Advanced Biofuels Overview

January 21, 2009

Factors to Consider

- Utility
  - Who is the end user?
- Production scalability
  - US gasoline consumption: 390 million gallons/day...
- Environmental friendliness

Liquid Fuels

Table 1

Types of liquid fuels.

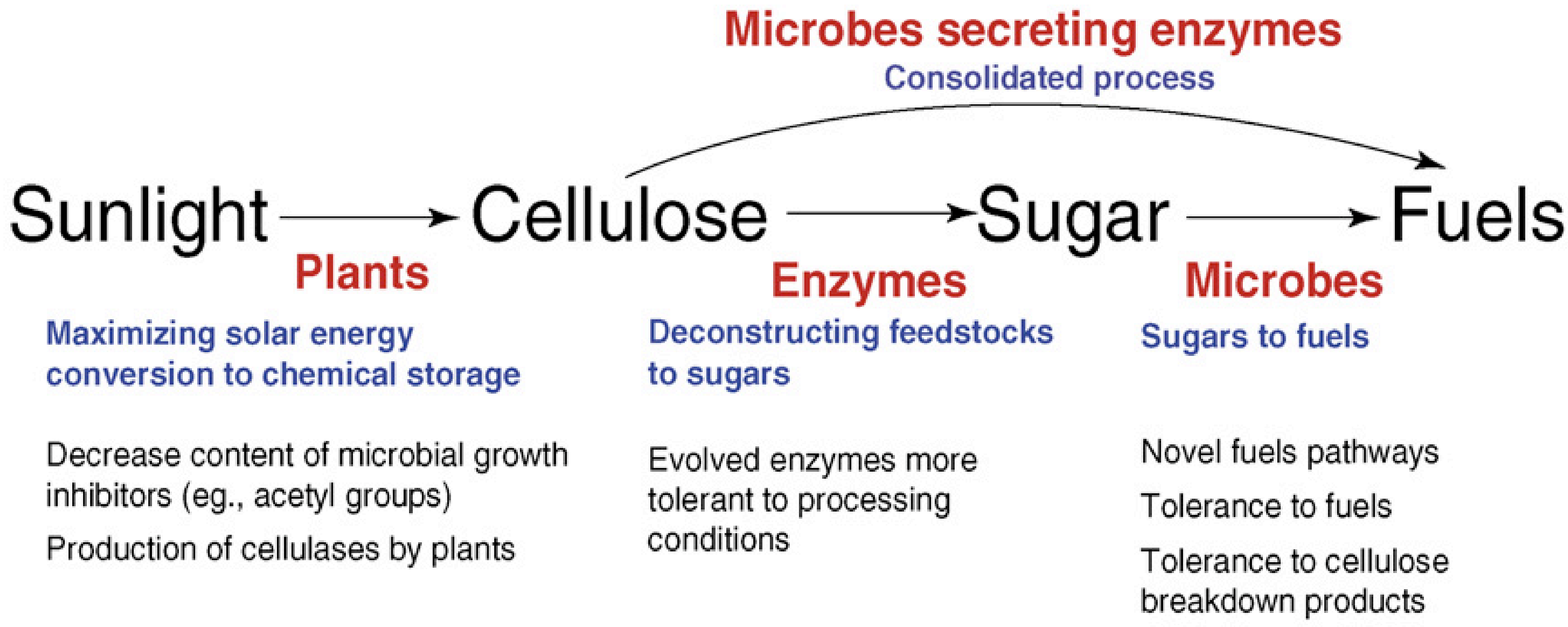
Fuel type	Major components	Important property	Biosynthetic alternatives
Gasoline	C_4–C_{12} hydrocarbons	Octane number[a]	Ethanol, *n*-butanol and *iso*-butanol
	Linear, branched, cyclic, aromatics	Energy content[b]	Short chain alcohols
	Anti-knock additives	Transportability	Short chain alkanes
Diesel	C_9–C_{23} (average C_{16})	Cetane number[c]	Biodiesel (FAMEs)
	Linear, branched, cyclic, aromatic	Low freezing temperature	Fatty alcohols, alkanes
	Anti-freeze additives	Low vapor pressure	Linear or cyclic isoprenoids
Jet fuel	C_8–C_{16} hydrocarbons	Very low freezing temperature	Alkanes
	Linear, branched, cyclic, aromatic	Net heat of combustion	Biodiesel
	Anti-freeze additives	Density	Linear or cyclic isoprenoids

[a] A measurement of its resistance to knocking. Knocking occurs when the fuel/air mixture spontaneously ignites before it reaches the optimum pressure and temperature for spark ignition.
[b] The amount of energy produced during combustion. The number of C–H and C–C bonds in a molecule is a good indication of how much energy a particular fuel will produce.
[c] A measurement of the combustion quality of diesel fuel during compression ignition. A shorter ignition delay, the time period between the start of injection and start of combustion of the fuel is preferred, and the ignition delay is indexed by the cetane number.

Lee et al. Curr Opin Biotechnol. 2008 Dec;19(6):556-63. Epub 2008 Nov 10.

Advanced Biofuel

Feedstock --> conversion technology --> End product



Lee et al. Curr Opin Biotechnol. 2008 Dec;19(6):556-63. Epub 2008 Nov 10.

Major Avenues of R&D

- Feedstock optimization
    - Fermentation Technology
        - Improving the economy of glucose
    - Photosynthetic Processes

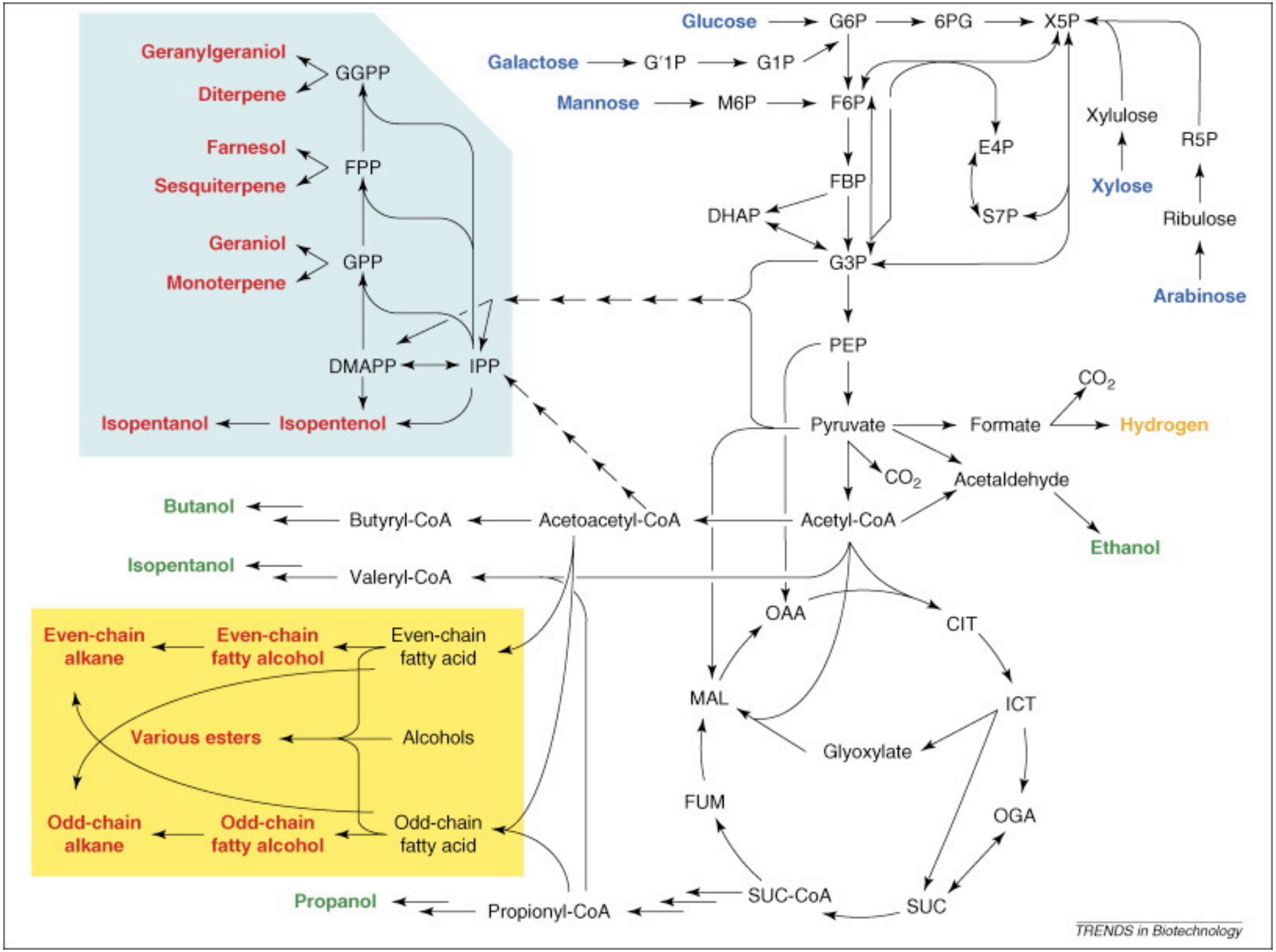
Synthetic Biology in Biofuel Production

- Host
  - *E. coli*, *S. cerevisiae*, Microalgae, etc.
- Pathway
  - Major metabolic pathways: amino acid, fatty acid, isoprenoid,
- Fuels

Popular Hosts

Organism	Fermentative/ Photosynthetic	Genetically Modifiable	Generation Time	Pathways Explored
E. coli	Fermentative	Yes	0.33 hr	Amino Acid, Fatty Acid, Isoprenoid
S. cerevisiae	Fermentative	Yes	1.5 hr	Amino Acid, Fatty Acid, Isoprenoid
Cyanobacteria	Photosynthetic	Yes	6 - 8 hr	
Green Microalgae	Fermentative/ Photosynthetic	Yes	8 hr	

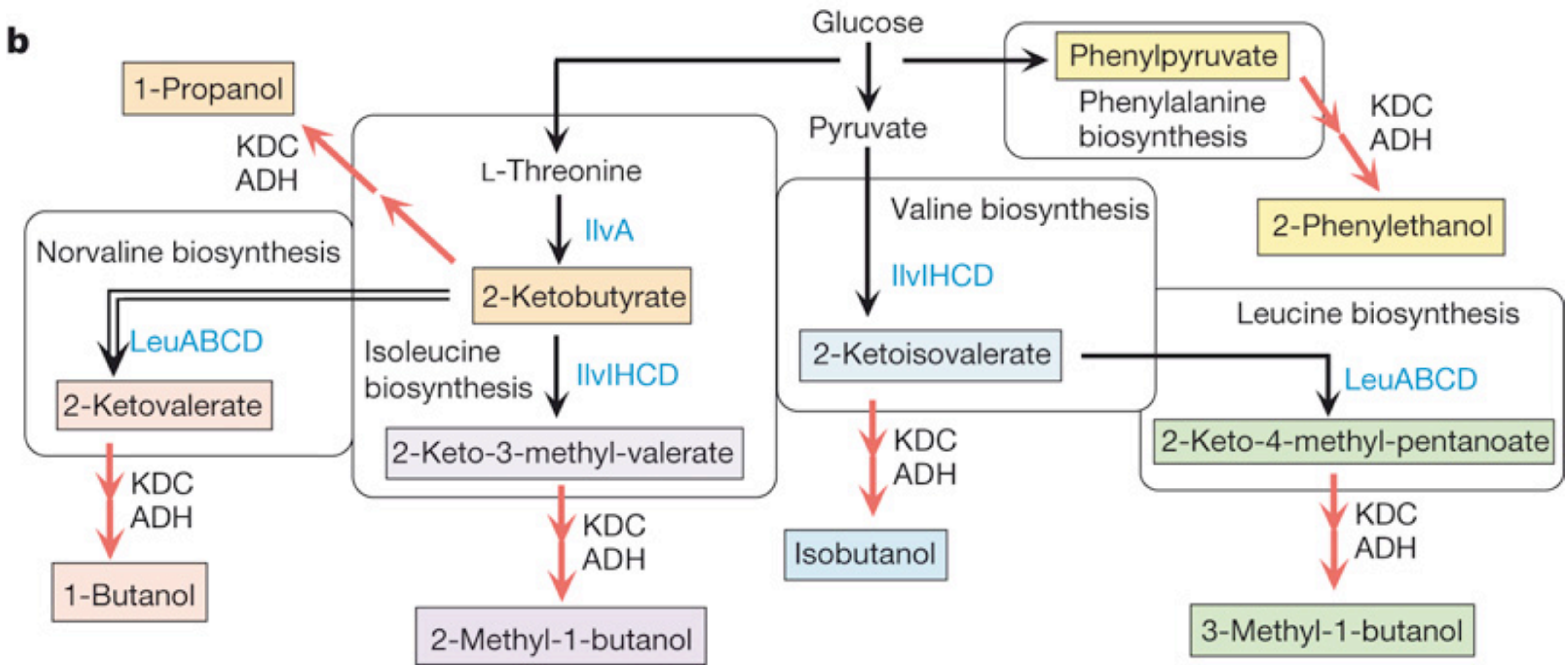
Metabolic Engineering



Fortman et al. Trends Biotechnol. 2008 Jul;26(7):375-81. Epub 2008 May 28.

Amino Acid Pathway

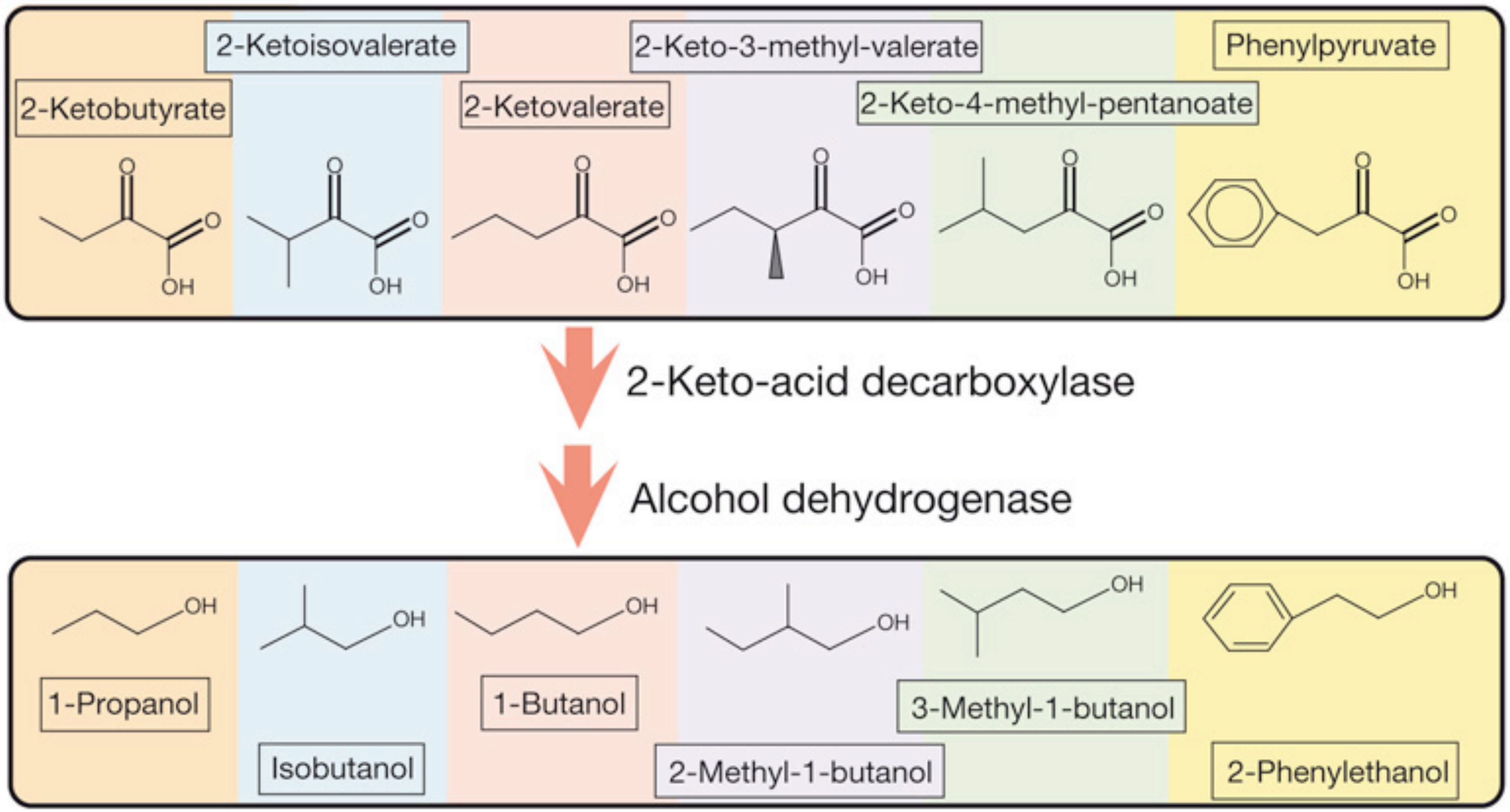
James Liao, UCLA



gevo™ NEXT GENERATION BIOFUELS

Atsumi et al. Nature. 2008 Jan 3;451(7174):86-9.

Amino Acid Pathway



Renewable Gasoline, Host: *E. coli*, *S. cerevisiae*

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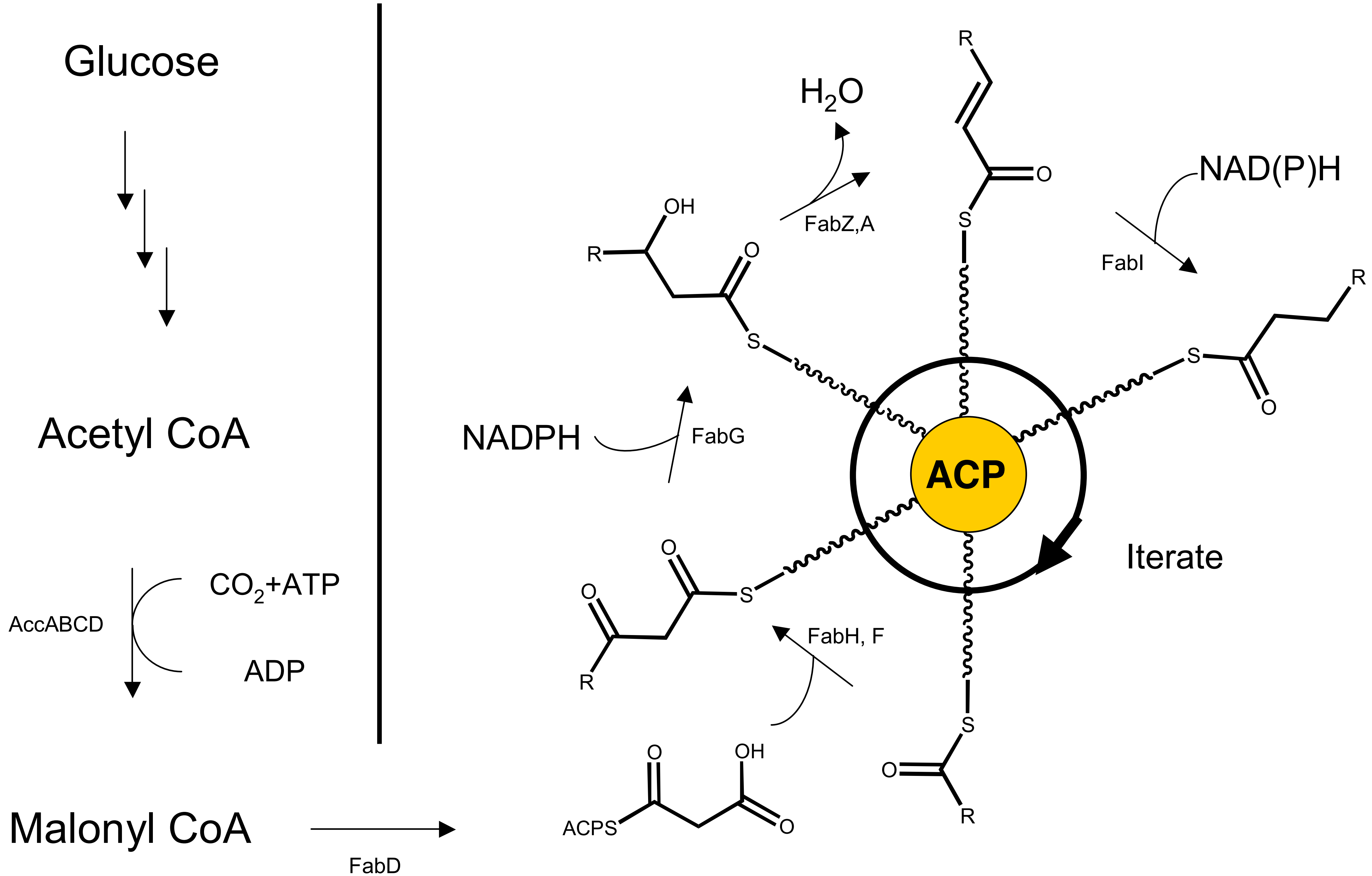
Atsumi et al. Nature. 2008 Jan 3;451(7174):86-9.

Fatty Acid Pathway



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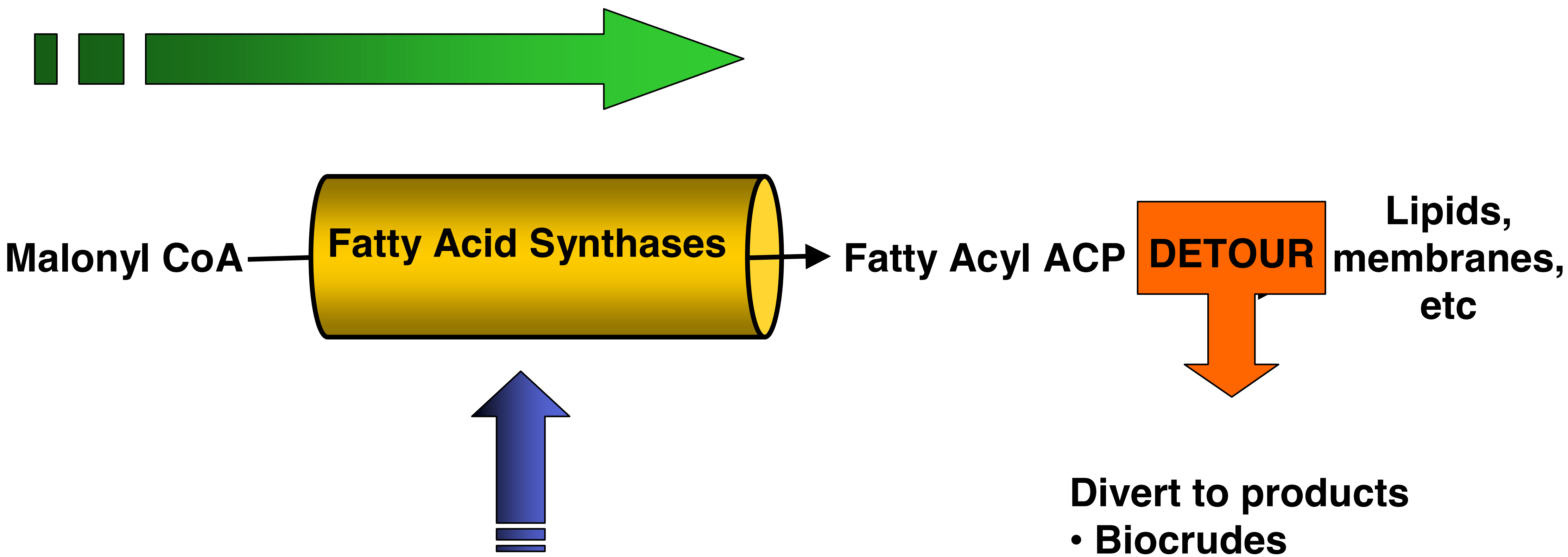
Fatty Acid Pathway

Improve Flux
- precursor supply
- regulation
- competing pathways



Control structure
- molecular weight
- modification

Divert to products
- Biocrudes
- Biofuels
- Chemicals

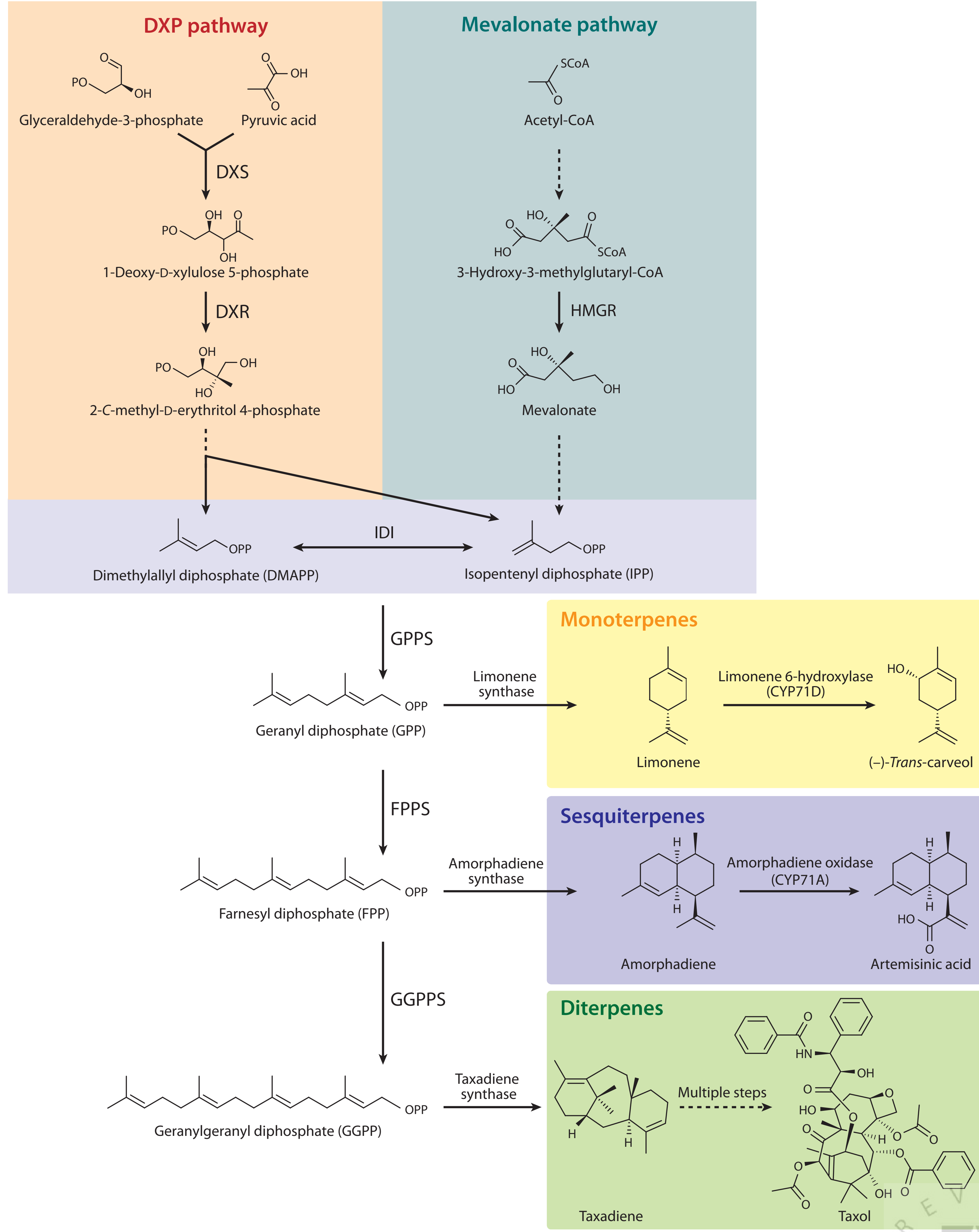
LS9, INC.

Isoprenoid Pathways

Jay Keasling,
UC Berkeley

Hosts: *E. coli* and *S. cerevisiae*

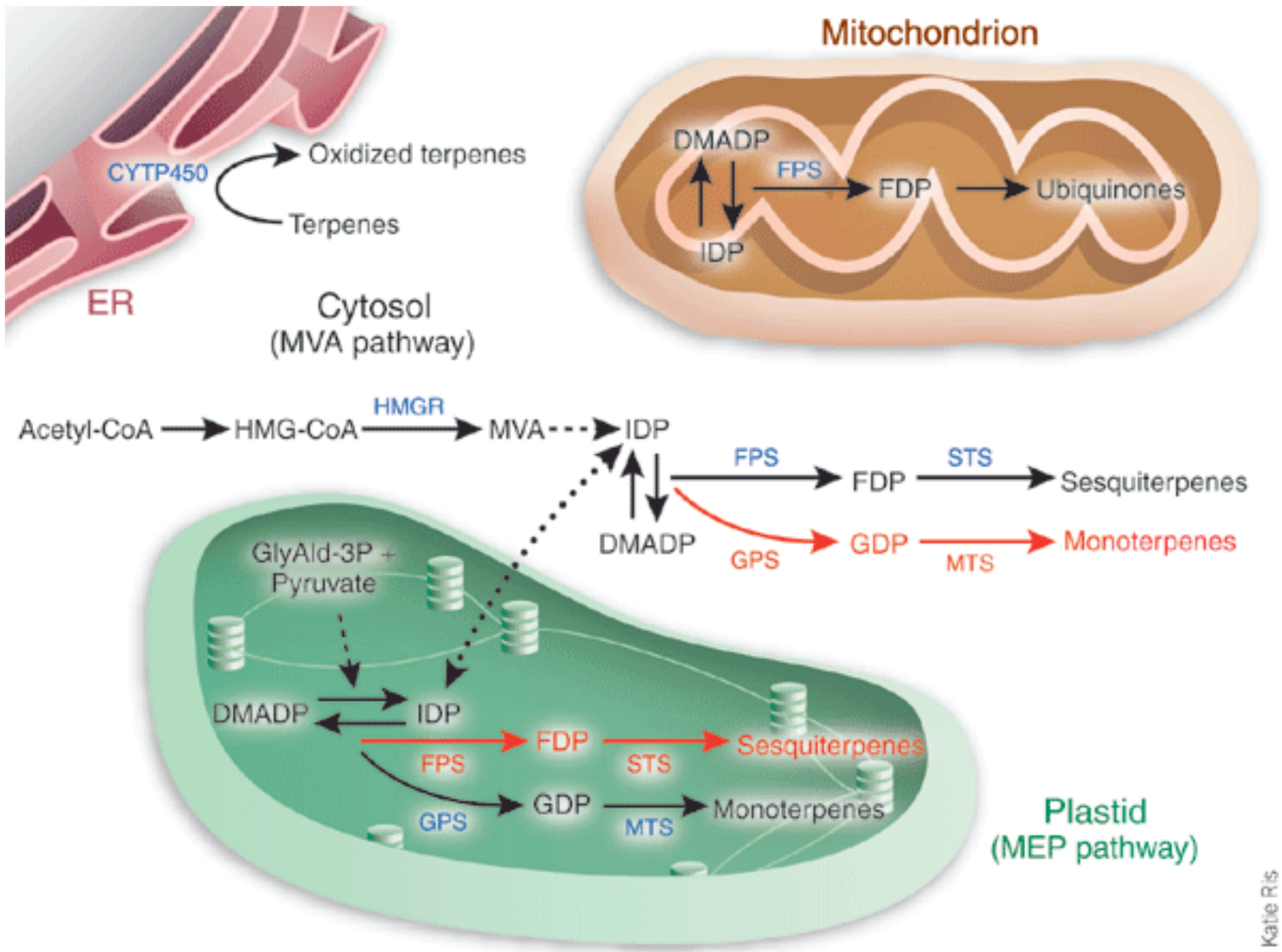
Fermentative Approach



Kirby et al. Annu Rev Plant Biol. 2009 Jan 9.

Isoprenoids from Plants

Joe Chappell, U. Kentucky



Chloroplast: 1000X Increase in Yield

Nature Biotechnology - 24, 1359 - 1361 (2006)

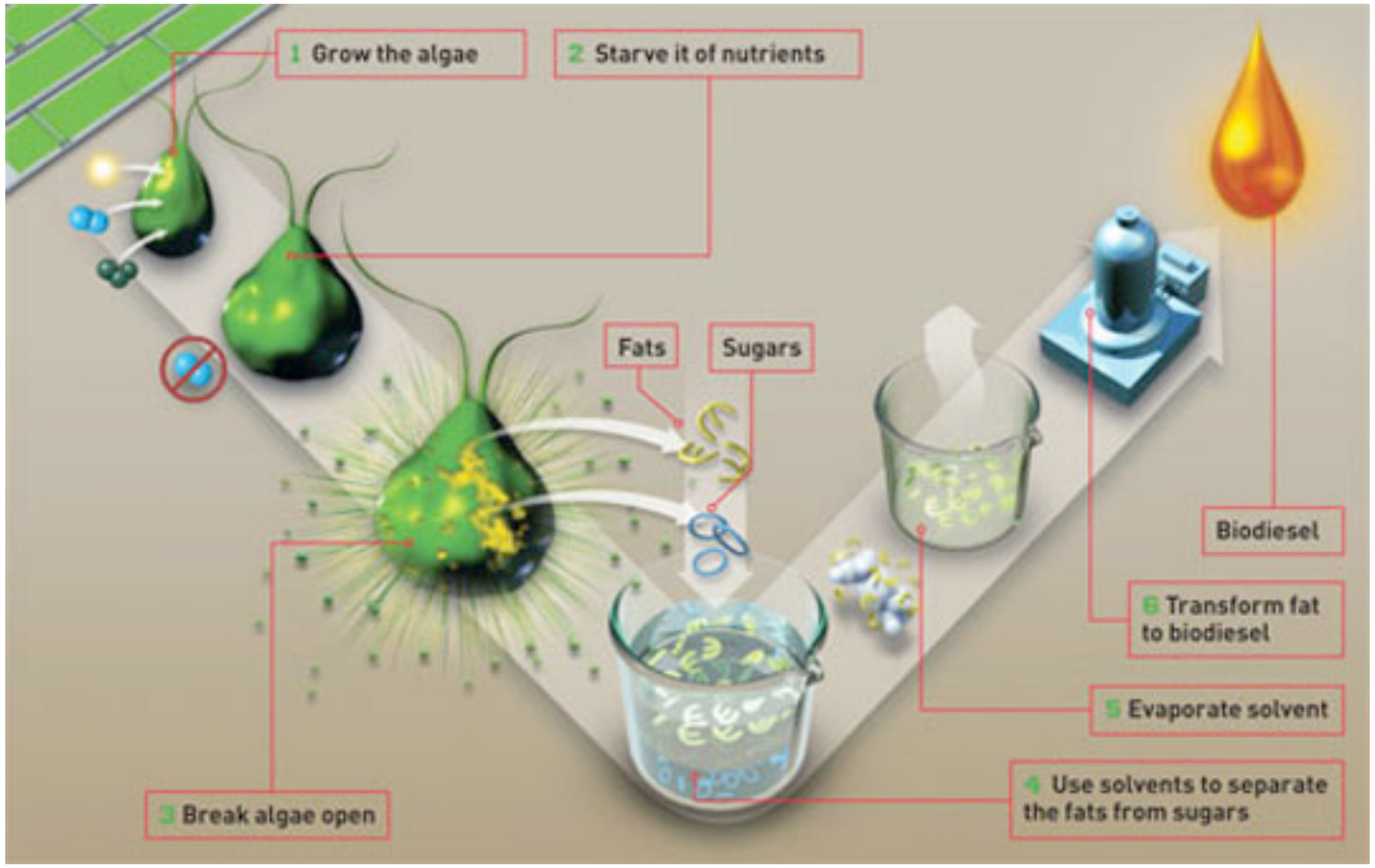
Yield Considerations

- How much fuel can be produced and how much is achieved to date?

- What are the bottlenecks?

Microalgae

- Similar to plants, photosynthetic
- High oil content
- Carbon sequenstration



LiveFuels Inc.

Algae Yield

Crop	US gal / acre-yr
Corn (Yellow #2)	18
Soybeans	48
Mustard seed	61*
Corn (Mavera™ Hybrid)	66*
Sunflowers	102
Peanuts	113
Rapeseed (canola)	127
Jatropha	202*
Oil palm	635
Micro-algae	>1,500*

Source data:

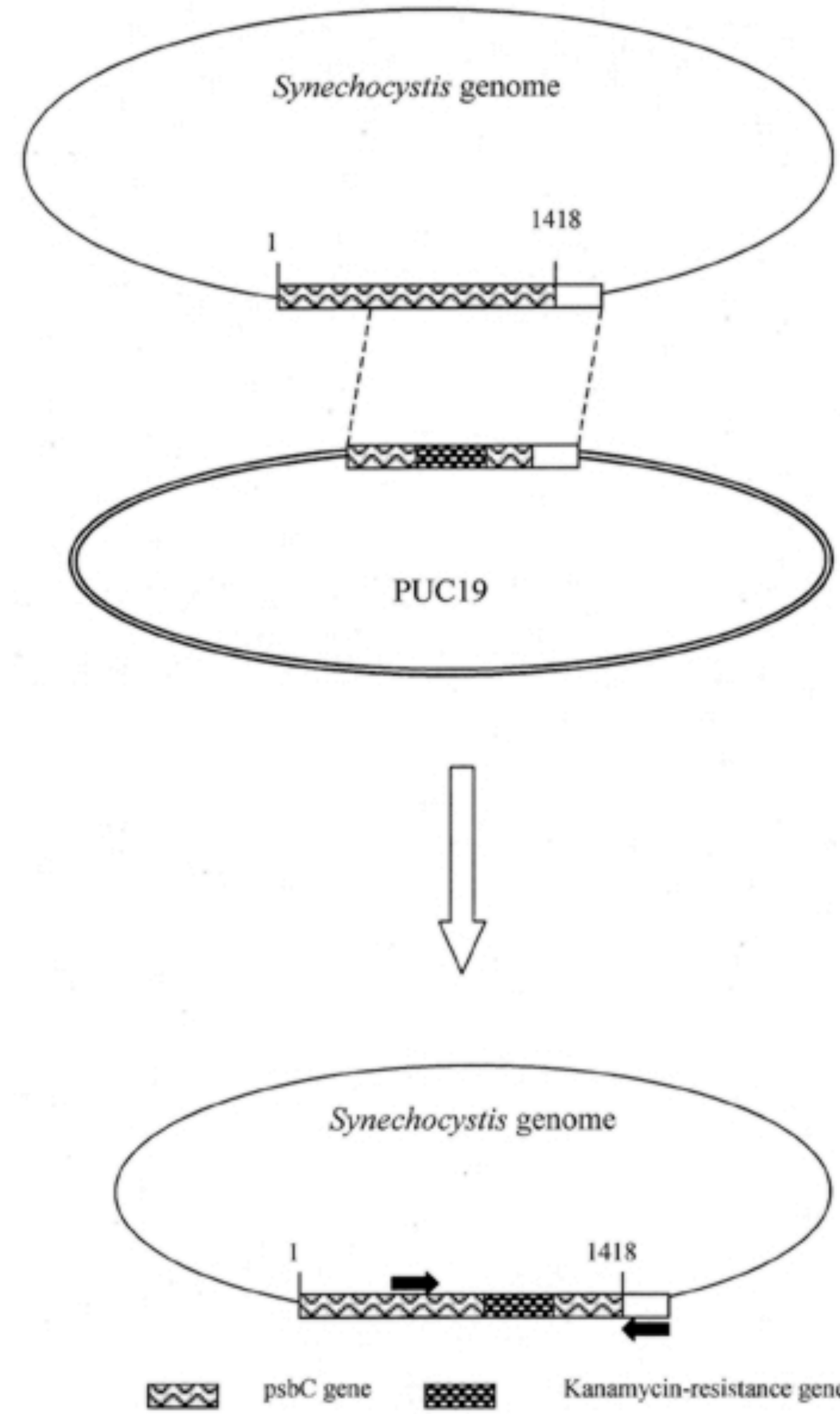
Consultant Consensus

Model Microalga

- Green Algae
    - *Chlamydomonas reinhardtii*
- Cyanobacteria (Blue-green Algae)
    - *Synechococcus PCC 7942*
    - *Synechocystis PC 6803*
    - *Synechococcus PCC 7002*
- Experts at Stanford
    - Arthur Grossman and Devaki Bhaya

Genetic Techniques

- Cyanobacteria
  - Many are naturally competent
  - Transformation via homologous recombination
  - Bacterial expression



http://photoscience.la.asu.edu/PHOTOSYN/courses/BIO_343/lab/Experiment-II.html

Genetic Techniques

- Green Algae
  - Particle bombardment
  - Electroporation
  - Glass bead transformation

Box 1. Nuclear versus chloroplastic transformation

Integration of transgenes into the chloroplast has important advantages. It enables controlled site-directed recombination of constructs and results in high expression levels with no silencing drawbacks (Table I). However, nuclear transformation might enable a wider range of possibilities both for transgenic protein expression (e.g. excretion, different cell-compartment expression, and glycosylation) and for manipulation of algal metabolism (gene inactivation or overexpression, and gain of additional pathways) (Table I).

Table I. Main characteristics of nuclear and chloroplastic transformations

	Nuclear	Chloroplastic
Cell compartment of expression	Extracellular, cytosol and chloroplast, among others	Chloroplast
Recombination machinery for integration of exogenous DNA	Mostly non-homologous	Homologous
Gene silencing	Probable	Not probable
Inheritance of integrated gene	Mendelian	Maternal
Level of expression (gene copy number)	Low to intermediate	High
Co-transformation of different markers	High	High
Versatility to express genes from different organisms	Intermediate to low	High
Glycosylation pattern of proteins	Similar to plants and animals	None

Leon-Banares et al. Trends in Biotechnology, Volume 22, Issue 1, January 2004, Pages 45-52

Considerations

- Genetic tractability
- Development and testing cycle
- Technical skill requirements