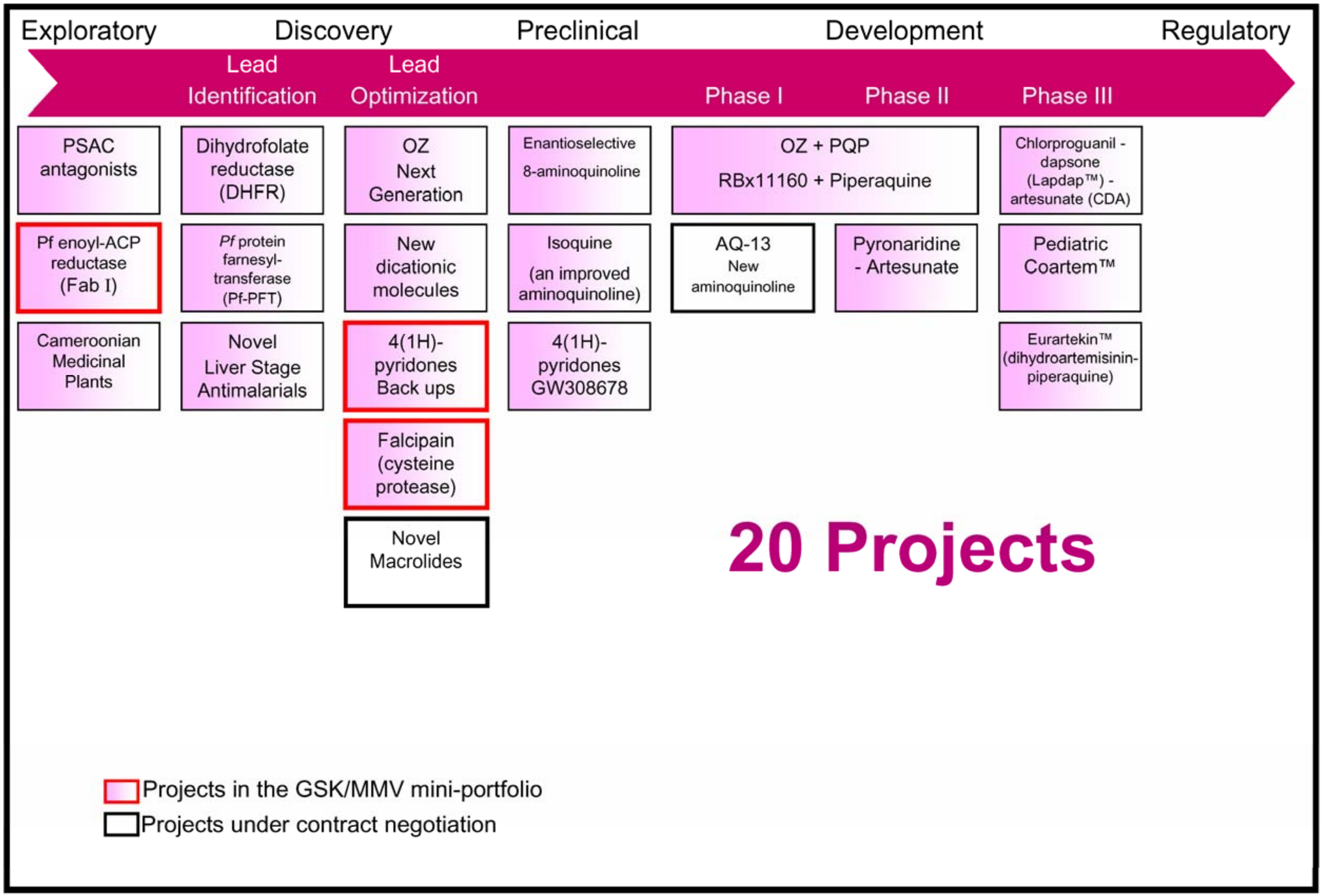
JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

New Drug Targets

Sean T. Prigge, PhD

MMV

MMV Portfolio 2nd Quarter 2006



Attritrion

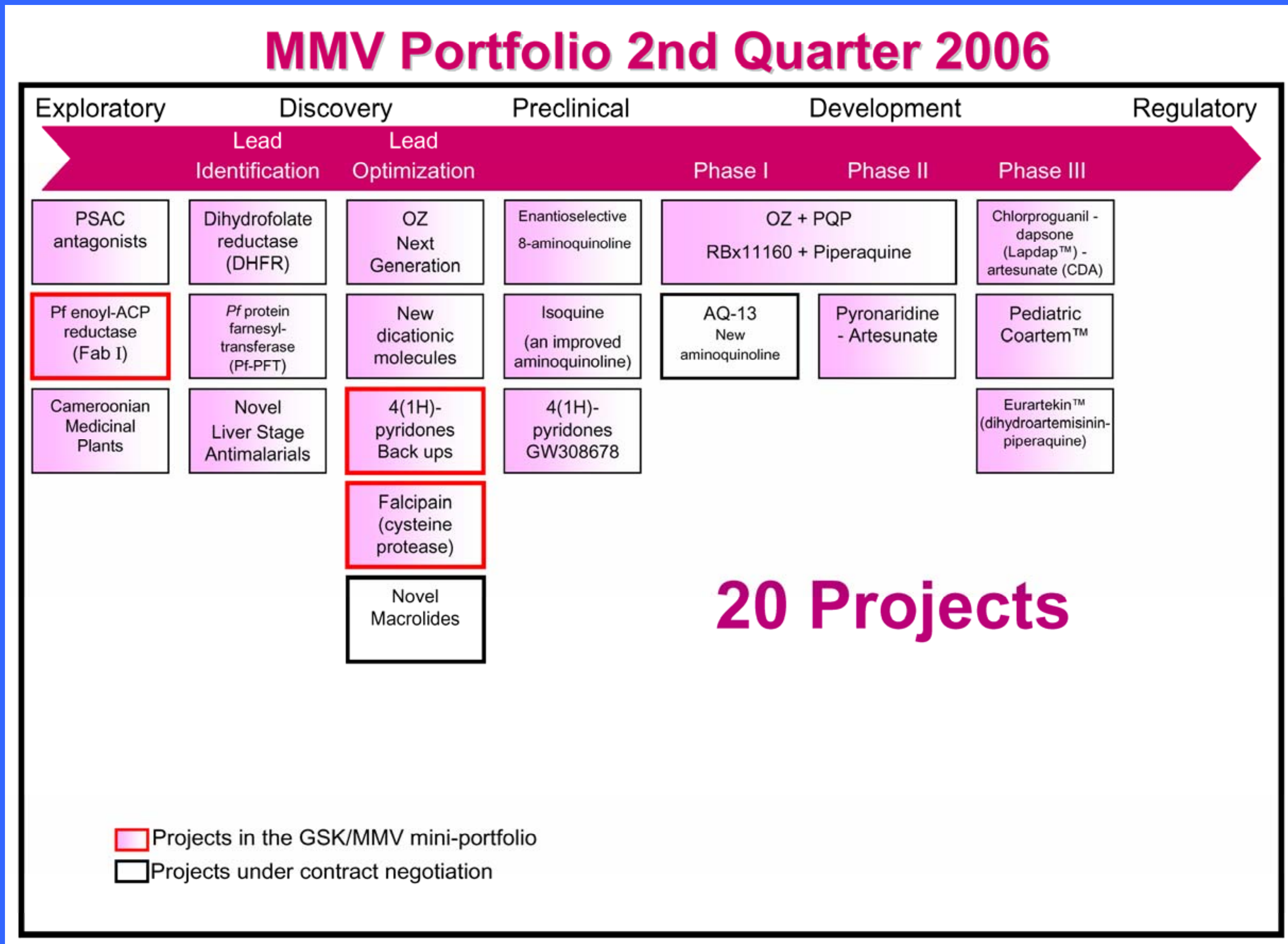
Projects	Objectives	Success Rates
Discovery		
• Exploratory	Target identification Target biochemistry Develop screening assay Develop X-ray crystallography	30% of projects will move to the next stage
• Lead Identification	High throughput screening for identification of Hits	65% of projects will move to the next stage
• Lead Optimization	SAR and molecular modeling to increase activity against parasite Improve pharmacokinetics Decrease toxicity	55% of projects will move to the next stage
Development		
• **Preclinical Transition**	Evaluation of toxicology Absorption, distribution, metabolism and excretion	55% of projects will move to the next stage
• Phase 1	First time in humans Safety and tolerability Pharmacokinetics 20 to 80 subjects exposed	70% of projects will move to the next stage
• Phase 2	Dose determining studies Early side-effect profile 200 to 300 subjects exposed	50% of projects will move to the next stage
• Phase 3	Large safety and efficacy studies Risk benefit 1000 to 3000 subjects exposed	65% of projects will move to registration
• Registration/Regulatory Approval	Drug available to patients	95% go on to drugs

Attrition

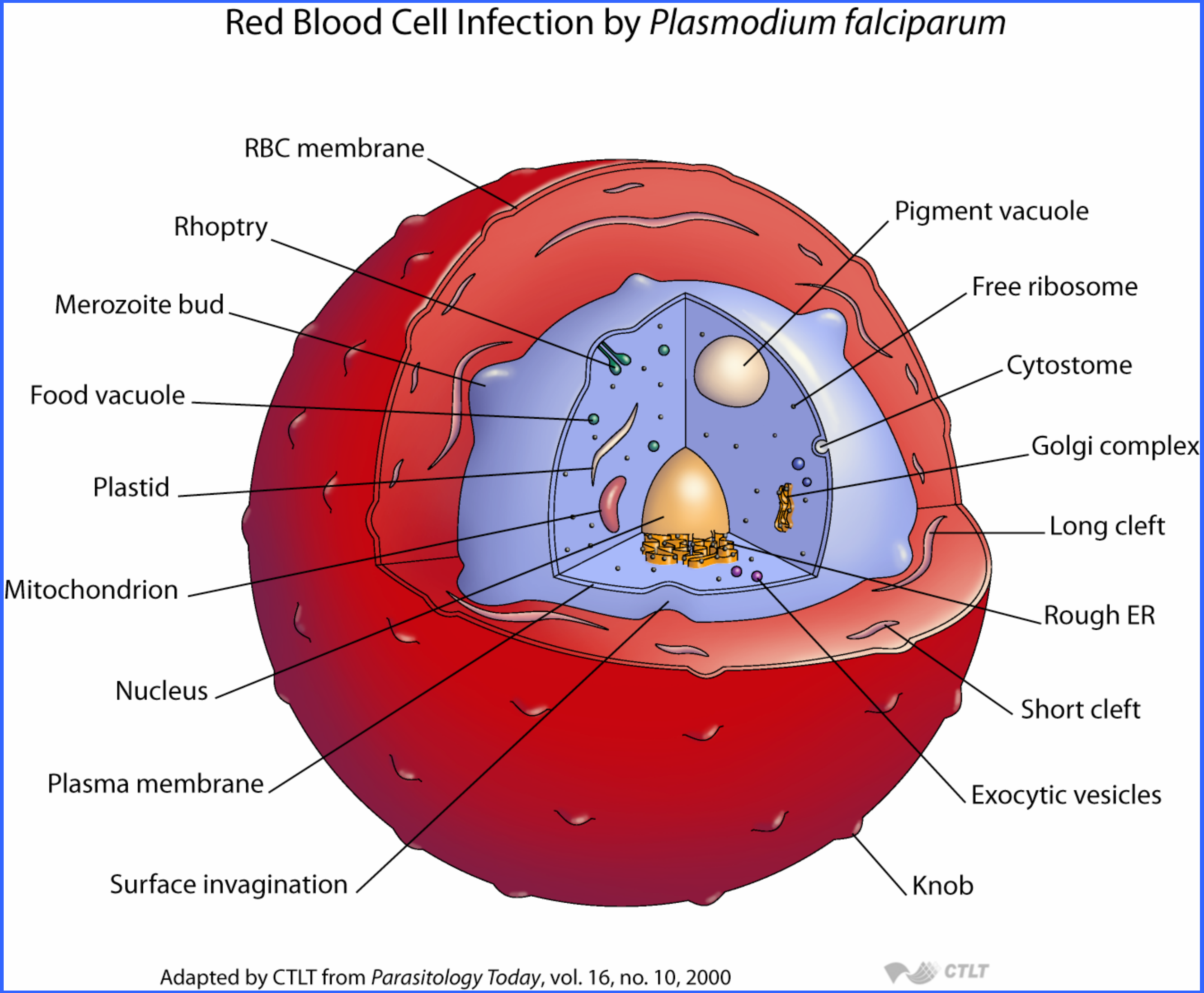
There are several reasons for the high dropout rate:

- a biologically poor target
- lack of activity against the target or parasite
- toxicity
- tolerability
- cost of goods

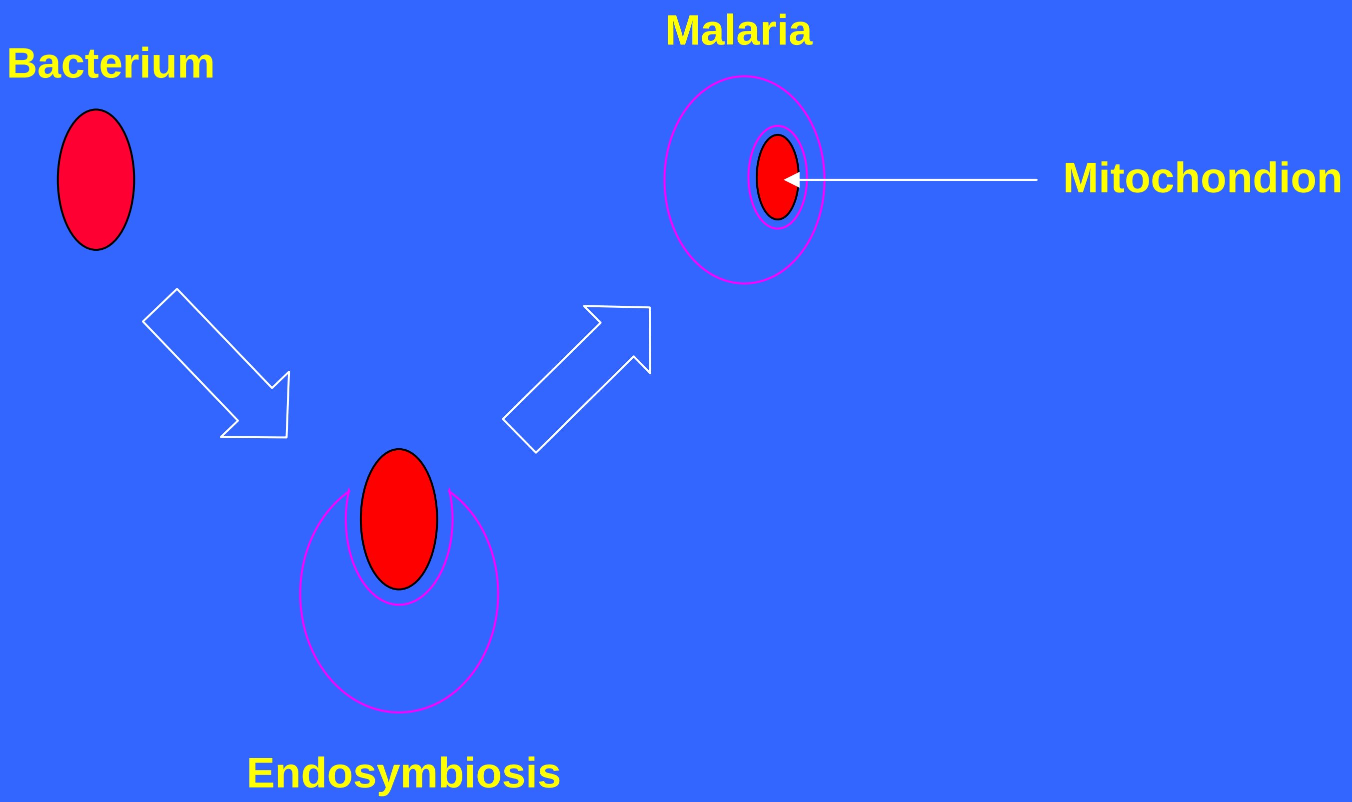
MMV


MMV Portfolio 2nd Quarter 2006
Exploratory
Discovery
Preclinical
Development
Regulatory
Lead Identification
Lead Optimization
Phase I
Phase II
Phase III
PSAC antagonists
Dihydrofolate reductase (DHFR)
OZ Next Generation
Enantioselective 8-aminoquinoline
OZ + PQP RBx11160 + Piperaquine
Chlorproguanil - dapsone (Lapdap™) - artesunate (CDA)
Pf enoyl-ACP reductase (Fab I)
Pf protein farnesyl-transferase (Pf-PFT)
New dicationic molecules
Isoquine (an improved aminoquinoline)
AQ-13 New aminoquinoline
Pyronaridine - Artesunate
Pediatric Coartem™
Cameroonian Medicinal Plants
Novel Liver Stage Antimalarials
4(1H)-pyridones Back ups
4(1H)-pyridones GW308678
Eurartekin™ (dihydroartemisinin-piperaquine)
Falcipain (cysteine protease)
Novel Macrolides
20 Projects
Projects in the GSK/MMV mini-portfolio
Projects under contract negotiation

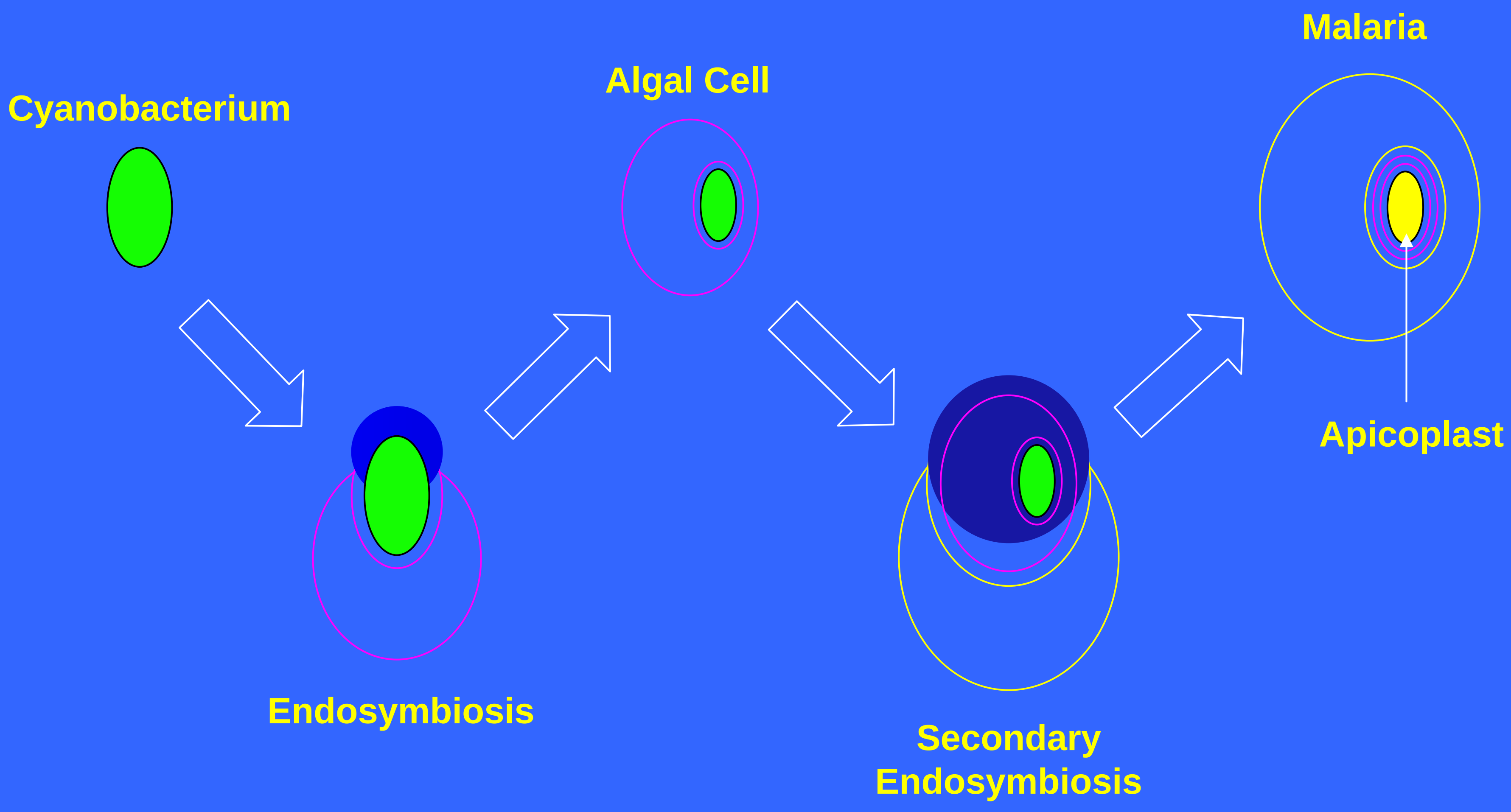
Malaria Parasite



Mitochondrial Origin



Apicoplast Origin



Oranellar Genomes

Mitochondrion (6 Kbp)

cytochrome oxidase I
cytochrome oxidase I
cytochrome oxidase III

Apicoplast (35 Kbp)

Full set of tRNAs
Clp protease
Elongation Factor TU
ABC transport involved
in Fe-S assembly
6 ORFs of less than 100 AA

Nuclear Genome

5300 genes

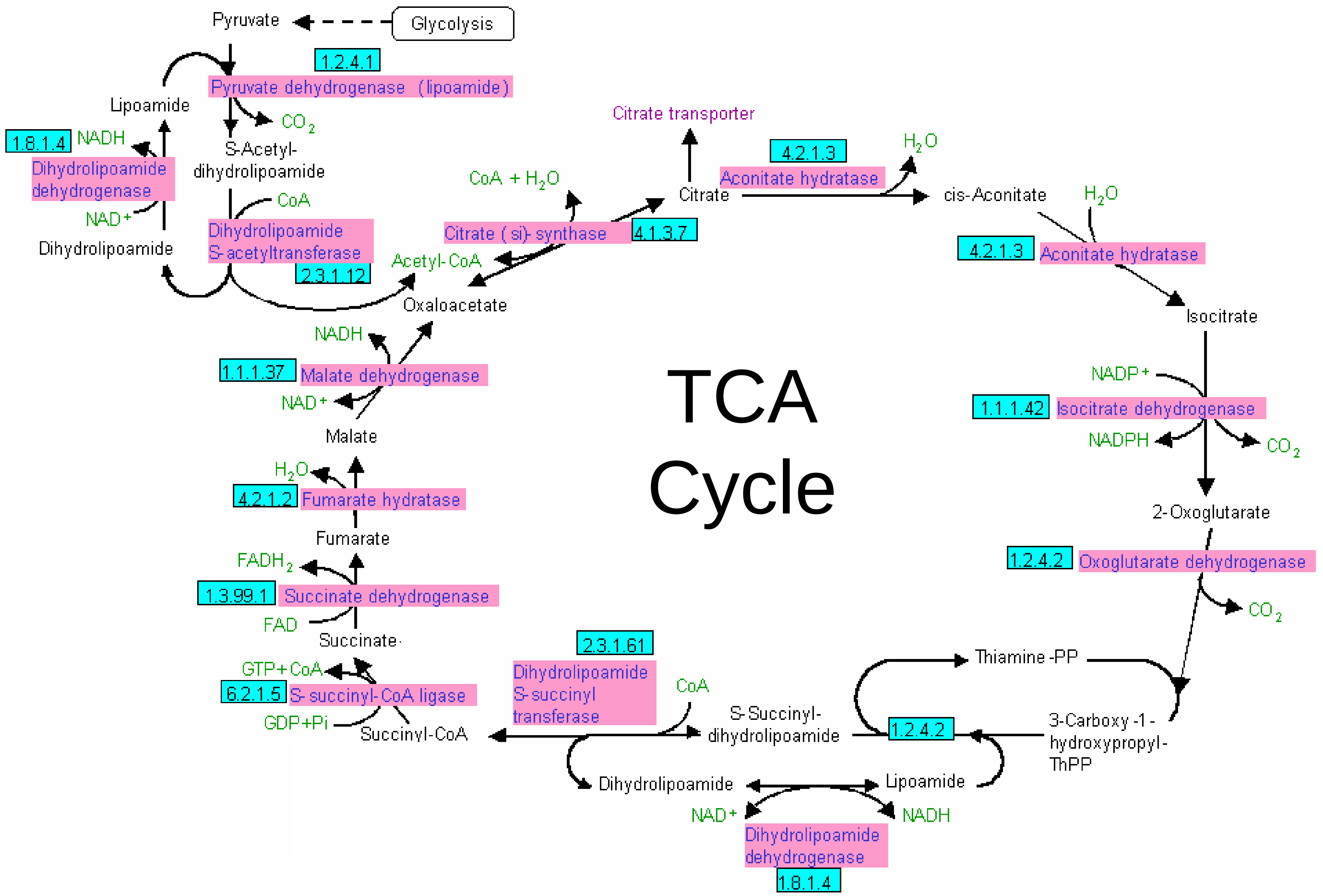
Mitochondial Import	**246 possible** **148 likely**
Apicoplast Import	**551 possilbe** **126 likely**

(<1000 plastid proteins in *A. thal*)

Metabolic Pathways

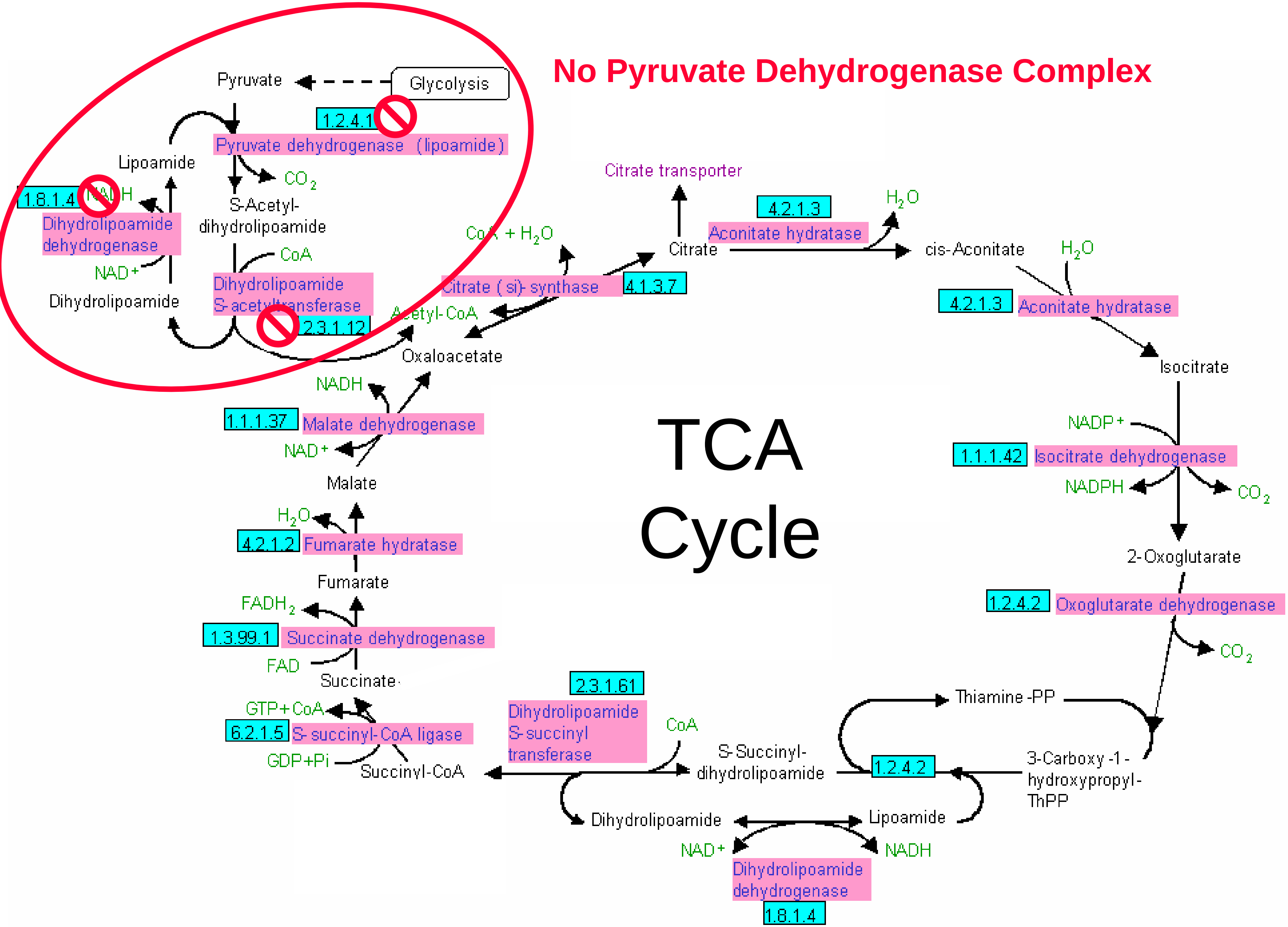
	Human	Plant	Malaria
Tricarboxylic Acid Cycle	Mito	Mito	?
Porphyrin Biosynthesis	Mito	Mito + Chlor	?
Shikimate Pathway	No	Chlor	?
Isoprenoid Biosyntheis Mevalonate	Cytosol	Cytosol	?
DOXP	No	Chlor	?
Fatty Acid Biosynthesis Type I	Cytosol	Cytosol	?
Type II	No	Chlor	?

http://www.genome.jp/kegg/pathway.html

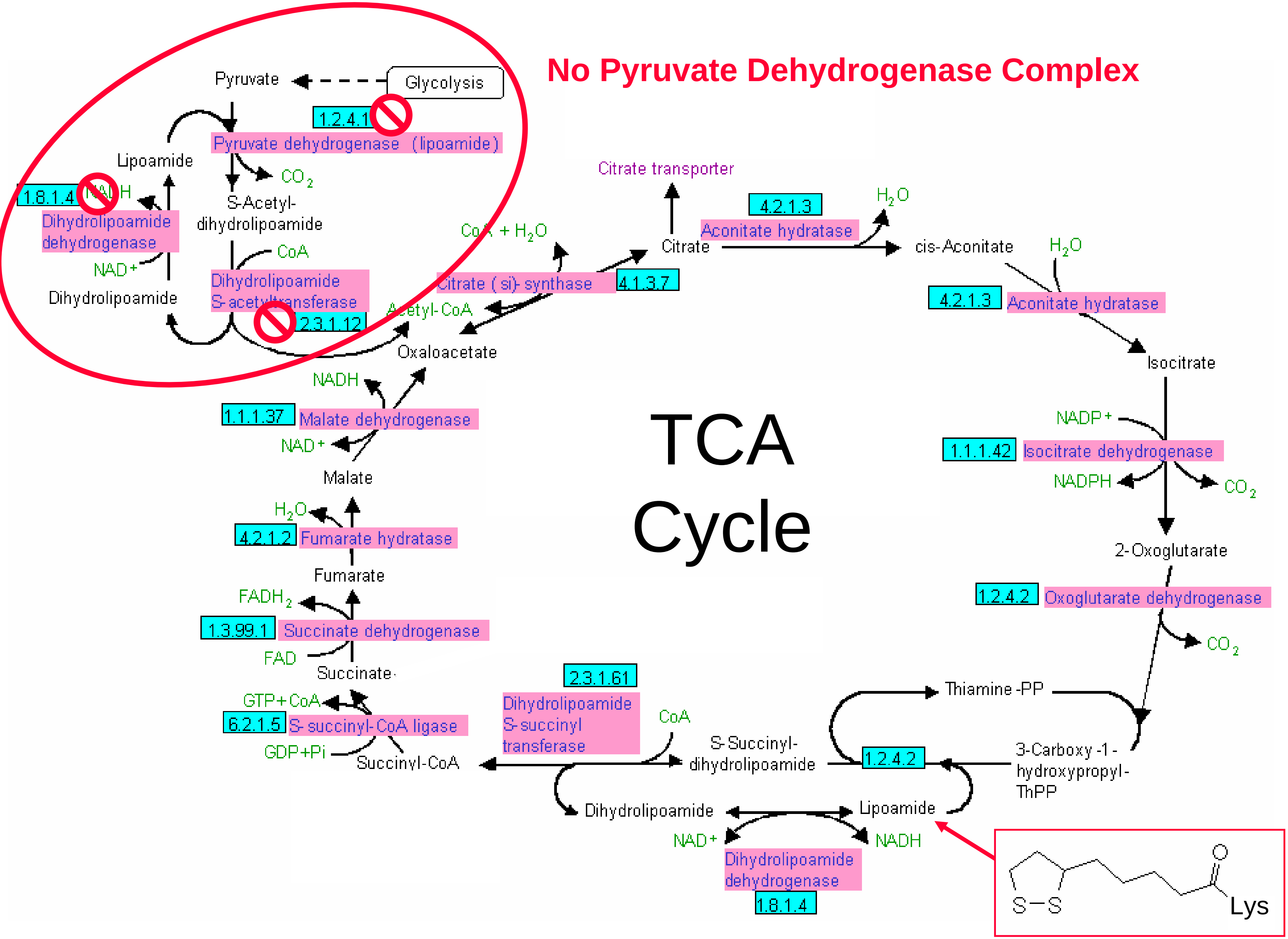


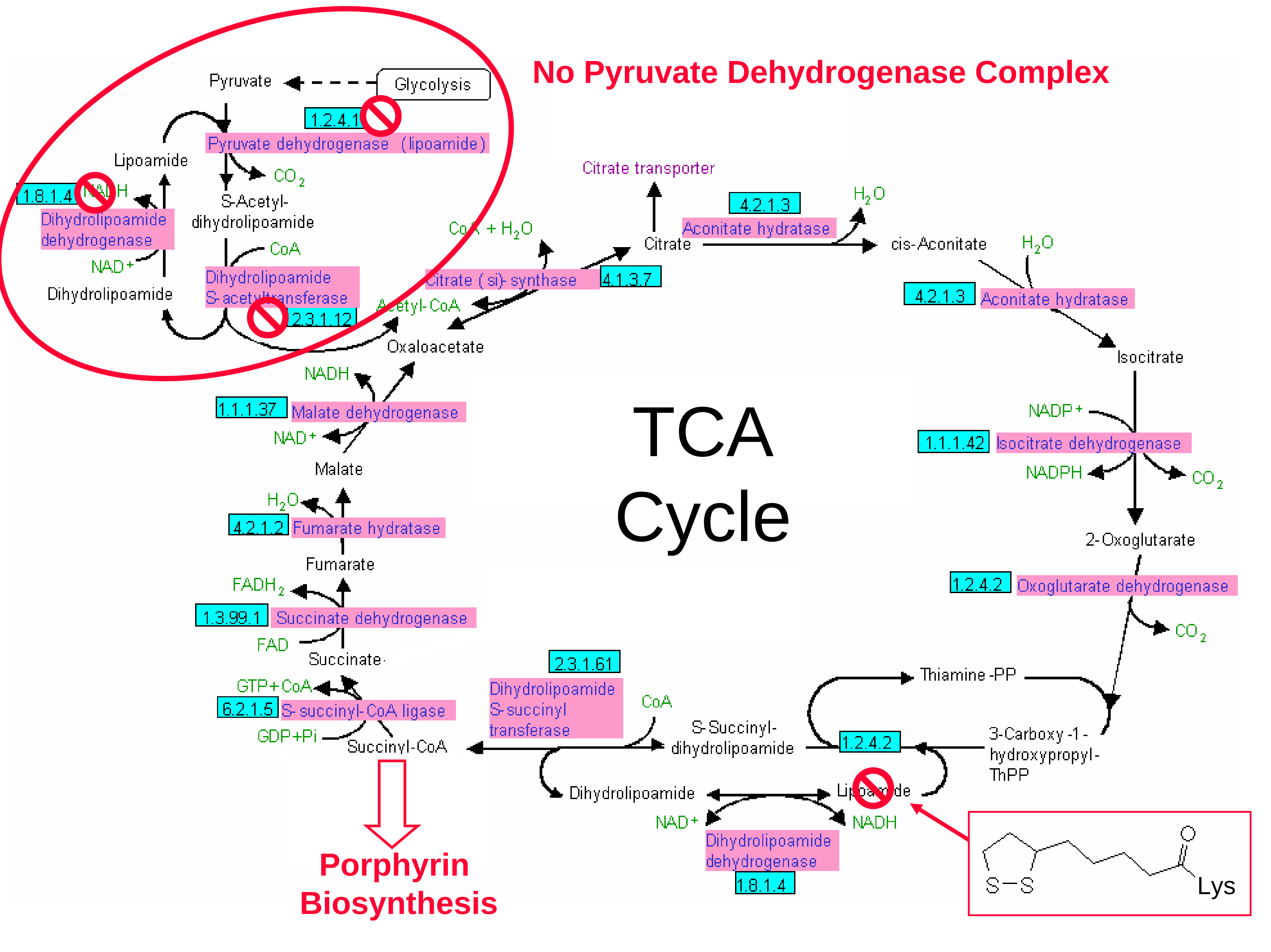
Pyruvate
Glycolysis
1.2.4.1
Pyruvate dehydrogenase (lipoamide)
Lipoamide
CO2
1.8.1.4
NADH
S-Acetyl-dihydrolipoamide
Dihydrolipoamide dehydrogenase
CoA
NAD+
Dihydrolipoamide
Dihydrolipoamide S-acetyltransferase
2.3.1.12
Citrate transporter
4.2.1.3
H2O
Aconitate hydratase
CoA + H2O
Citrate
cis-Aconitate
H2O
Citrate (si)-synthase
4.1.3.7
Acetyl-CoA
4.2.1.3
Aconitate hydratase
Oxaloacetate
Isocitrate
NADH
1.1.1.37
Malate dehydrogenase
NAD+
Malate
TCA Cycle
NADP+
1.1.1.42
Isocitrate dehydrogenase
NADPH
CO2
H2O
4.2.1.2
Fumarate hydratase
Fumarate
2-Oxoglutarate
FADH2
1.3.99.1
Succinate dehydrogenase
FAD
1.2.4.2
Oxoglutarate dehydrogenase
CO2
Succinate
2.3.1.61
GTP+CoA
6.2.1.5
S-succinyl-CoA ligase
GDP+Pi
Succinyl-CoA
Dihydrolipoamide S-succinyl transferase
CoA
S-Succinyl-dihydrolipoamide
Thiamine-PP
1.2.4.2
3-Carboxy-1-hydroxypropyl-ThPP
Dihydrolipoamide
Lipoamide
NAD+
NADH
Dihydrolipoamide dehydrogenase
1.8.1.4

No Pyruvate Dehydrogenase Complex



No Pyruvate Dehydrogenase Complex





No Pyruvate Dehydrogenase Complex
Pyruvate
Glycolysis
1.2.4.1
Pyruvate dehydrogenase (lipoamide)
Lipoamide
CO2
1.8.1.4
NADH
Dihydrolipoamide dehydrogenase
S-Acetyl-dihydrolipoamide
NAD+
CoA
Dihydrolipoamide
Dihydrolipoamide S-acetyltransferase
2.3.1.12
Citrate transporter
4.2.1.3
H2O
Aconitate hydratase
CoA + H2O
Citrate
cis-Aconitate
Citrate (si)-synthase
4.1.3.7
Acetyl-CoA
4.2.1.3
Aconitate hydratase
Oxaloacetate
Isocitrate
NADH
1.1.1.37
Malate dehydrogenase
NAD+
TCA Cycle
NADP+
1.1.1.42
Isocitrate dehydrogenase
NADPH
CO2
Malate
H2O
4.2.1.2
Fumarate hydratase
2-Oxoglutarate
Fumarate
1.2.4.2
Oxoglutarate dehydrogenase
FADH2
1.3.99.1
Succinate dehydrogenase
CO2
FAD
Succinate
2.3.1.61
Thiamine-PP
GTP+CoA
Dihydrolipoamide S-succinyl transferase
6.2.1.5
S-succinyl-CoA ligase
CoA
S-Succinyl-dihydrolipoamide
1.2.4.2
3-Carboxy-1-hydroxypropyl-ThPP
GDP+Pi
Succinyl-CoA
Dihydrolipoamide
Lipoamide
NAD+
NADH
Dihydrolipoamide dehydrogenase
1.8.1.4
S–S
O
Lys
Porphyrin Biosynthesis

Metabolic Pathways

	Human	Plant	Malaria
Tricarboxylic Acid Cycle	Mito	Mito	Mito
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Fatty Acid Biosynthesis Type I	Cytosol	Cytosol	?
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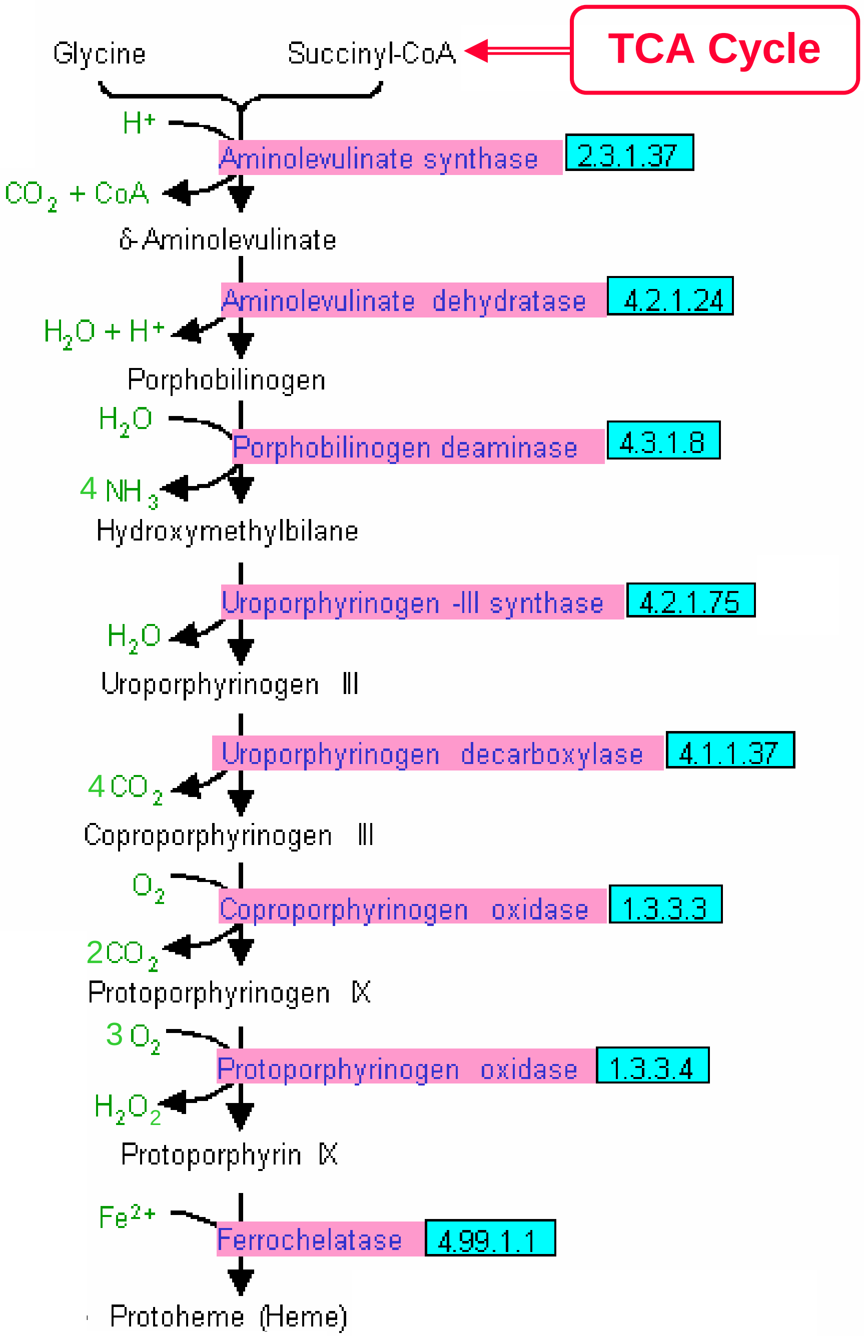
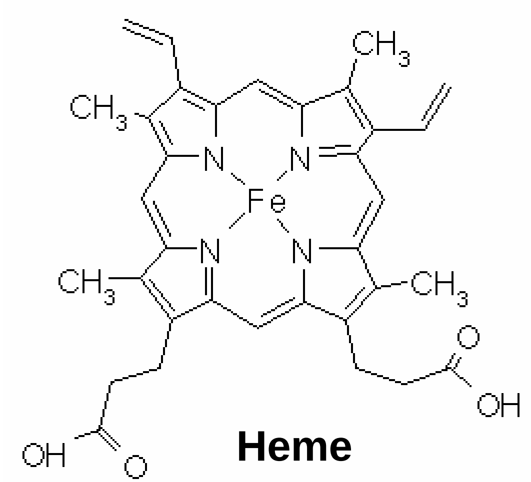
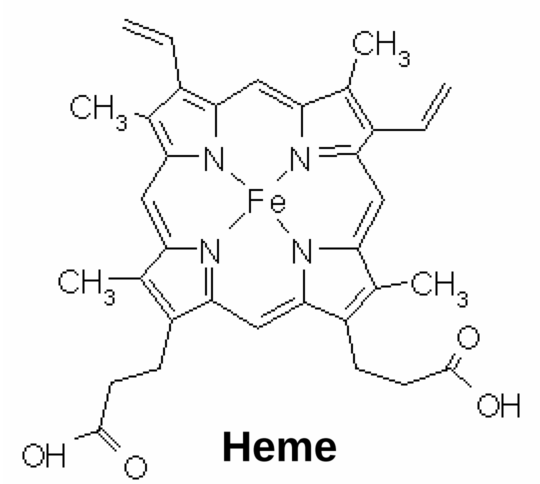
Porphyrin Biosynthesis

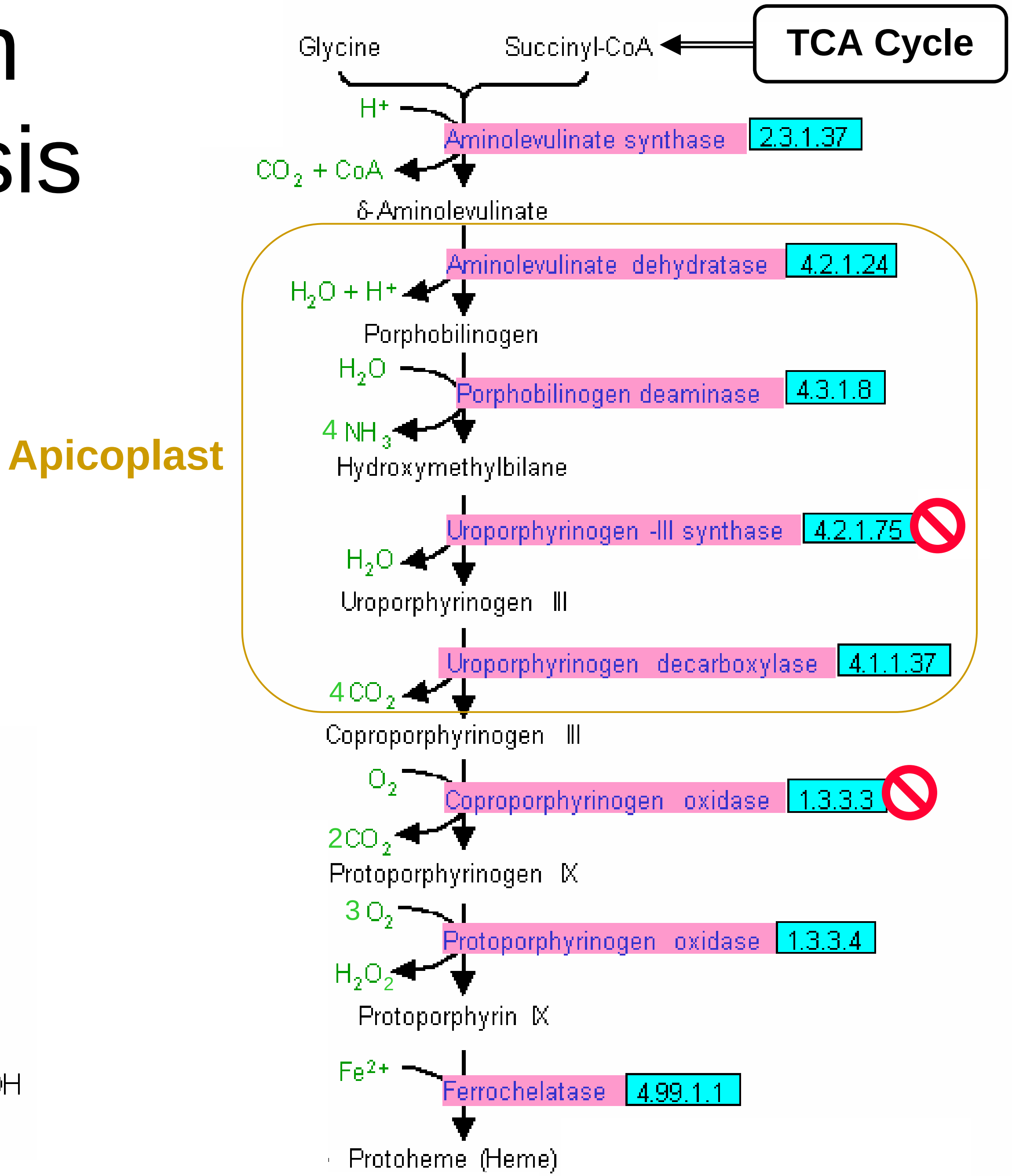


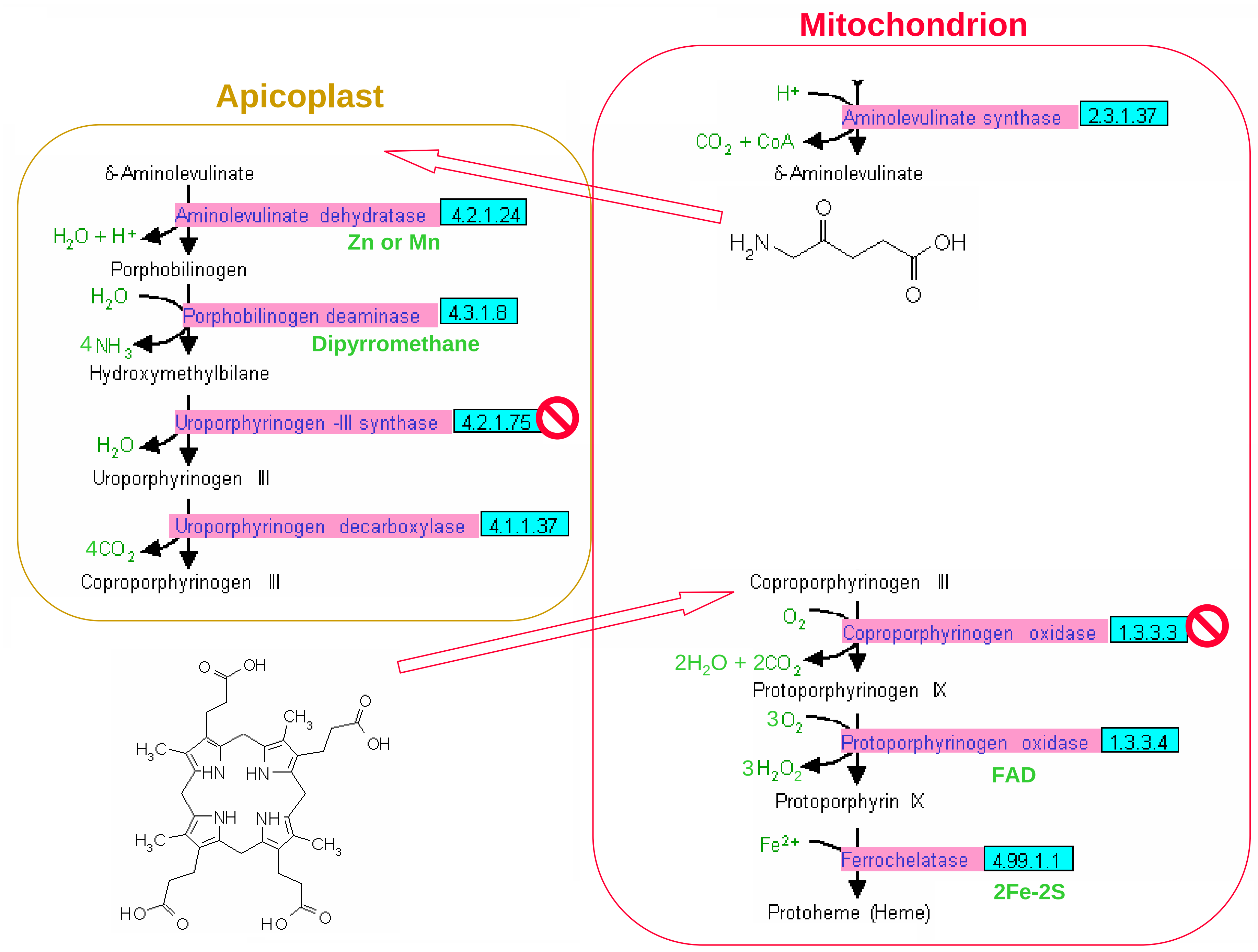


Heme

Porphyrin Biosynthesis





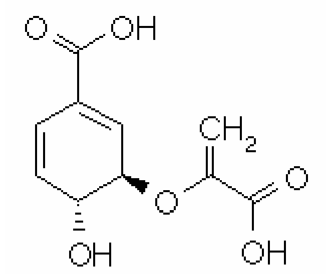


Mitochondrion
Apicoplast
H+
Aminolevulinate synthase
2.3.1.37
CO2 + CoA
δ-Aminolevulinate
δ-Aminolevulinate
Aminolevulinate dehydratase
4.2.1.24
H2O + H+
Zn or Mn
Porphobilinogen
H2O
Porphobilinogen deaminase
4.3.1.8
4 NH3
Dipyrromethane
Hydroxymethylbilane
Uroporphyrinogen -III synthase
4.2.1.75
H2O
Uroporphyrinogen III
Uroporphyrinogen decarboxylase
4.1.1.37
4CO2
Coproporphyrinogen III
Coproporphyrinogen III
O2
Coproporphyrinogen oxidase
1.3.3.3
2H2O + 2CO2
Protoporphyrinogen IX
3 O2
Protoporphyrinogen oxidase
1.3.3.4
3 H2O2
FAD
Protoporphyrin IX
Fe2+
Ferrochelatase
4.99.1.1
2Fe-2S
Protoheme (Heme)

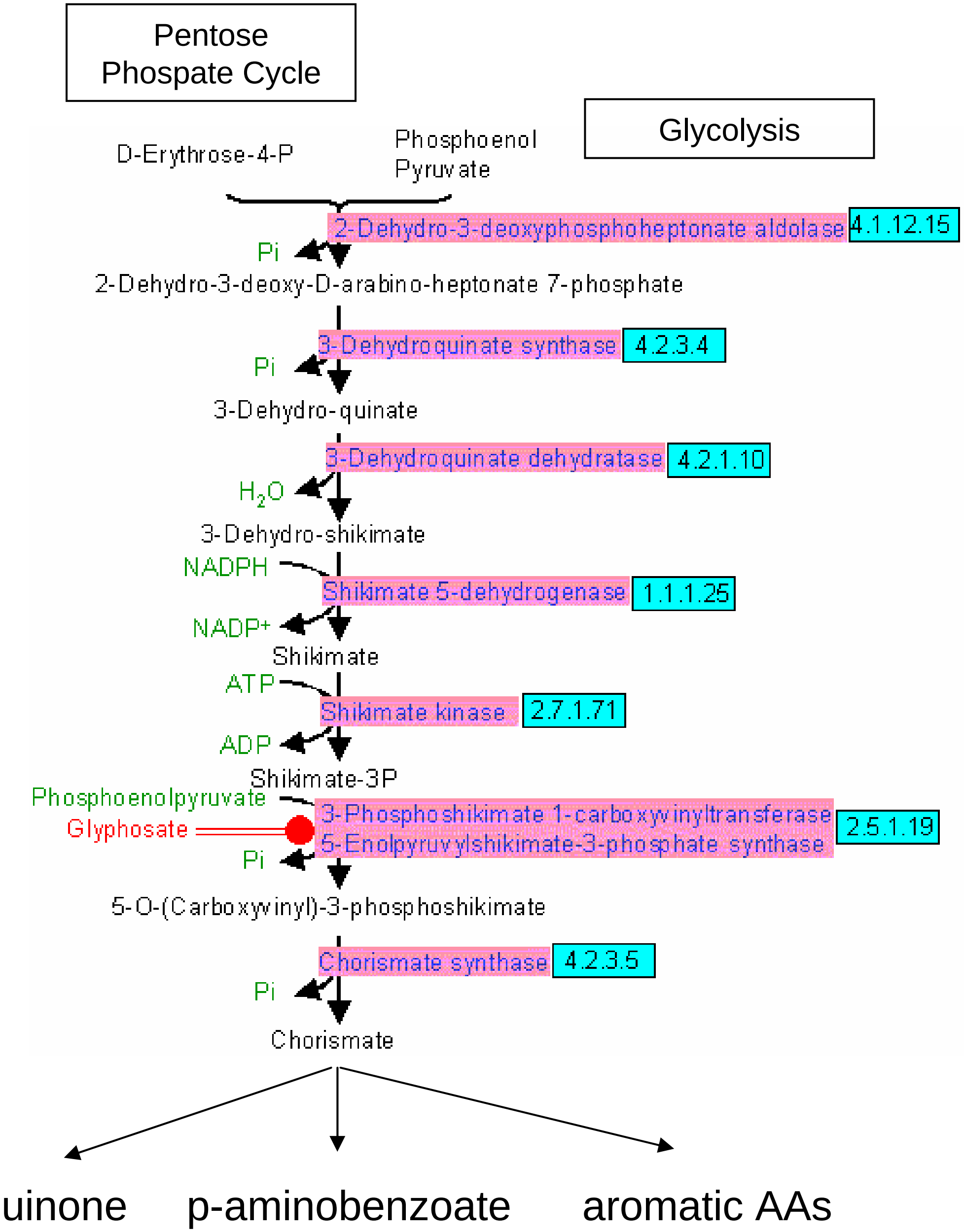
Metabolic Pathways

	Human	Plant	Malaria
Tricarboxylic Acid Cycle	Mito	Mito	Mito
Porphyrin Biosynthesis	Mito	Mito + Chlor	½ Mito ½ Apico
Shikimate Pathway	No	Chlor	?
Isoprenoid Biosyntheis Mevalonate	Cytosol	Cytosol	?
DOXP	No	Chlor	?
Fatty Acid Biosynthesis Type I	Cytosol	Cytosol	?
Type II	No	Chlor	?

Shikimate Pathway

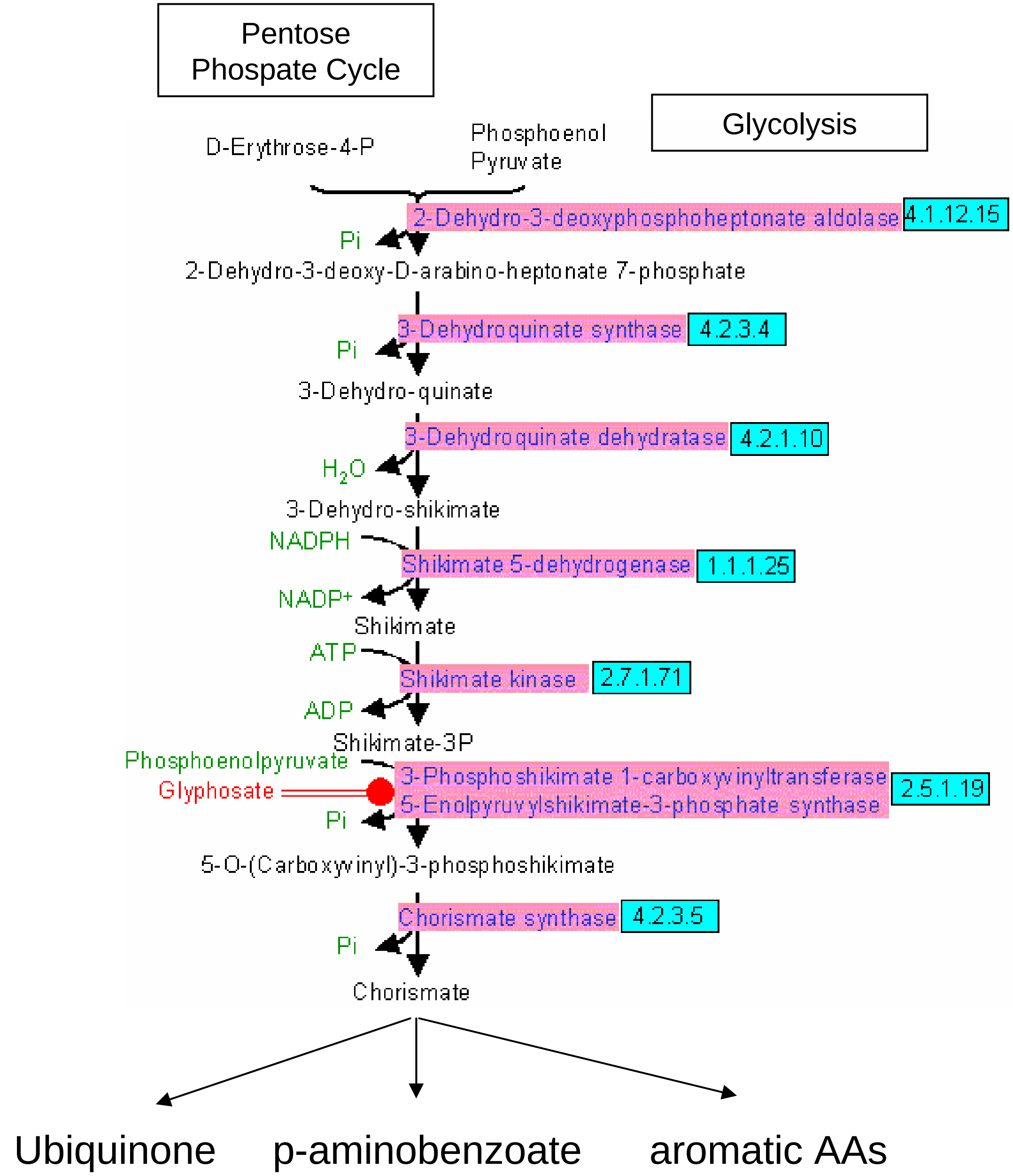


Chorismate



Shikimate Pathway

- No predicted oranellar targeting.
-CS localized to cytosol by immunomicroscopy



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DOXP	No	Chlor	?
Fatty Acid Biosynthesis Type I	Cytosol	Cytosol	?
Type II	No	Chlor	?

Isoprenoid Biosynthesis

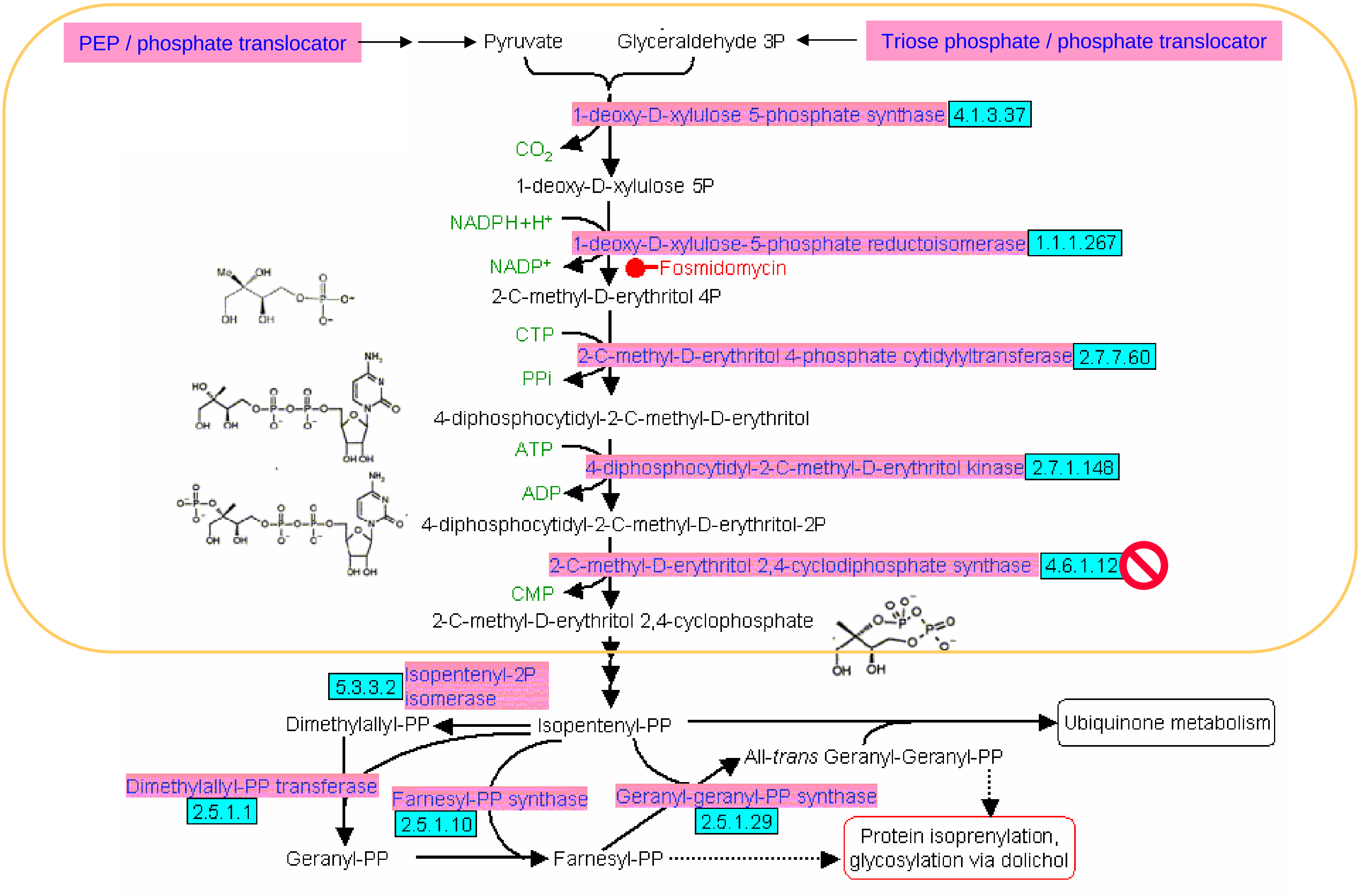
Mevalonate-dependent

4 steps from 3-hydroxy methylglutaryl-Coa to
Isopentenyl-PP (V,L,I degradation)
Typically supports terpenoid and sterol biosynthesis

Mevalonate-independent

DOXP (1-deoxy D-xylulose 5-phosphate)

Isoprenoid Biosynthesis



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Fatty Acid Biosynthesis Type I	Cytosol	Cytosol	?
Type II	No	Chlor	?

Fatty Acid Biosynthesis

Type I Fatty Acid Synthase

Multifuctional enzyme (one or two polypeptides)
Found in most eukaryotic cells

Type II Fatty Acid Synthase

Enzymes expressed as separate proteins
Found in plants and microorganisms

Fatty Acid Biosynthesis

⊘

Type I Fatty Acid Synthase

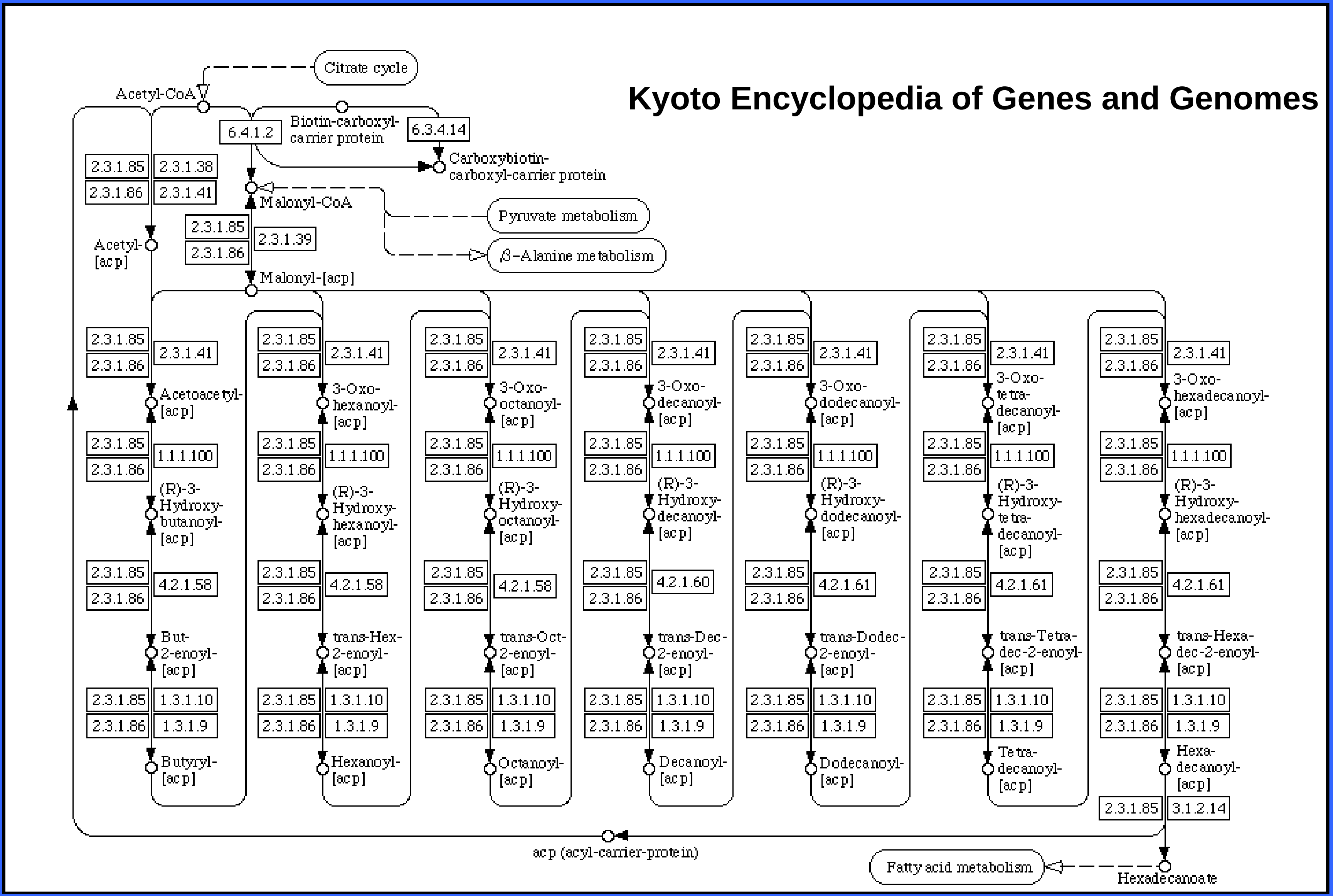
Multifuctional enzyme (one or two polypeptides)
Found in most eukaryotic cells

7 genes found

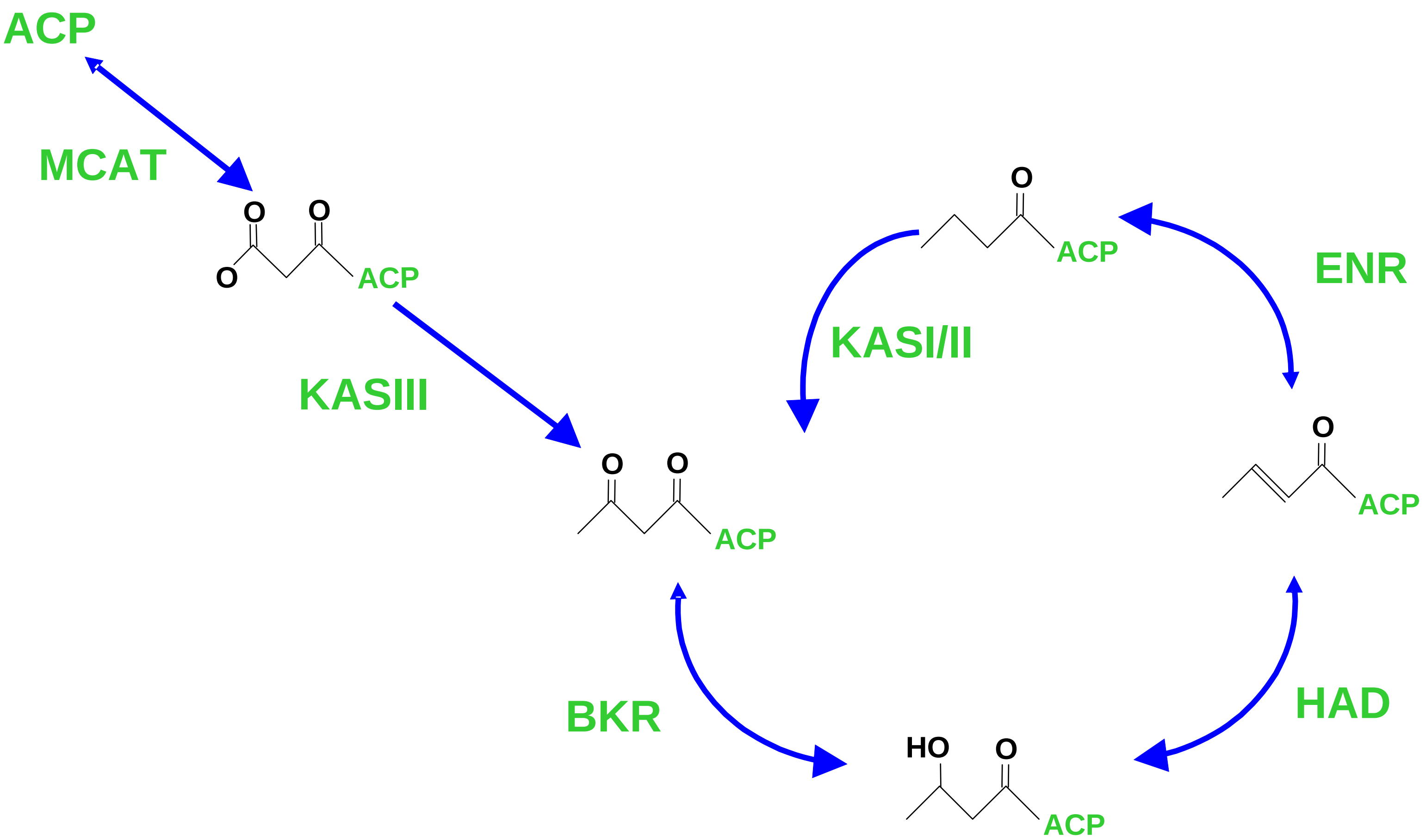
Type II Fatty Acid Synthase

Enzymes expressed as separate proteins
Found in plants and microorganisms

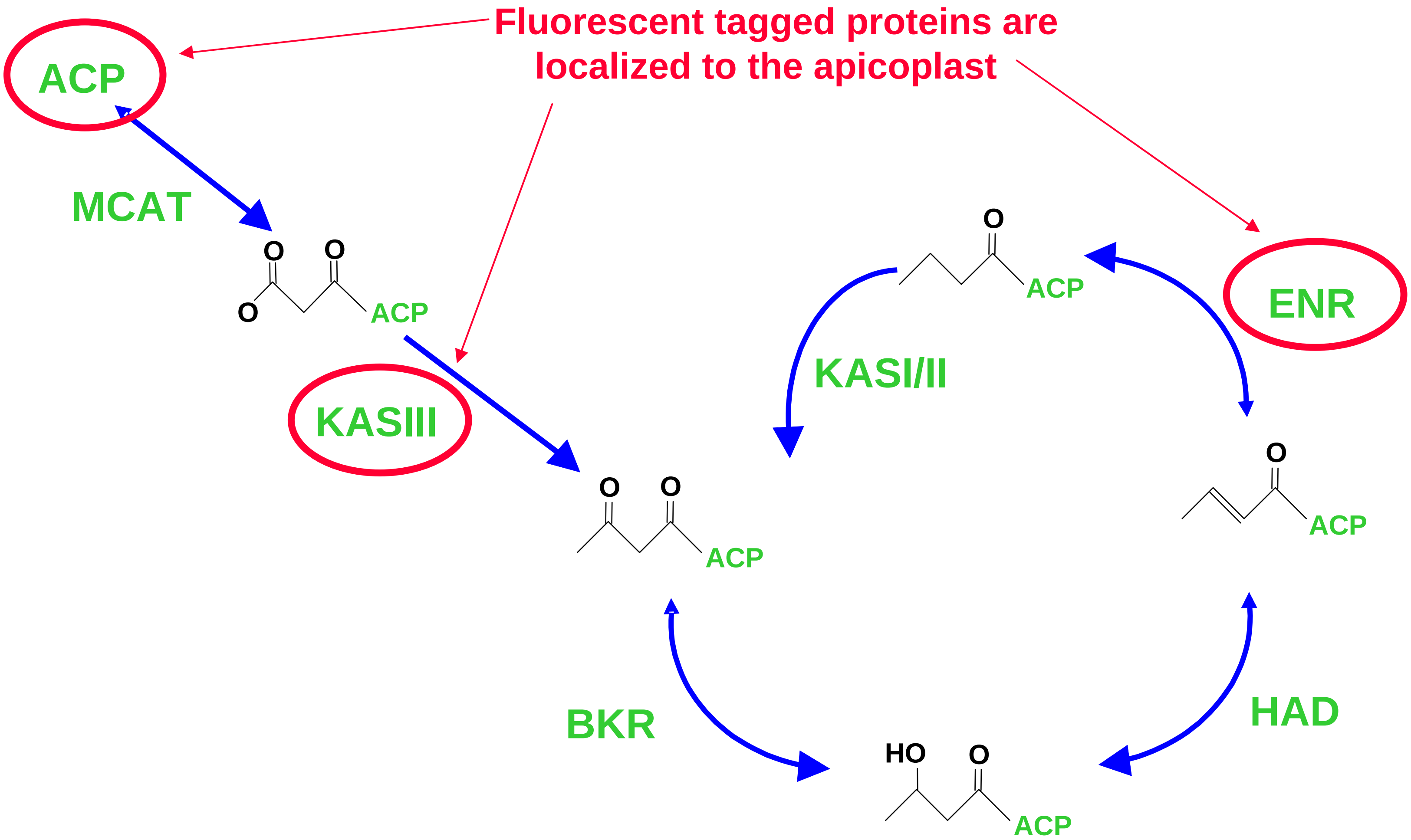
Type II FAS


Kyoto Encyclopedia of Genes and Genomes
Citrate cycle
Acetyl-CoA
6.4.1.2
Biotin-carboxyl-carrier protein
6.3.4.14
Carboxybiotin-carboxyl-carrier protein
2.3.1.85
2.3.1.86
2.3.1.38
2.3.1.41
Malonyl-CoA
Pyruvate metabolism
β-Alanine metabolism
2.3.1.39
Acetyl-[acp]
Malonyl-[acp]
Acetoacetyl-[acp]
1.1.1.100
(R)-3-Hydroxy-butanoyl-[acp]
4.2.1.58
But-2-enoyl-[acp]
1.3.1.10
1.3.1.9
Butyryl-[acp]
3-Oxo-hexanoyl-[acp]
(R)-3-Hydroxy-hexanoyl-[acp]
trans-Hex-2-enoyl-[acp]
Hexanoyl-[acp]
3-Oxo-octanoyl-[acp]
(R)-3-Hydroxy-octanoyl-[acp]
trans-Oct-2-enoyl-[acp]
Octanoyl-[acp]
3-Oxo-decanoyl-[acp]
(R)-3-Hydroxy-decanoyl-[acp]
4.2.1.60
trans-Dec-2-enoyl-[acp]
Decanoyl-[acp]
3-Oxo-dodecanoyl-[acp]
(R)-3-Hydroxy-dodecanoyl-[acp]
4.2.1.61
trans-Dodec-2-enoyl-[acp]
Dodecanoyl-[acp]
3-Oxo-tetra-decanoyl-[acp]
(R)-3-Hydroxy-tetra-decanoyl-[acp]
trans-Tetra-dec-2-enoyl-[acp]
Tetra-decanoyl-[acp]
3-Oxo-hexadecanoyl-[acp]
(R)-3-Hydroxy-hexadecanoyl-[acp]
trans-Hexa-dec-2-enoyl-[acp]
Hexa-decanoyl-[acp]
3.1.2.14
acp (acyl-carrier-protein)
Fatty acid metabolism
Hexadecanoate

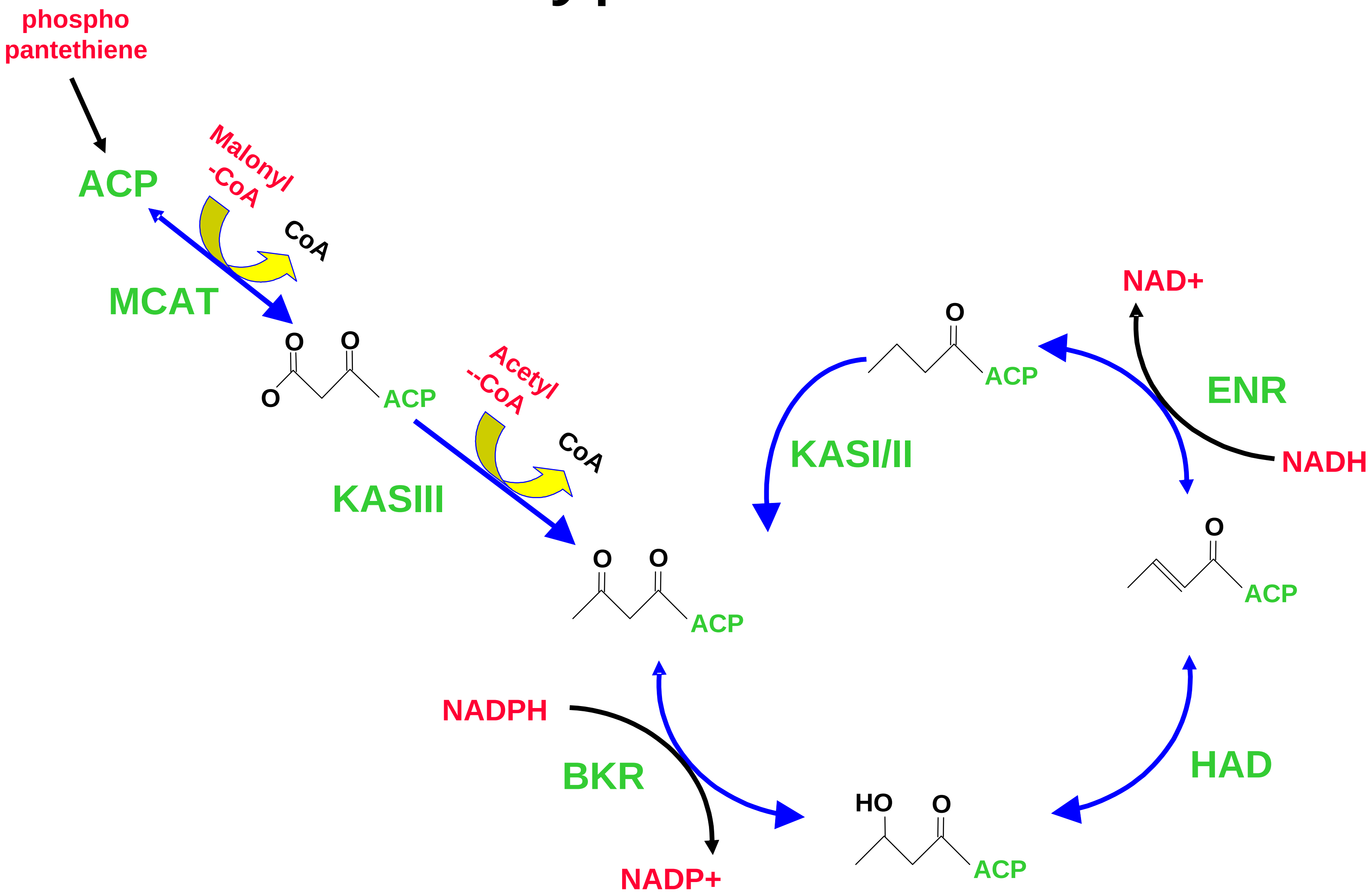
Type II FAS


ACP
MCAT
O
O
O
ACP
KASIII
O
O
ACP
KASI/II
O
ACP
ENR
O
ACP
BKR
HO
O
ACP
HAD

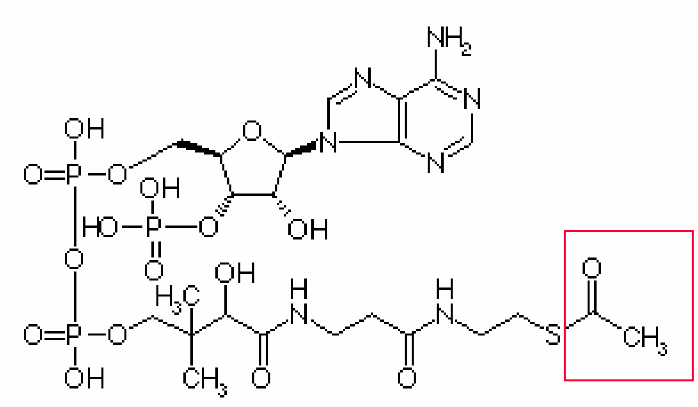
Type II FAS


Fluorescent tagged proteins are localized to the apicoplast
ACP
MCAT
KASIII
KASI/II
ENR
BKR
HAD
O
O
O
ACP
O
O
ACP
O
ACP
O
ACP
HO
O
ACP

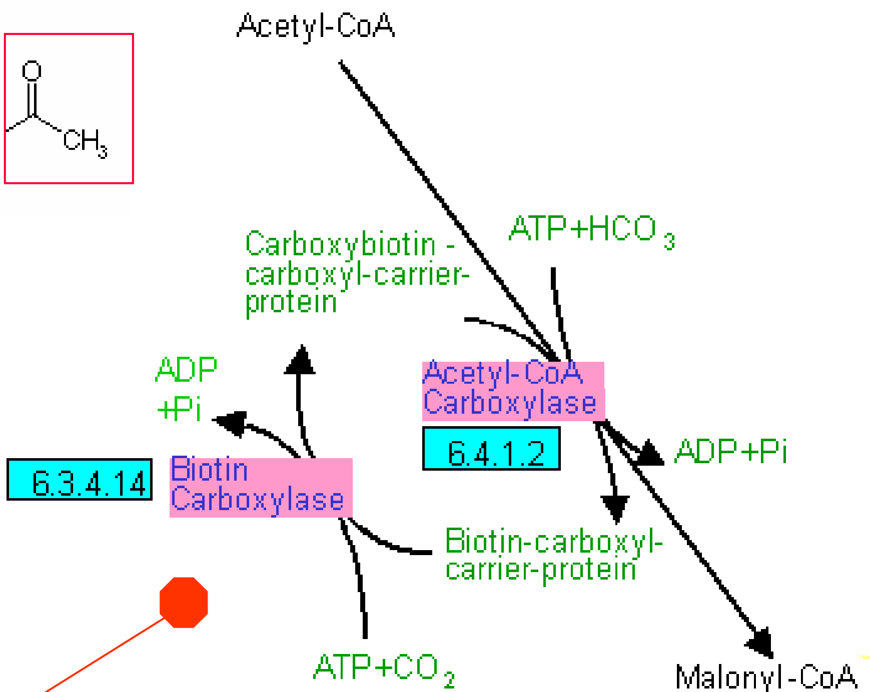
Type II FAS

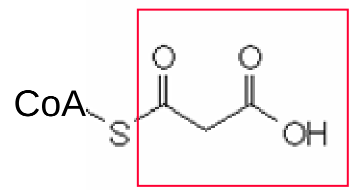

phospho
pantethiene
ACP
Malonyl
-CoA
CoA
MCAT
O
O
O
ACP
Acetyl
--CoA
CoA
KASIII
O
O
ACP
NAD+
O
ACP
ENR
KASI/II
NADH
O
ACP
NADPH
BKR
NADP+
HAD
HO
O
ACP

Type II FAS



Acetyl-CoA

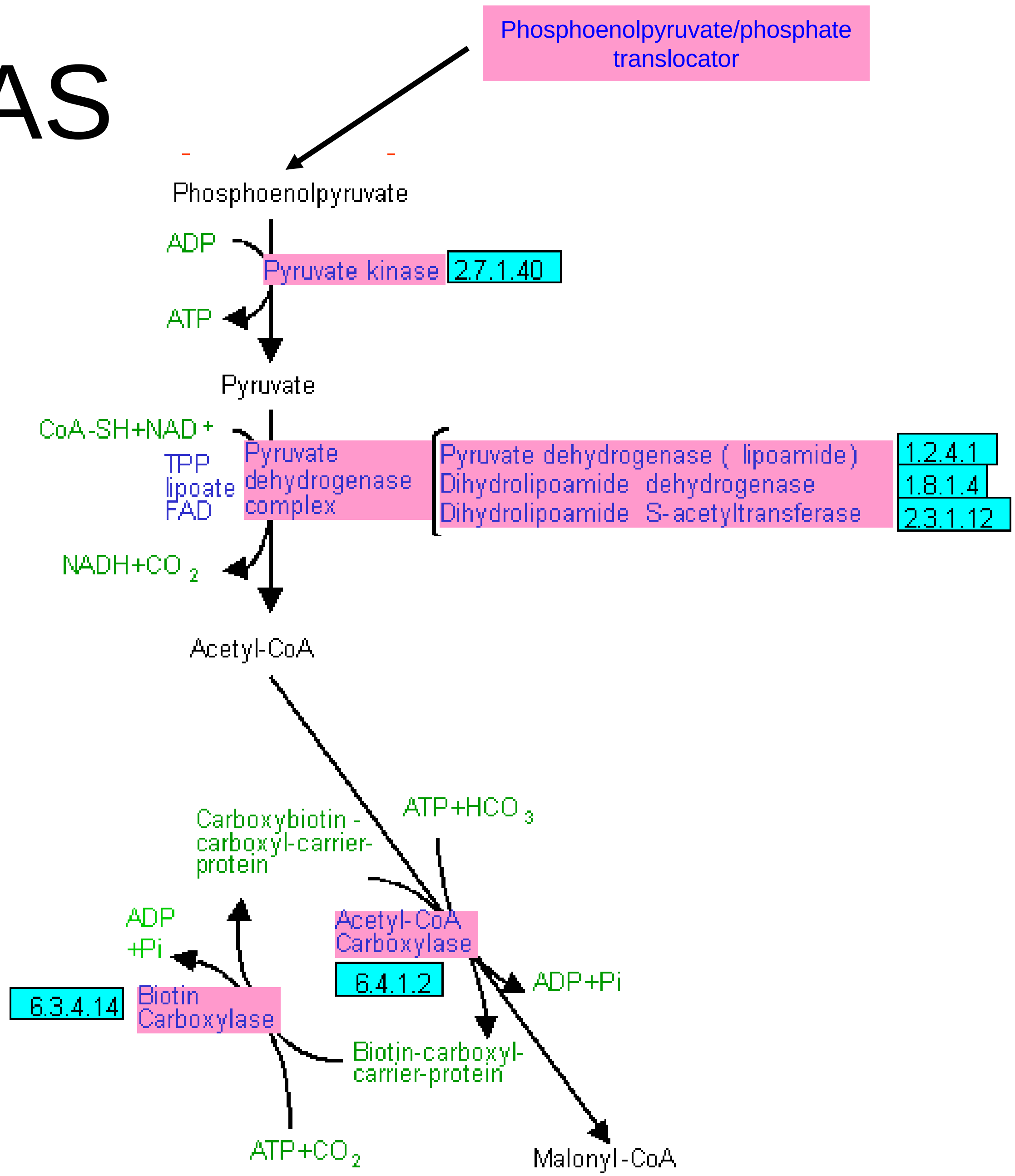




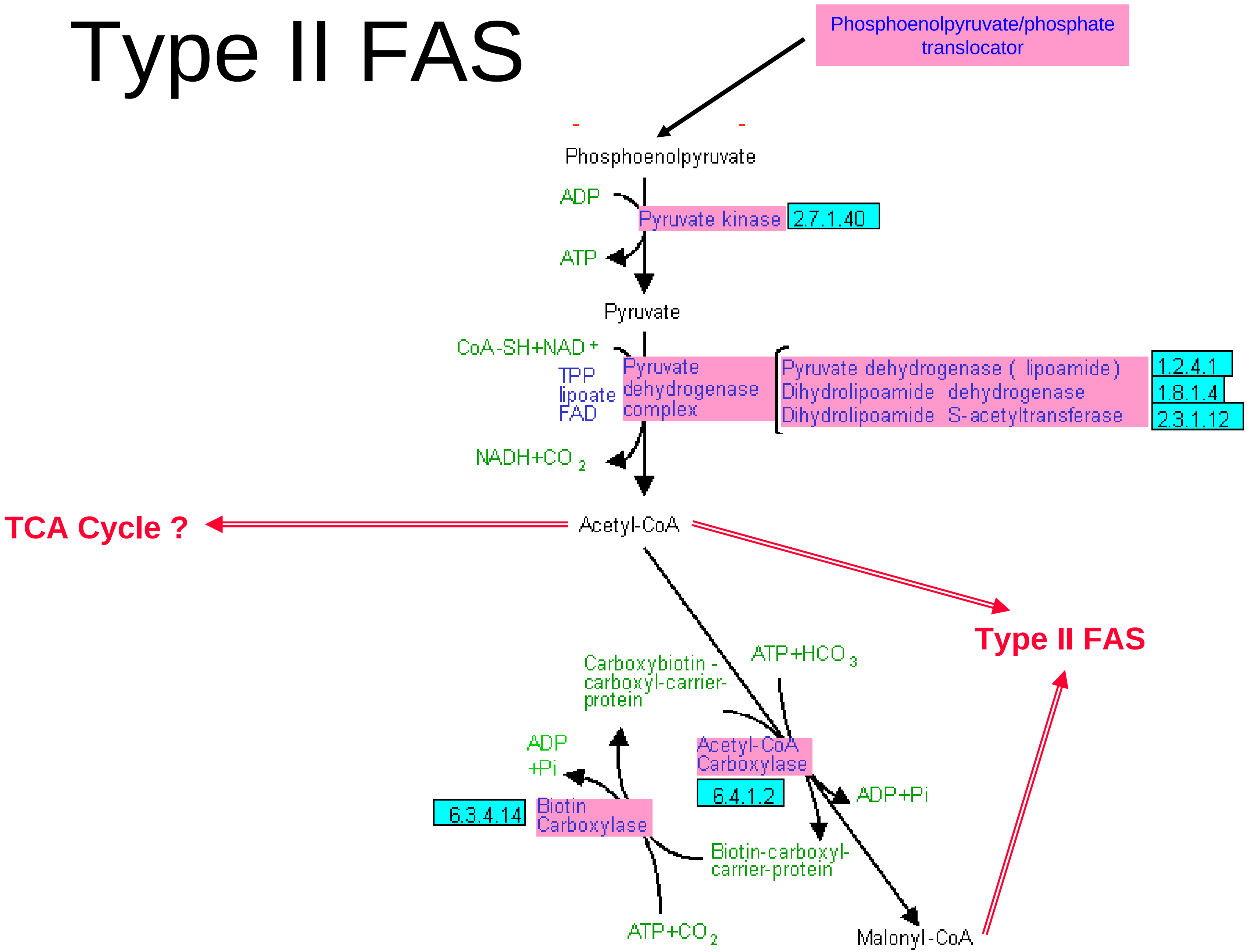
Malonyl-CoA

Dims, Fops

Type II FAS


Phosphoenolpyruvate/phosphate translocator
Phosphoenolpyruvate
ADP
Pyruvate kinase
2.7.1.40
ATP
Pyruvate
CoA-SH+NAD +
TPP
lipoate
FAD
Pyruvate dehydrogenase complex
Pyruvate dehydrogenase (lipoamide)
Dihydrolipoamide dehydrogenase
Dihydrolipoamide S-acetyltransferase
1.2.4.1
1.8.1.4
2.3.1.12
NADH+CO 2
Acetyl-CoA
Carboxybiotin - carboxyl-carrier-protein
ATP+HCO 3
ADP +Pi
Acetyl-CoA Carboxylase
6.4.1.2
ADP+Pi
6.3.4.14
Biotin Carboxylase
Biotin-carboxyl-carrier-protein
ATP+CO 2
Malonyl-CoA

Type II FAS



Phosphoenolpyruvate/phosphate translocator
Phosphoenolpyruvate
ADP
Pyruvate kinase
2.7.1.40
ATP
Pyruvate
CoA-SH+NAD +
TPP
lipoate
FAD
Pyruvate dehydrogenase complex
Pyruvate dehydrogenase (lipoamide)
Dihydrolipoamide dehydrogenase
Dihydrolipoamide S-acetyltransferase
1.2.4.1
1.8.1.4
2.3.1.12
NADH+CO 2
TCA Cycle ?
Acetyl-CoA
Type II FAS
Carboxybiotin -carboxyl-carrier-protein
ATP+HCO 3
ADP
+Pi
Acetyl-CoA Carboxylase
6.4.1.2
ADP+Pi
6.3.4.14
Biotin Carboxylase
Biotin-carboxyl-carrier-protein
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Two Conclusions

1. Apicoplast = reducing environment
   Reactions sensitive to oxidizing environment

2. Close association between mitochondrion and apicoplast
   Heme biosynthesis
   Sharing of:
      Acetyl-CoA
      tRNA-Ligases
      Fe-S
      Lipoate