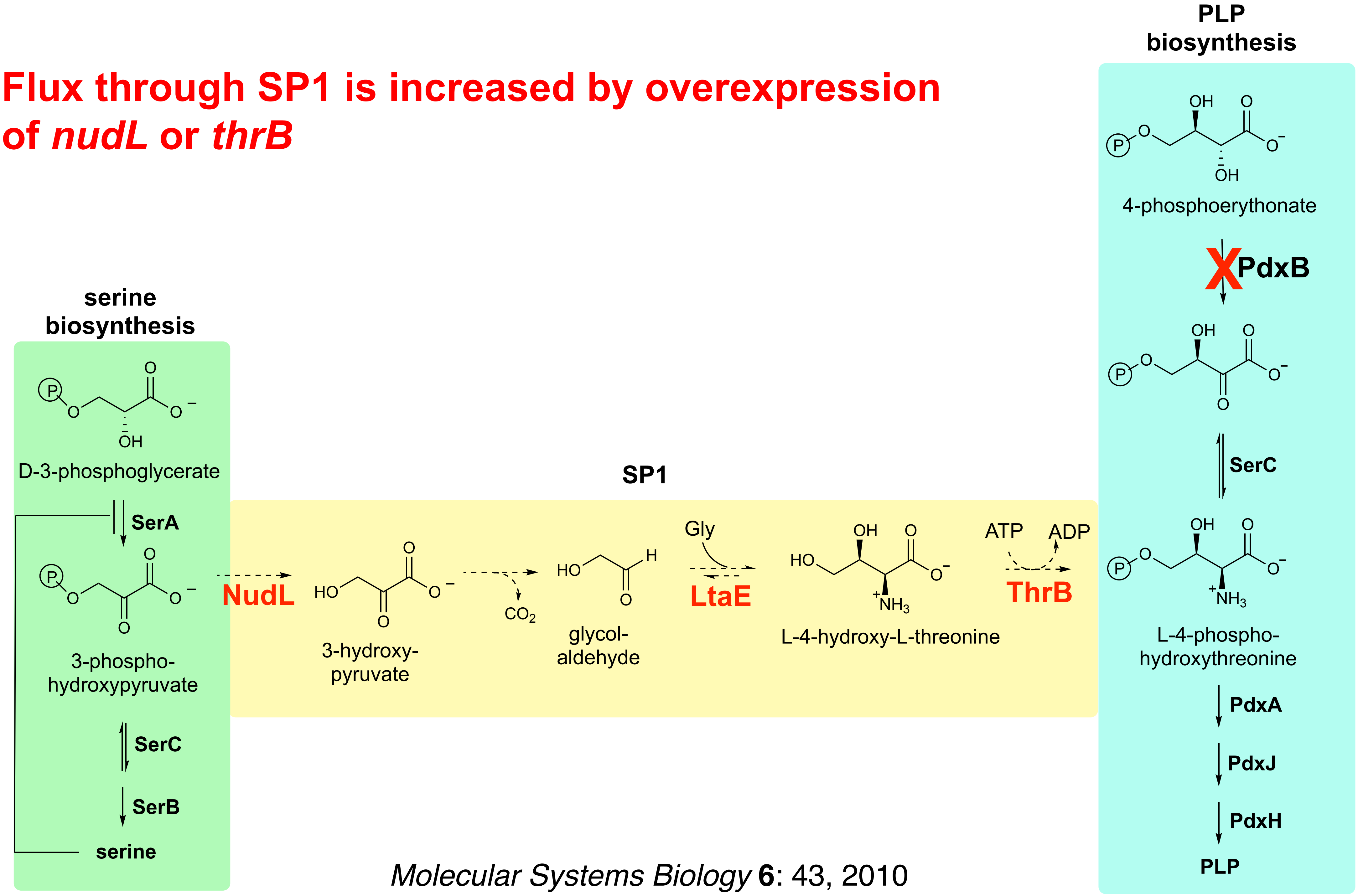

(e)
thrB
(+2)
homoserine kinase
OH
O
HO
O⁻
+NH3
ATP
ADP
P
L-4-hydroxy-L-threonine
L-4-phospho-
hydroxythreonine

SP1 requires three promiscuous activities and one non-enzymatic reaction



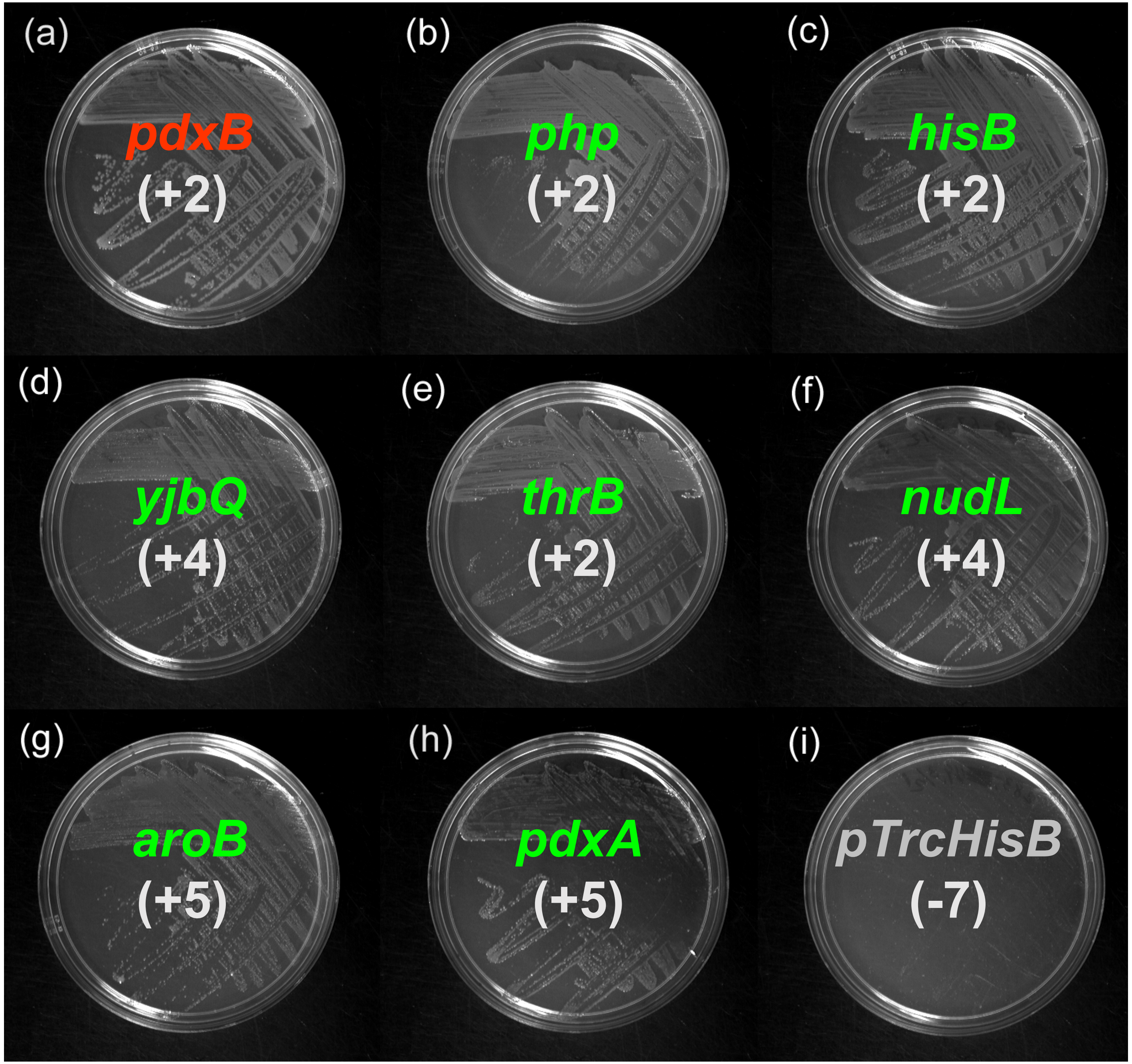
Molecular Systems Biology **6**: 43, 2010

Flux through SP1 is increased by overexpression of *nudL* or *thrB*

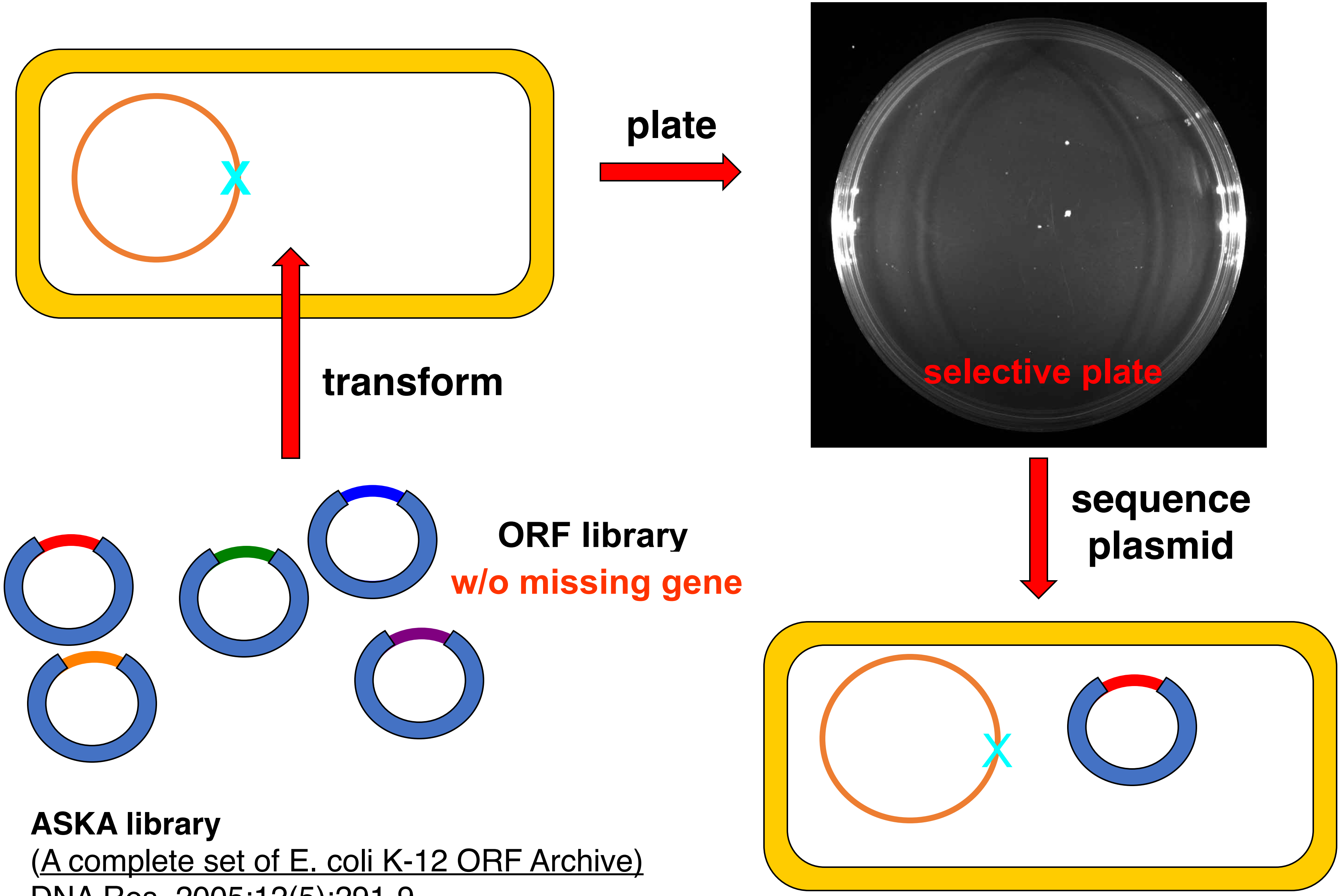


Molecular Systems Biology **6**: 43, 2010

At least two other SPs are facilitated by overexpression of single enzymes



Multicopy suppression



ASKA library
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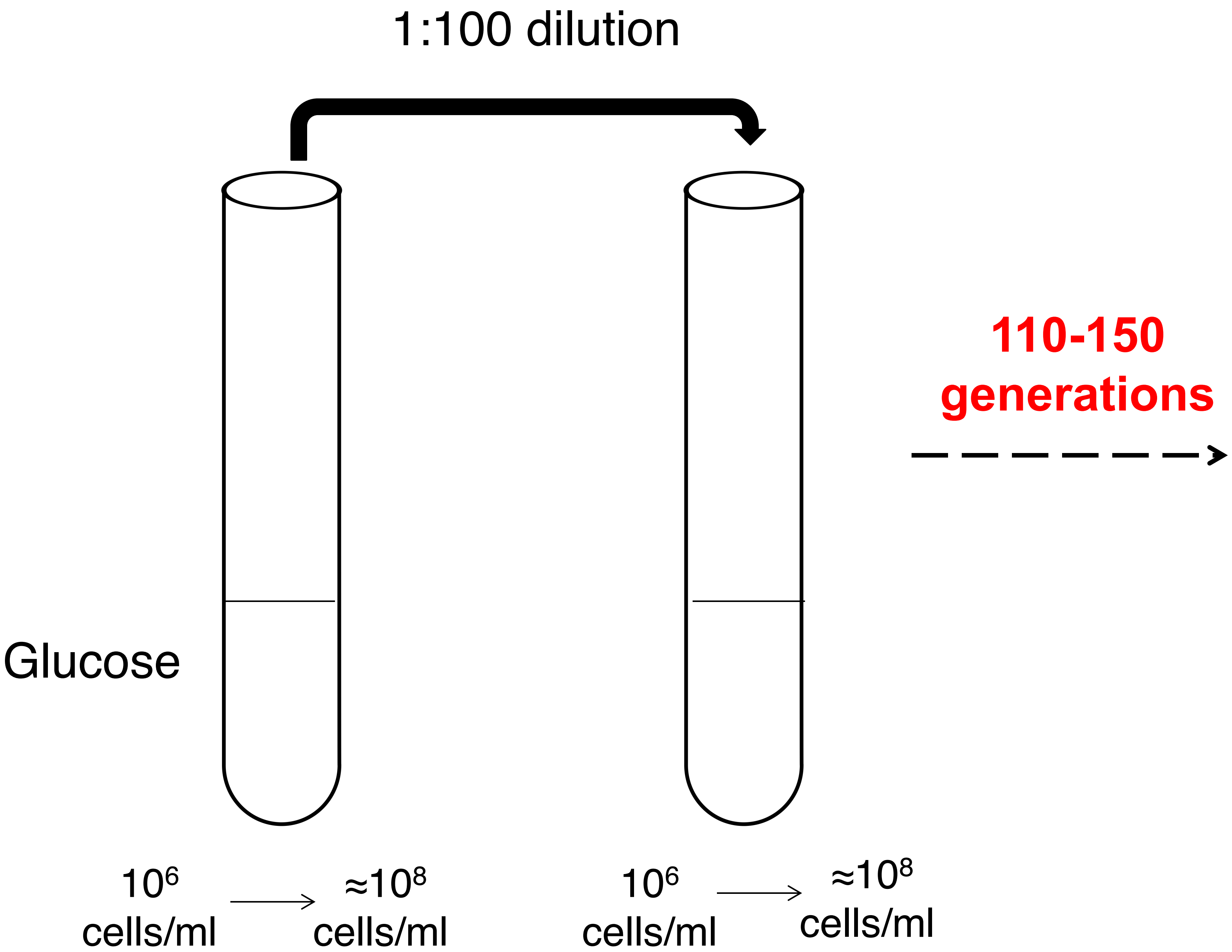
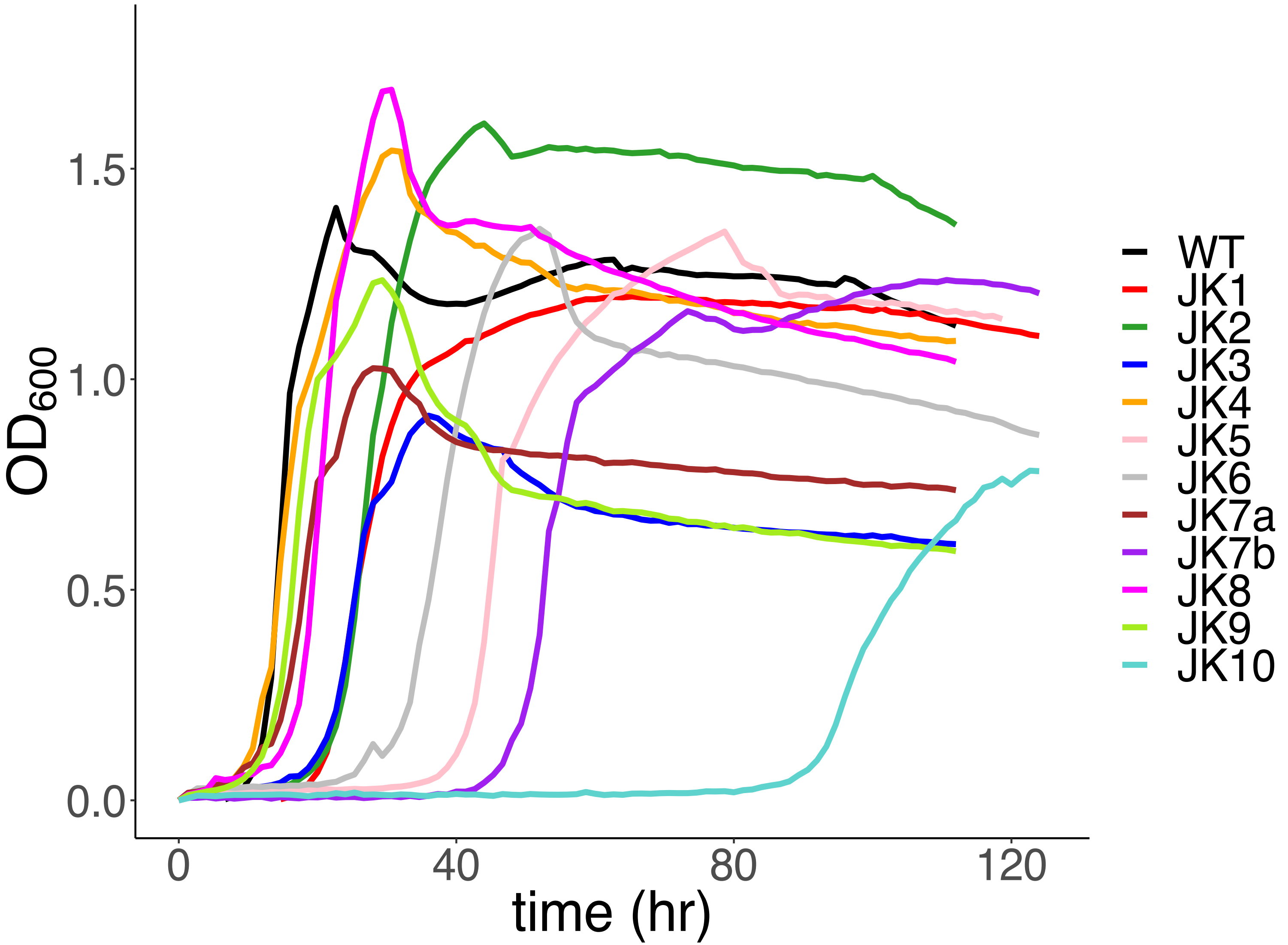
DNA Res. 2005;12(5):291-9

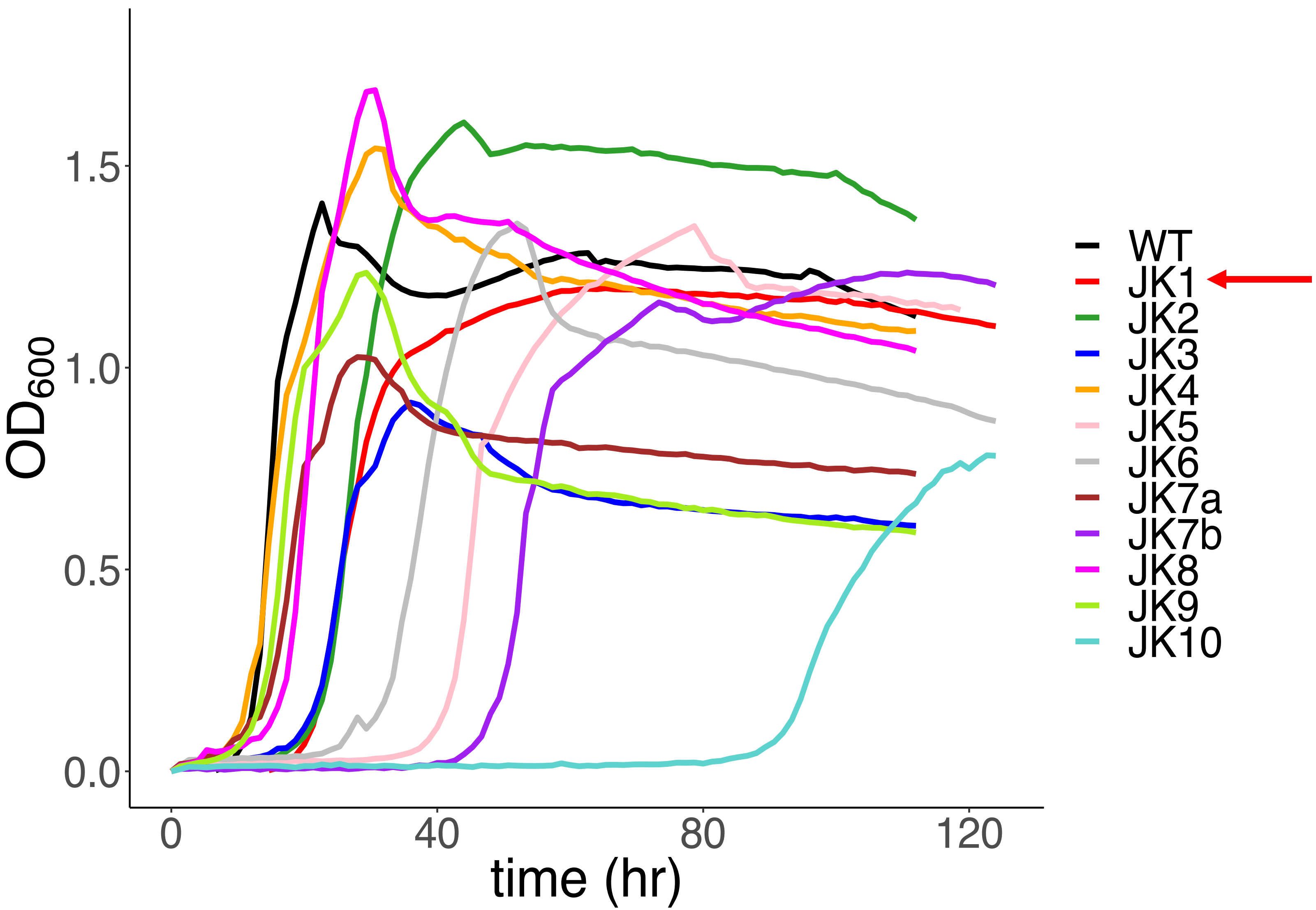

1:100 dilution
110-150
generations
Glucose
10^6
cells/ml
≈10^8
cells/ml
10^6
cells/ml
≈10^8
cells/ml



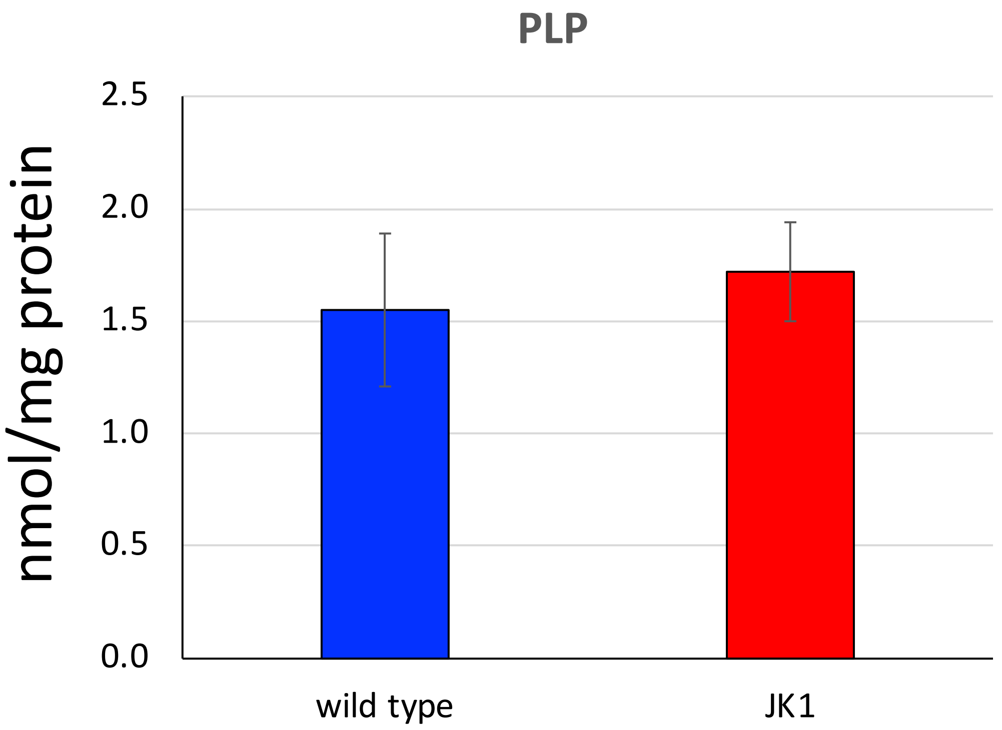
Kim, Flood, et al *PNAS* 2019

1) **How are the evolved strains making PLP?**

2) **How do mutations improve PLP synthesis?**

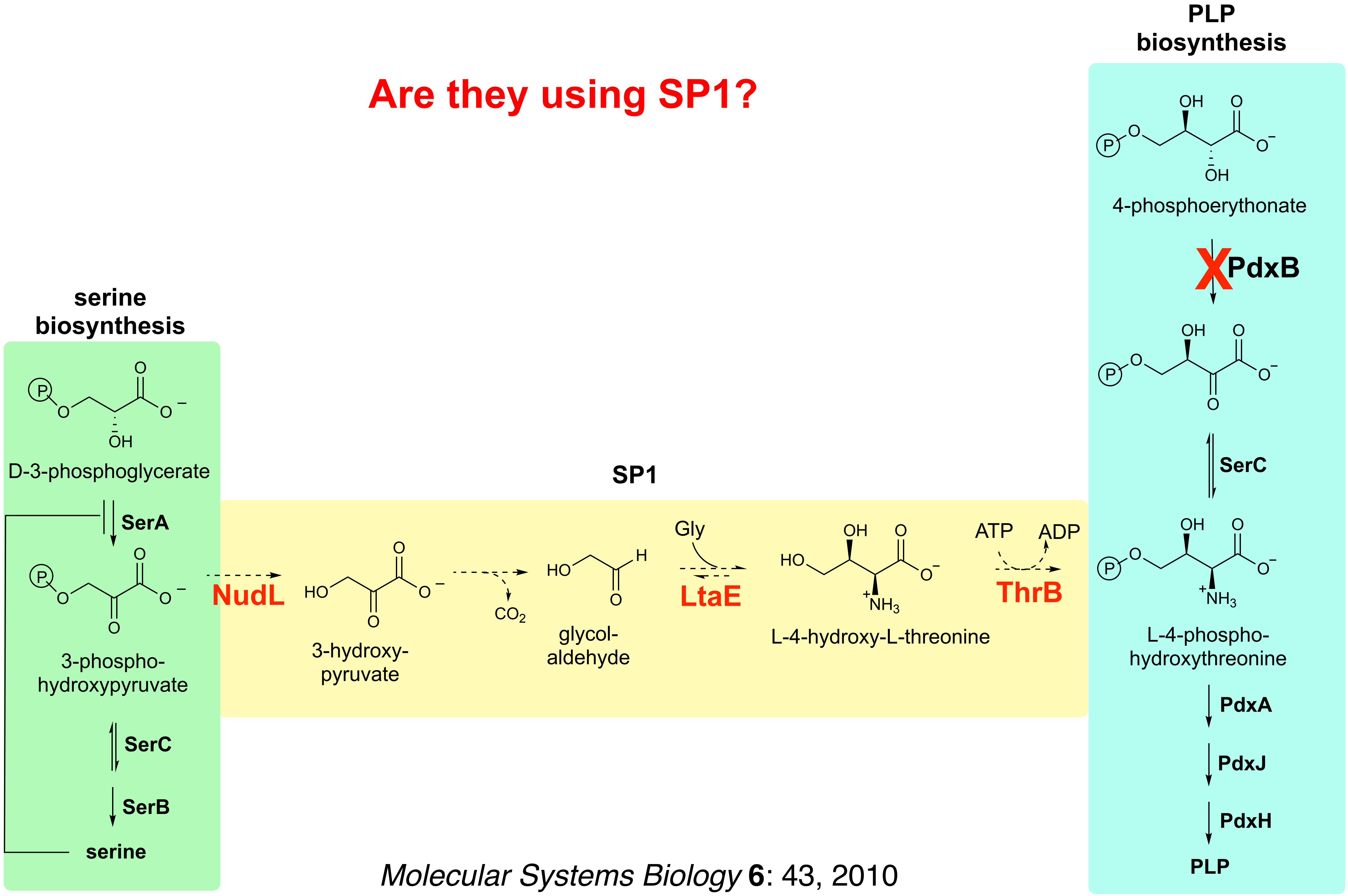


Kim, Flood, et al *PNAS* 2019



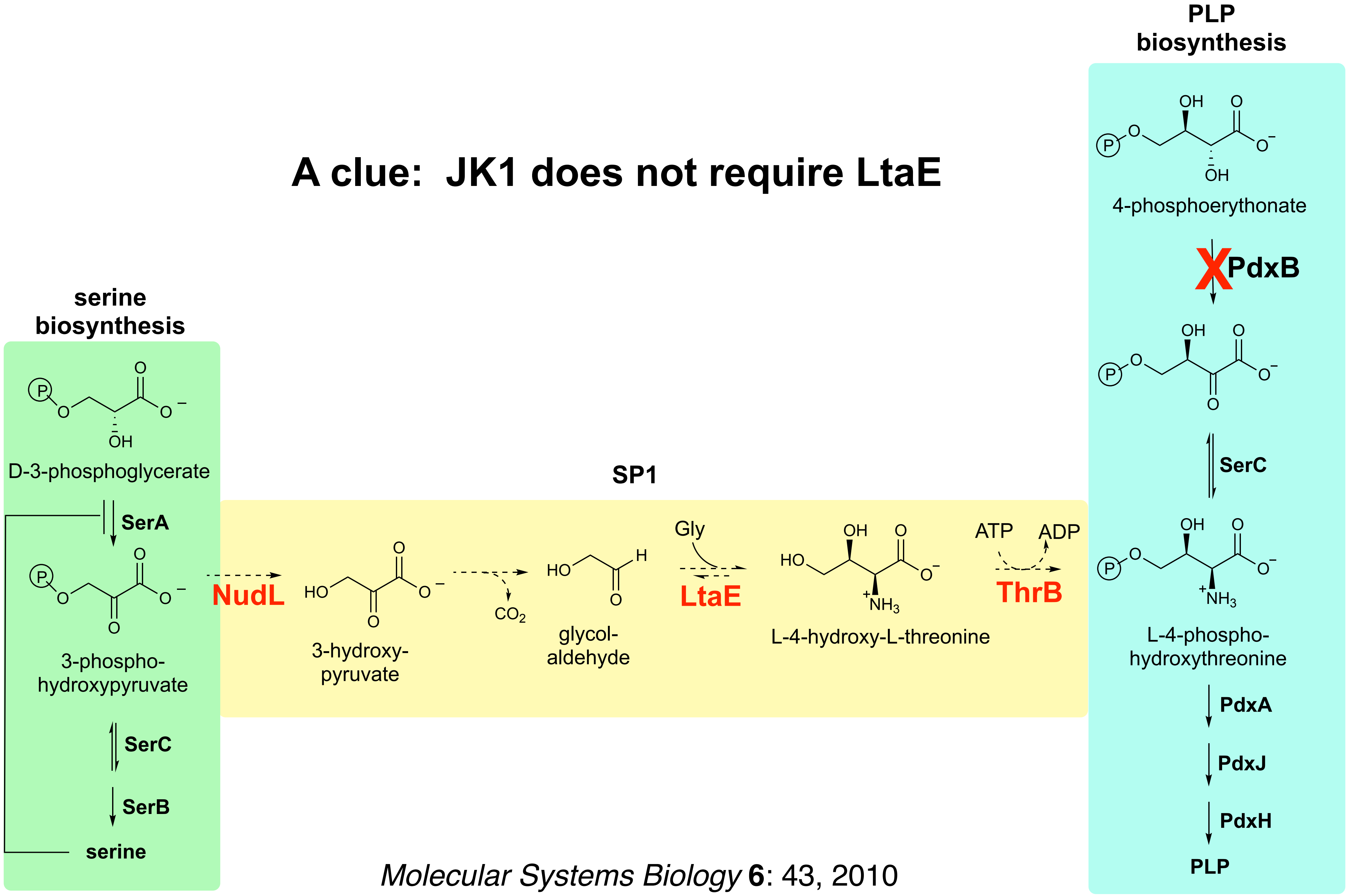
Kim, Flood, et al *PNAS* 2019

Are they using SP1?



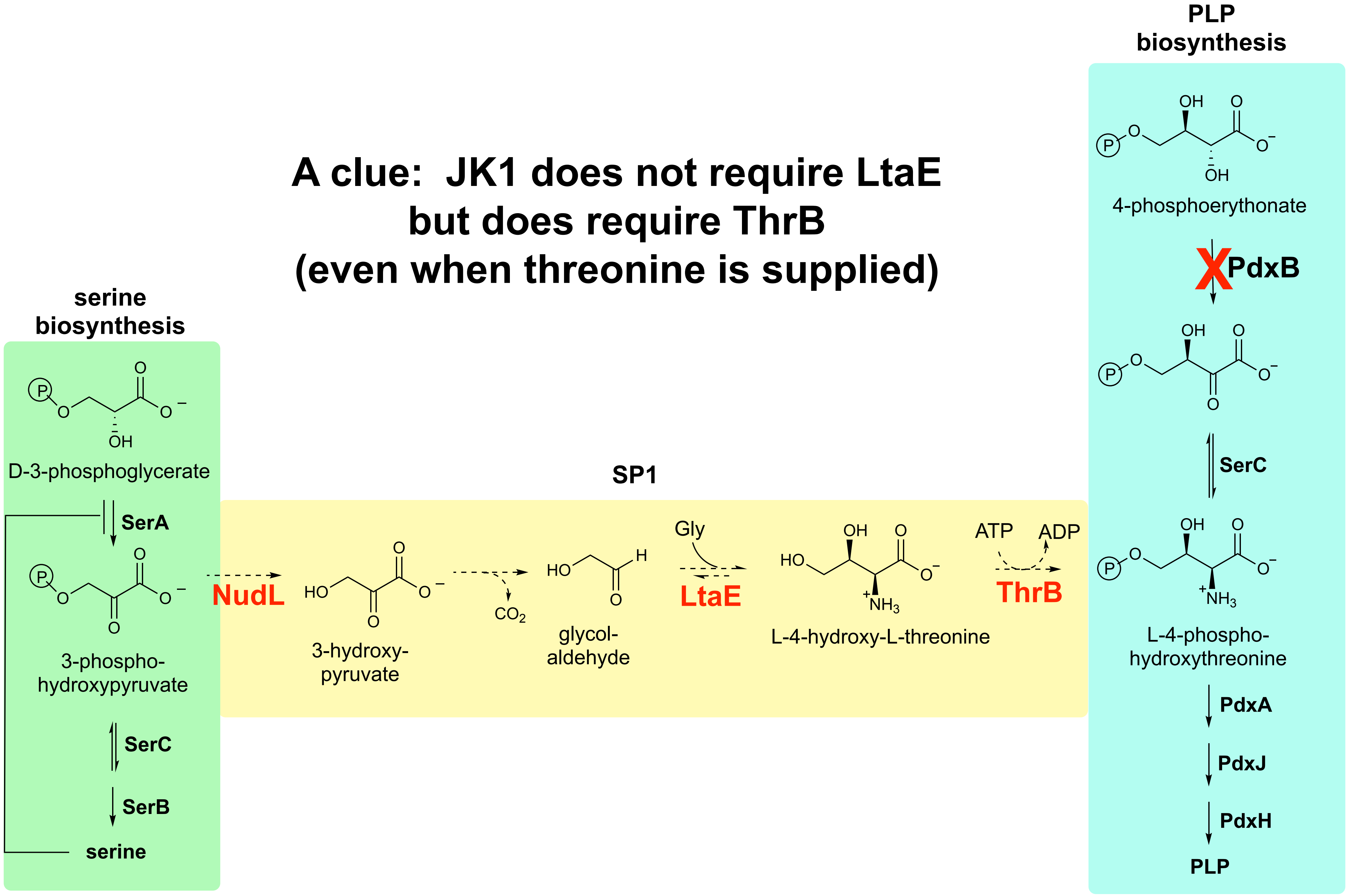
Molecular Systems Biology **6**: 43, 2010

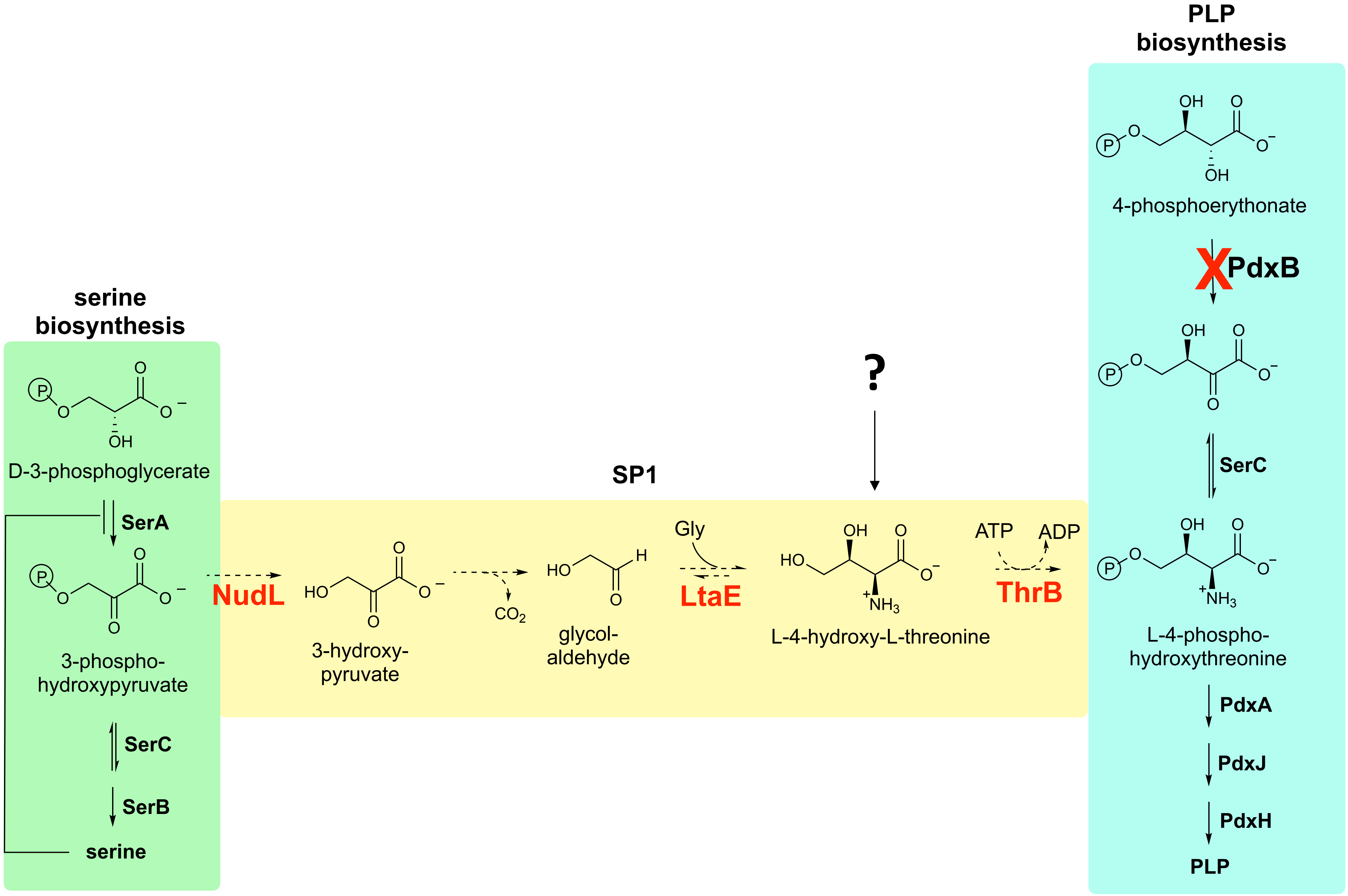
A clue: JK1 does not require LtaE



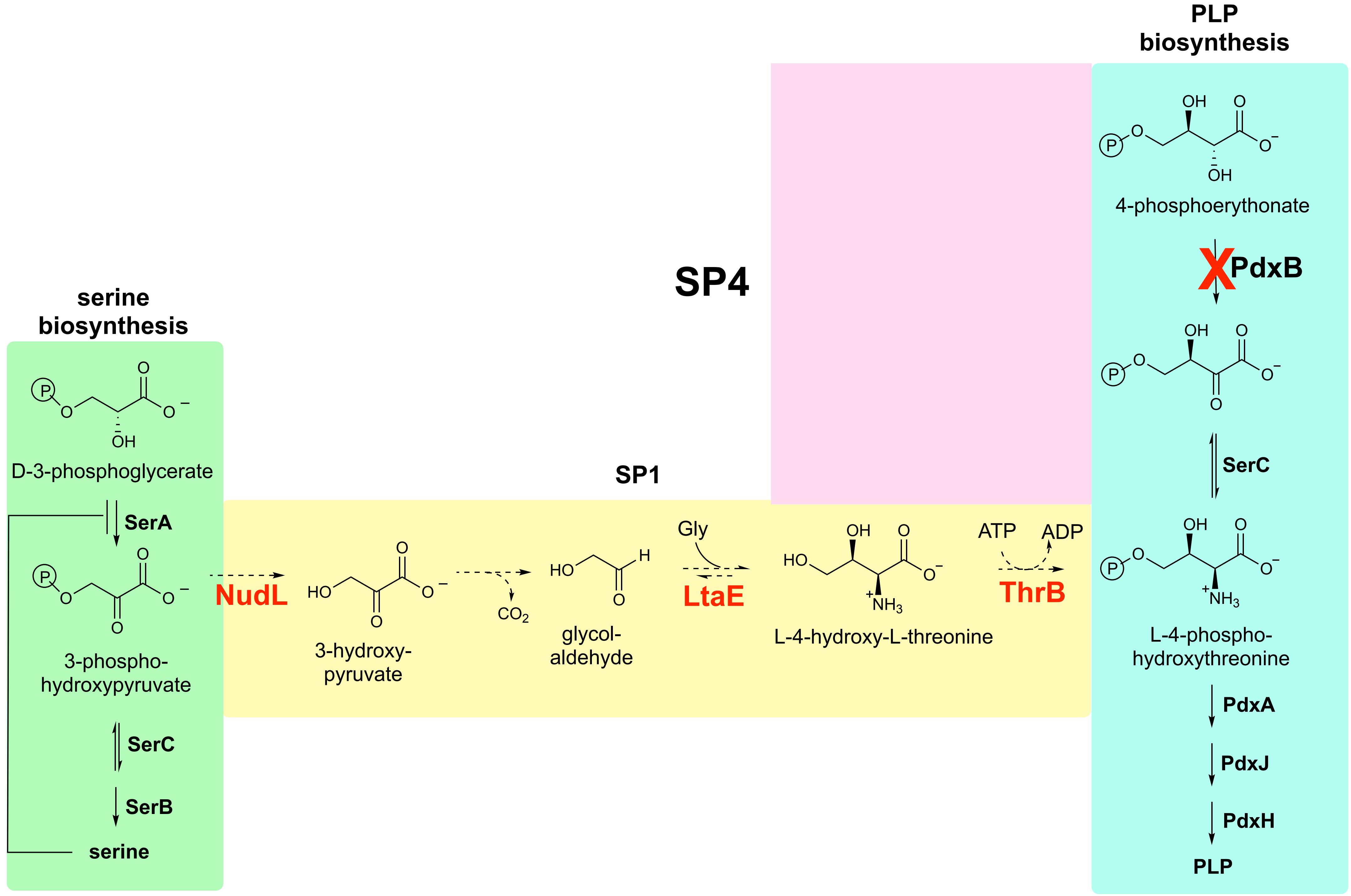
Molecular Systems Biology **6**: 43, 2010

A clue: JK1 does not require LtaE but does require ThrB (even when threonine is supplied)

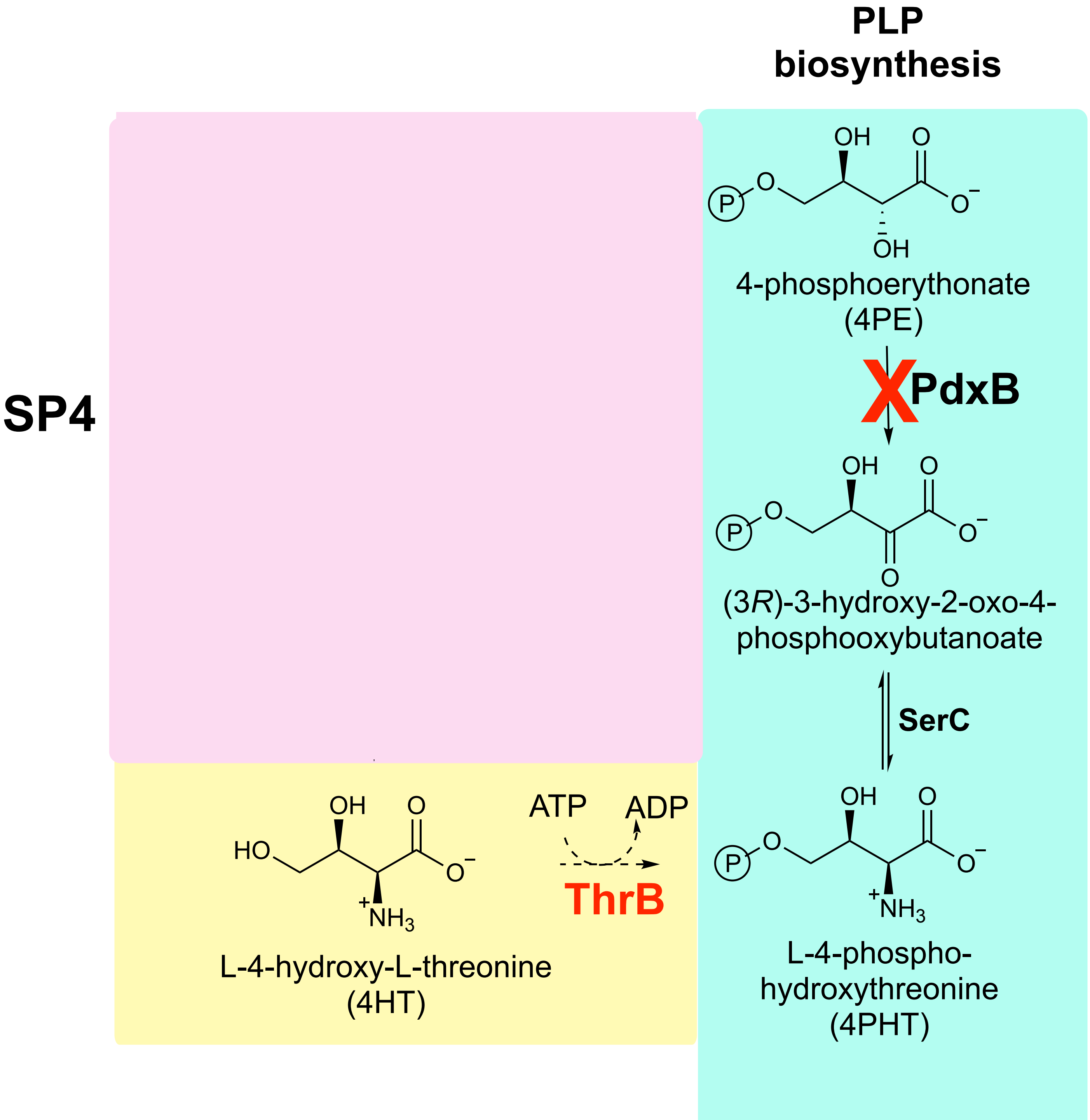


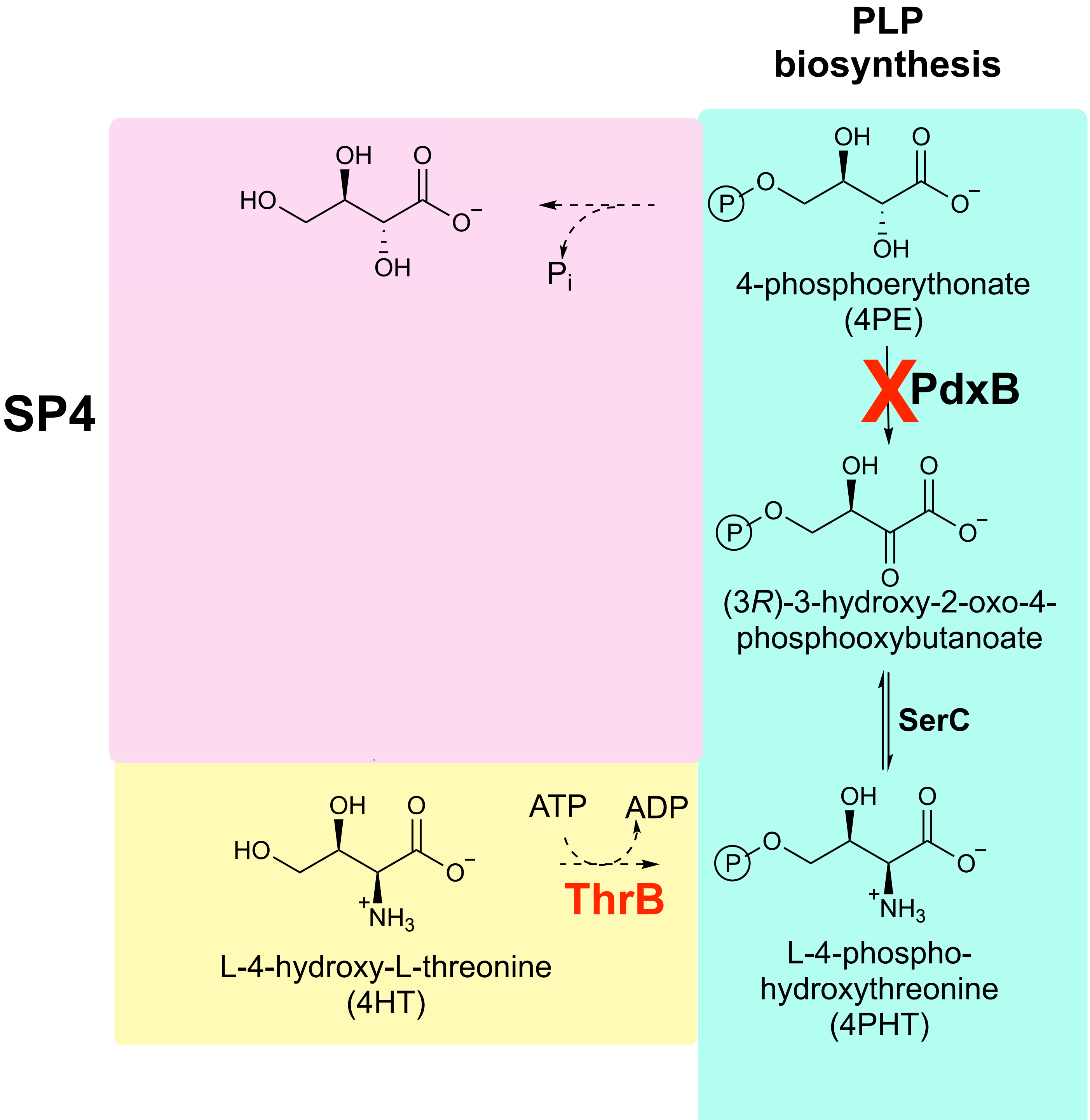


PLP biosynthesis
4-phosphoerythonate
PdxB
SerC
L-4-phospho-hydroxythreonine
PdxA
PdxJ
PdxH
PLP
serine biosynthesis
D-3-phosphoglycerate
SerA
3-phospho-hydroxypyruvate
SerC
SerB
serine
SP1
?
NudL
3-hydroxy-pyruvate
CO2
glycol-aldehyde
Gly
LtaE
L-4-hydroxy-L-threonine
ATP
ADP
ThrB

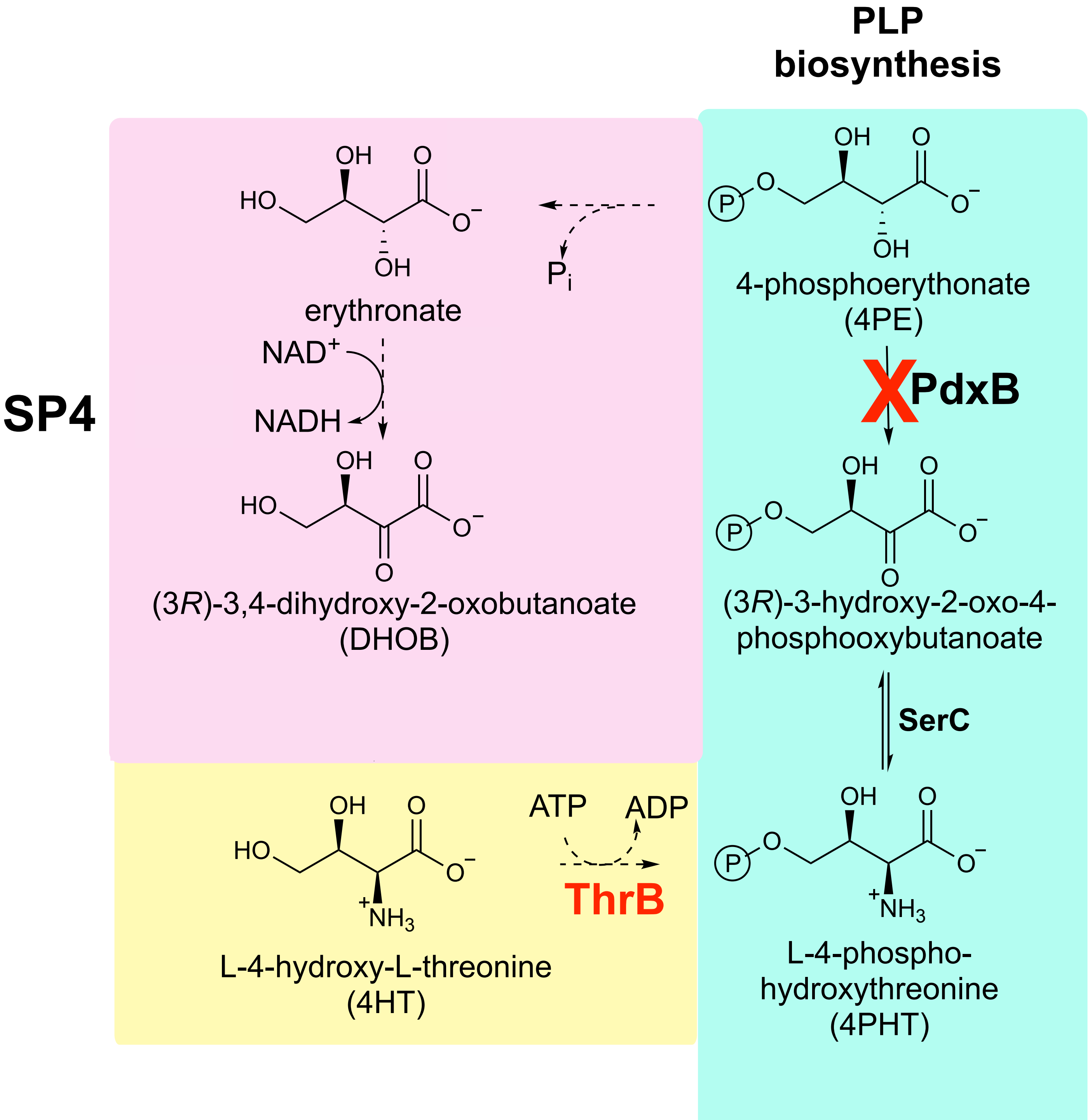


PLP biosynthesis
serine biosynthesis
SP4
SP1
D-3-phosphoglycerate
SerA
3-phospho-hydroxypyruvate
SerC
SerB
serine
NudL
3-hydroxy-pyruvate
CO_2
glycol-aldehyde
Gly
LtaE
L-4-hydroxy-L-threonine
ATP
ADP
ThrB
4-phosphoerythonate
PdxB
SerC
L-4-phospho-hydroxythreonine
PdxA
PdxJ
PdxH
PLP

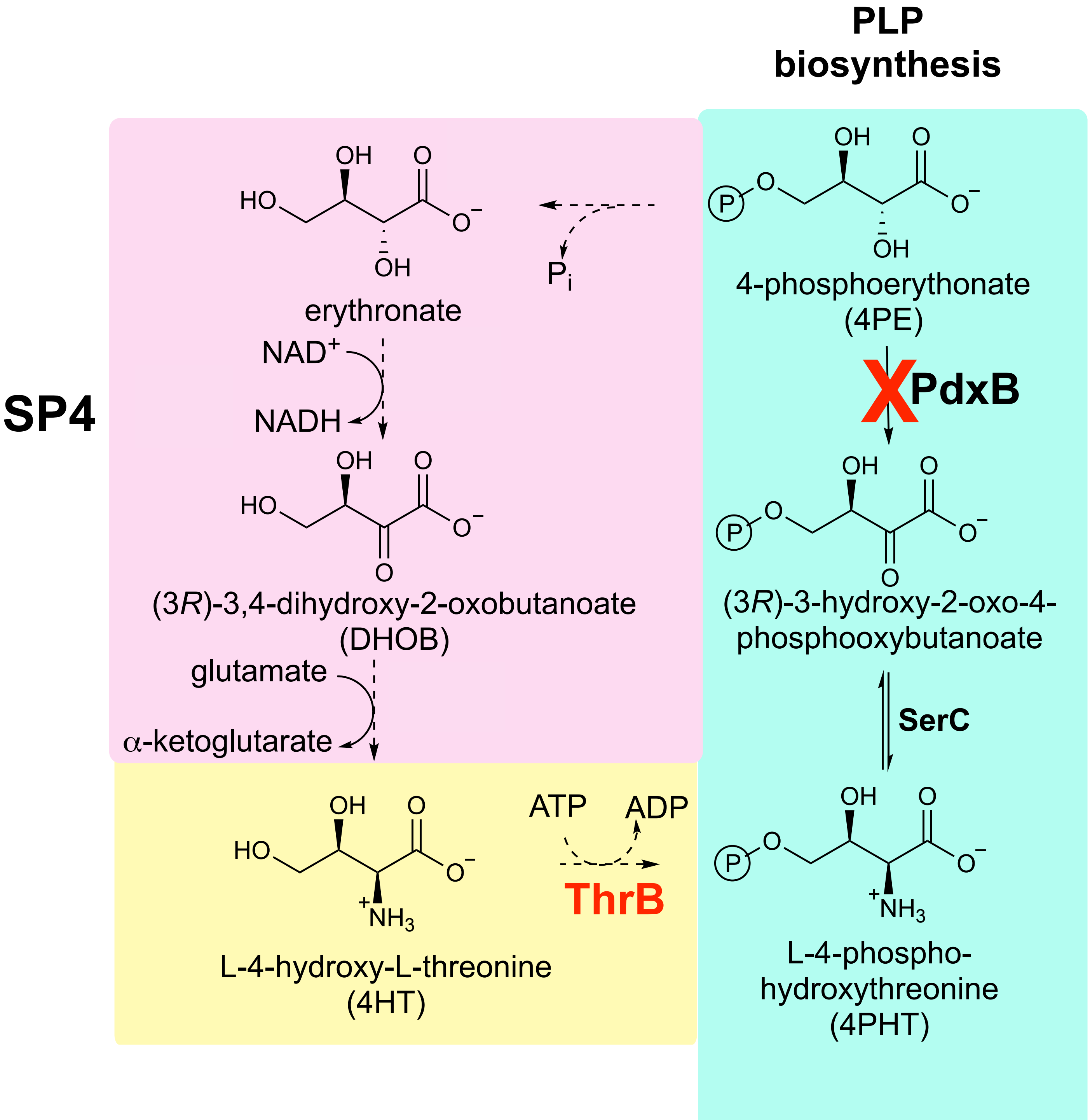

PLP
biosynthesis
SP4
4-phosphoerythonate
(4PE)
PdxB
(3R)-3-hydroxy-2-oxo-4-
phosphooxybutanoate
SerC
L-4-hydroxy-L-threonine
(4HT)
ATP
ADP
ThrB
L-4-phospho-
hydroxythreonine
(4PHT)

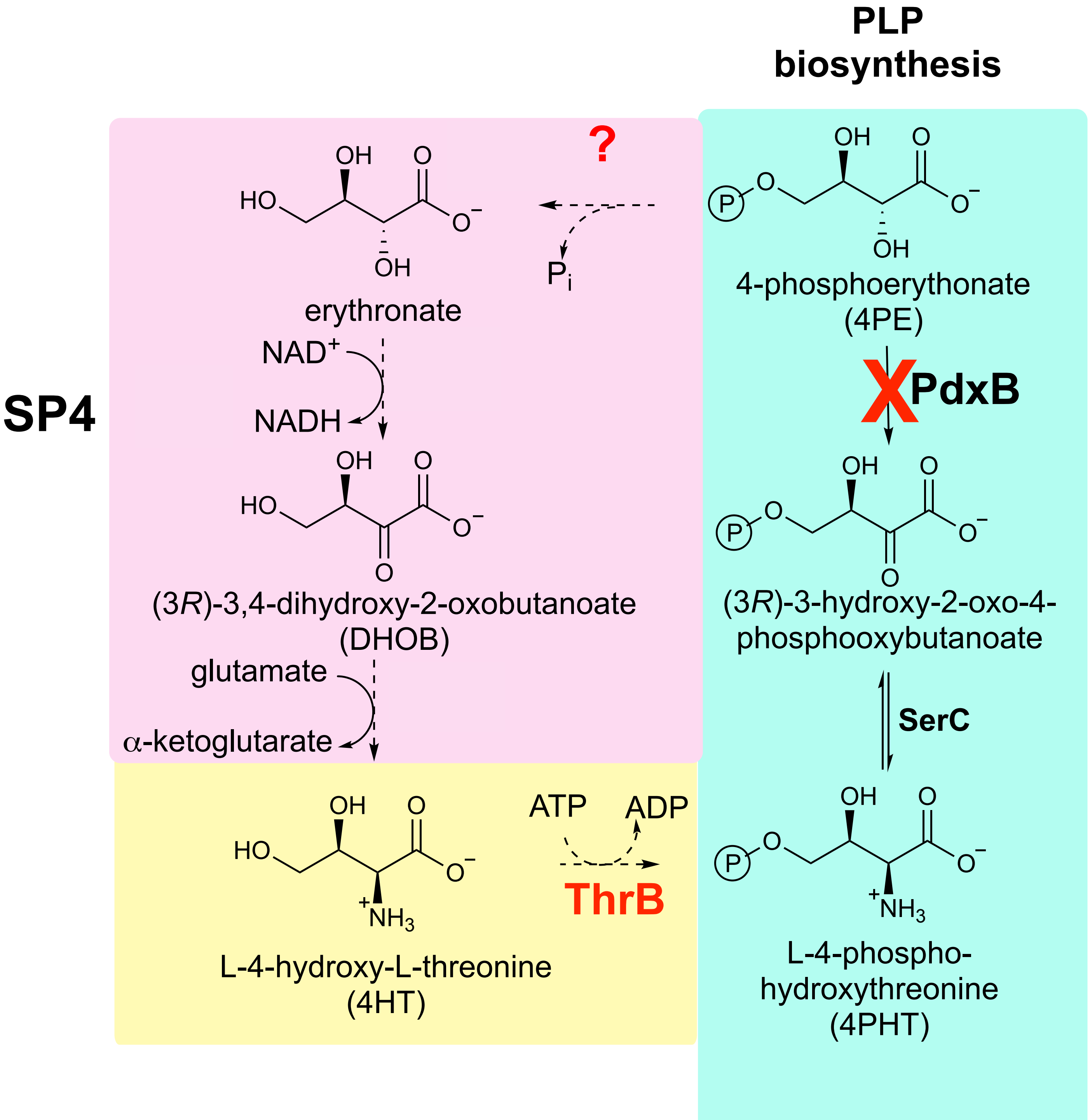


PLP
biosynthesis
SP4
OH
O
HO
O−
OH
Pi
4-phosphoerythonate
(4PE)
X
PdxB
(3R)-3-hydroxy-2-oxo-4-
phosphooxybutanoate
SerC
ATP
ADP
ThrB
+NH3
L-4-hydroxy-L-threonine
(4HT)
L-4-phospho-
hydroxythreonine
(4PHT)

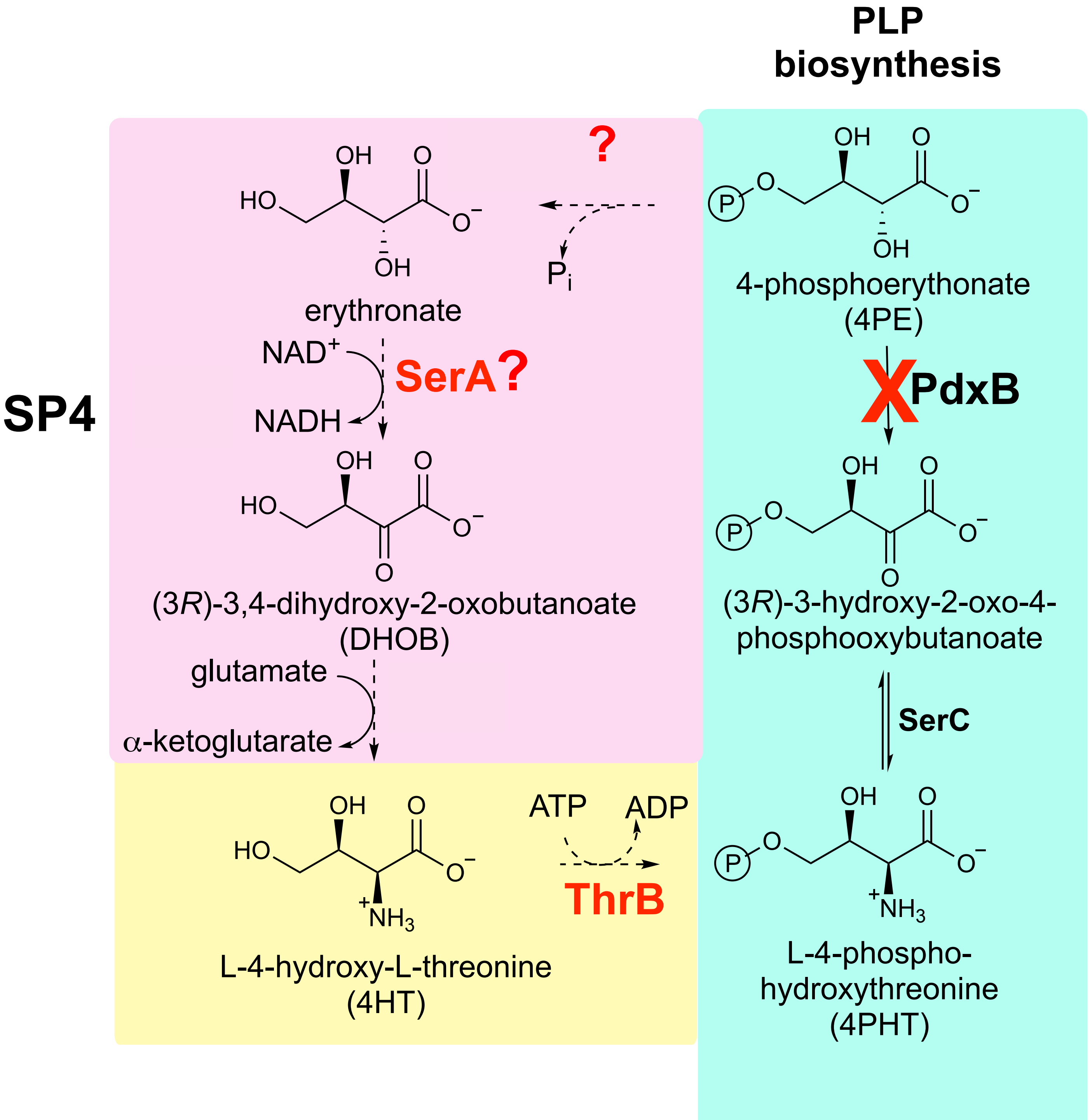


PLP biosynthesis
SP4
erythronate
Pi
NAD+
NADH
(3R)-3,4-dihydroxy-2-oxobutanoate (DHOB)
L-4-hydroxy-L-threonine (4HT)
ATP
ADP
ThrB
4-phosphoerythonate (4PE)
PdxB
(3R)-3-hydroxy-2-oxo-4-phosphooxybutanoate
SerC
L-4-phospho-hydroxythreonine (4PHT)


PLP
biosynthesis
SP4
erythronate
NAD+
NADH
Pi
4-phosphoerythonate
(4PE)
PdxB
(3R)-3,4-dihydroxy-2-oxobutanoate
(DHOB)
(3R)-3-hydroxy-2-oxo-4-
phosphooxybutanoate
glutamate
α-ketoglutarate
SerC
ATP
ADP
ThrB
L-4-hydroxy-L-threonine
(4HT)
L-4-phospho-
hydroxythreonine
(4PHT)



PLP
biosynthesis
SP4
?
HO
OH
O
O−
OH
Pi
erythronate
NAD+
NADH
(3R)-3,4-dihydroxy-2-oxobutanoate
(DHOB)
glutamate
α-ketoglutarate
NH3
L-4-hydroxy-L-threonine
(4HT)
ATP
ADP
ThrB
P
4-phosphoerythonate
(4PE)
PdxB
(3R)-3-hydroxy-2-oxo-4-phosphooxybutanoate
SerC
L-4-phospho-hydroxythreonine
(4PHT)



PLP
biosynthesis
SP4
?
erythronate
NAD+
NADH
SerA?
(3R)-3,4-dihydroxy-2-oxobutanoate
(DHOB)
glutamate
α-ketoglutarate
L-4-hydroxy-L-threonine
(4HT)
ATP
ADP
ThrB
4-phosphoerythonate
(4PE)
PdxB
(3R)-3-hydroxy-2-oxo-4-phosphooxybutanoate
SerC
L-4-phospho-hydroxythreonine
(4PHT)

SerA = 3-phospho-glycerate dehydrogenase

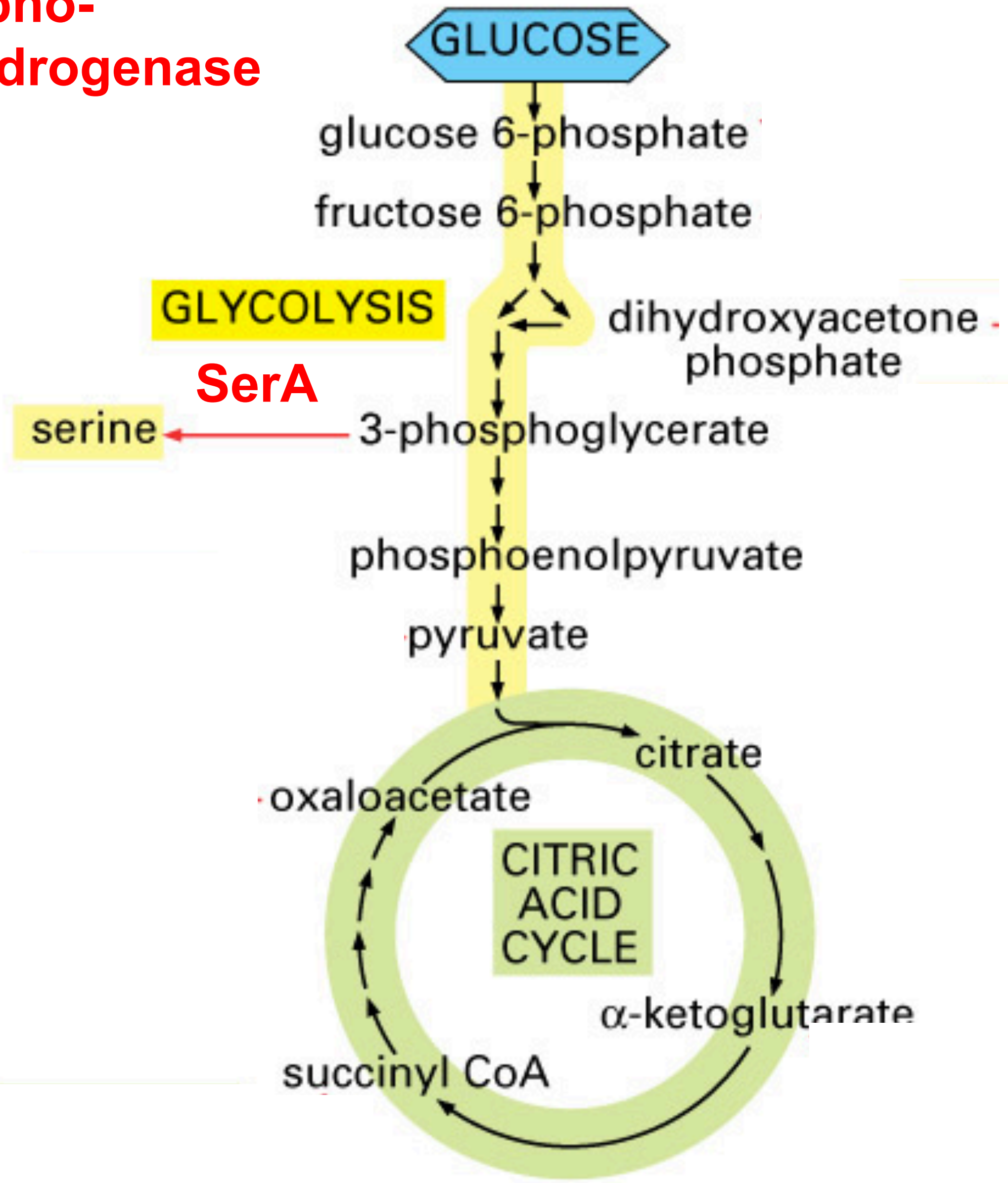


Figure 13-23 Essential Cell Biology, 2/e. (© 2004 Garland Science)

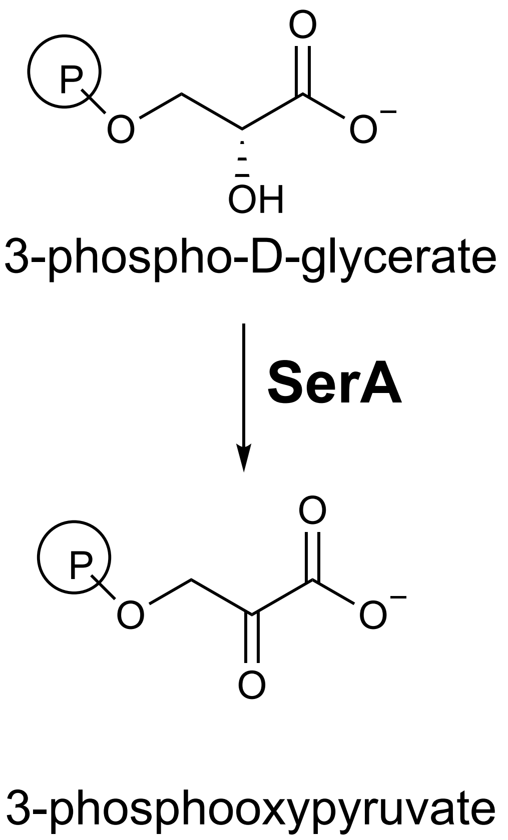
Mutations in *serA* were found in multiple strains

JK1	JK2	JK3	JK4	JK5	JK6
ybhA/pgl	*ybhA/pgl*	*ybhA/pgl*	*ybhA*	*ybhA/pgl*	*gapA*
gapA	*gapA*	*gapA*	*rpe*	*gapA*	***serA***
rpoS	*purF*	*ilvH*	*sdhA*	*yjjK*	*yjjK*
rpoC	*gltB*	*rng*	*rho*	*purF*	
	ypjA		*lon*	*ilvH*	
				nadR	

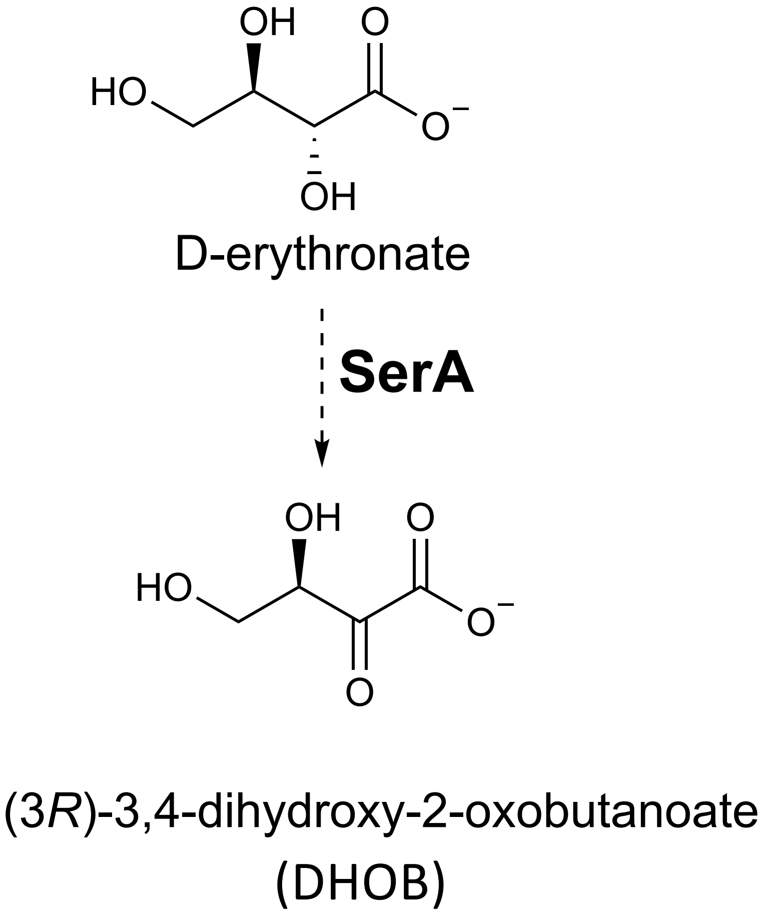
JK7a	JK7b	JK8	JK9	JK10
ybhA	*ybhA/pgl*	*gapA*	*ybhA/pgl*	*ybhA/pgl*
gapA	***serA***	***serA***	*gapA*	*gapA*
purF	*gapA*	*yjjK*	***serA***	*rpe*
nadR	*pykF*	*gltB*	*pykF*	*ilvH*
rpoS	*pyrE*	*livH*		*rng*

Both reactions require oxidation of an alcohol alpha to a carboxylate

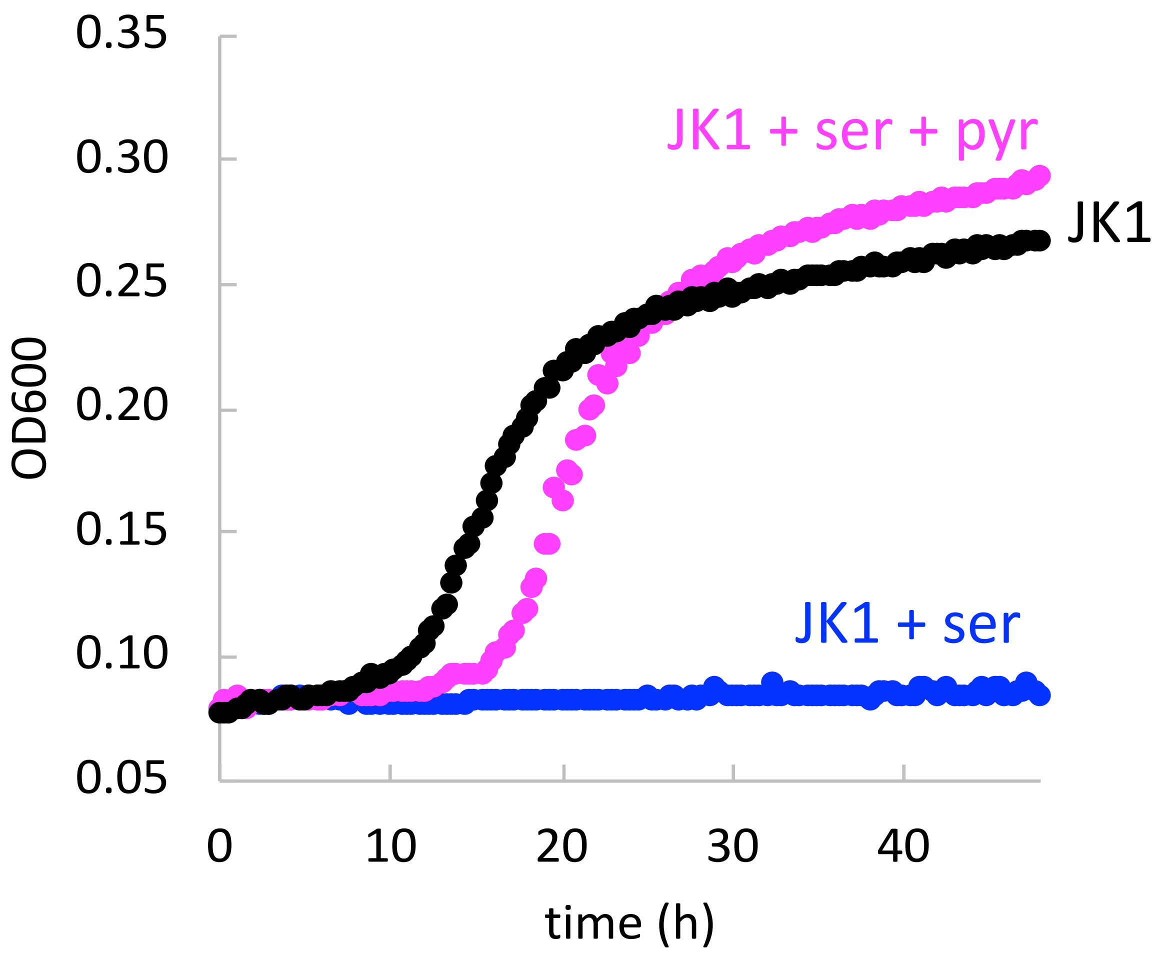
serine biosynthesis



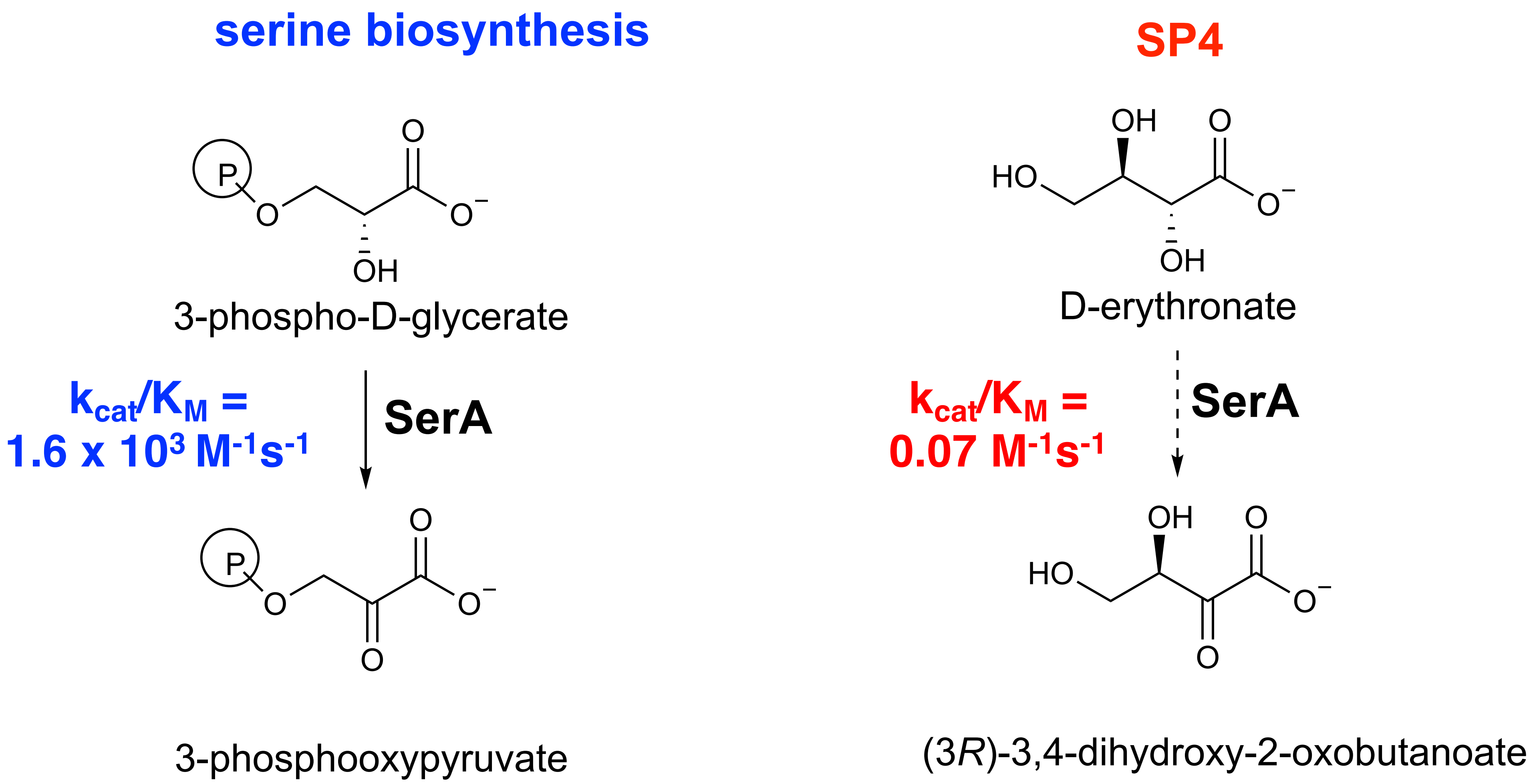
SP4



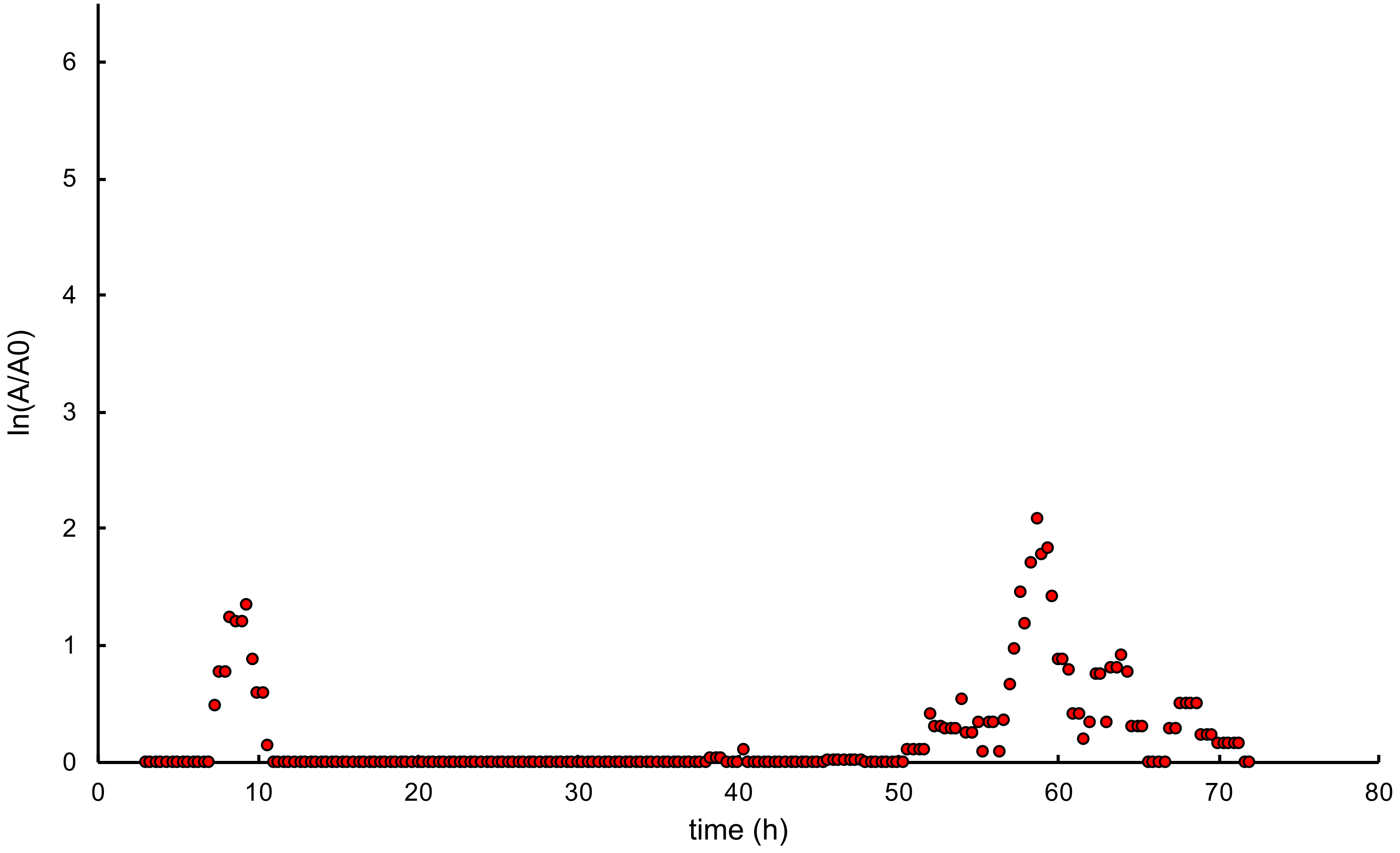
Serine inhibits growth of JK1



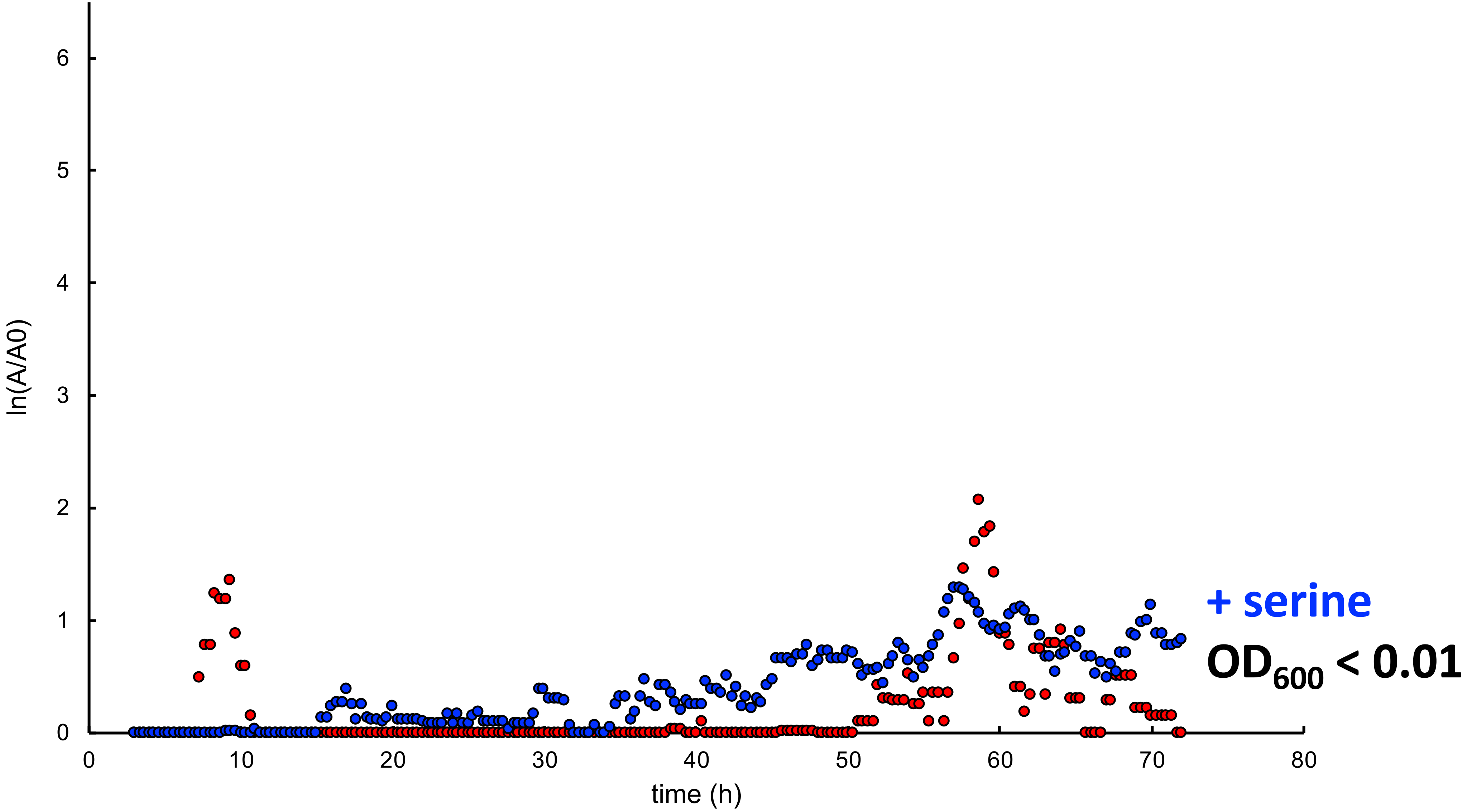
SerA has weak activity with erythronate



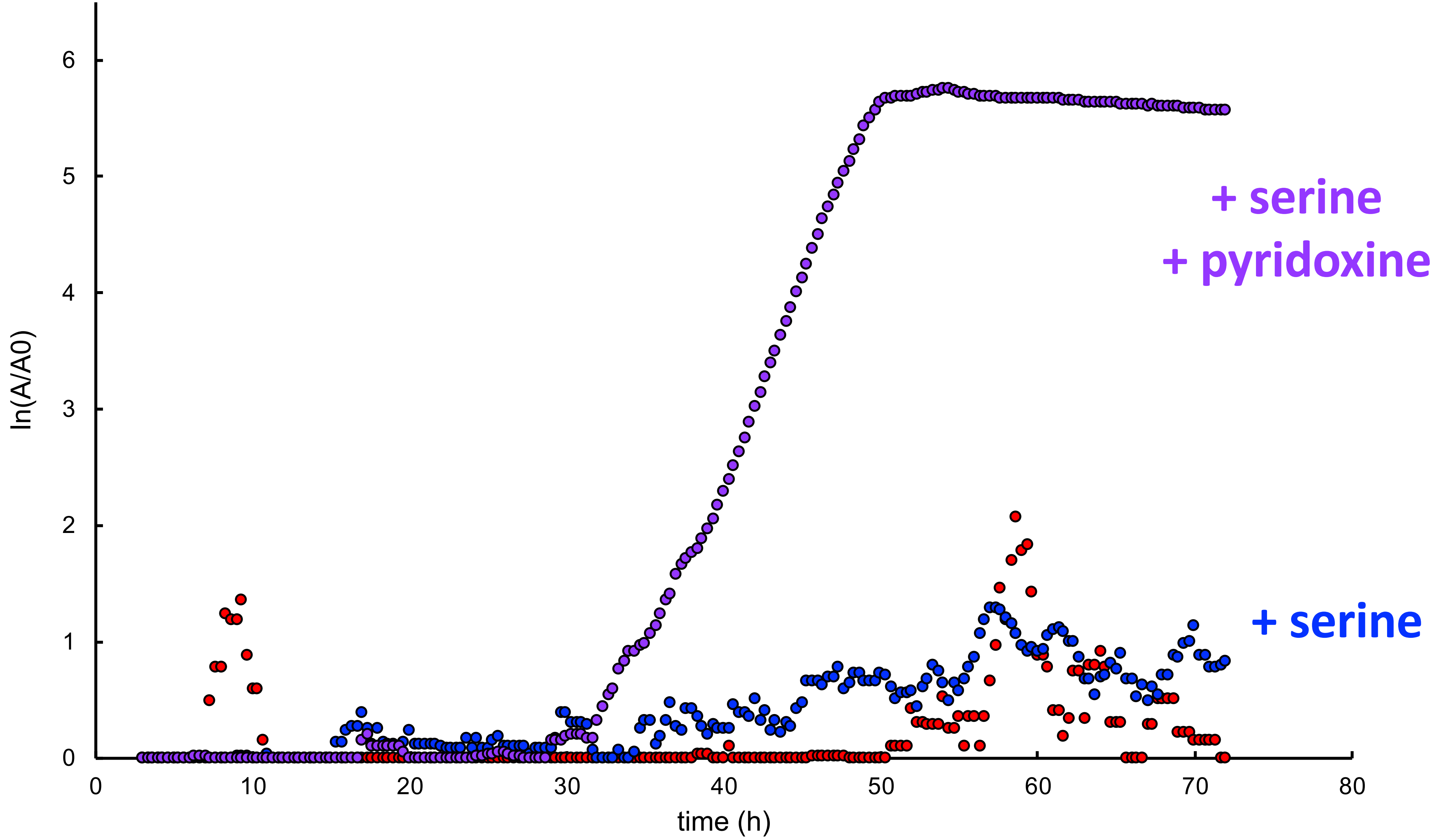
ΔserA JK1



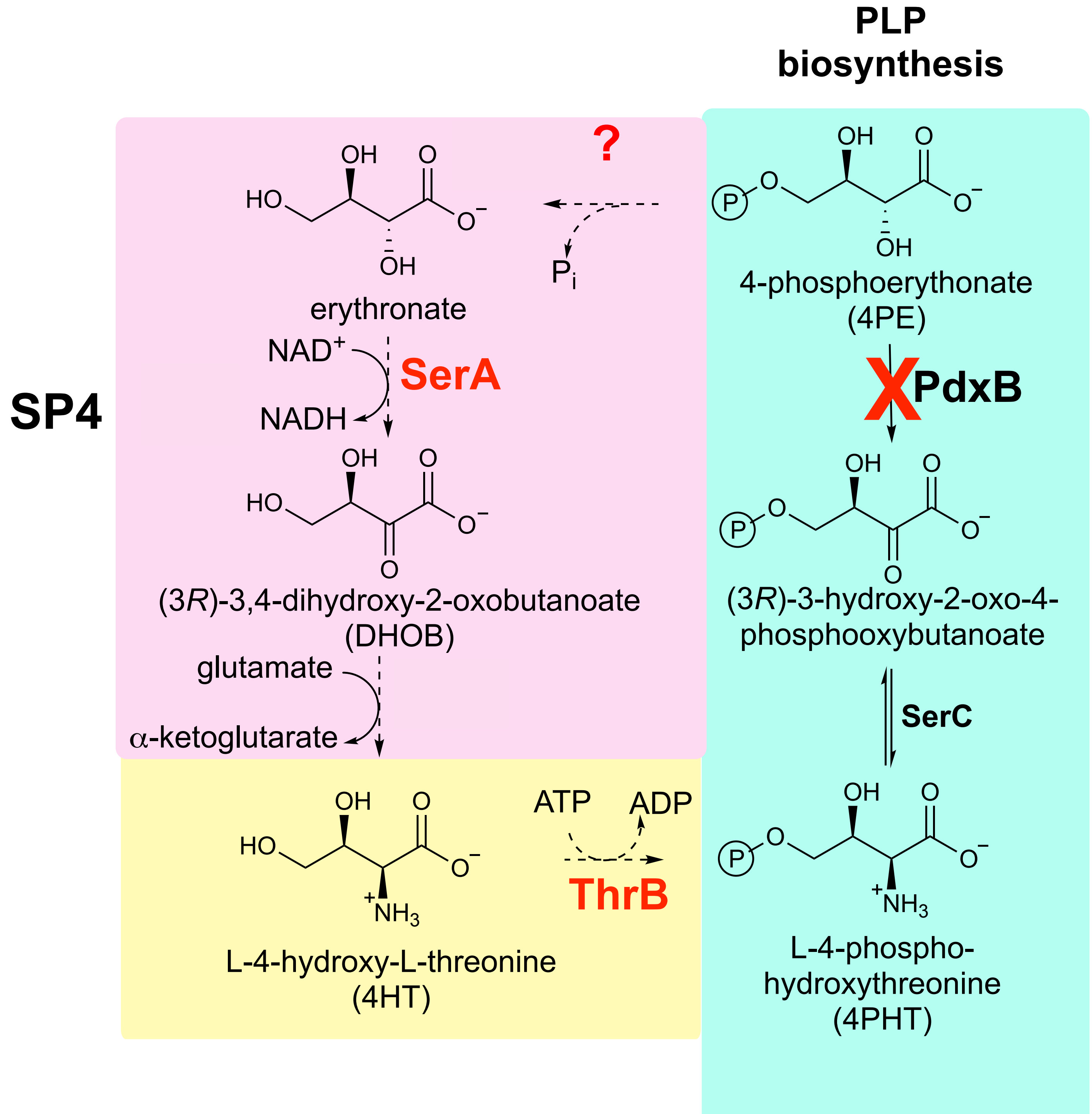
ΔserA JK1



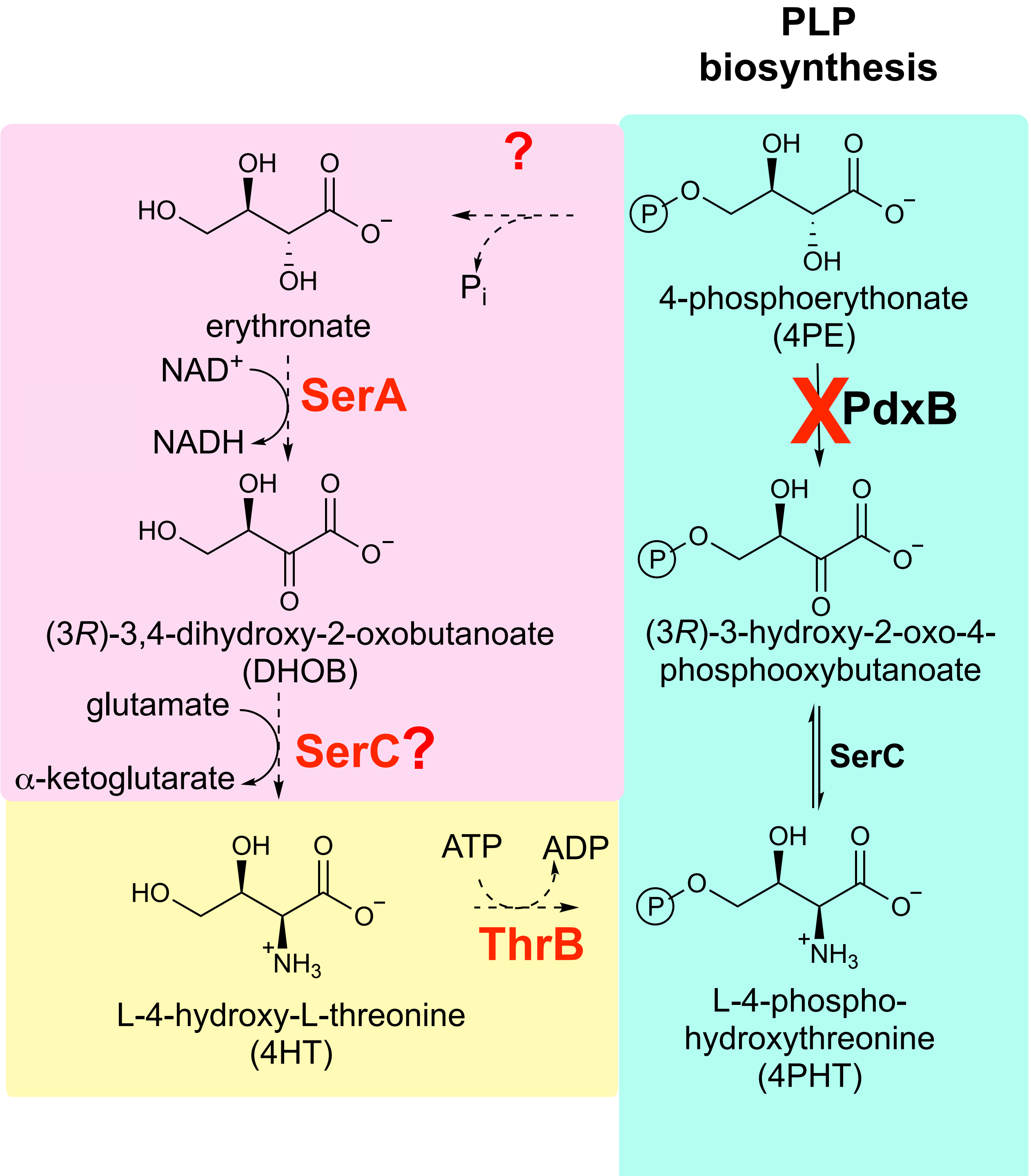
ΔserA JK1



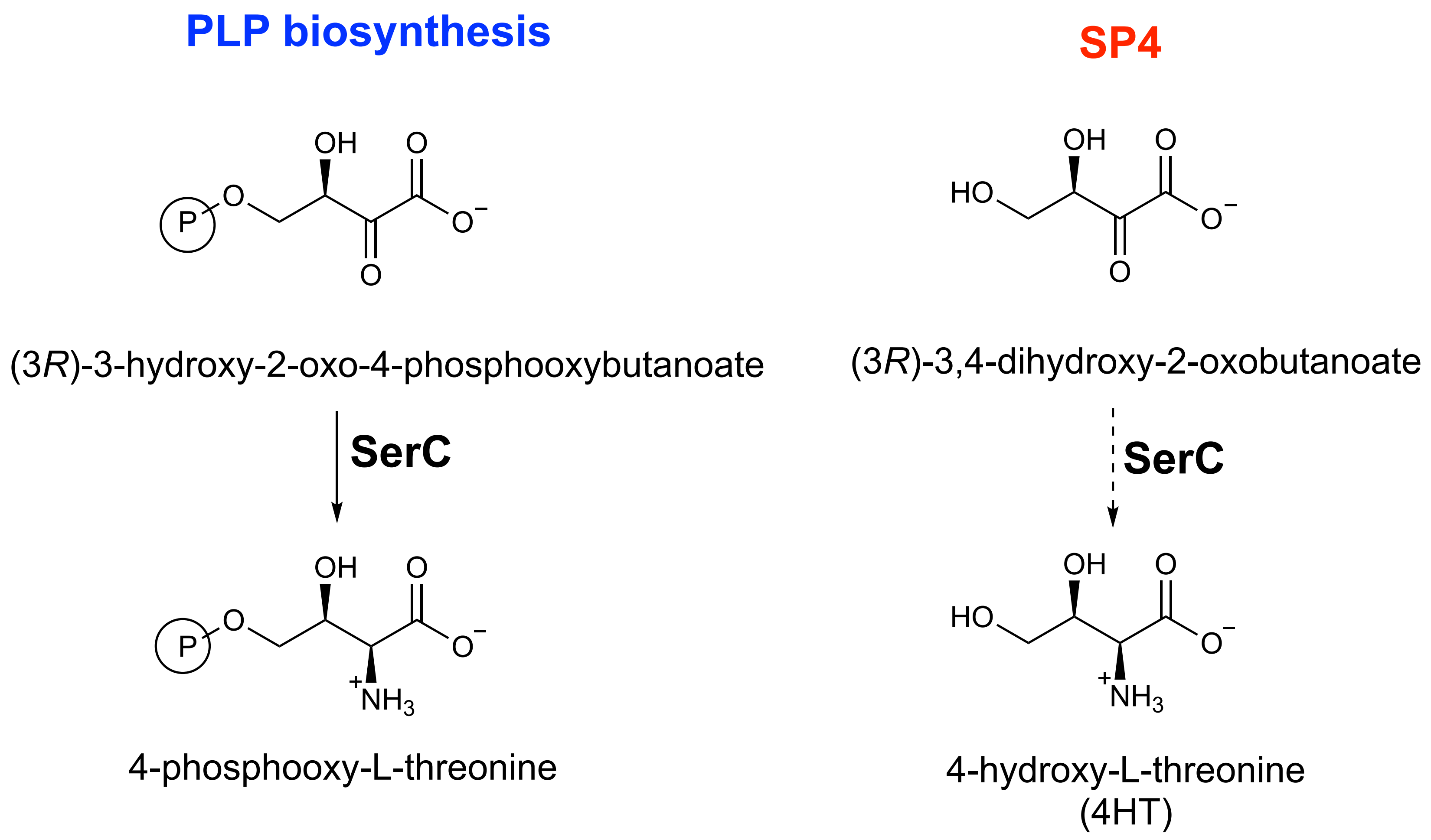
ln(A/A0)
0
1
2
3
4
5
6
time (h)
0
10
20
30
40
50
60
70
80
+ serine
+ pyridoxine
+ serine


PLP biosynthesis
SP4
erythronate
?
P_i
NAD^+
NADH
SerA
(3R)-3,4-dihydroxy-2-oxobutanoate (DHOB)
glutamate
α-ketoglutarate
L-4-hydroxy-L-threonine (4HT)
ATP
ADP
ThrB
4-phosphoerythonate (4PE)
PdxB
(3R)-3-hydroxy-2-oxo-4-phosphooxybutanoate
SerC
L-4-phospho-hydroxythreonine (4PHT)

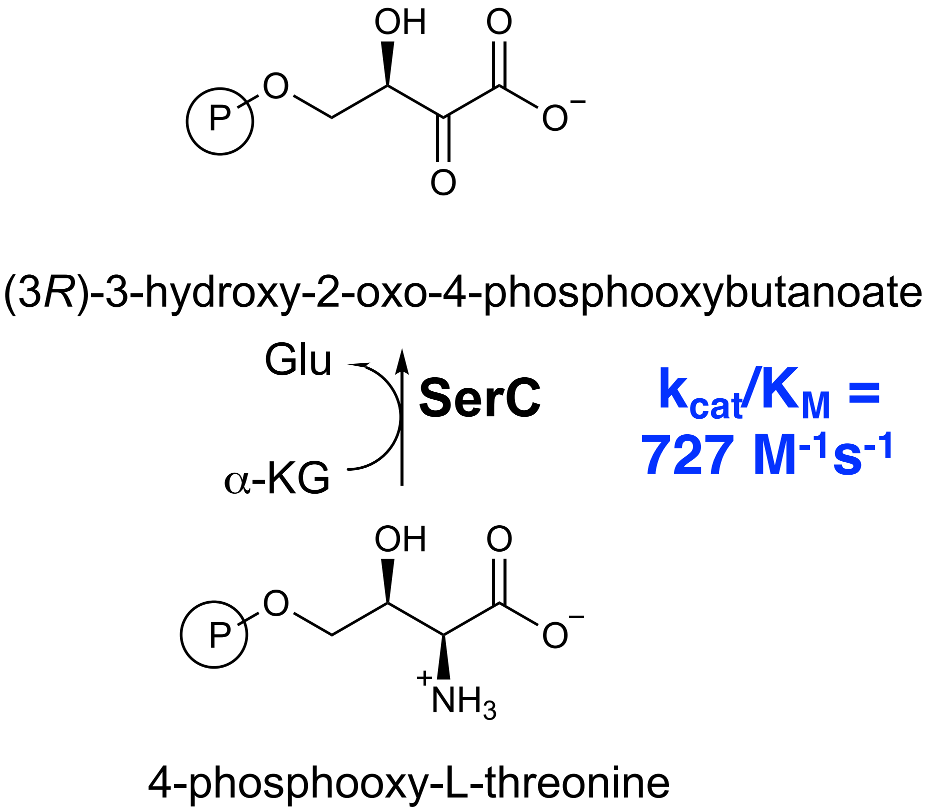
SP4



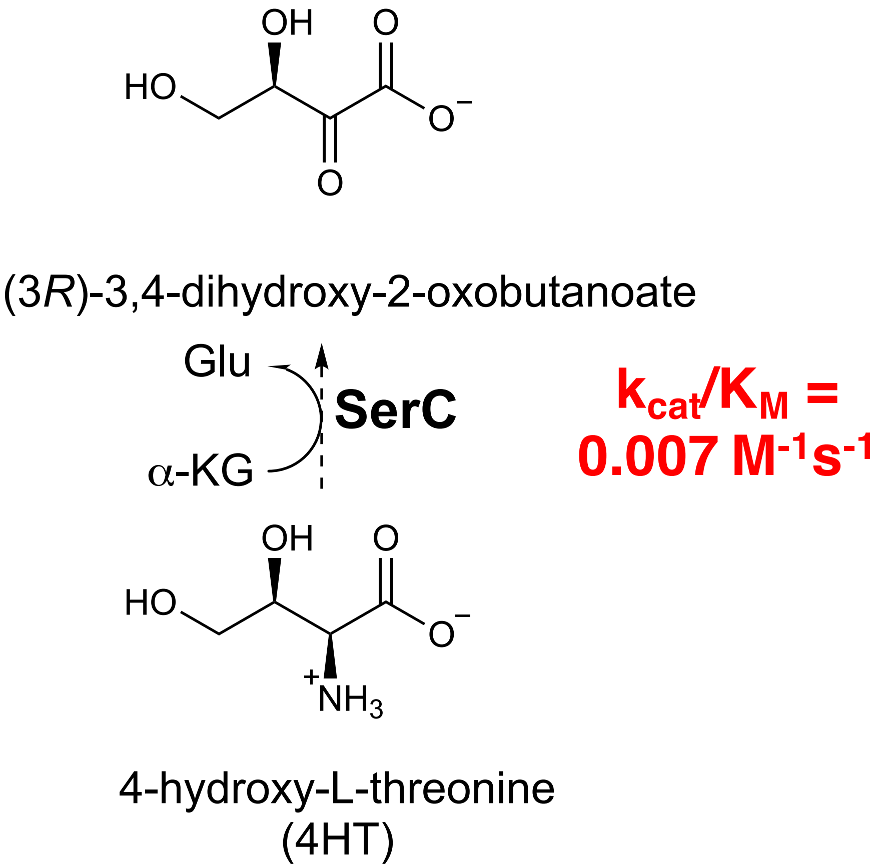
PLP biosynthesis
HO
OH
O
O−
OH
erythronate
?
Pi
P
O
OH
O
O−
OH
4-phosphoerythonate (4PE)
NAD+
NADH
SerA
PdxB
HO
OH
O
O−
O
(3R)-3,4-dihydroxy-2-oxobutanoate (DHOB)
P
O
OH
O
O−
O
(3R)-3-hydroxy-2-oxo-4-phosphooxybutanoate
glutamate
α-ketoglutarate
SerC?
SerC
HO
OH
O
O−
+NH3
L-4-hydroxy-L-threonine (4HT)
ATP
ADP
ThrB
P
O
OH
O
O−
+NH3
L-4-phospho-hydroxythreonine (4PHT)


PLP biosynthesis
SP4
OH
O
P
O
O
O⁻
HO
(3R)-3-hydroxy-2-oxo-4-phosphooxybutanoate
(3R)-3,4-dihydroxy-2-oxobutanoate
SerC
SerC
+NH3
4-phosphooxy-L-threonine
4-hydroxy-L-threonine
(4HT)

PLP biosynthesis

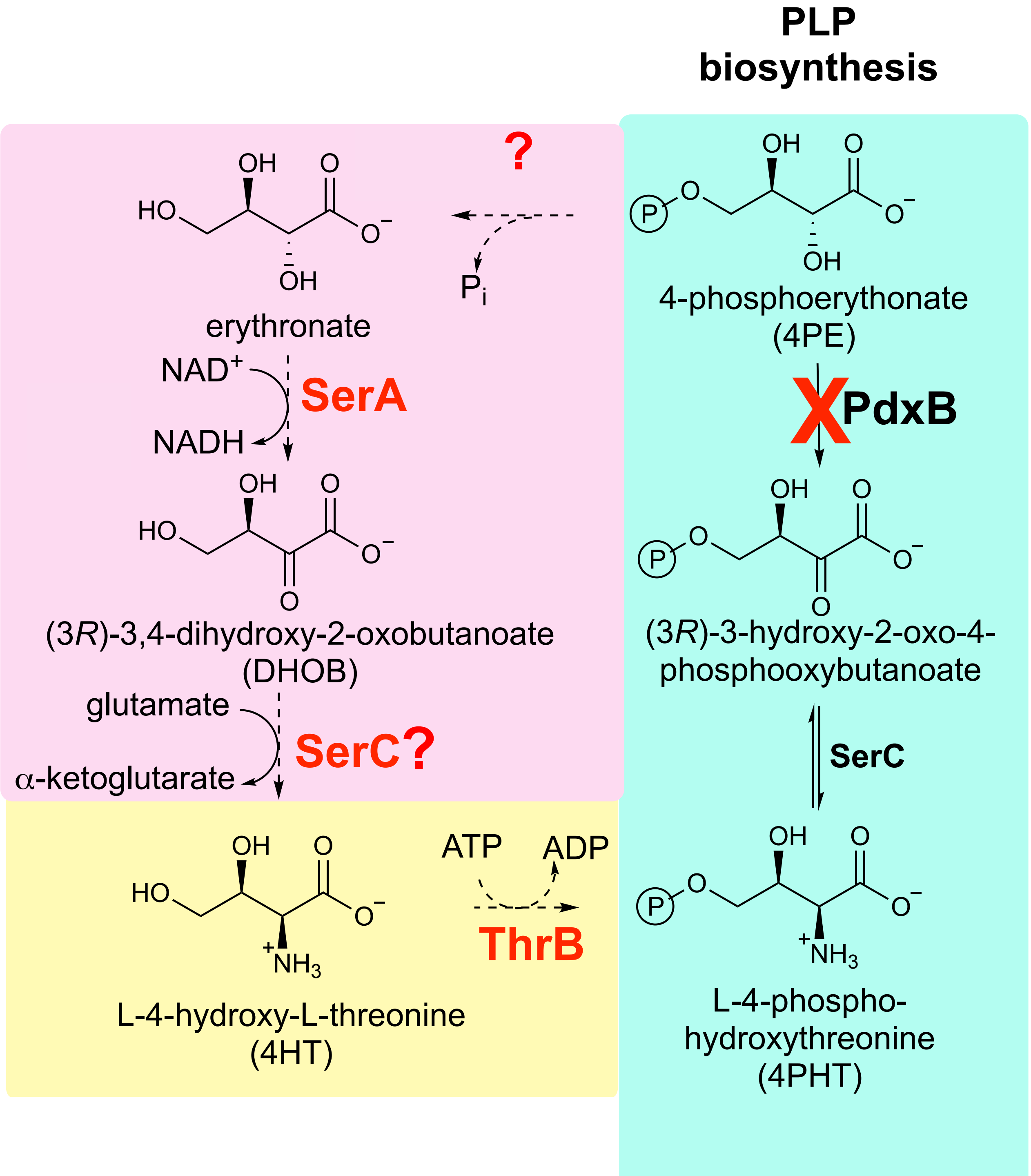


SP4

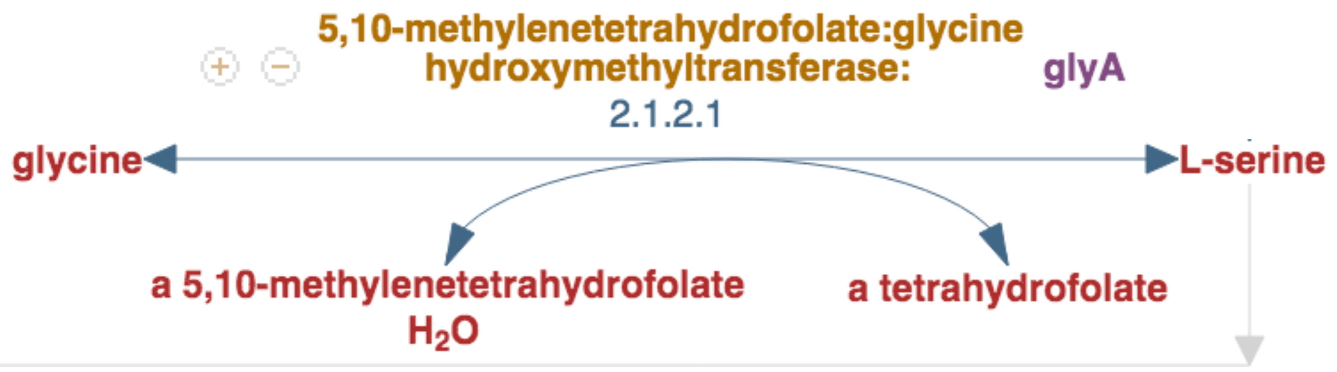


SP4

Can't just delete *serC*……



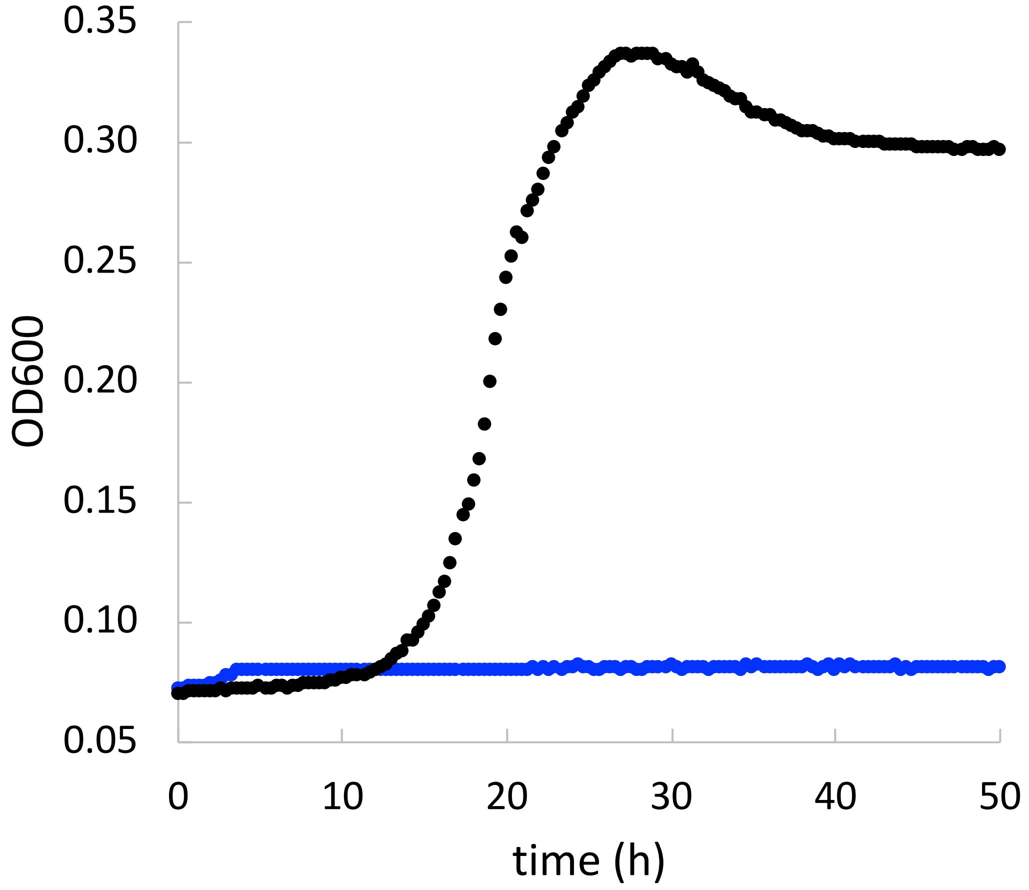
JK1 *serA**



5,10-methylenetetrahydrofolate:glycine hydroxymethyltransferase:
glyA
2.1.2.1
glycine
L-serine
a 5,10-methylenetetrahydrofolate
H_2O
a tetrahydrofolate

JK1 *serA** + gly

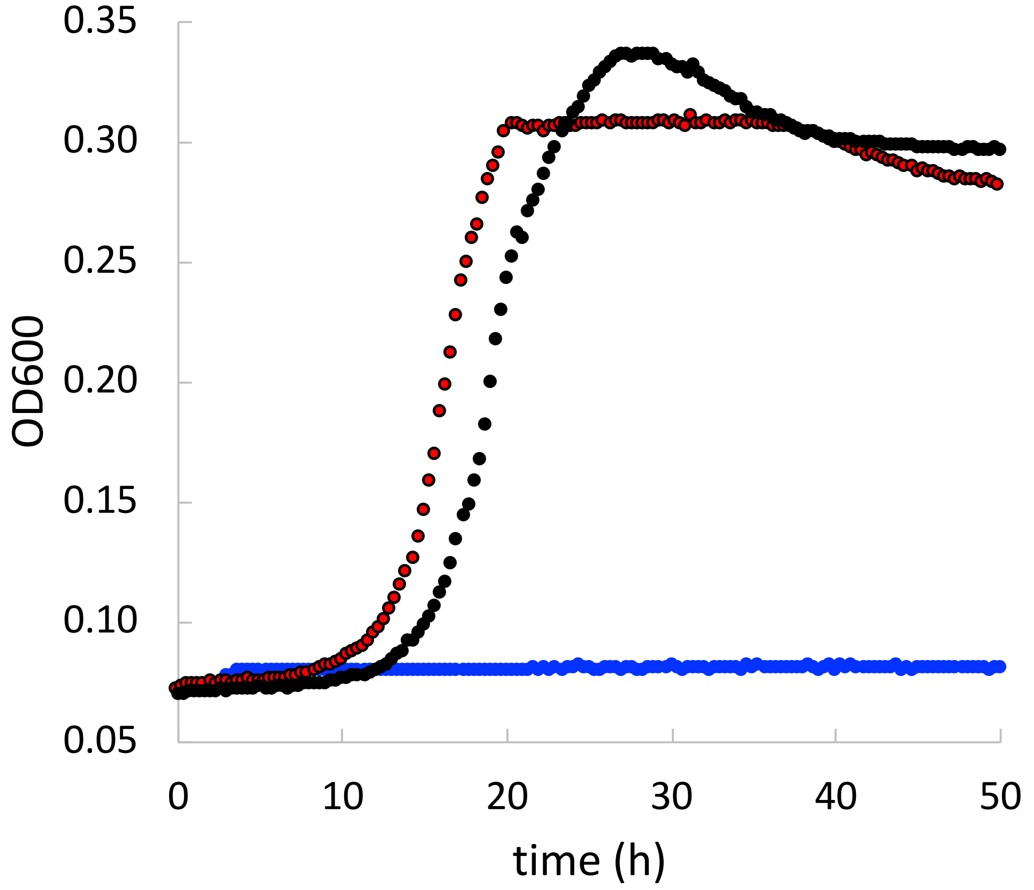
JK1 *serA* ΔserC* + gly

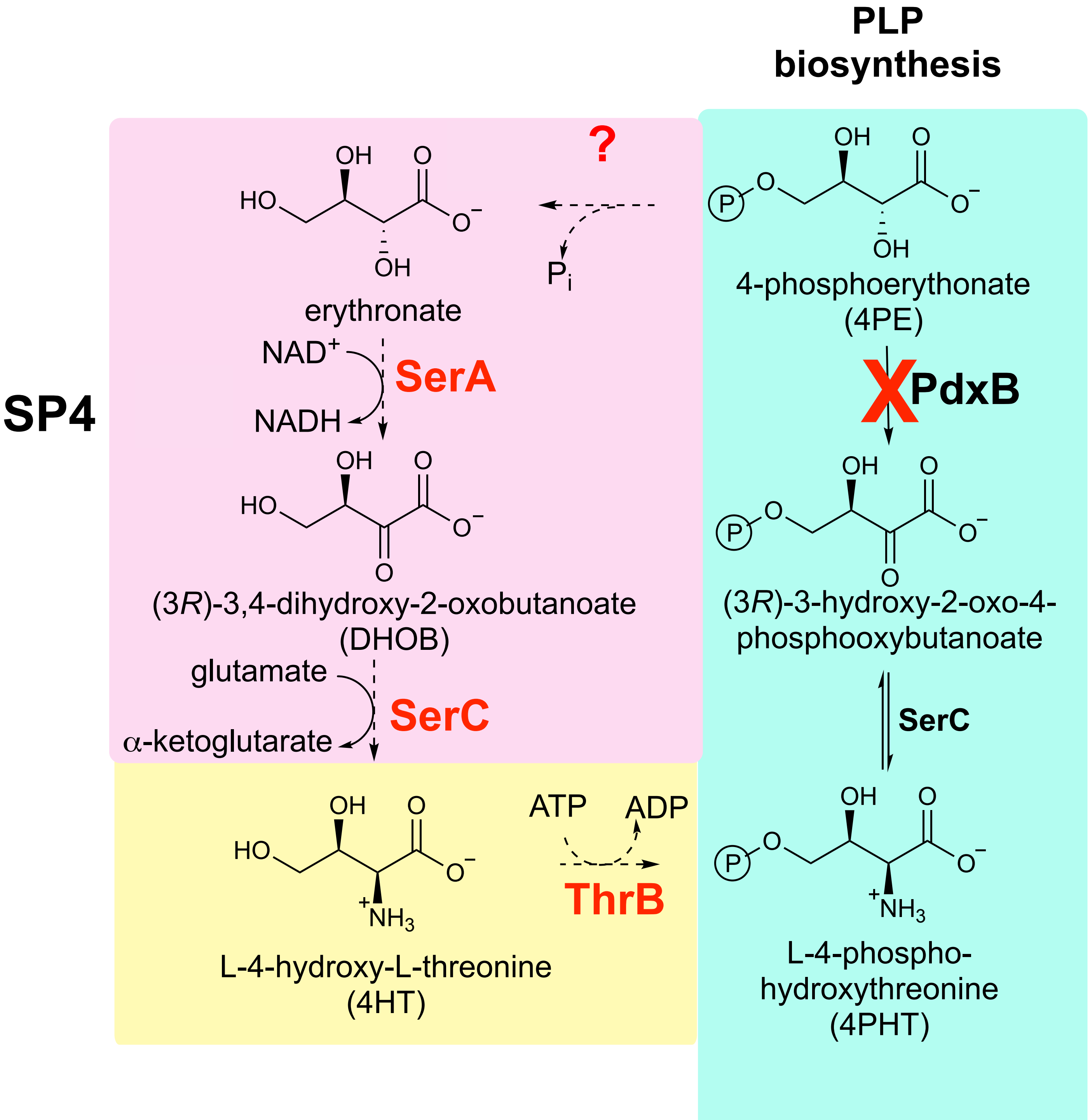


JK1 *serA** + gly

JK1 *serA* ΔserC* + gly

JK1 *serA* ΔserC* + gly + pyr





PLP
biosynthesis
SP4
?
erythronate
P
i
NAD+
NADH
SerA
(3R)-3,4-dihydroxy-2-oxobutanoate
(DHOB)
glutamate
α-ketoglutarate
SerC
L-4-hydroxy-L-threonine
(4HT)
ATP
ADP
ThrB
4-phosphoerythonate
(4PE)
PdxB
(3R)-3-hydroxy-2-oxo-4-
phosphooxybutanoate
SerC
L-4-phospho-
hydroxythreonine
(4PHT)

1) How are the evolved strains making PLP?

2) **How do mutations improve PLP synthesis?**

Mutations in evolved strains

JK1
ybhA/pgl
gapA
rpoS
rpoC

JK2
ybhA/pgl
gapA
purF
gltB
ypjA

JK3
ybhA/pgl
gapA
ilvH
rng

JK4
ybhA
rpe
sdhA
rho
lon

JK5
ybhA/pgl
gapA
yjjK
purF
ilvH
nadR

JK6
gapA
serA
yjjK

JK7a
ybhA
gapA
purF
nadR
rpoS

JK7b
ybhA/pgl
serA
gapA
pykF
pyrE

JK8
gapA
serA
yjjK
gltB
livH

JK9
ybhA/pgl
gapA
serA
pykF

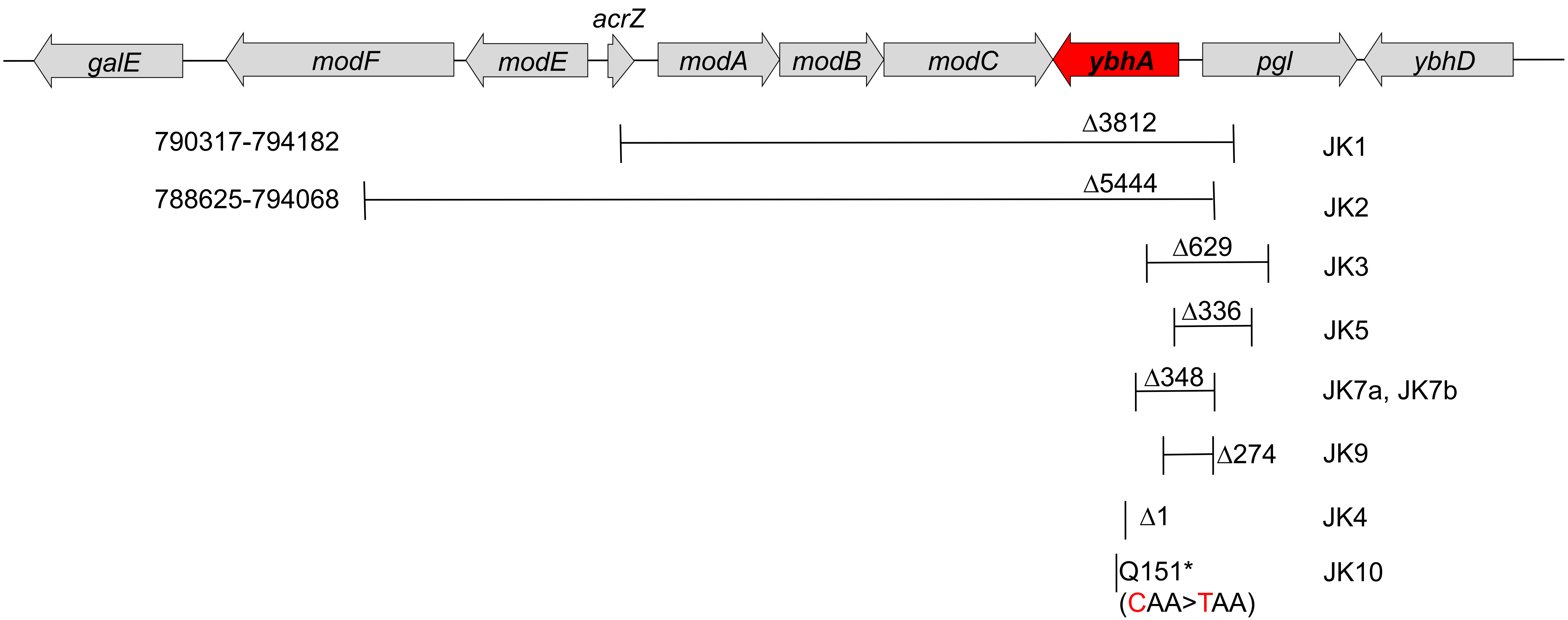
JK10
ybhA/pgl
gapA
rpe
ilvH
rng

Mutations in evolved strains

JK1	**JK2**	**JK3**	**JK4**	**JK5**	**JK6**
ybhA/pgl	***ybhA/pgl***	***ybhA/pgl***	***ybhA***	***ybhA/pgl***	***gapA***
gapA	***gapA***	***gapA***	*rpe*	***gapA***	***serA***
rpoS	*purF*	*ilvH*	*sdhA*	*yjjK*	*yjjK*
rpoC	*gltB*	*rng*	*rho*	*purF*	
	ypjA		*lon*	*ilvH*	
				nadR	

JK7a	**JK7b**	**JK8**	**JK9**	**JK10**
ybhA	***ybhA/pgl***	***gapA***	***ybhA/pgl***	***ybhA/pgl***
gapA	***serA***	***serA***	***gapA***	***gapA***
purF	***gapA***	*yjjK*	***serA***	*rpe*
nadR	*pykF*	*gltB*	*pykF*	*ilvH*
rpoS	*pyrE*	*livH*		*rng*

Mutations in most evolved strains cause loss of YbhA function



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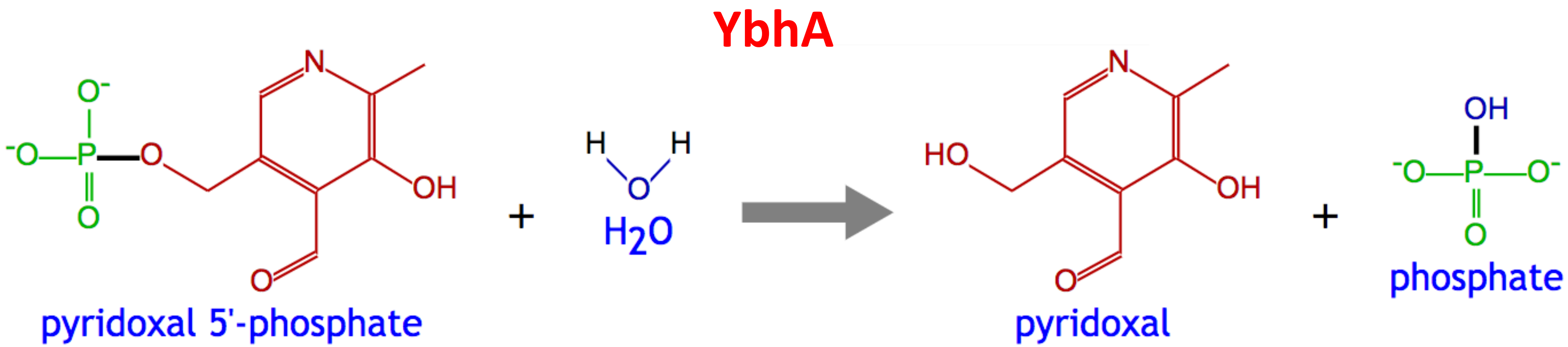
Genome-wide Analysis of Substrate Specificities of the *Escherichia coli* Haloacid Dehalogenase-like Phosphatase Family*[S]

Received for publication, June 7, 2006, and in revised form, September 21, 2006 Published, JBC Papers in Press, September 21, 2006, DOI 10.1074/jbc.M605449200

Ekaterina Kuznetsova[‡§¶], Michael Proudfoot[‡], Claudio F. Gonzalez[‡], Greg Brown[‡], Marina V. Omelchenko[||], Ivan Borozan[‡], Liran Carmel[||], Yuri I. Wolf[||], Hirotada Mori[**], Alexei V. Savchenko[‡§¶], Cheryl H. Arrowsmith[‡‡‡1], Eugene V. Koonin[||], Aled M. Edwards[‡§¶2], and Alexander F. Yakunin[‡¶3]



Mutations in evolved strains

JK1
ybhA/pgl
gapA
rpoS
rpoC

JK2
ybhA/pgl
gapA
purF
gltB
ypjA

JK3
ybhA/pgl
gapA
ilvH
rng

JK4
ybhA
rpe
sdhA
rho
lon

JK5
ybhA/pgl
gapA
yjjK
purF
ilvH
nadR

JK6
gapA
serA
yjjK

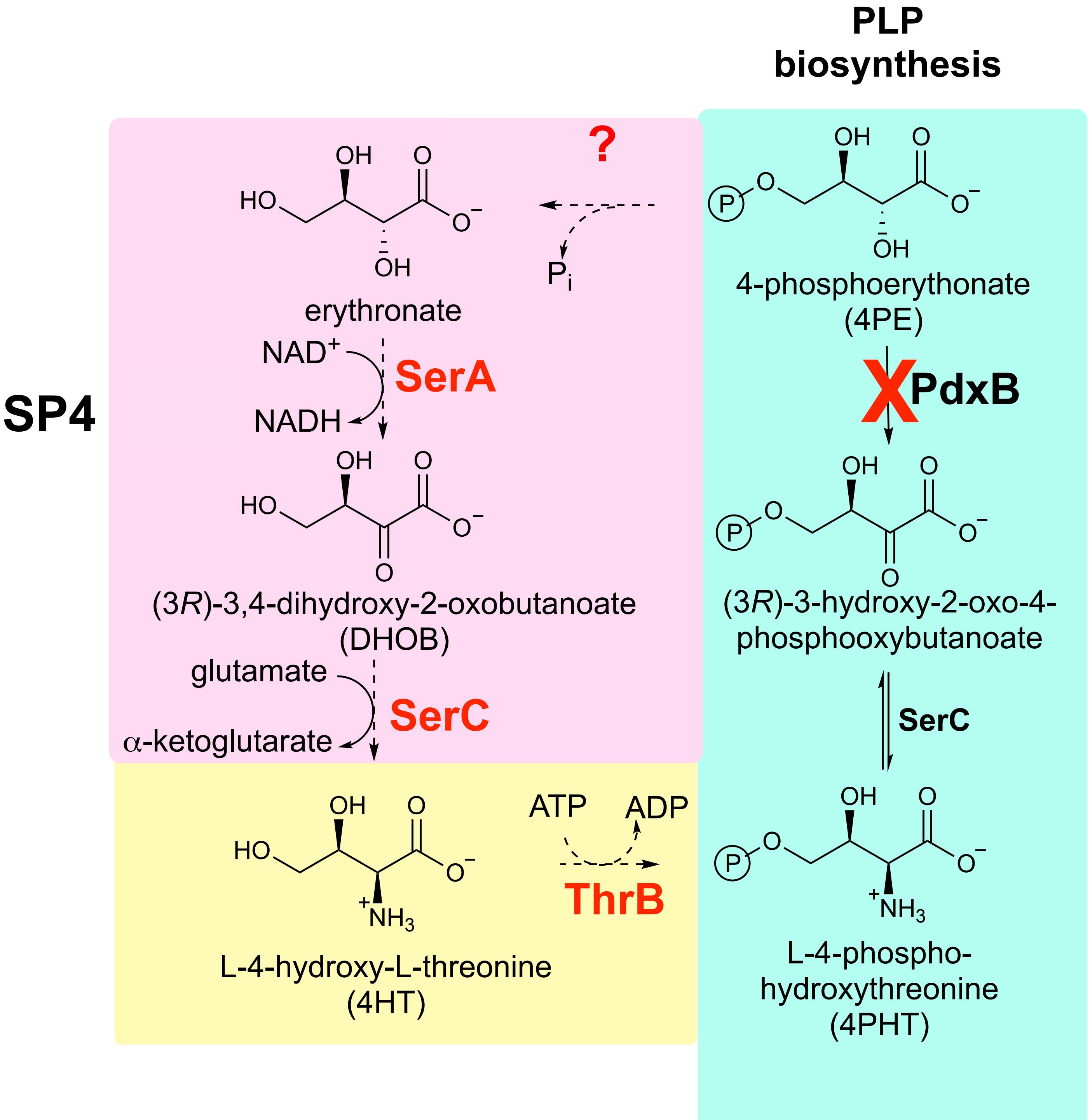
JK7a
ybhA
gapA
purF
nadR
rpoS

JK7b
ybhA/pgl
serA
gapA
pykF
pyrE

JK8
gapA
serA
yjjK
gltB
livH

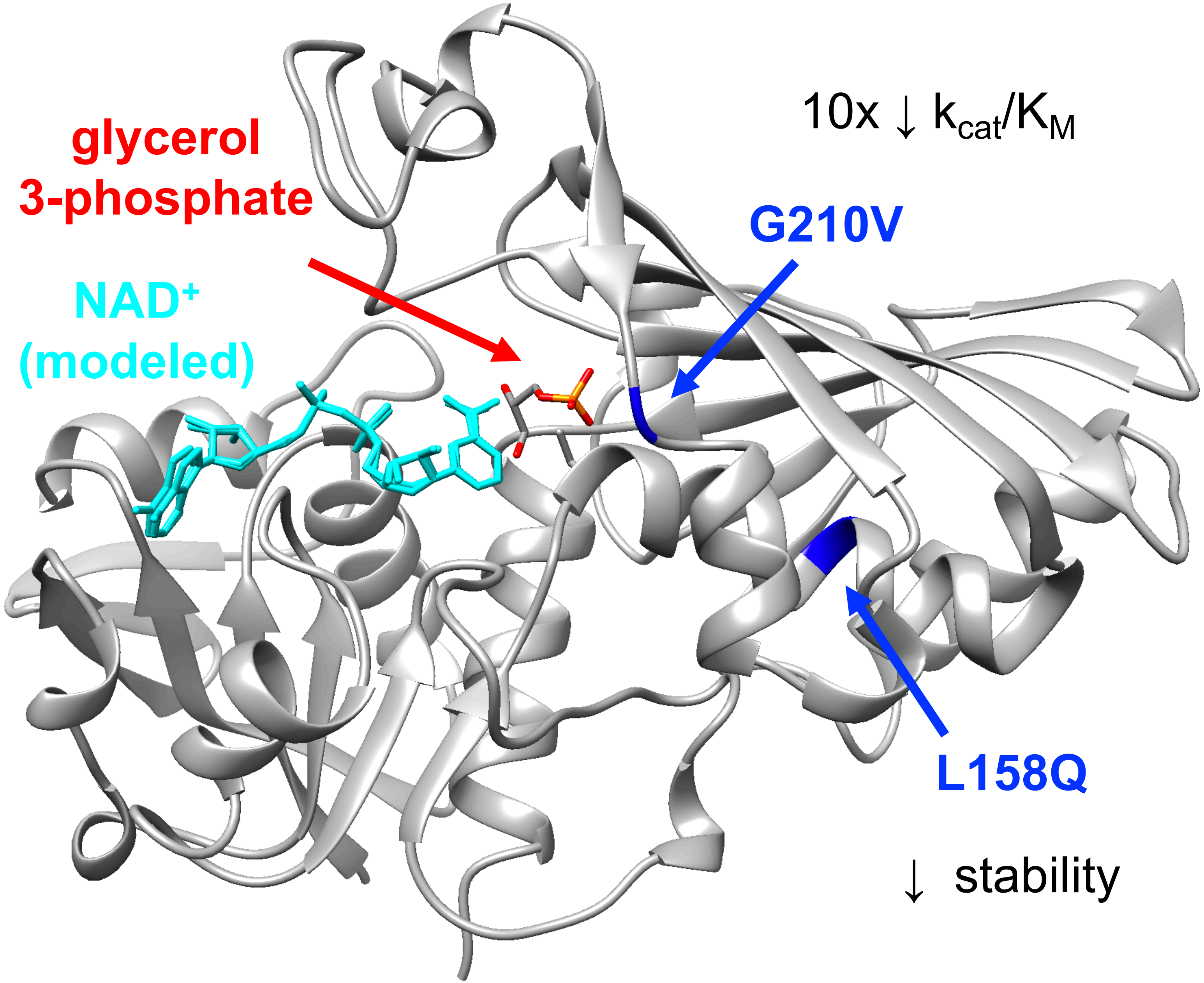
JK9
ybhA/pgl
gapA
serA
pykF

JK10
ybhA/pgl
gapA
rpe
ilvH
rng

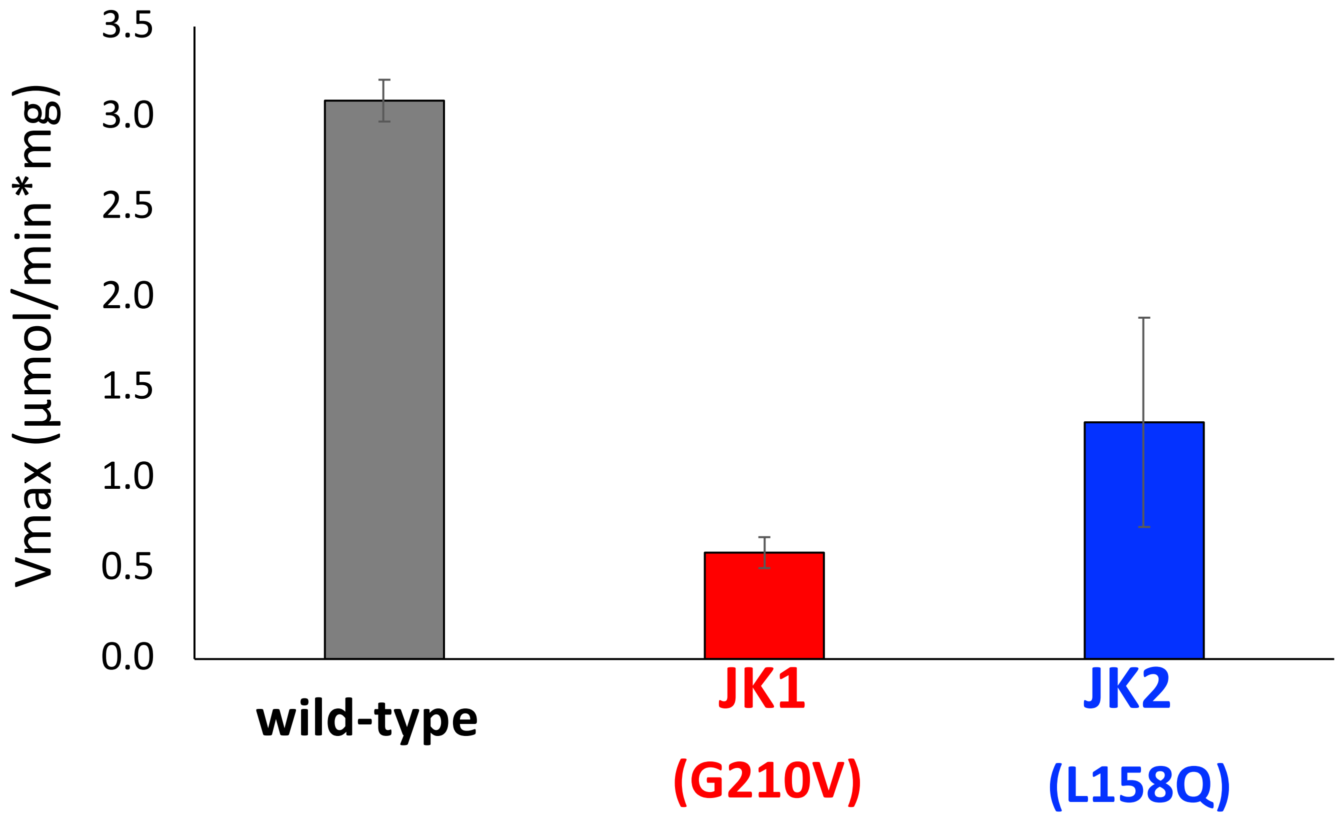


PLP biosynthesis
SP4
?
Pi
erythronate
NAD+
NADH
SerA
(3R)-3,4-dihydroxy-2-oxobutanoate (DHOB)
glutamate
α-ketoglutarate
SerC
ATP
ADP
ThrB
L-4-hydroxy-L-threonine (4HT)
4-phosphoerythonate (4PE)
PdxB
(3R)-3-hydroxy-2-oxo-4-phosphooxybutanoate
SerC
L-4-phospho-hydroxythreonine (4PHT)

E. coli GapA (glyceraldehyde 3-phosphate dehydrogenase, GAPDH)

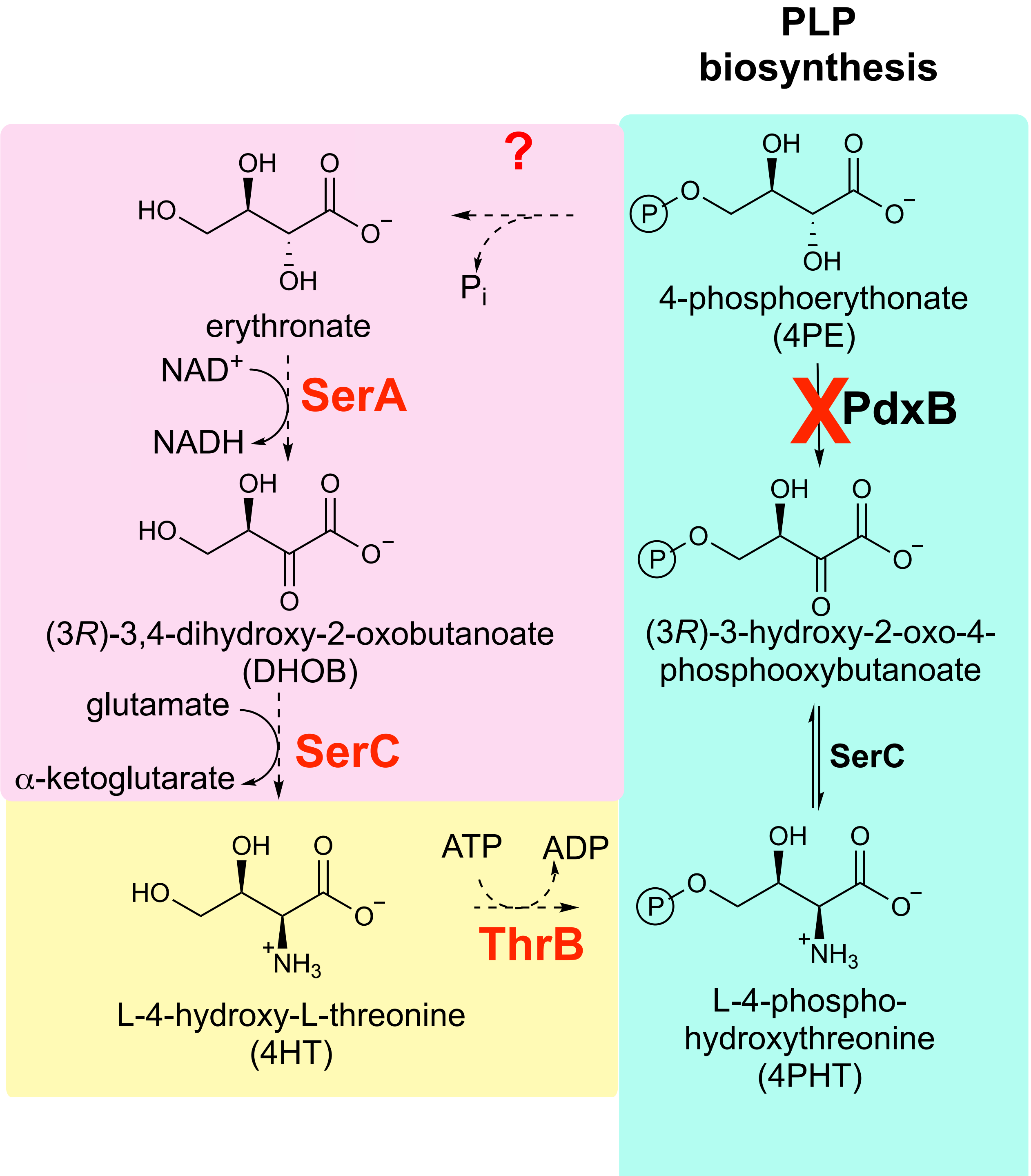


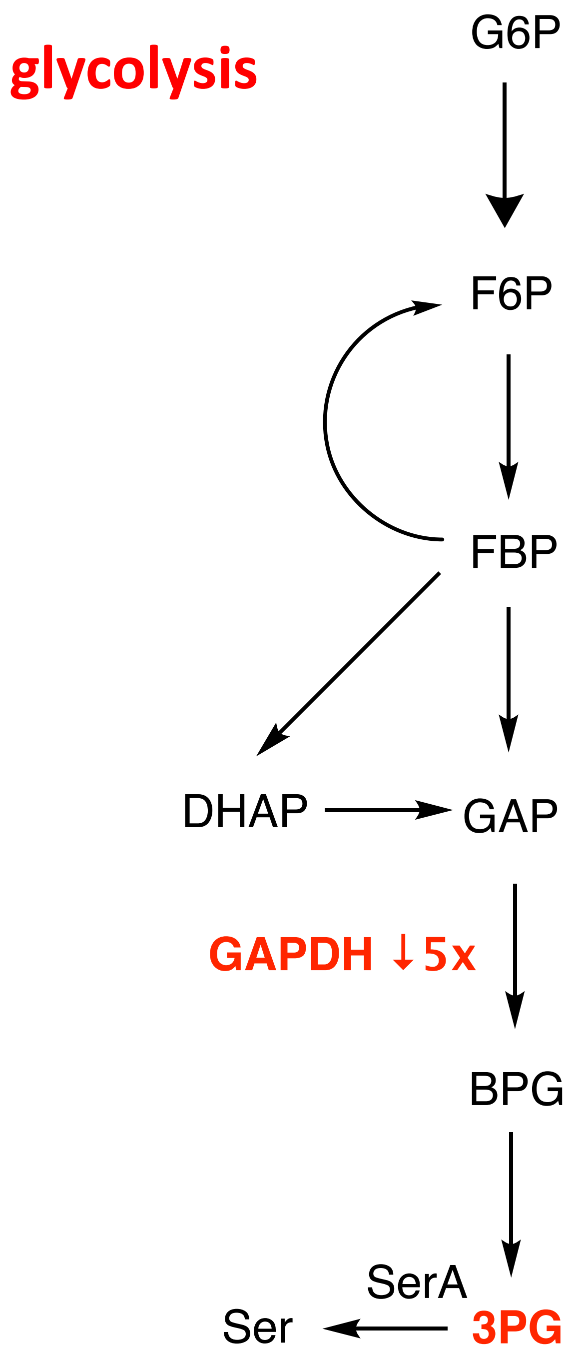
GAPDH activity in crude lysates

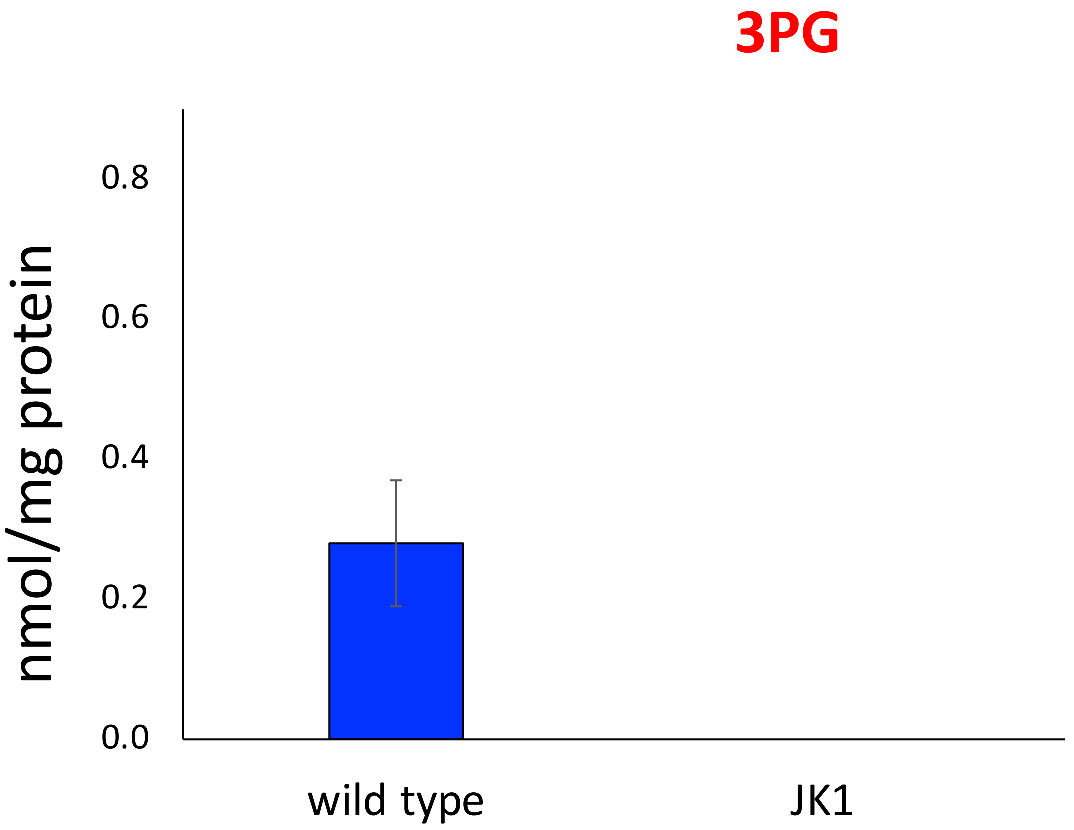


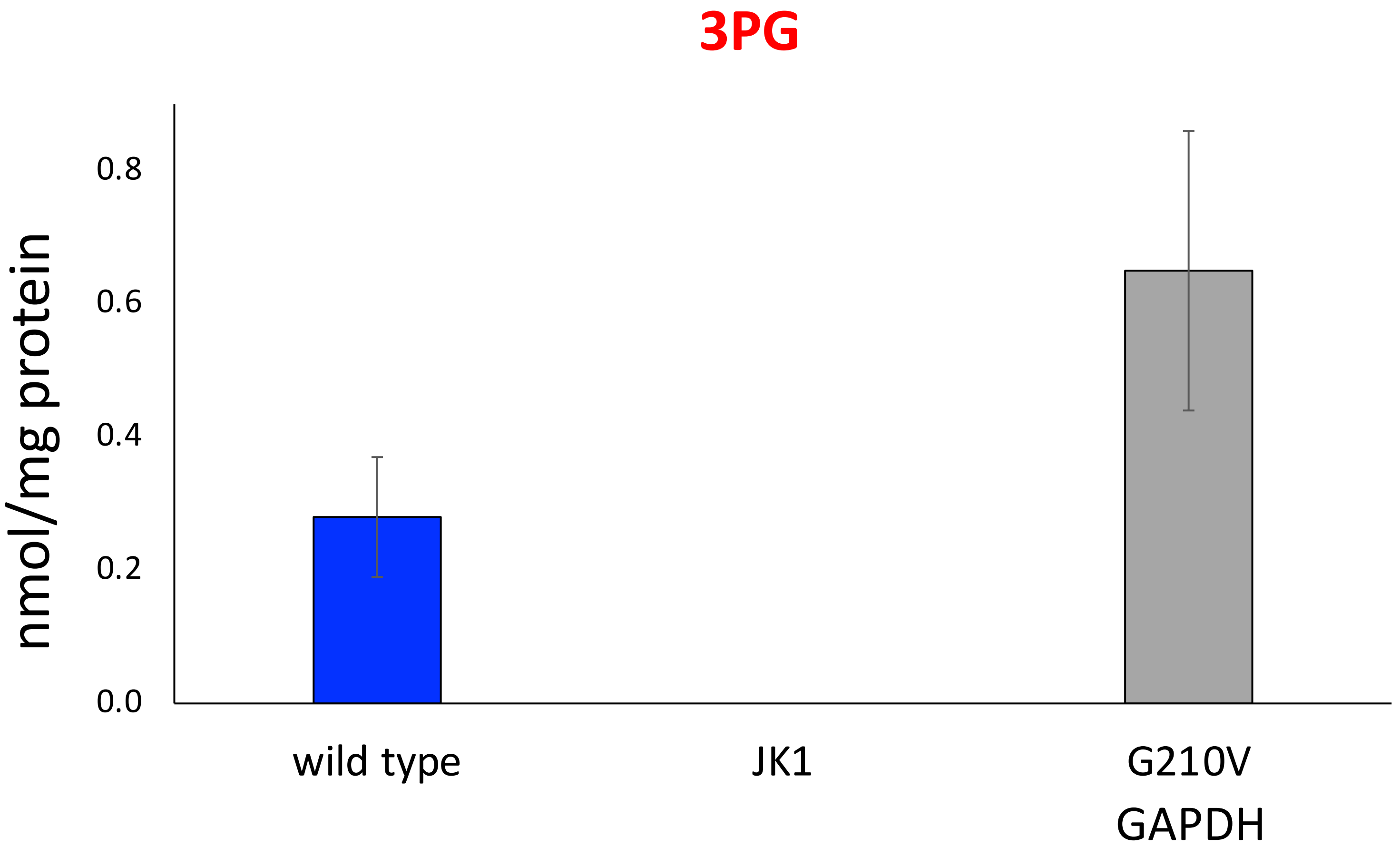
Why would decreased GAPDH activity improve PLP synthesis?

SP4

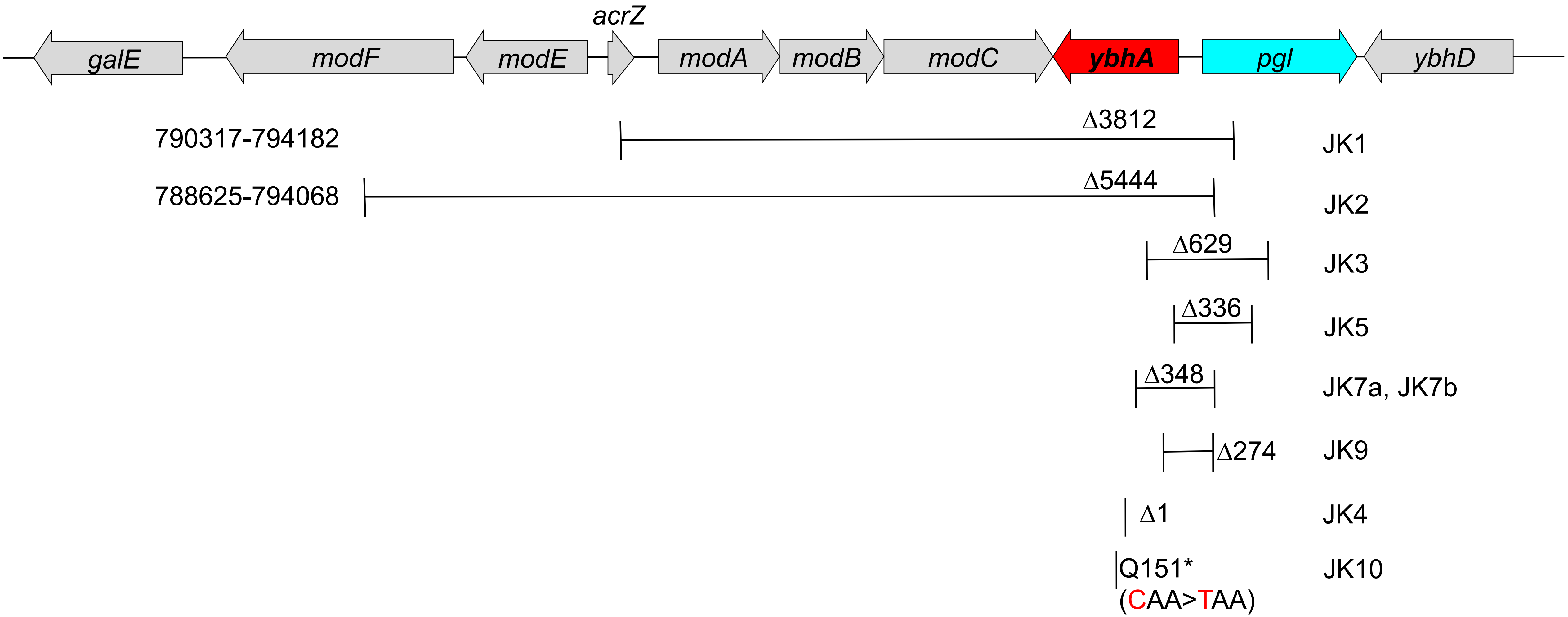



glycolysis
G6P
F6P
FBP
DHAP
GAP
GAPDH ↓5x
BPG
SerA
Ser
3PG

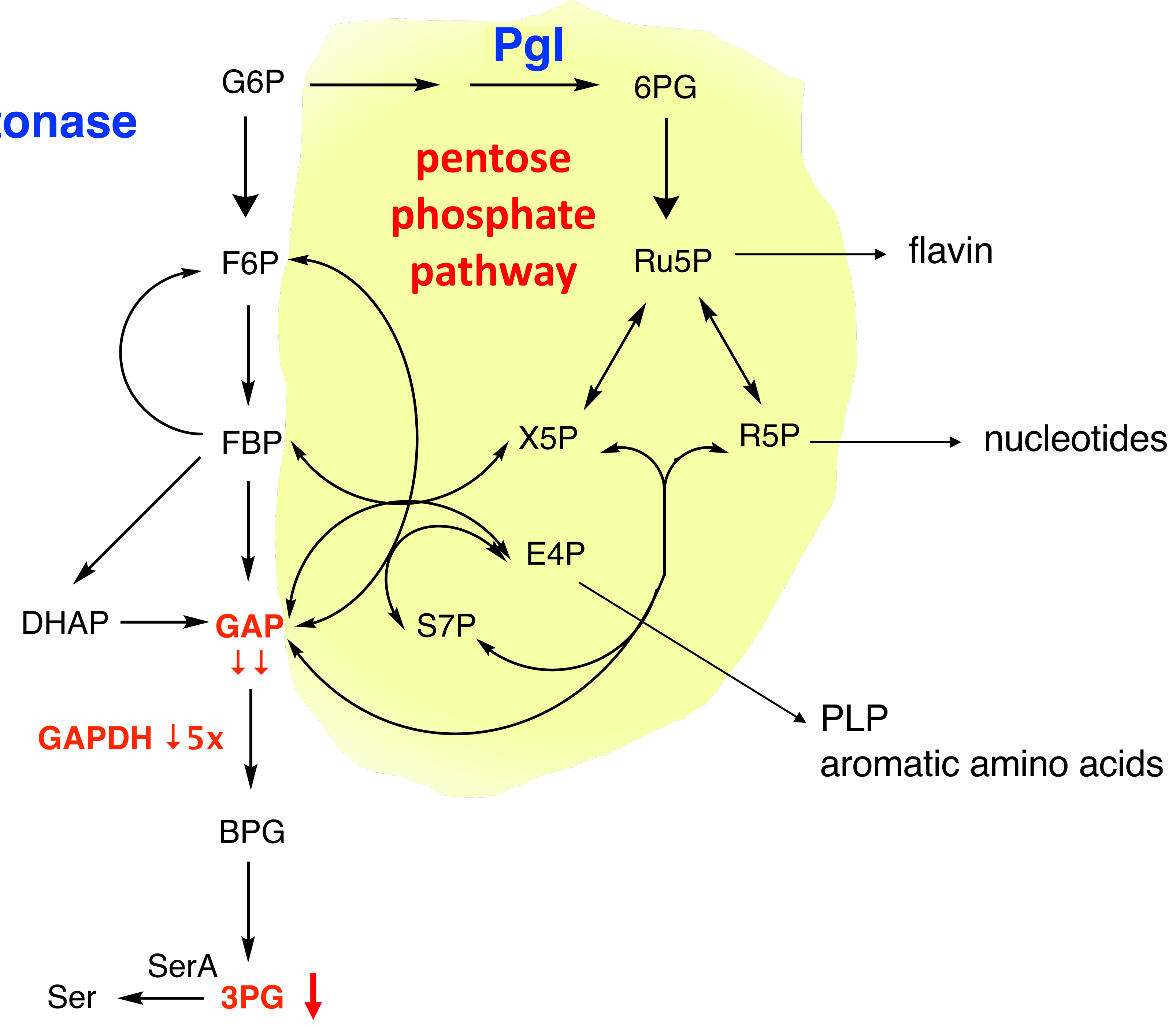

3PG
nmol/mg protein
0.8
0.6
0.4
0.2
0.0
wild type
JK1


3PG
nmol/mg protein
0.8
0.6
0.4
0.2
0.0
wild type
JK1
G210V
GAPDH

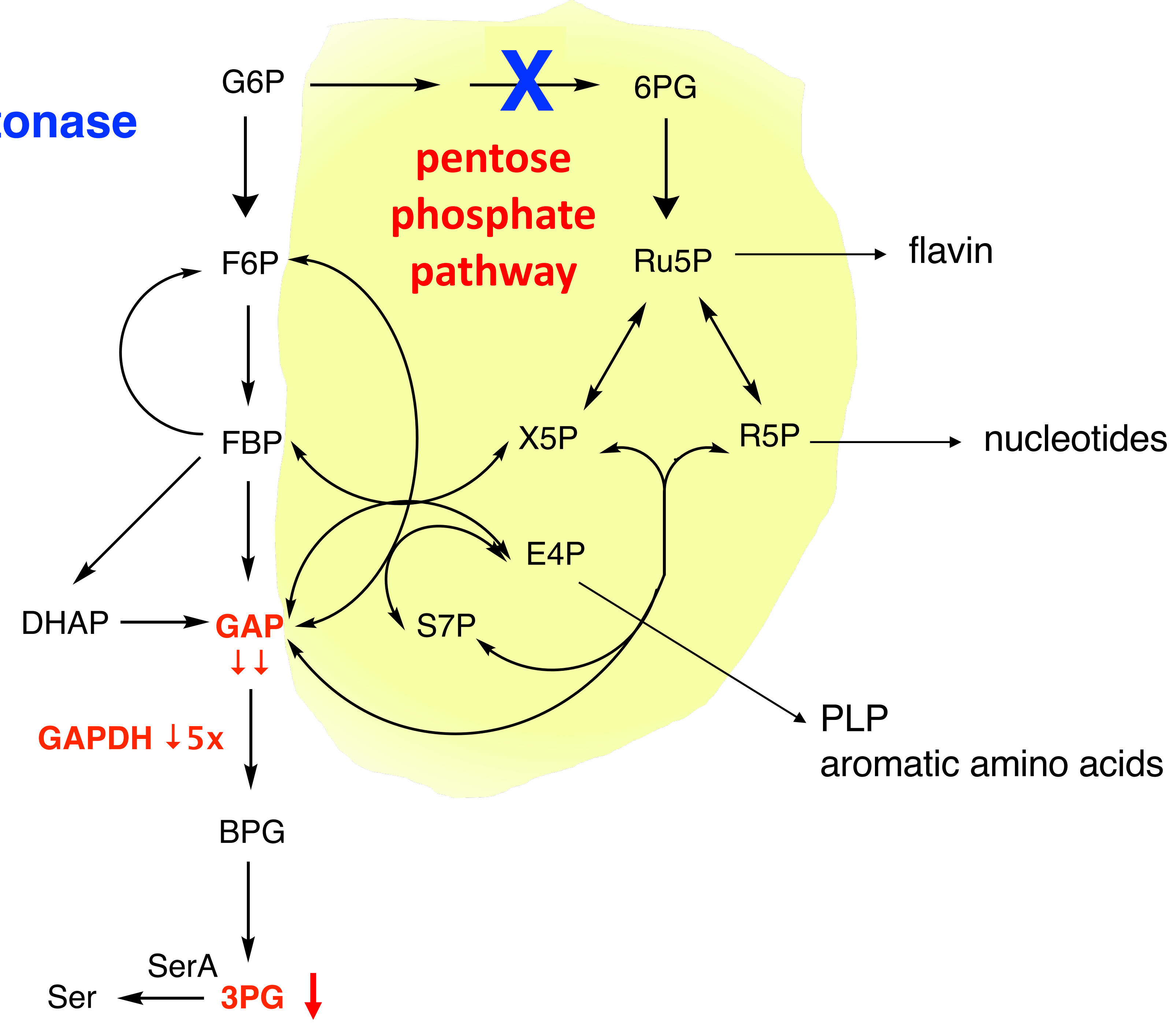
A closer look……



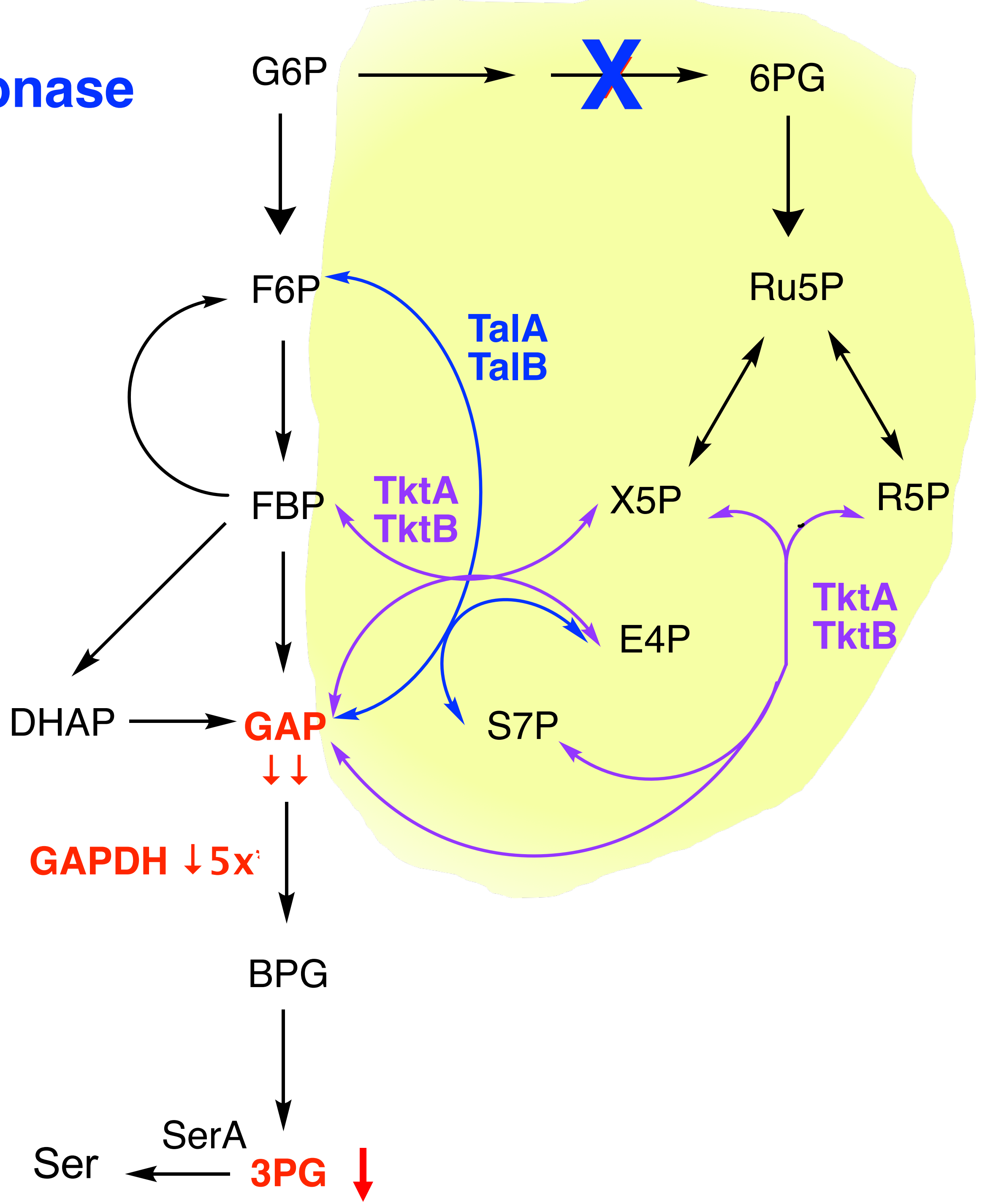
Pgl = 6-phosphogluconolactonase



Pgl = 6-phosphogluconolactonase



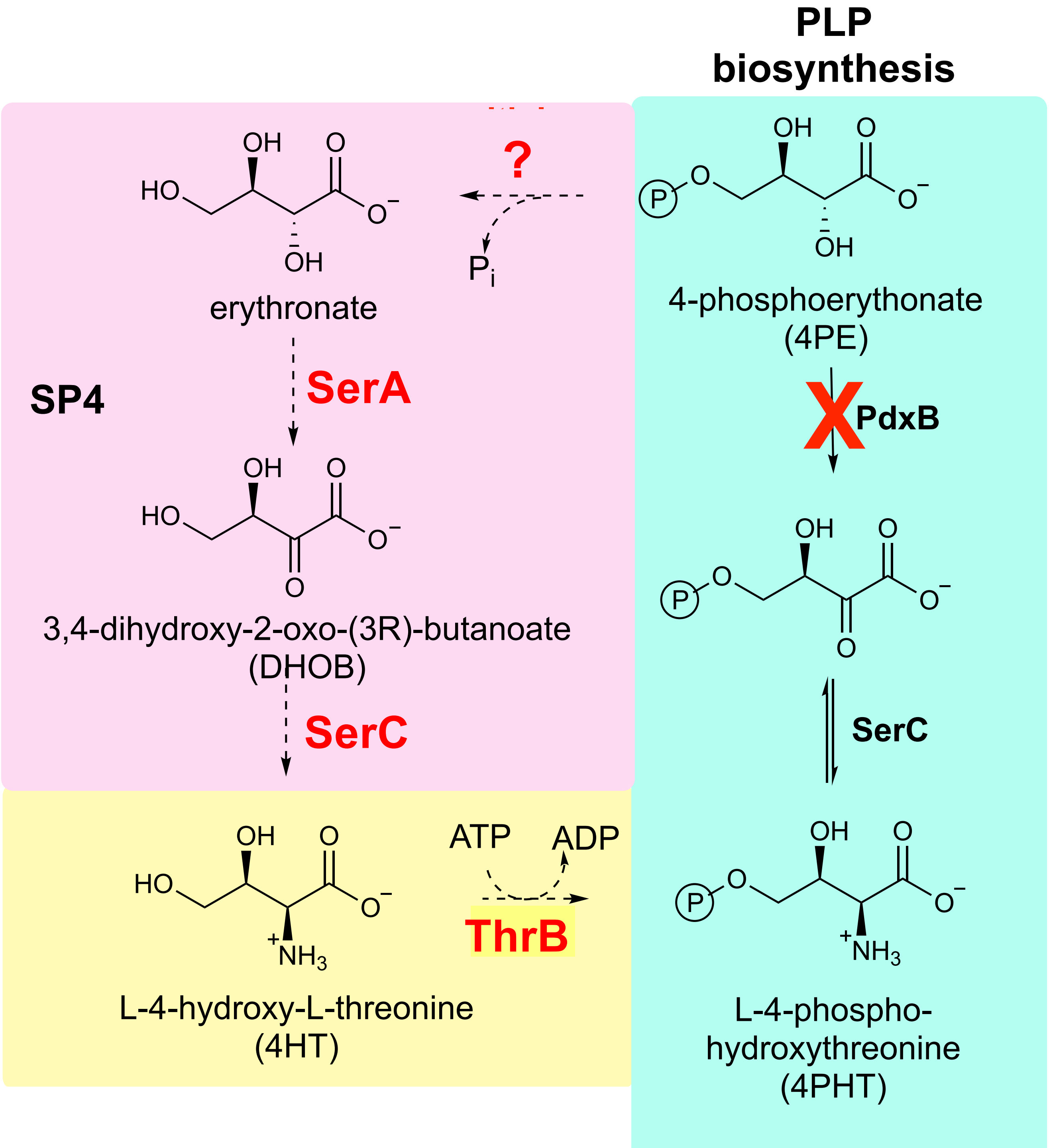
Pgl = 6-phosphogluconolactonase



Mutations in evolved strains

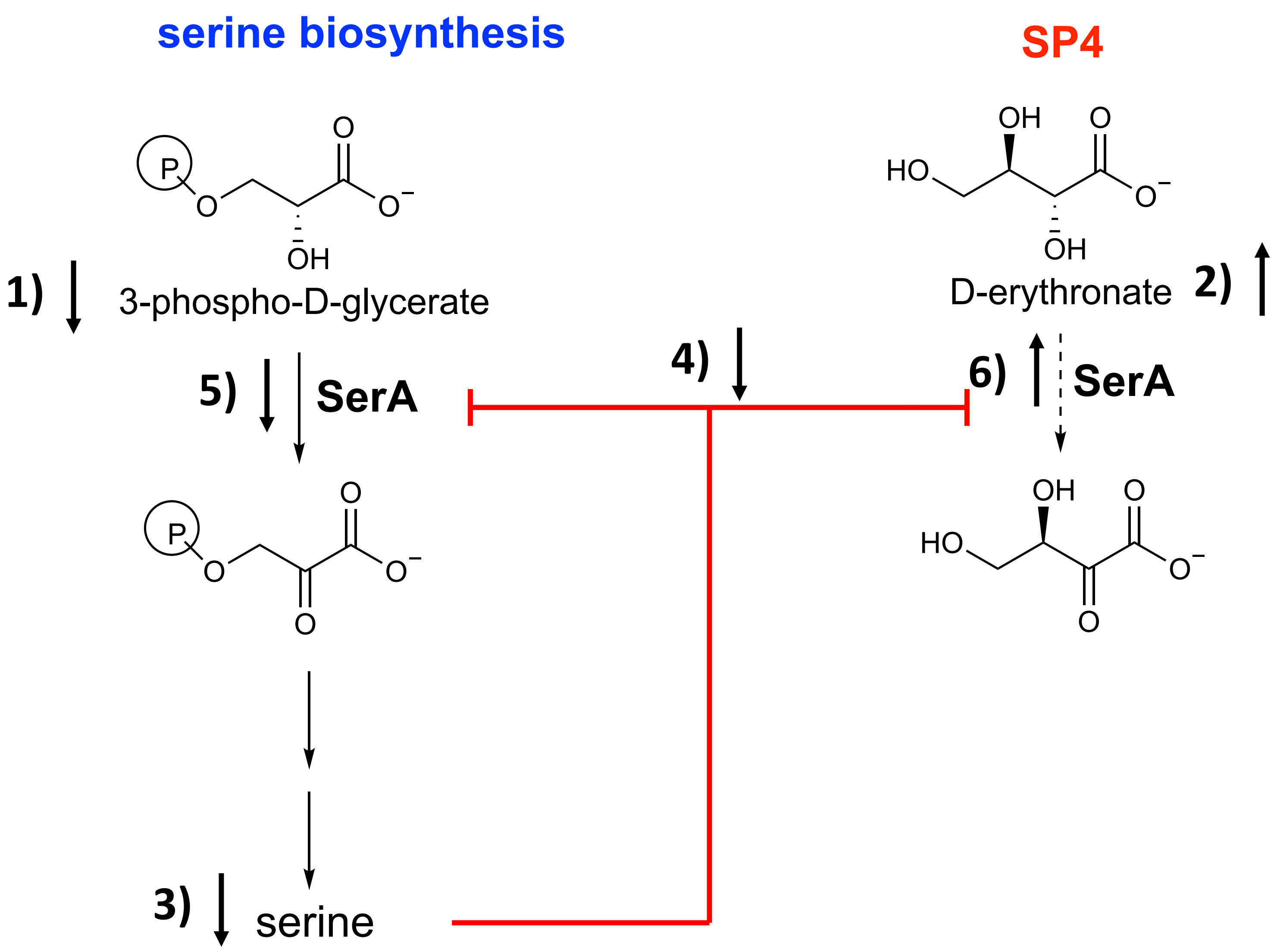
JK1	**JK2**	**JK3**	**JK4**	**JK5**	**JK6**
ybhA/pgl	*ybhA/pgl*	*ybhA/pgl*	*ybhA*	*ybhA/pgl*	*gapA*
gapA	*gapA*	*gapA*	*rpe*	*gapA*	***serA***
rpoS	*purF*	*ilvH*	*sdhA*	*yjjK*	*yjjK*
rpoC	*gltB*	*rng*	*rho*	*purF*	
	ypjA		*lon*	*ilvH*	
				nadR	

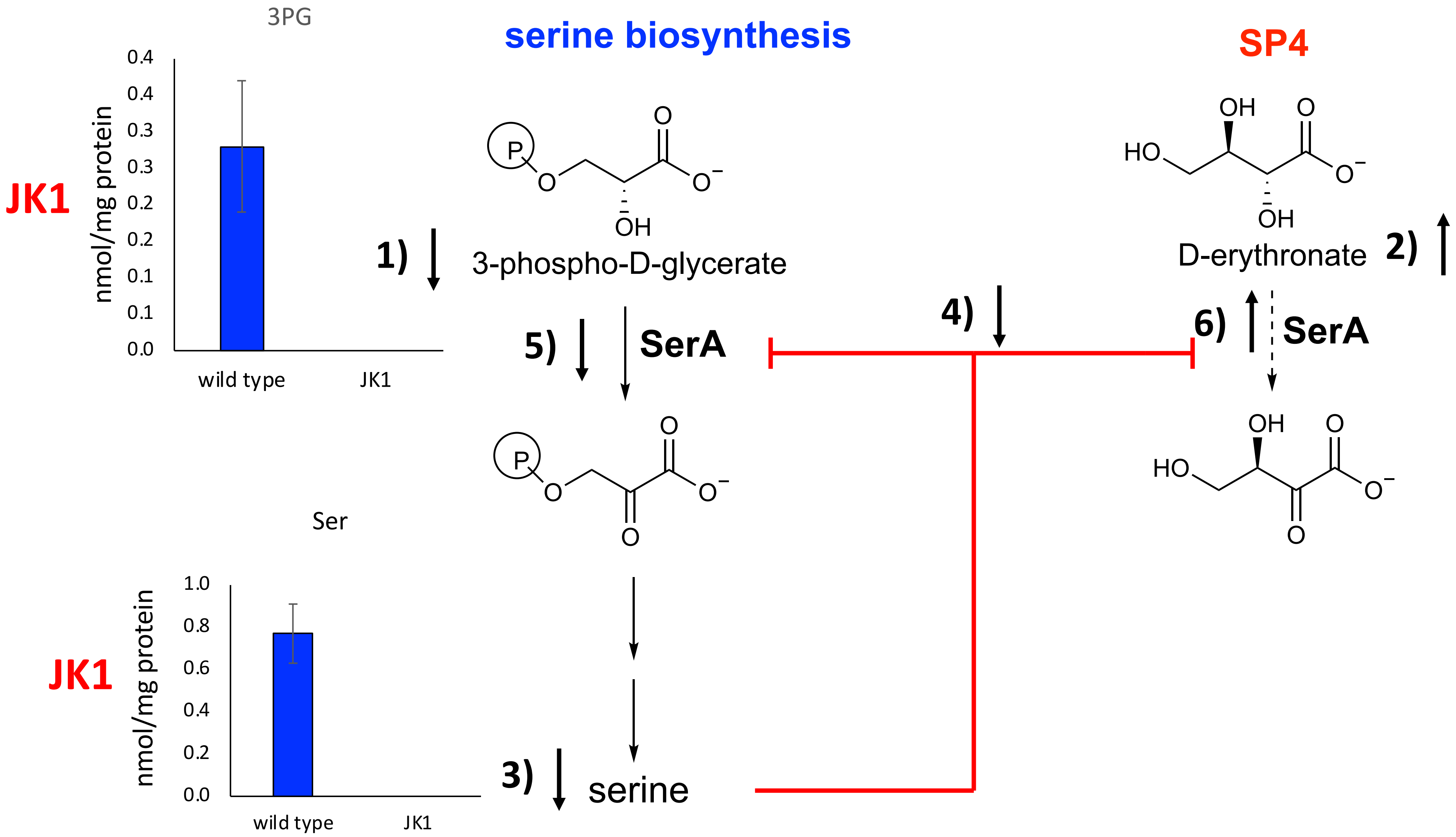
JK7a	**JK7b**	**JK8**	**JK9**	**JK10**
ybhA	*ybhA/pgl*	*gapA*	*ybhA/pgl*	*ybhA/pgl*
gapA	***serA***	***serA***	*gapA*	*gapA*
purF	*gapA*	*yjjK*	***serA***	*rpe*
nadR	*pykF*	*gltB*	*pykF*	*ilvH*
rpoS	*pyrE*	*livH*		*rng*



PLP biosynthesis
SP4
erythronate
?
P_i
SerA
3,4-dihydroxy-2-oxo-(3R)-butanoate (DHOB)
SerC
L-4-hydroxy-L-threonine (4HT)
ATP
ADP
ThrB
4-phosphoerythonate (4PE)
PdxB
SerC
L-4-phospho-hydroxythreonine (4PHT)

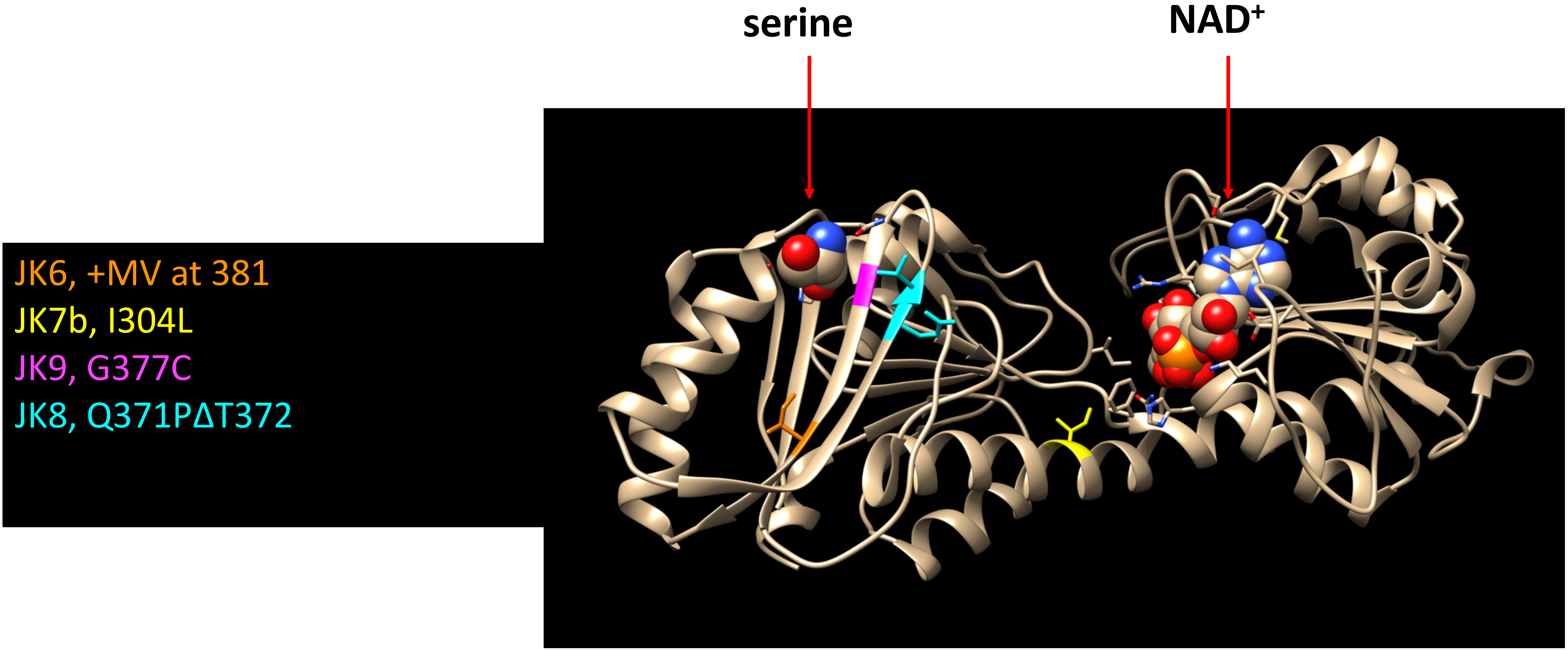
Six (!) ways to improve oxidation of erythronate

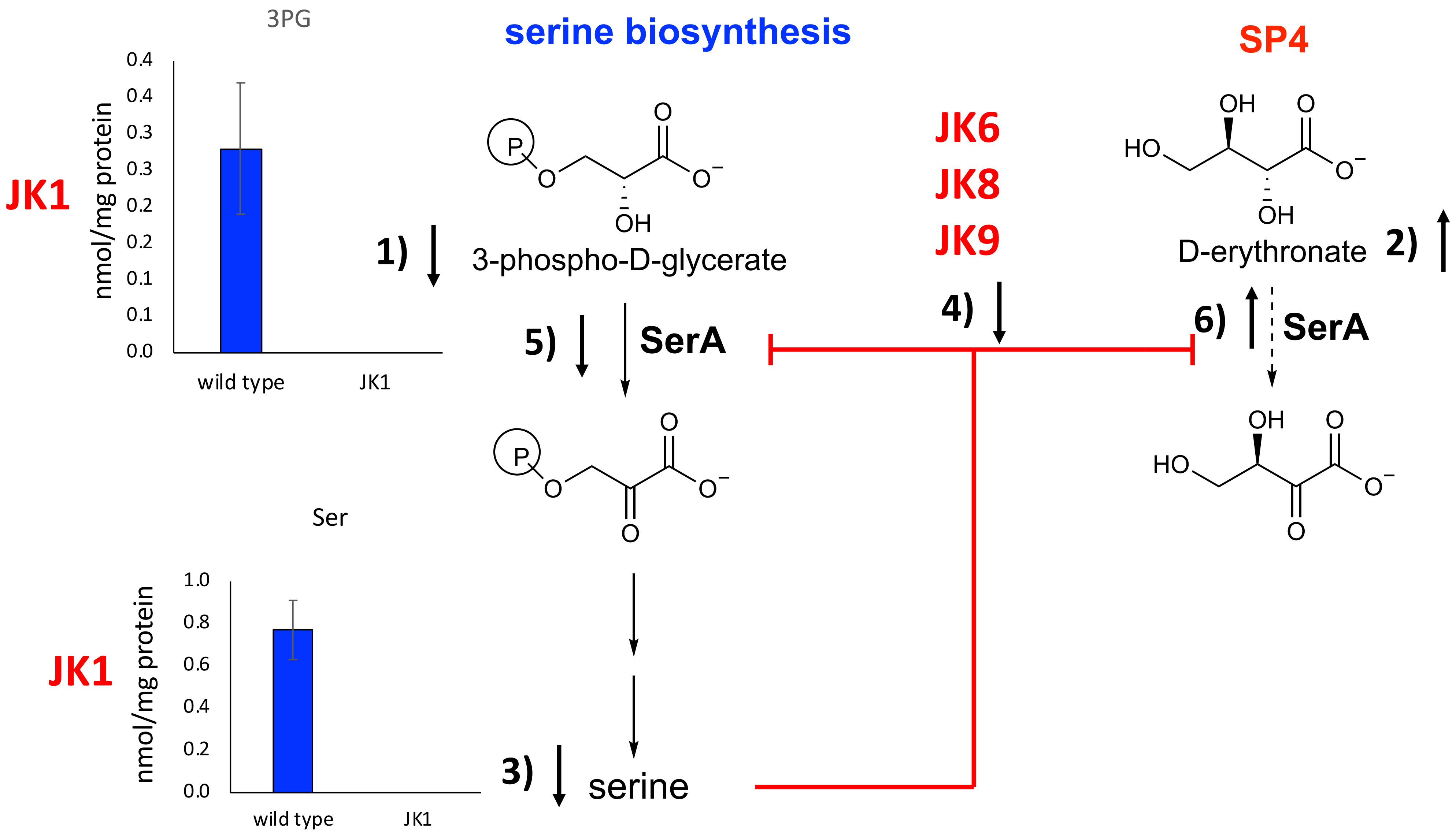


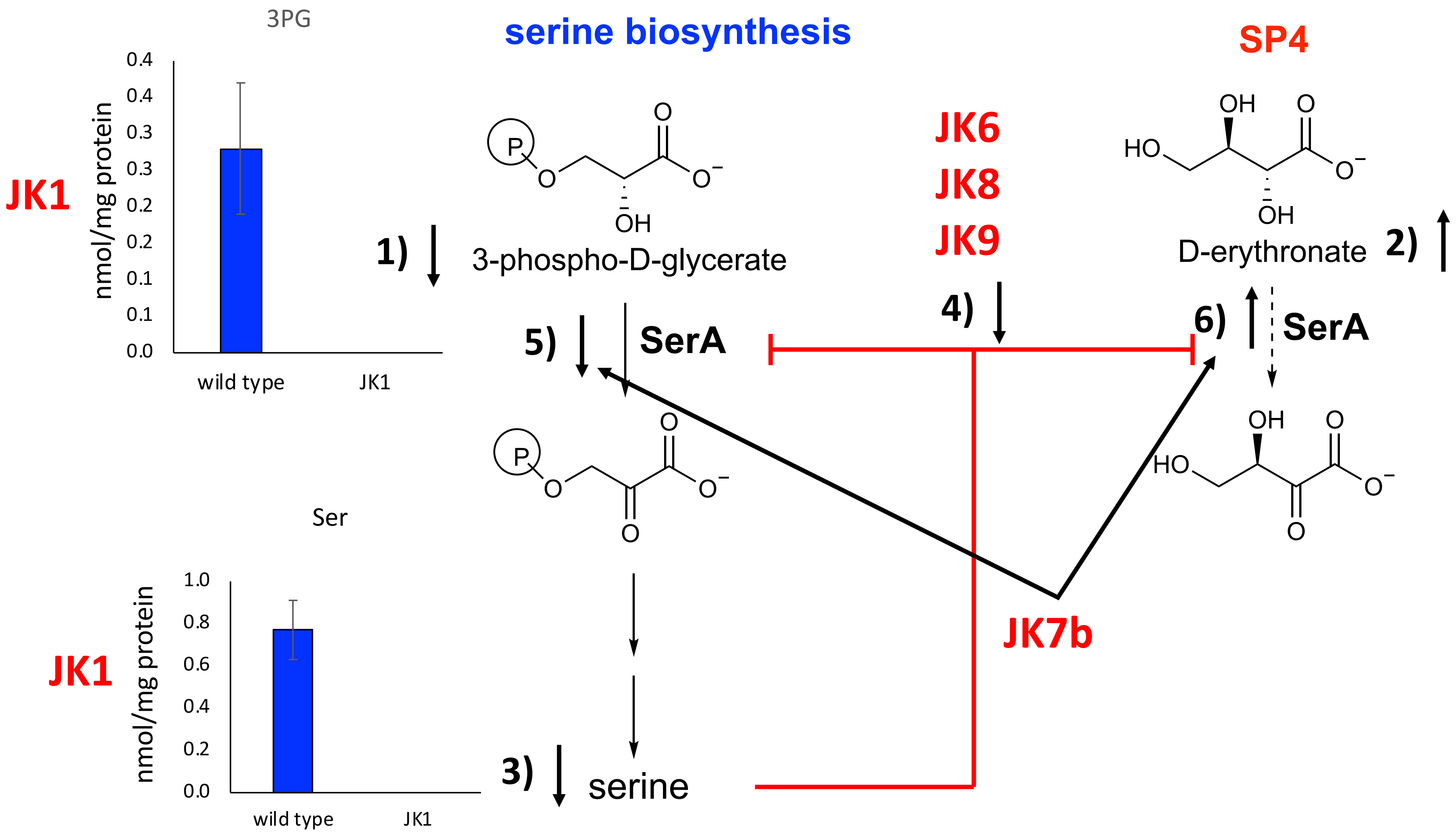


3PG
JK1
nmol/mg protein
0.4
0.4
0.3
0.3
0.2
0.2
0.1
0.1
0.0
wild type
JK1
serine biosynthesis
SP4
P
O
OH
O⁻
3-phospho-D-glycerate
HO
D-erythronate
1)
2)
3)
4)
5)
6)
SerA
SerA
Ser
JK1
nmol/mg protein
1.0
0.8
0.6
0.4
0.2
0.0
wild type
JK1
serine

SerA (3-phosphoglycerate dehydrogenase)

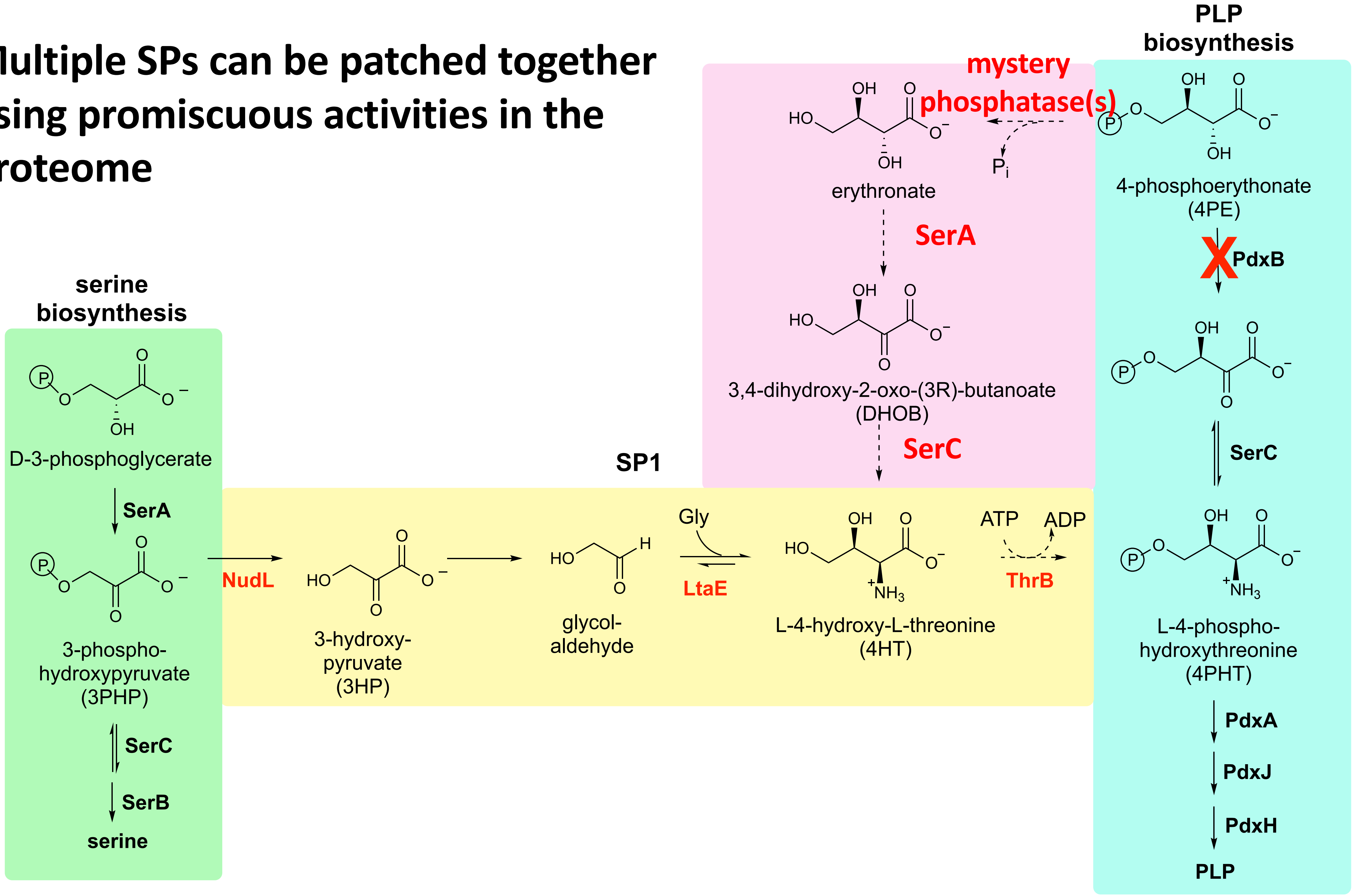



3PG
0.4
0.4
0.3
0.3
0.2
0.2
0.1
0.1
0.0
nmol/mg protein
wild type
JK1
JK1
serine biosynthesis
P
O
O
O−
OH
1)
3-phospho-D-glycerate
5)
SerA
P
O
O
O−
O
3)
serine
Ser
1.0
0.8
0.6
0.4
0.2
0.0
nmol/mg protein
wild type
JK1
JK1
JK6
JK8
JK9
4)
SP4
HO
OH
O
O−
OH
D-erythronate
2)
6)
SerA
HO
OH
O
O−
O



serine biosynthesis
SP4
3PG
nmol/mg protein
0.4
0.4
0.3
0.3
0.2
0.2
0.1
0.1
0.0
wild type
JK1
JK1
1)
3-phospho-D-glycerate
JK6
JK8
JK9
4)
D-erythronate
2)
6)
SerA
5)
SerA
JK7b
Ser
1.0
0.8
0.6
0.4
0.2
0.0
wild type
JK1
JK1
3)
serine

Multiple SPs can be patched together using promiscuous activities in the proteome

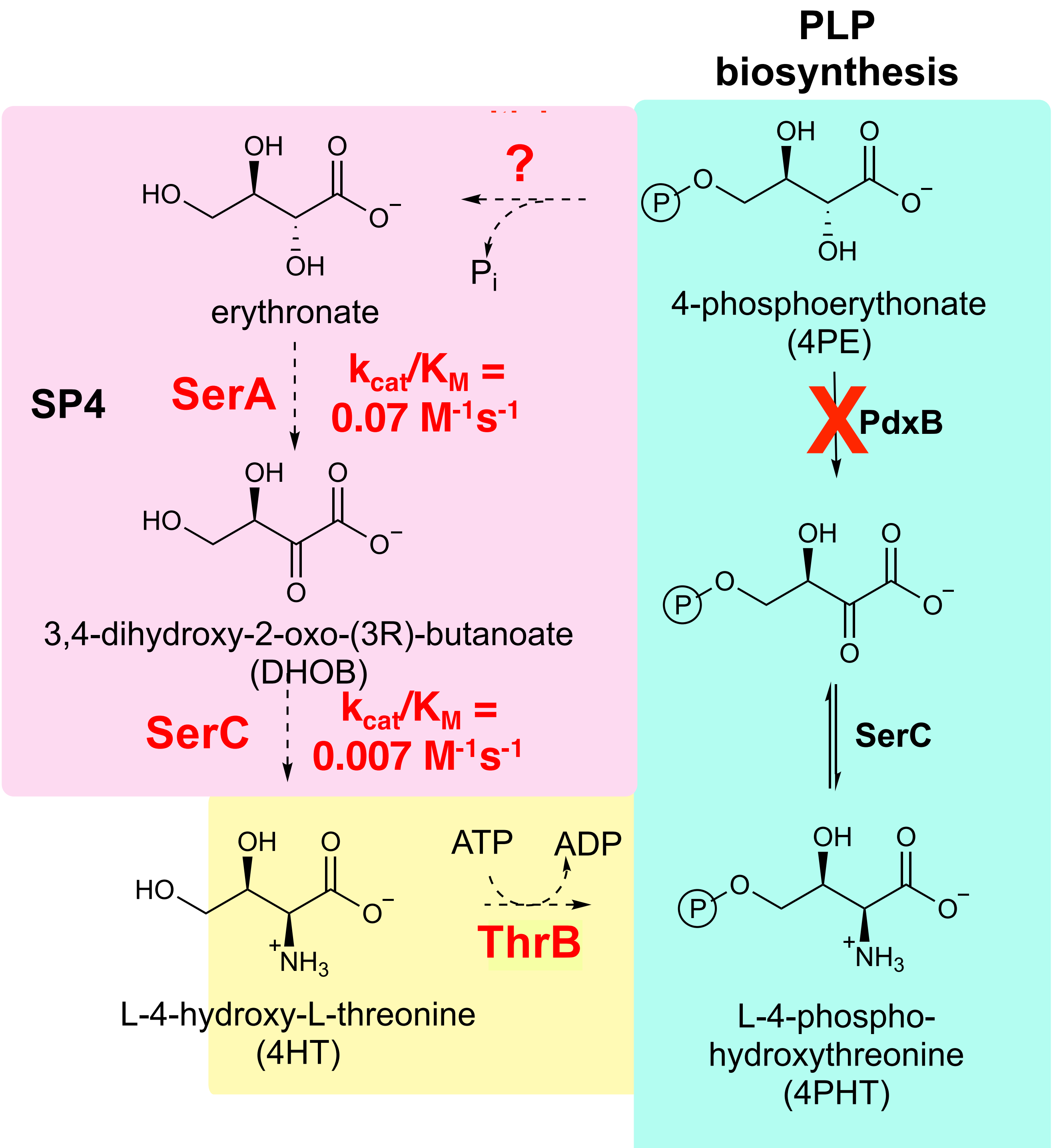


Mutations that elevate flux through a SP need not occur in genes encoding enzymes in the SP

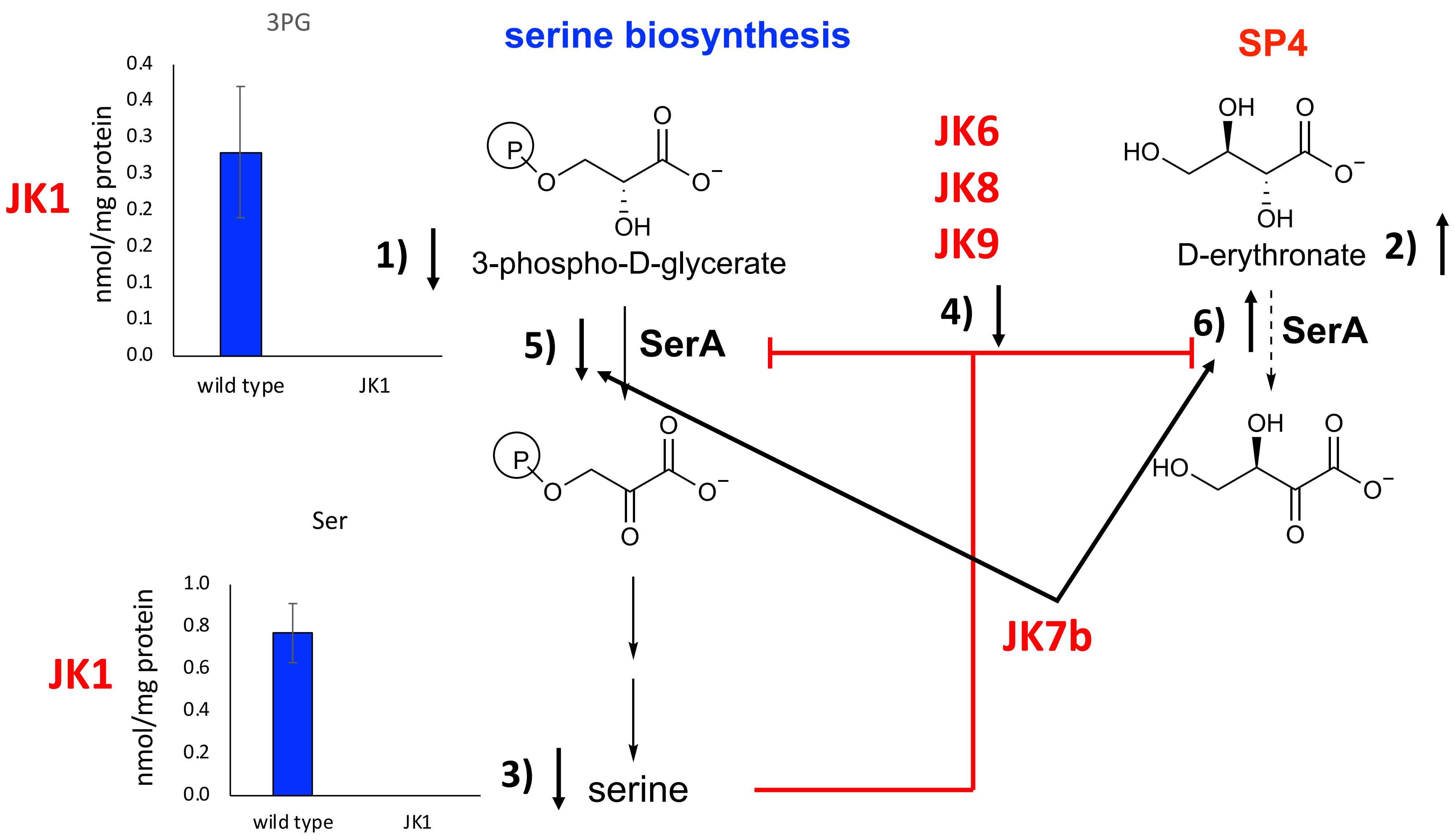
M117A01	M116B01	M215A01	M214B01	M317A02	M314B01
ybhA/pgl	*ybhA/pgl*	*ybhA/pgl*	*ybhA*	*ybhA/pgl*	*gapA*
gapA	*gapA*	*gapA*	*rpe*	*gapA*	***serA***
rpoS	*purF*	*ilvH*	*sdhA*	*yjjK*	*yjjK*
rpoC	*gltB*	*rng*	*rho*	*purF*	
	ypjA		*lon*	*ilvH*	
				nadR	

M414B02	M514A01	M514B01	M614A02	M414B01
ybhA/pgl	*gapA*	*ybhA/pgl*	*ybhA/pgl*	*ybhA*
serA	***serA***	*gapA*	*gapA*	*gapA*
gapA	*yjjK*	***serA***	*rpe*	*purF*
pykF	*gltB*	*pykF*	*ilvH*	*nadR*
pyrE	*livH*		*rng*	*rpoS*

Inefficient promiscuous activities may be sufficient to launch a new SP



The same phenotypic result can be achieved in multiple ways



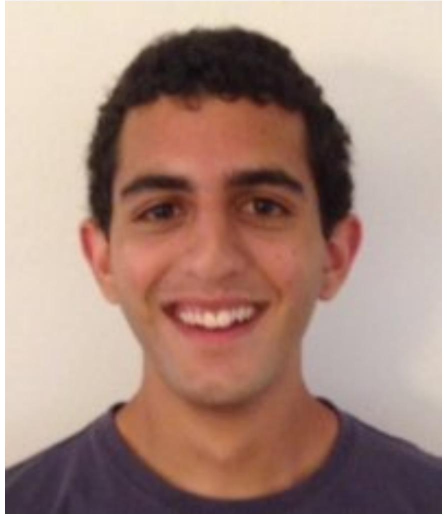
Jake Flood

Dr. Juhan Kim

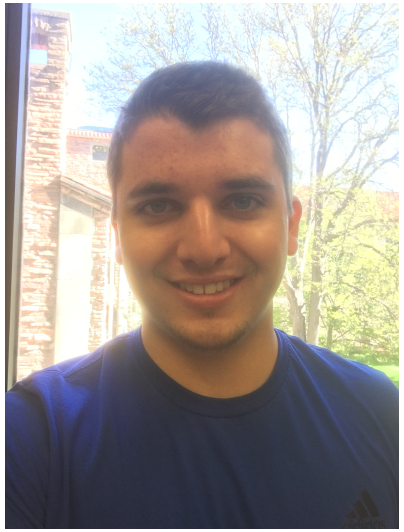
Andrew Morgenthaler

NIGMS

NASA Astrobiology Institute



Cyrus Gidfar



Michael Kristofich

Proteomics: Will Old, University of Colorado Boulder
Chris Ebmeier

Metabolomics: Uwe Sauer, ETH Zurich
Tobias Fuhrer

Sequencing: Vaughn Cooper, University of Pittsburgh
Dan Snyder