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Identification of amino acid synthesis pathways in *Desulfovibrio vulgaris* by isotopic labeling, metabolite analysis, and genome sequence analysis

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Conclusions

We propose the following pathways:

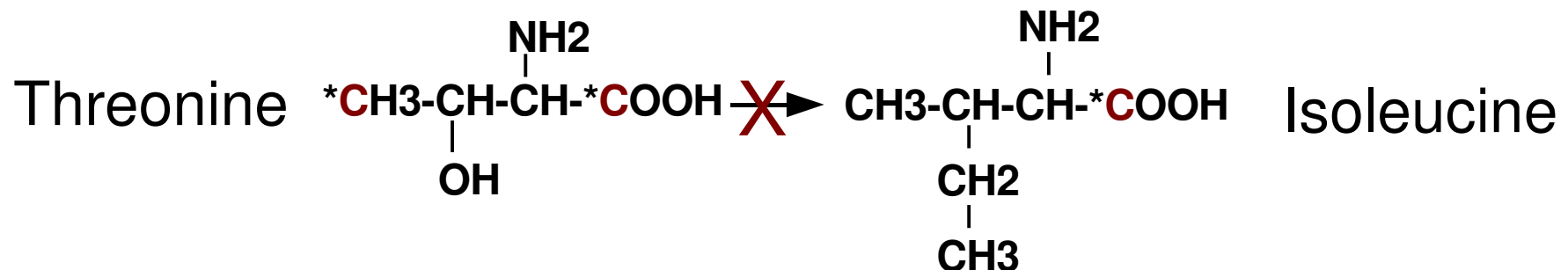
- Incomplete TCA cycle with an atypical Re-citrate synthase
 - role of DVU0398 verified in vitro and by knockout
- Synthesis of isoleucine via citramalate synthase
- Synthesis of methionine via O-succinyl-homoserine, cystathionine, and homocysteine, but the genes involved could not be identified
- Synthesis of lysine via L,L-diaminopimelate aminotransferase

Background

- *D. vulgaris Hildenborough* (DvH) is a model sulfate-reducing bacterium (δ -Proteobacteria)
 - oxidizes lactate to acetate
- Gaps in annotated pathways
 - for synthesis of Ile, Met, Lys
 - but it grows in minimal media
- TCA cycle unclear
 - often incomplete in anaerobes
 - DvH known to have Re-citrate synthase (not annotated)

Isotopic Labeling Analysis

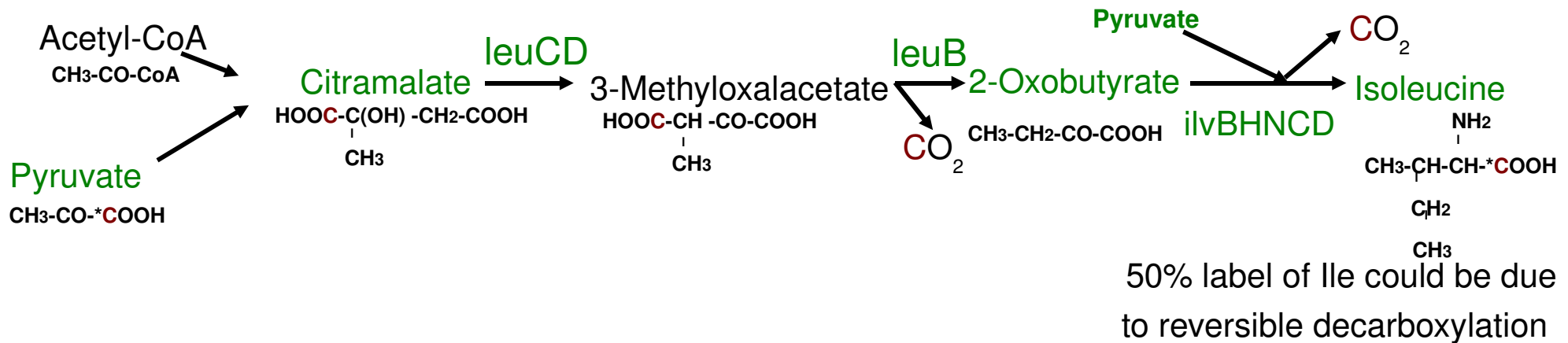
- Grow DvH on 1-¹³C lactate
- Analyze amino acids (GC/MS, FT-ICR/MS)
- Infer sources of amino acids & metabolic fluxes
 - TCA cycle ends at α -ketoglutarate & succinate
 - labeling of glutamate confirms Re-citrate synthase
 - isoleucine does not originate from threonine + acetyl-CoA



DVU0398 is Re-Citrate Synthase

- Identified by proximity to aconitase in *Clostridia*
 - enzyme was recently characterized in *C. kluyveri*¹
- In vitro, cleaves acetyl-CoA in presence of oxaloacetate
 - requires Mn^{2+} for activity
 - inactivated by O_2
- DVU0398 knockout requires α -ketoglutarate, glutamate, or glutamine to grow
 - confirms TCA cycle is broken at α -ketoglutarate dehydr.

Predicted Citramalate Synthase DVU1914

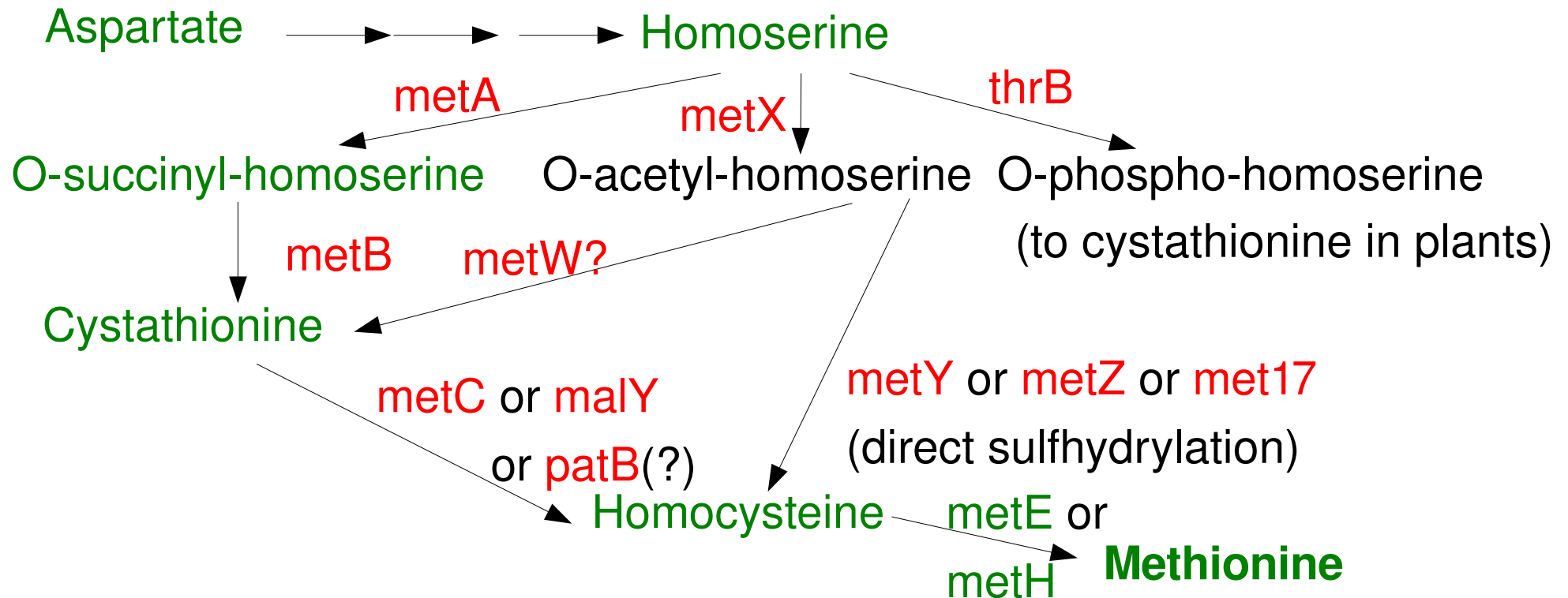


DVU1914 is a member of the IPM synthase family

- includes leuA, homocitrate, citramalate, Re-citrate synthase
- close homologs of DVU1914 are often near leuA, ilvCDBH
 - trees suggest DVU2981 is correctly annotated as leuA
- >50% identical to recently identified citramalate synthase in *G. sulfurreducens* (C. Risso et al., J Bact 190:2266-74)

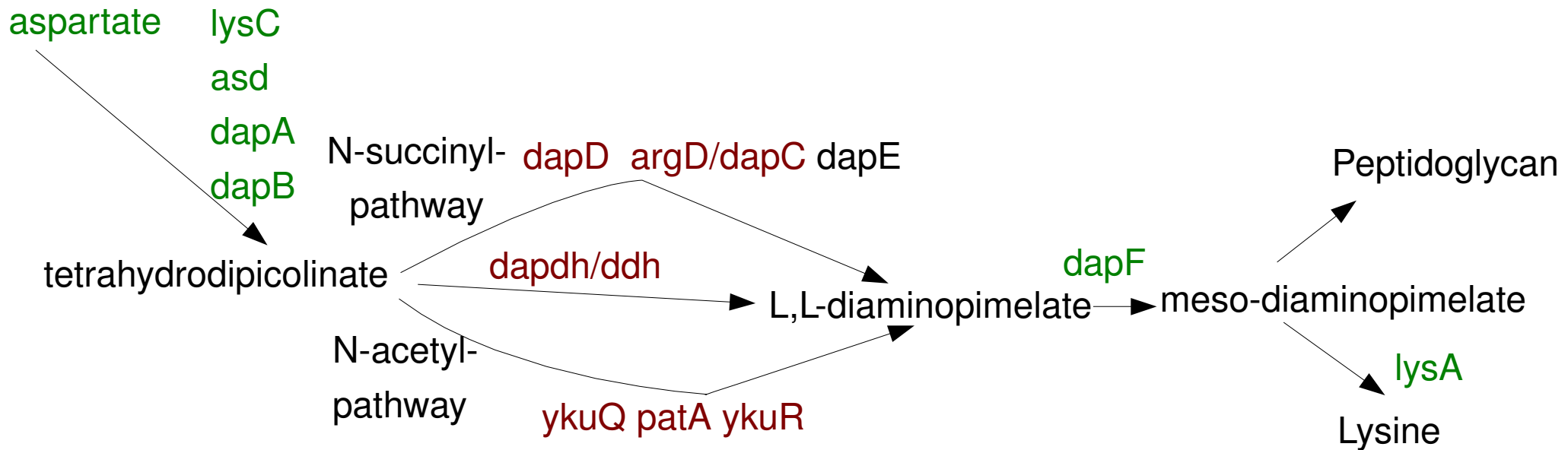
Methionine Synthesis Mystery

- No annotated genes to convert homoserine to homocysteine
- Metabolomics supports the trans-sulfhydration pathway
- Candidate genes identified, but knockouts still grow w/o external methionine (“metW”, DVU3369; “patB”; DVU0171)



Diaminopimelate Synthesis via L,L-DAP aminotransferase

Standard bacterial pathways are absent



L,L-DAP aminotransferase recently identified in bacteria

DVU1655 has close homologs in proximity to **dapF** in other organisms

54% identical to **dapL1** from *Moorella thermoacetica*

A. Hudson et al., J. Bact 2008, 190:3256-63