Alternative Pathways for CO_2 Assimilation in Photosynthetic Microorganisms

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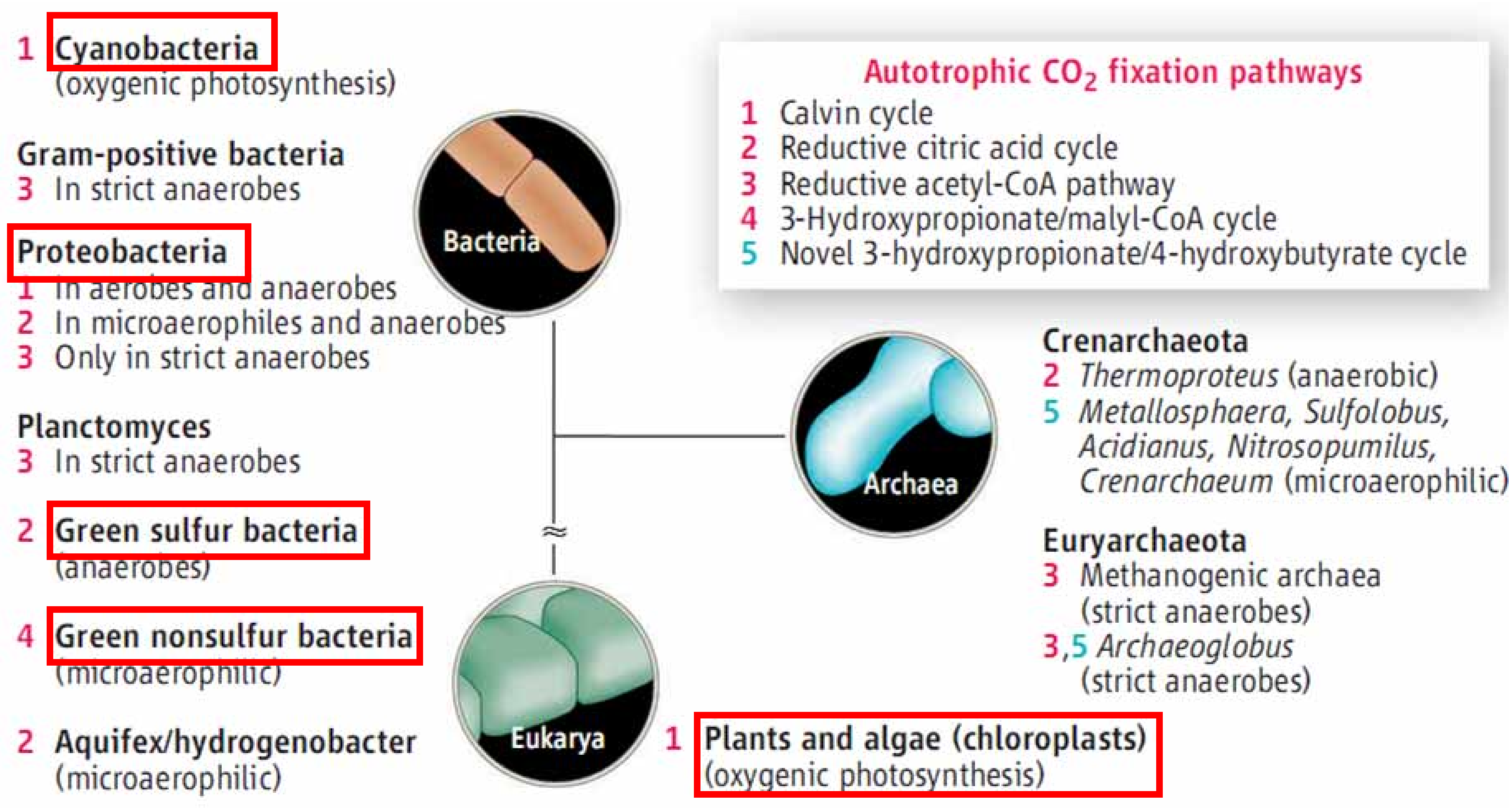
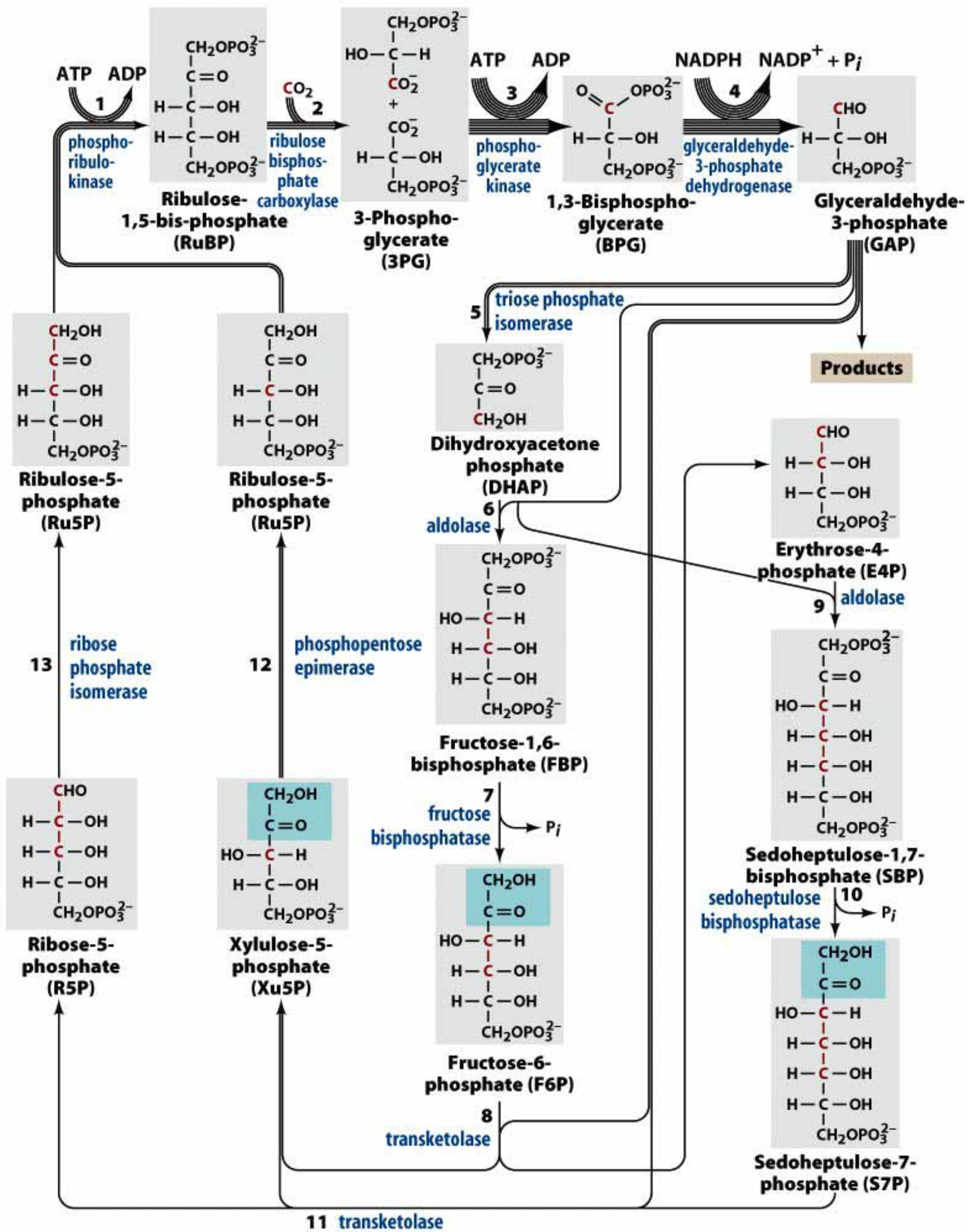
Biological Capture & Utilization of CO_2
Sept. 2, 2009

Reported autotrophic carbon fixation pathways



Thauer *Science* (2007)



Calvin Cycle

- Also called the reductive pentose phosphate cycle.
- Phases are carboxylation, reduction and regeneration.
- Many steps are identical to steps in the oxidative pentose phosphate pathway, but use chloroplast specific enzymes.

Carboxylation and phosphorylation

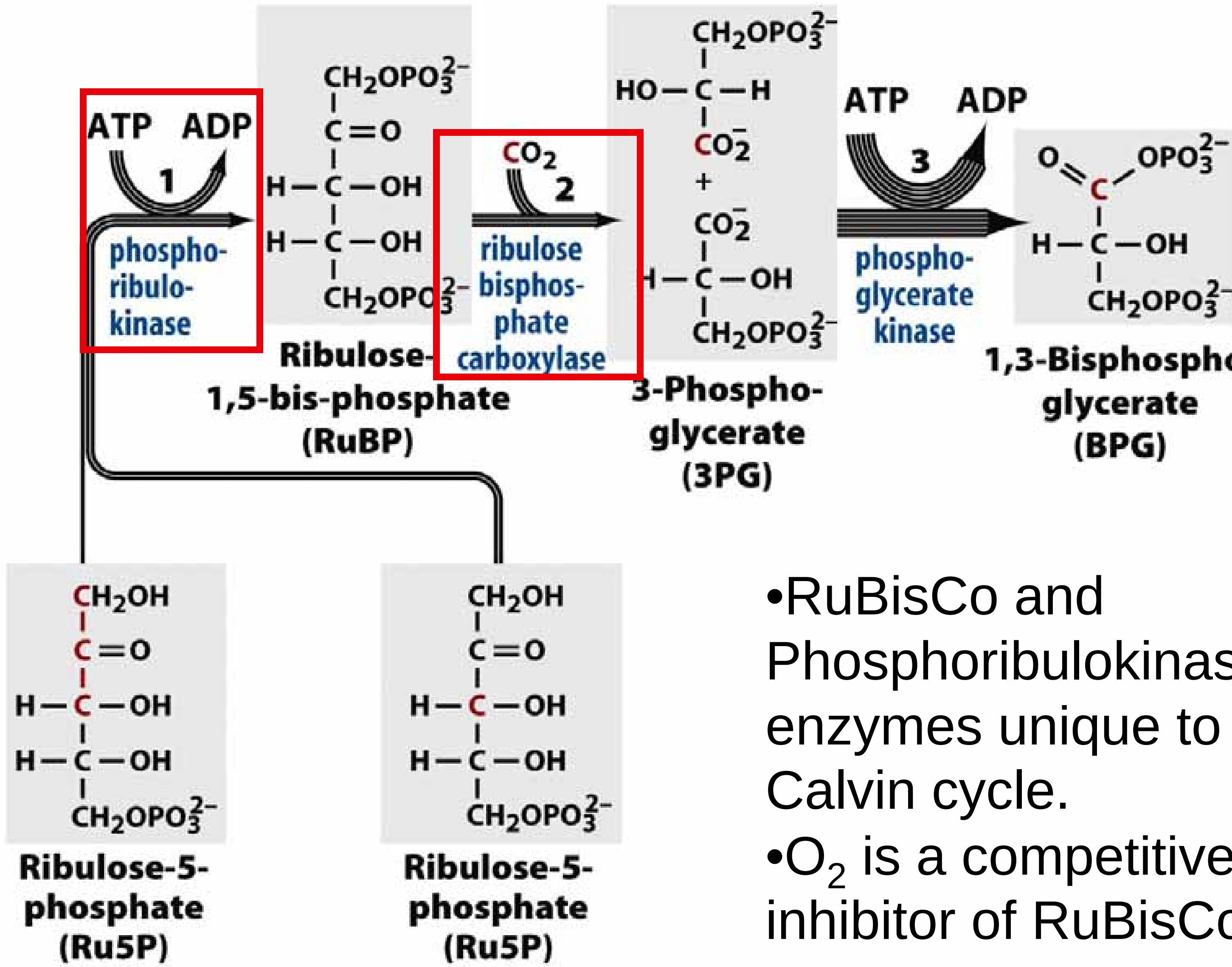
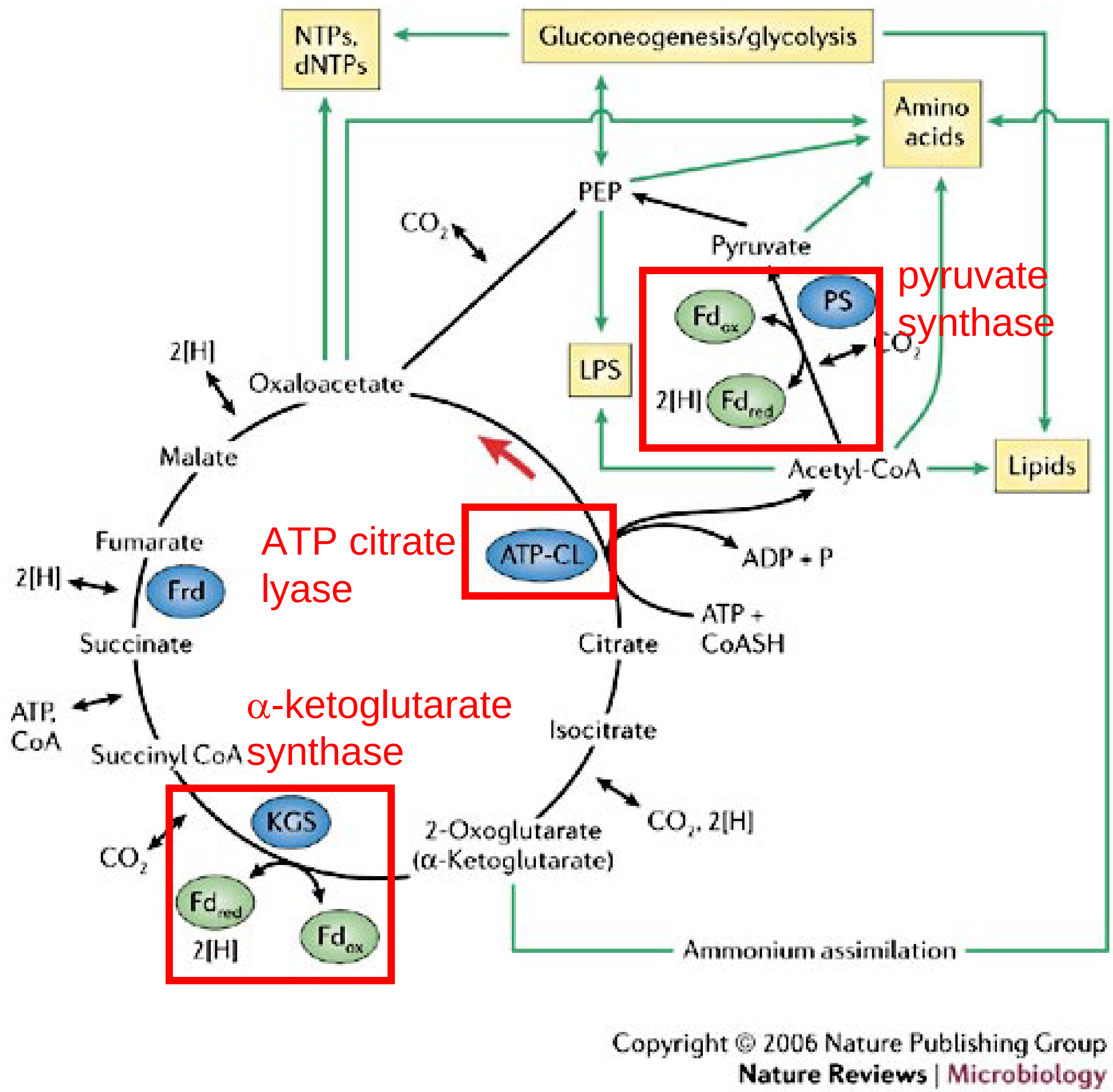


Figure 19-26 part 1

- RuBisCo and Phosphoribulokinase are enzymes unique to the Calvin cycle.
- O_2 is a competitive inhibitor of RuBisCo

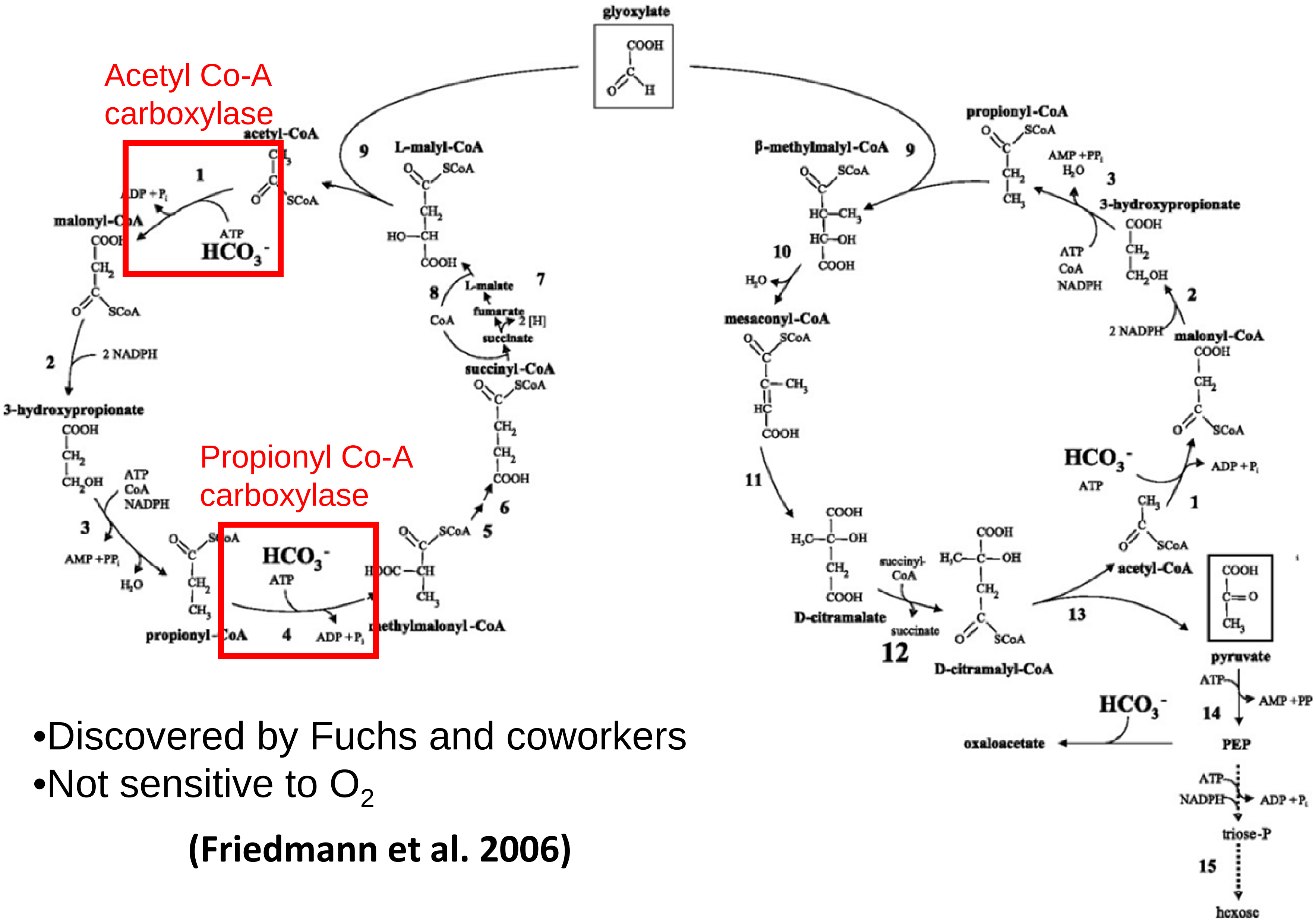
Reductive Citric Acid Cycle



- The R-CAC was originally discovered by Evans, Buchanan and Arnon in green sulfur bacteria.
- It is now known to operate in a wide range of strictly anaerobic bacteria and archaea.
- The ferredoxin-dependent enzymes are very sensitive to O_2.

Campbell *et al. Nature Reviews Microbiology* May 2006 | doi:10.1038/nrmicro1414

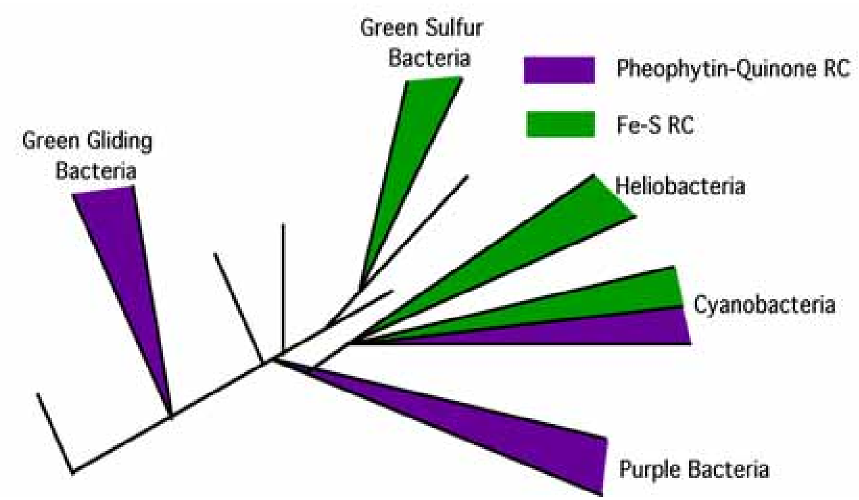
3-hydroxypropionate cycle



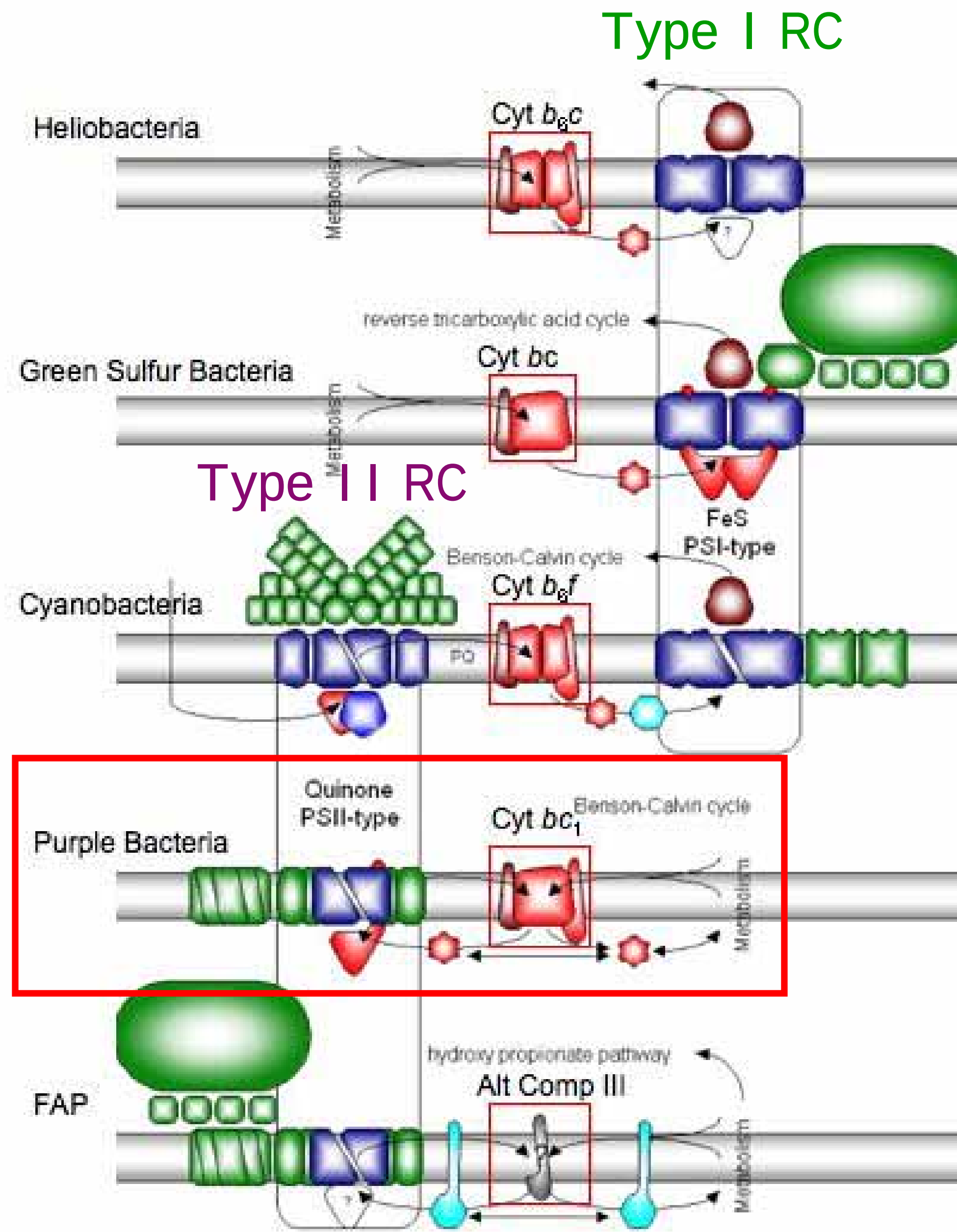
- Discovered by Fuchs and coworkers
- Not sensitive to O_2

(Friedmann et al. 2006)

Photosynthetic Proteobacteria



Most proteobacteria are not PS. All PS proteobacteria have a type II RC and cyclic ET chain. Most have Calvin cycle for carbon fixation.



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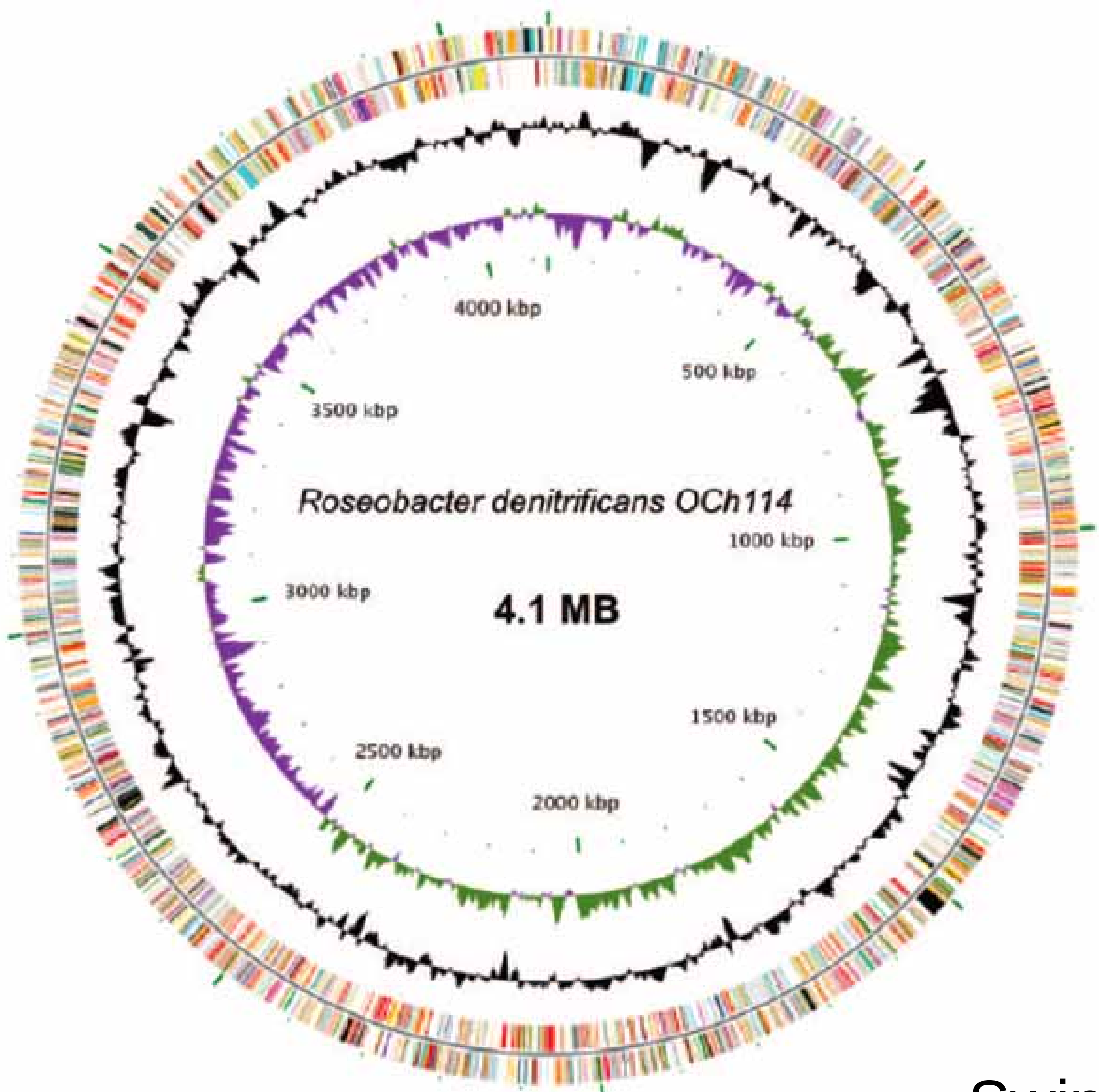
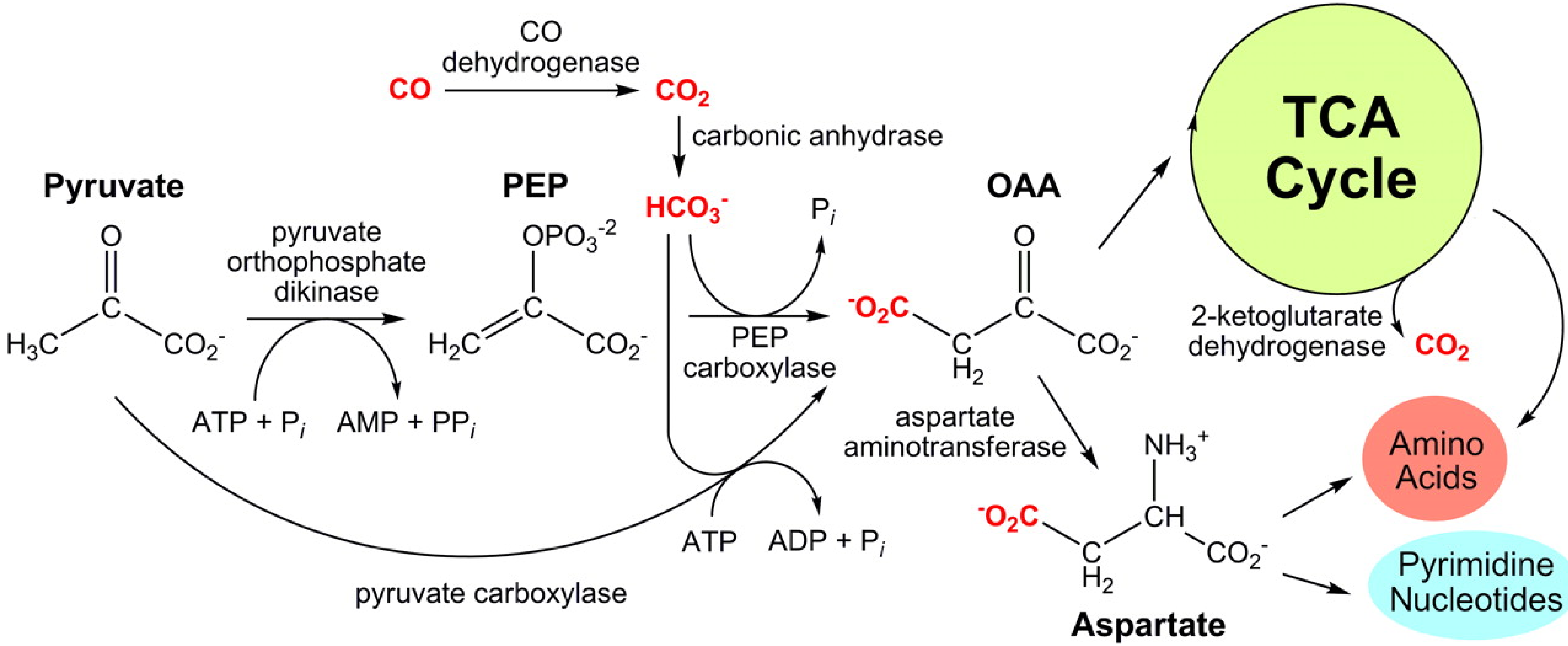
Roseobacter denitrificans



- *R. denitrificans* is a ubiquitous aerobic, anoxygenic PS proteobacterium.
- Grows photoheterotrophically in upper layers of marine ecosystems.
- It can only do PS under aerobic conditions.
- Reported to fix CO_2
- No RuBisCo or PRK genes found in genome.

Swingley et al. *J. Bact.* (2007)

Genomic data suggested anaplerotic enzymes fixing carbon in *R. denitrificans*



No evidence of any autotrophic carbon fixation pathways in genome, but organism does fix some CO_2.

Swingley et al. *J. Bact.* (2007)

Experimental Approach

- Development of minimal defined growth medium
- Labeling with 13C labeled organic compounds and HCO_3^-
- Measurements of ^{13}C-isotopomer labeling patterns in protein-derived amino acids
- Gene expression profiles
- Enzymatic activity assays

Kuo-Hsiang (Joseph) Tang

Collaborators:
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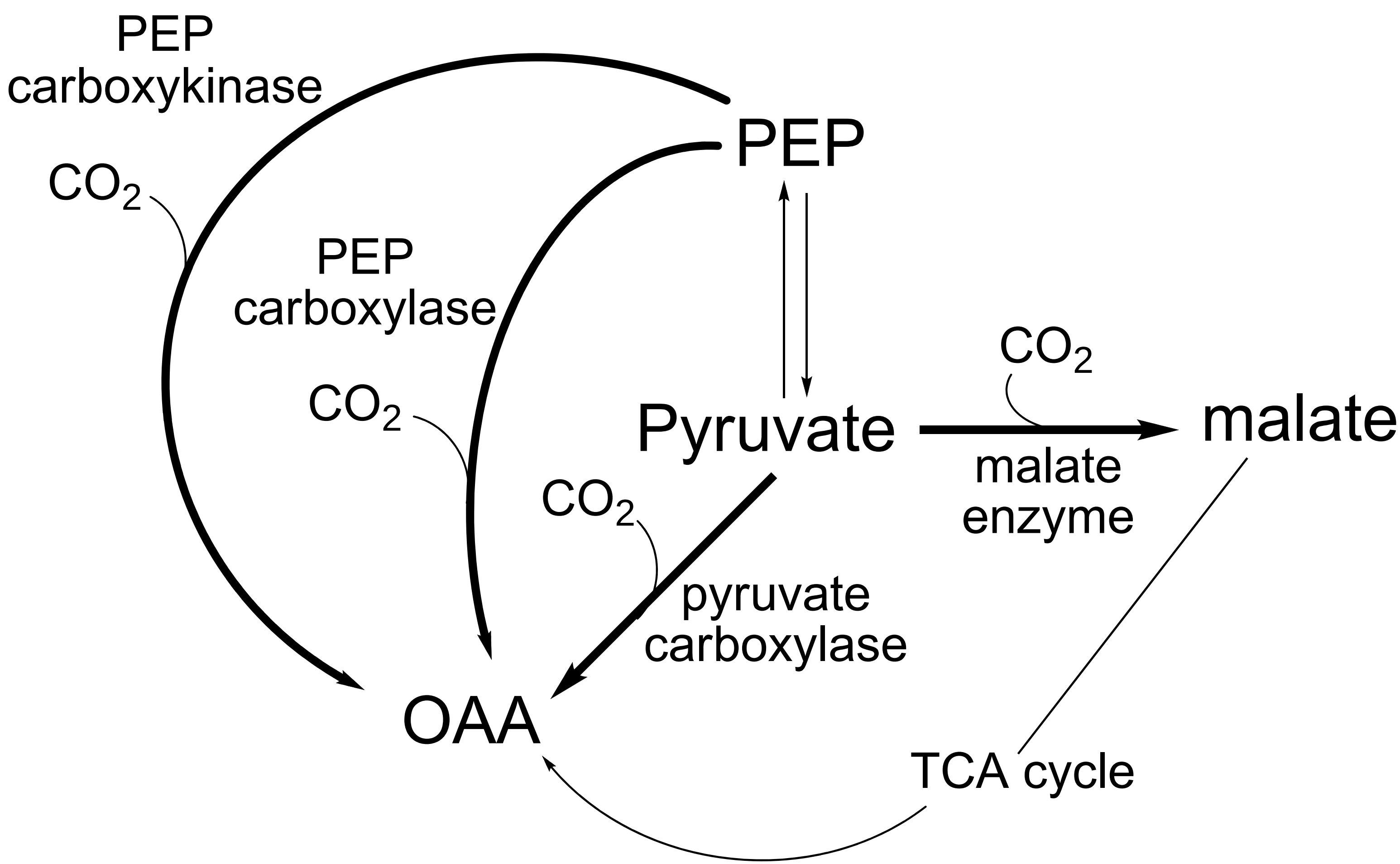
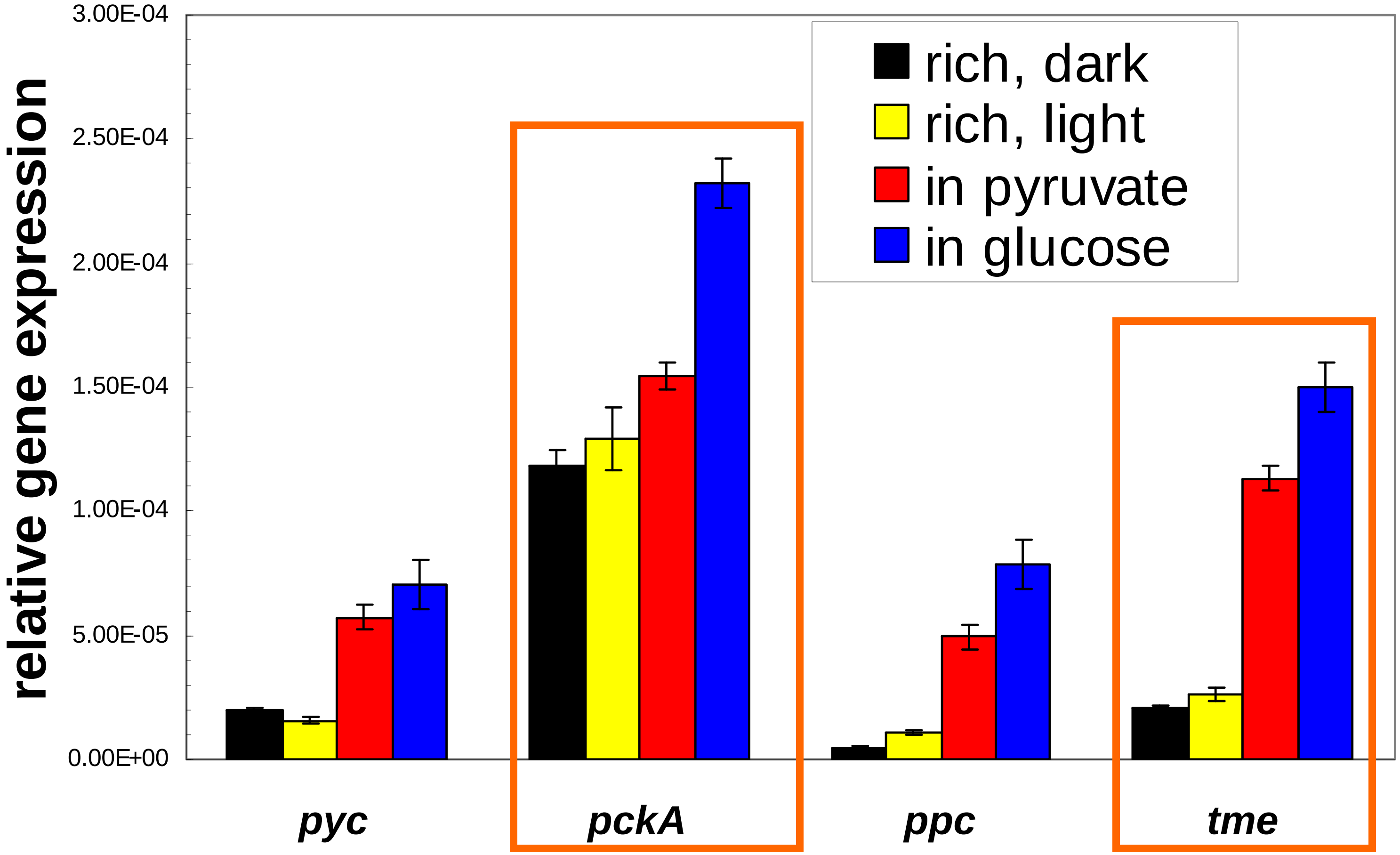
Possible anaplerotic pathways for fixing CO_2



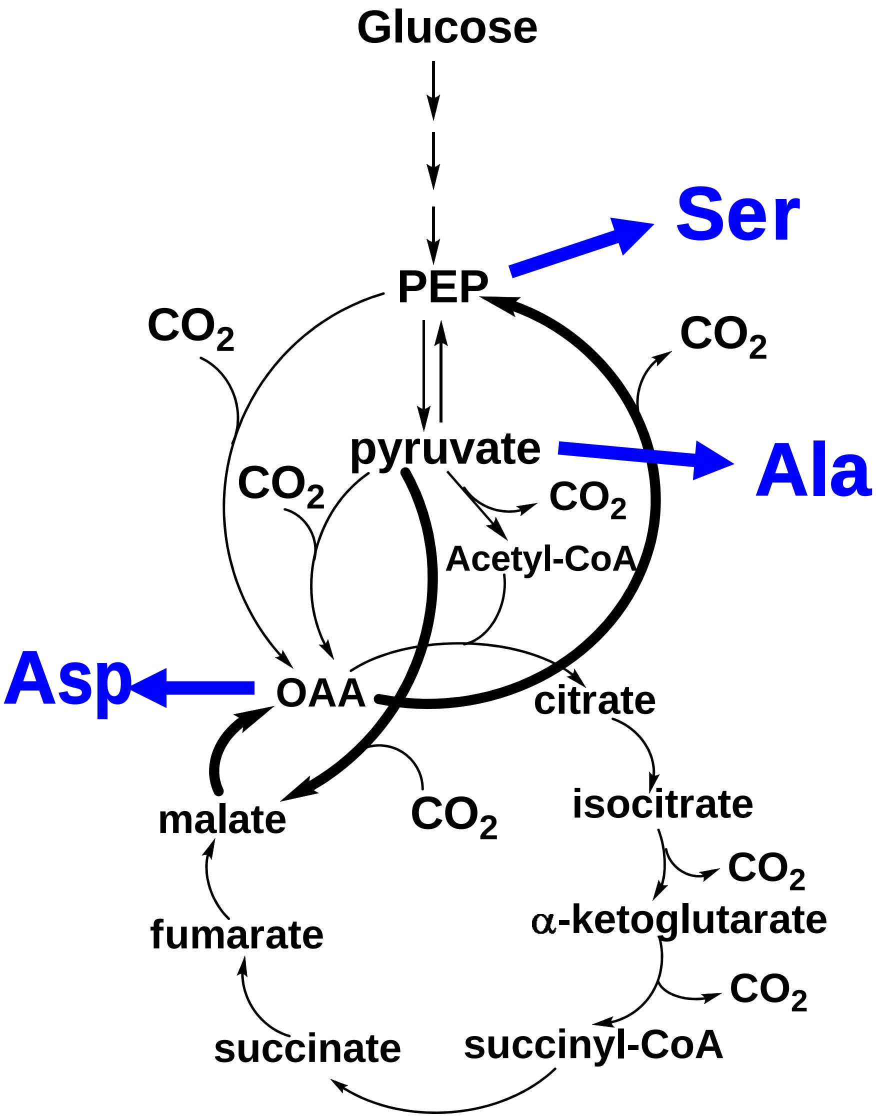
All genes found; enzymatic activity detected.

Gene expression profiles



pyc (pyruvate carboxylase), *pckA* (PEP carboxykinase), *ppc* (PEP carboxylase), and *tme* (malic enzyme)

Proposed CO_2 fixation in *R. denitrificans*



Different ^{13}C-labeling patterns in Ala vs. Ser

Experimental data suggest major flux to OAA from the TCA cycle and pyruvate

Summary of *R. denitrificans*

- Metabolic studies of carbohydrate metabolism, carbon fixation, and amino acid biosynthesis in *R. denitrificans*.

- *R. denitrificans* uses:

  - the anaplerotic pathways to fix 10-15% protein-based carbon

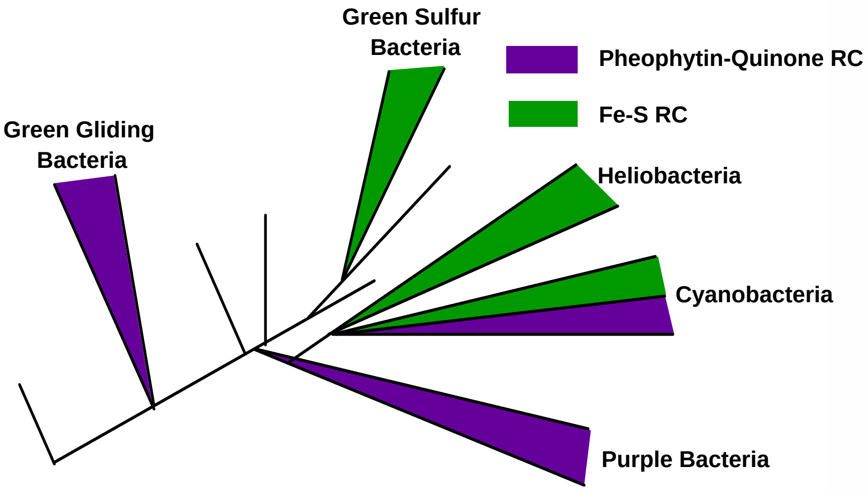
  - the Emden-Douderoff pathway predominantly for utilizing carbohydrate.

  - non-oxidative PP pathway for synthesizing ATP, nucleic acids, coenzymes and histidine.

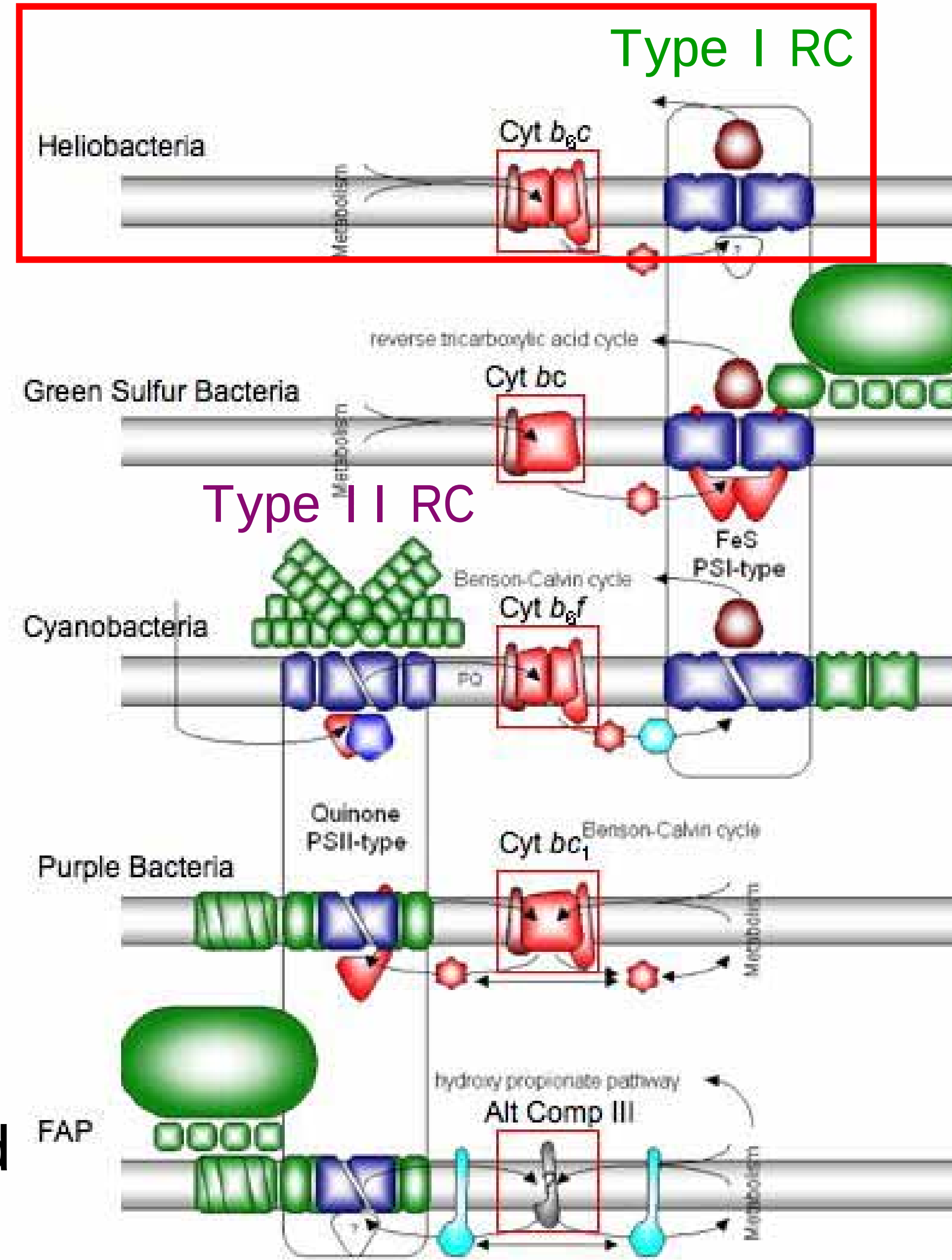
Tang et al. *PLoS One* (2009)

(Accepted pending minor revisions)

Photosynthetic Heliobacteria



Heliobacteria are the only group of Gram positive phototrophs. They have an extremely simple photosynthetic apparatus, and are not capable of photoautotrophic metabolism.



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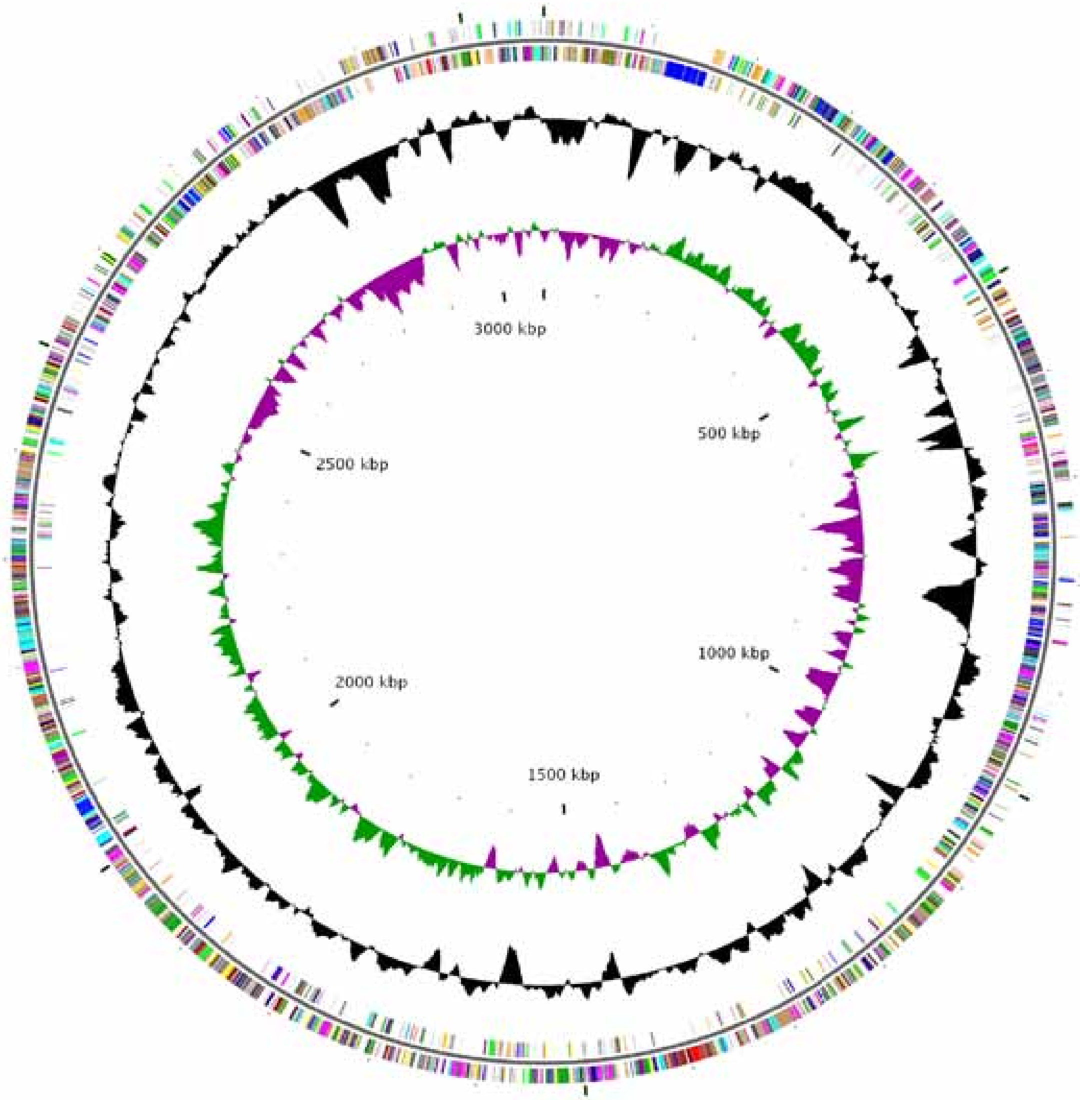
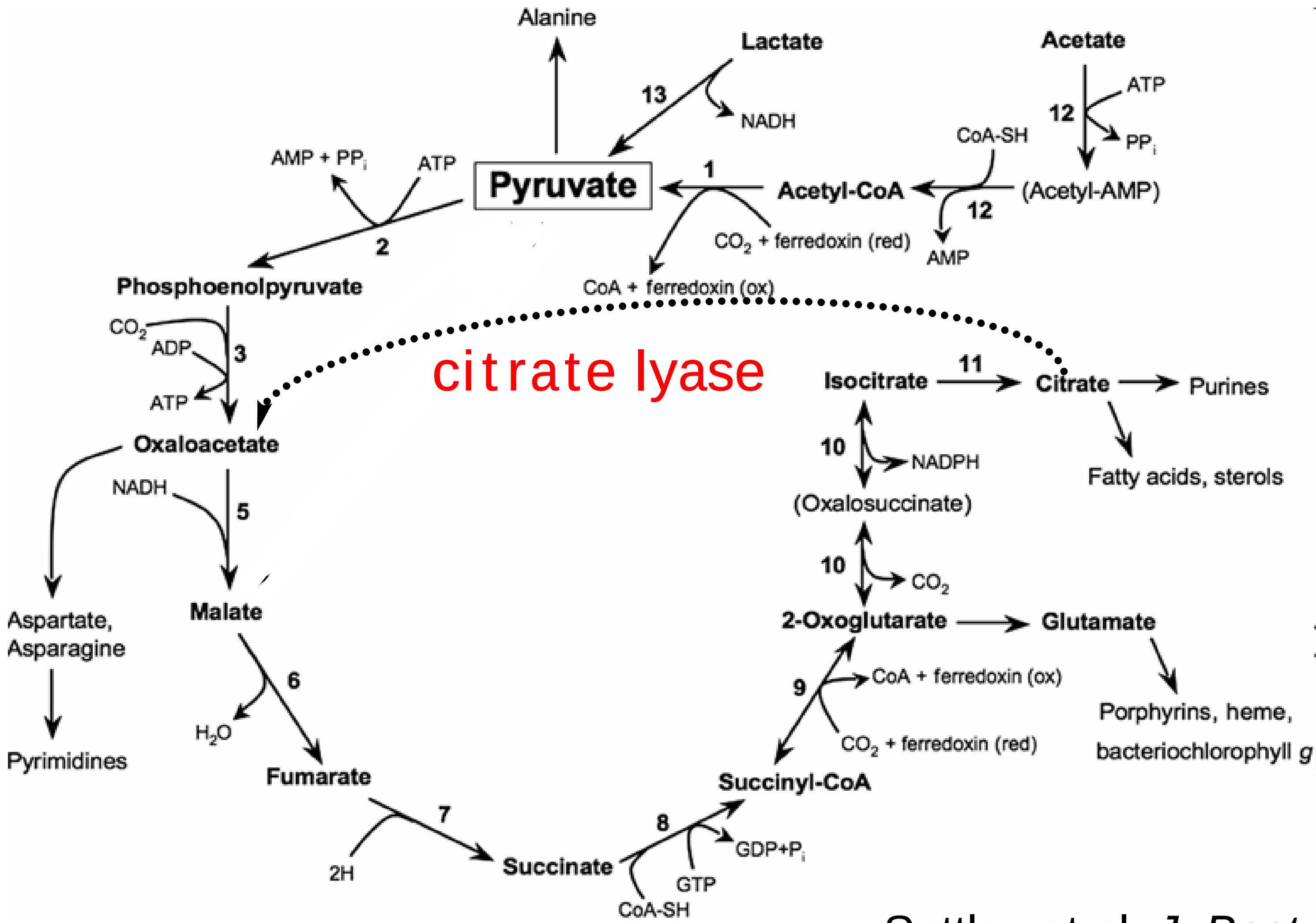
Heliobacterium modesticaldum



- Heliobacterium genome 3 Mb.
- Exhibits extreme strand coding bias.
- Has genes for N_2 fixation and a truncated set of genes for spore formation.
- No evidence of autotrophic carbon fixation.

Sattley et al.
J. Bact. 2008

All genes encoding the reverse TCA cycle found in *H. modesticaldum* except for citrate lyase



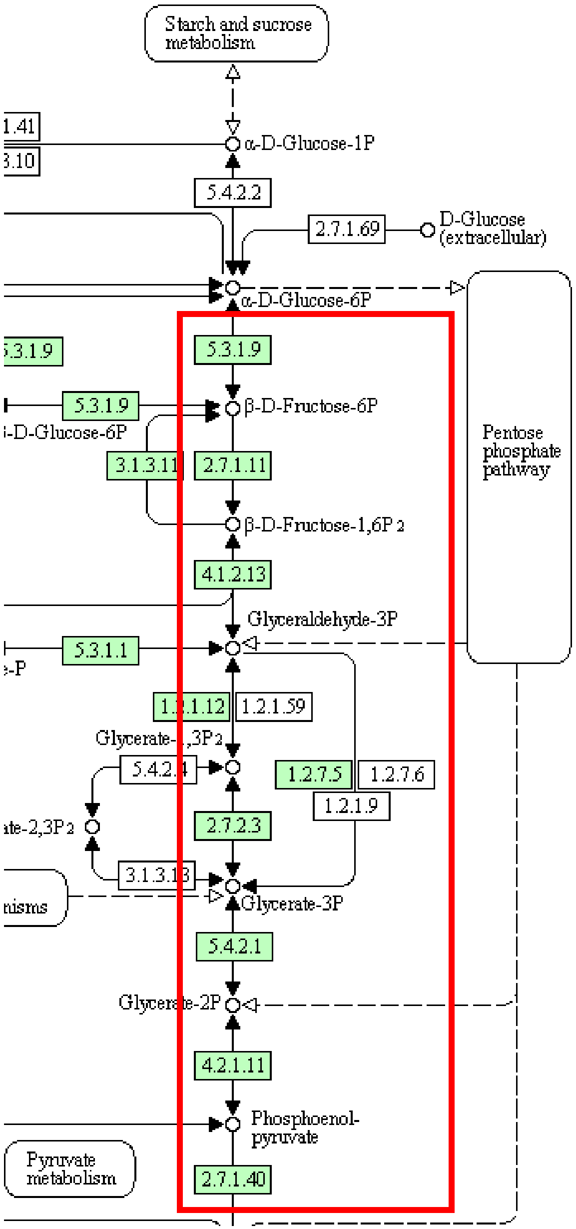
Sattley et al. *J. Bact.* (2008)

Experimental data support the genomic information

- No reductive TCA cycle
- Activity assays: citrate lyase activity cannot be detected in *H. modesticaldum*, but detected in *Cbl. tepidum* (with complete reductive TCA cycle).
- Metabolic analysis: ^{13}C-labeling patterns in protein-based amino acids
- Pyruvate carboxykinase fixing CO_2 (PEP + CO_2 -> OAA) supported by activity assay and metabolic analysis.

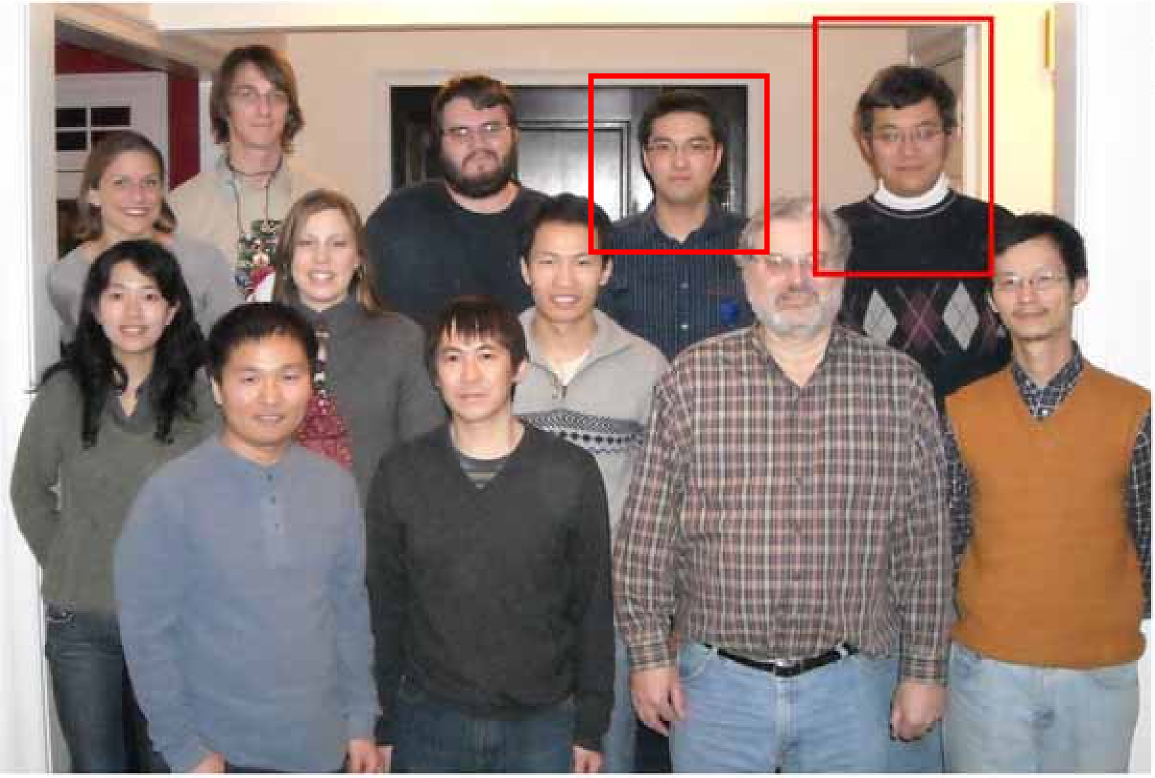
Tang et al. unpublished

Heliobacterial Carbohydrate metabolism



- Genomic information: complete glycolysis/ gluconeogenesis pathway
- Cannot grow with glucose
- The enzymatic activity of 6-phosphofructokinase and pyruvate kinase detected & glycolysis pathway is active.

Wash. U. Research Group-2009



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Supported by:

NASA Exobiology

NSF Microbial Genome Program

Mike Madigan

Jeff Touchman

Tom Beatty

Yinjie Tang