

# 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 <sub>4</sub> –C <sub>12</sub> hydrocarbons Linear, branched, cyclic, aromatics Anti-knock additives	Octane number <sup>a</sup> Energy content <sup>b</sup> Transportability	Ethanol, <i>n</i> -butanol and <i>iso</i> -butanol Short chain alcohols Short chain alkanes
Diesel	C <sub>9</sub> –C <sub>23</sub> (average C <sub>16</sub> ) Linear, branched, cyclic, aromatic Anti-freeze additives	Cetane number <sup>c</sup> Low freezing temperature Low vapor pressure	Biodiesel (FAMEs) Fatty alcohols, alkanes Linear or cyclic isoprenoids
Jet fuel	C <sub>8</sub> –C <sub>16</sub> hydrocarbons Linear, branched, cyclic, aromatic Anti-freeze additives	Very low freezing temperature Net heat of combustion Density	Alkanes Biodiesel Linear or cyclic isoprenoids

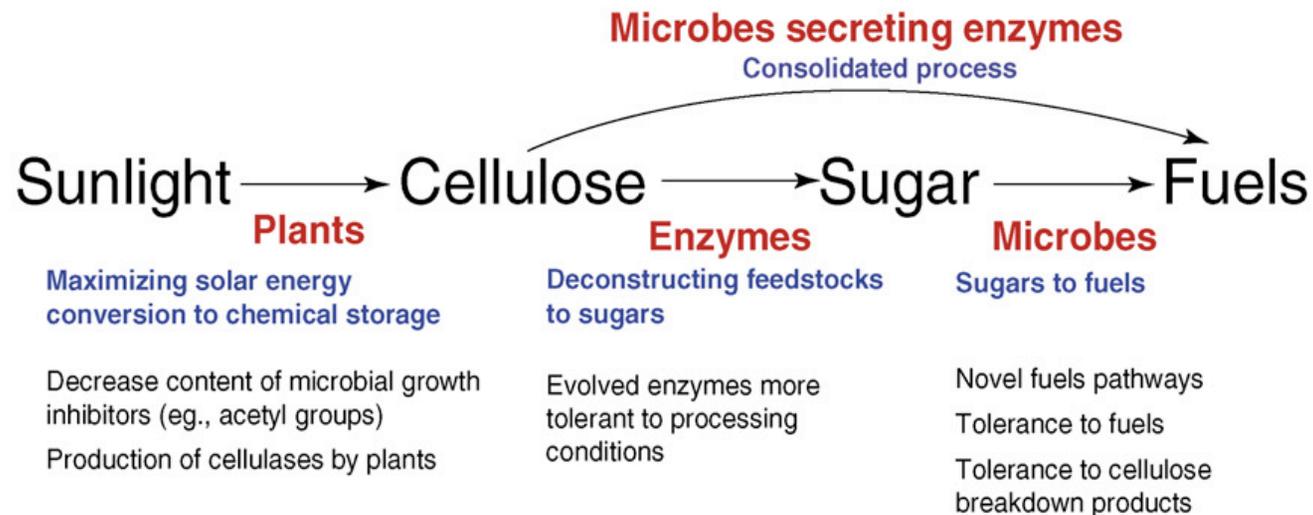
<sup>a</sup> A measurement of its resistance to knocking. Knocking occurs when the fuel/air mixture spontaneously ignites before it reaches the optimum pressure and temperature for spark ignition.

<sup>b</sup> 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.

<sup>c</sup> 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.

# Advanced Biofuel

Feedstock --> conversion technology --> End product



# Major Avenues of R&D

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

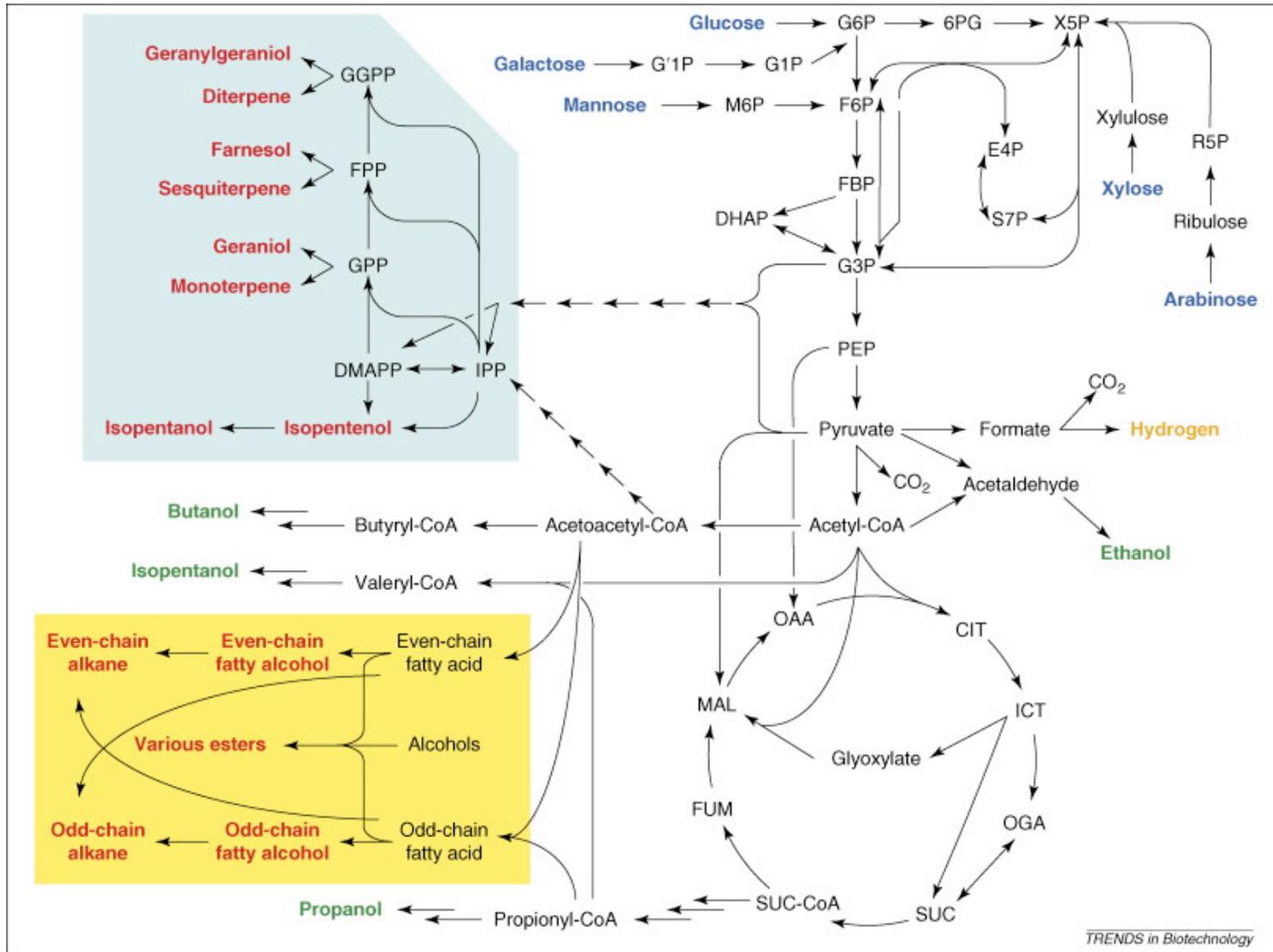
# Synthetic Biology in Biofuel Production

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

# Popular Hosts

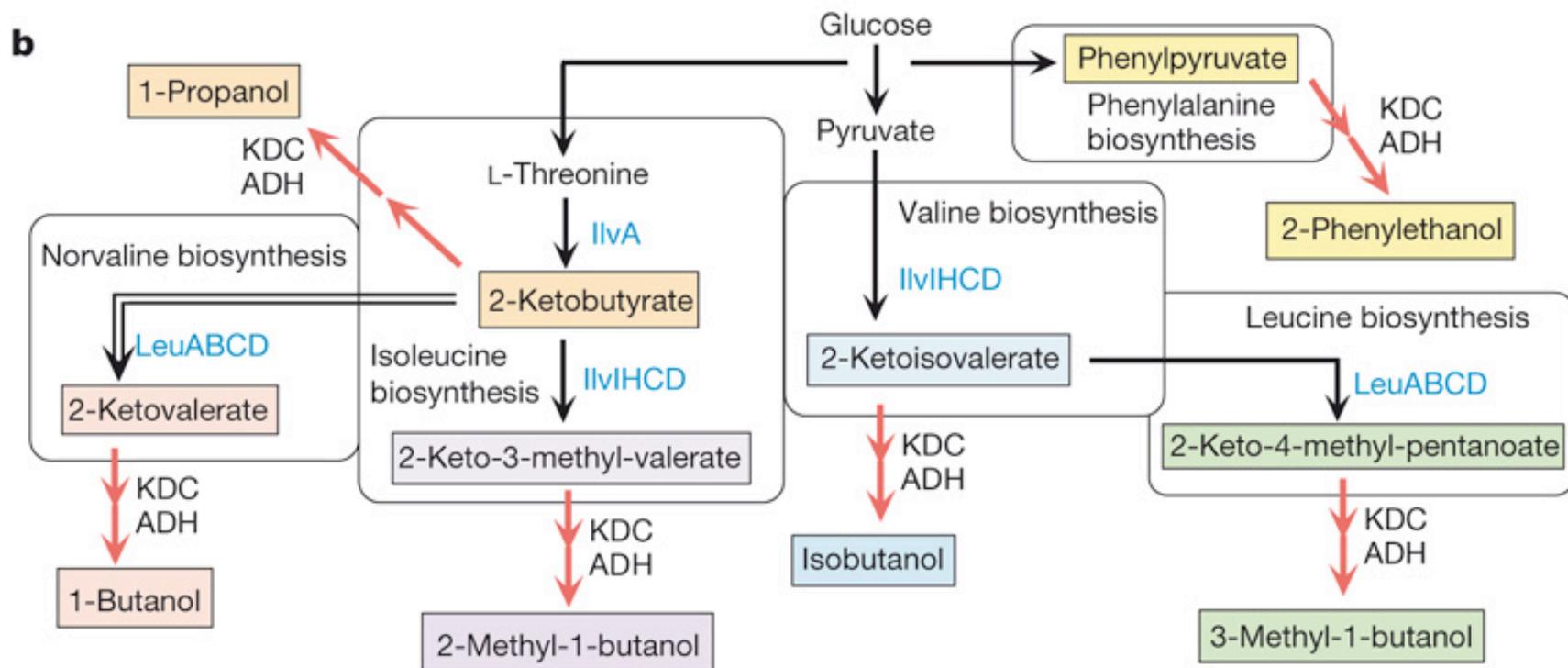
Organism	Fermentative/ Photosynthetic	Genetically Modifiable	Generation Time	Pathways Explored
E. coli	Fermentative	Yes	0.33 hr	Amino Acid, Fatty Acid, Isoprenoid
S. cerevisiae	Fermentative	Yes	1.5 hr	Amino Acid, Fatty Acid, Isoprenoid
Cyanobacteria	Photosynthetic	Yes	6 - 8 hr	
Green Microalgae	Fermentative/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

# Metabolic Engineering

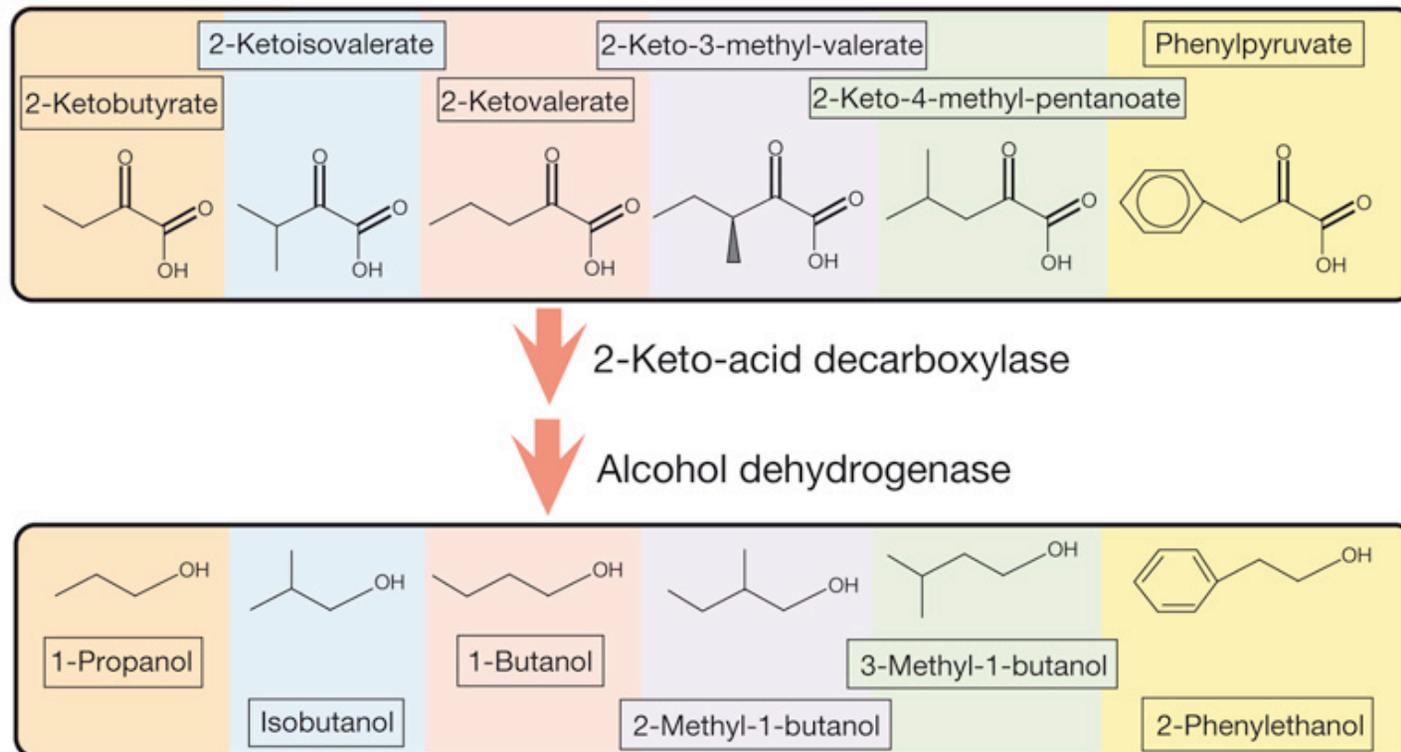


# Amino Acid Pathway

James Liao, UCLA

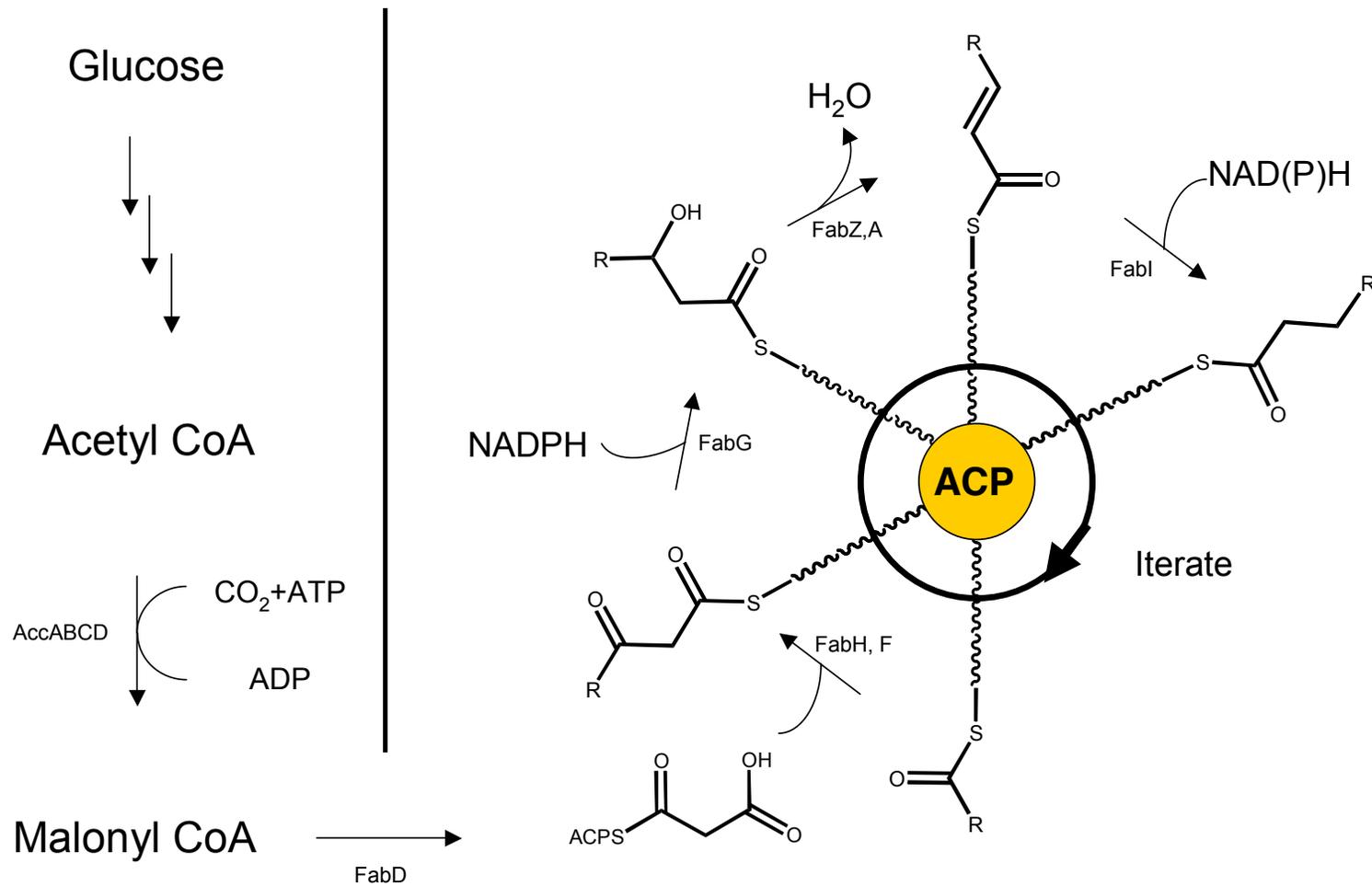


# Amino Acid Pathway



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

# Fatty Acid Pathway

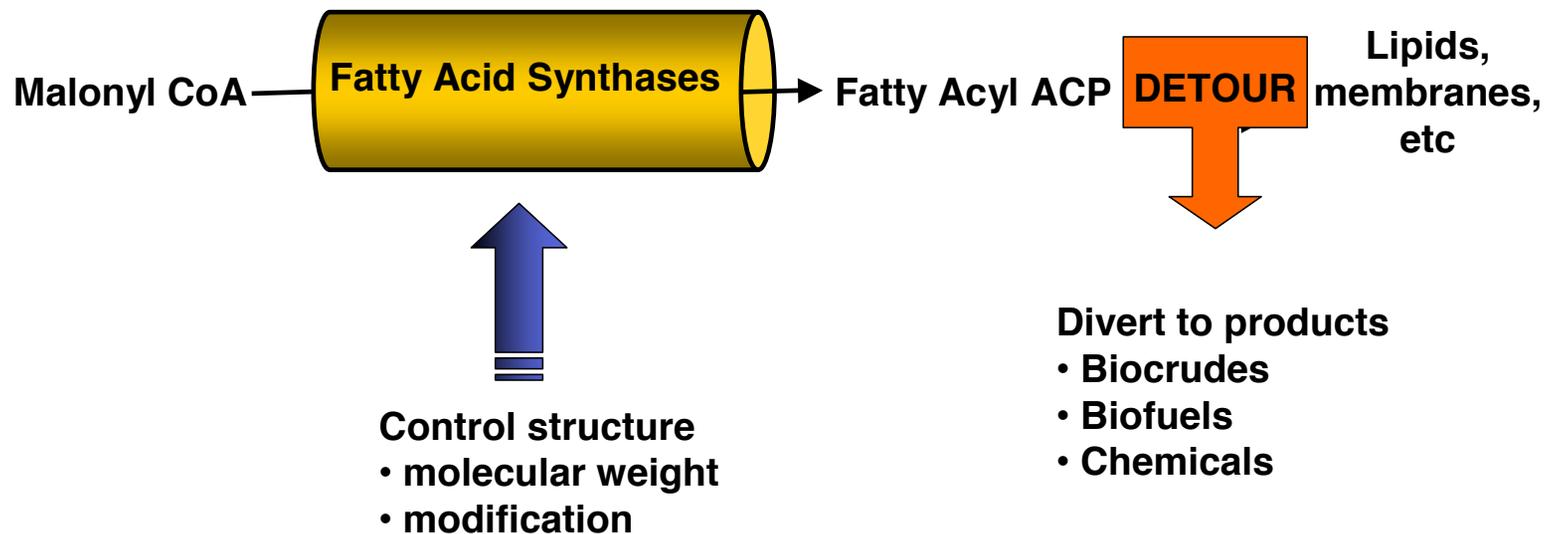


LS9, INC.

# Fatty Acid Pathway

Improve Flux

- precursor supply
- regulation
- competing pathways



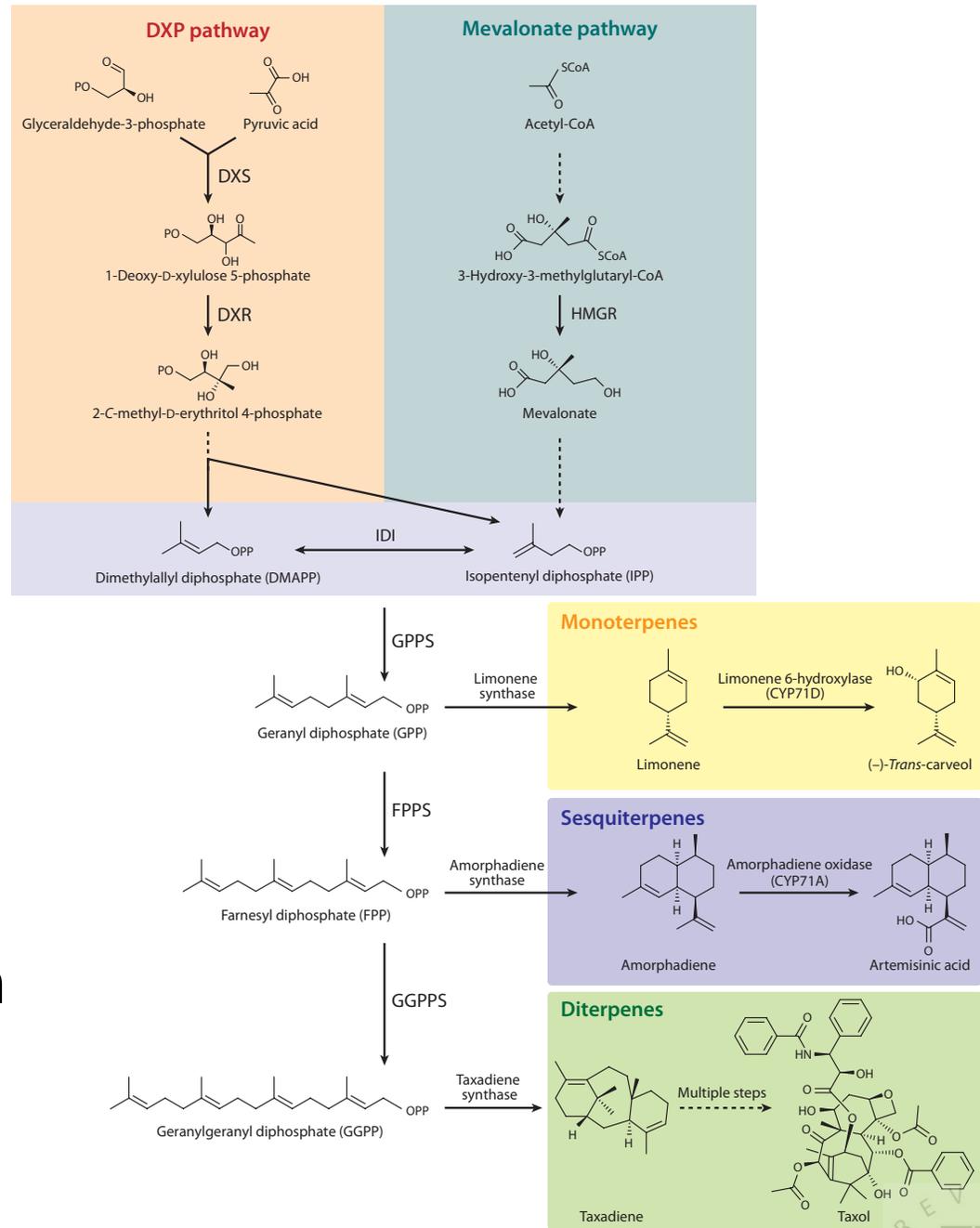
LS9, INC.

# Isoprenoid Pathways

Jay Keasling,  
UC Berkeley

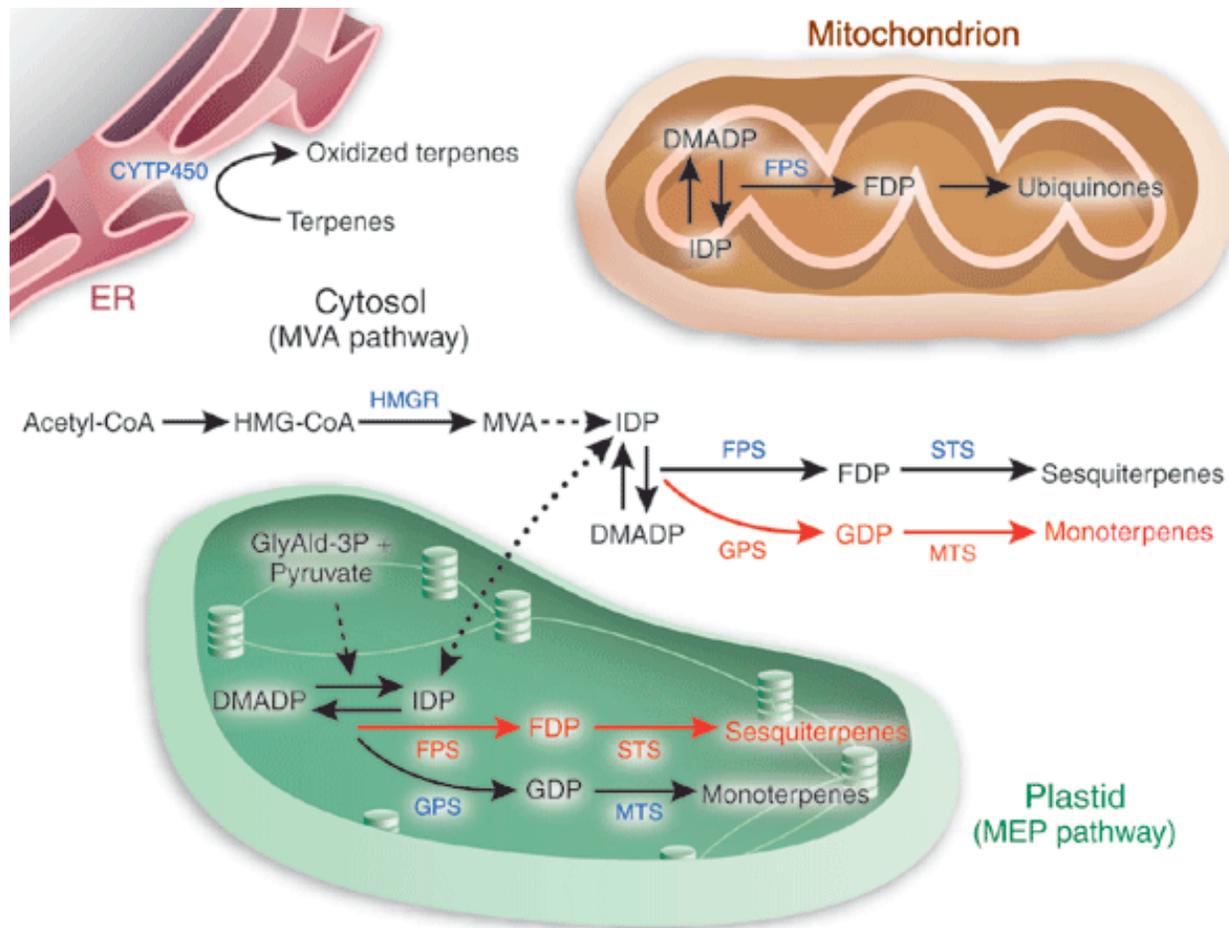
Hosts: *E. coli* and  
*S. cerevisiae*

Fermentative Approach



# Isoprenoids from Plants

Joe Chappell, U. Kentucky



Chloroplast: 1000X Increase in Yield

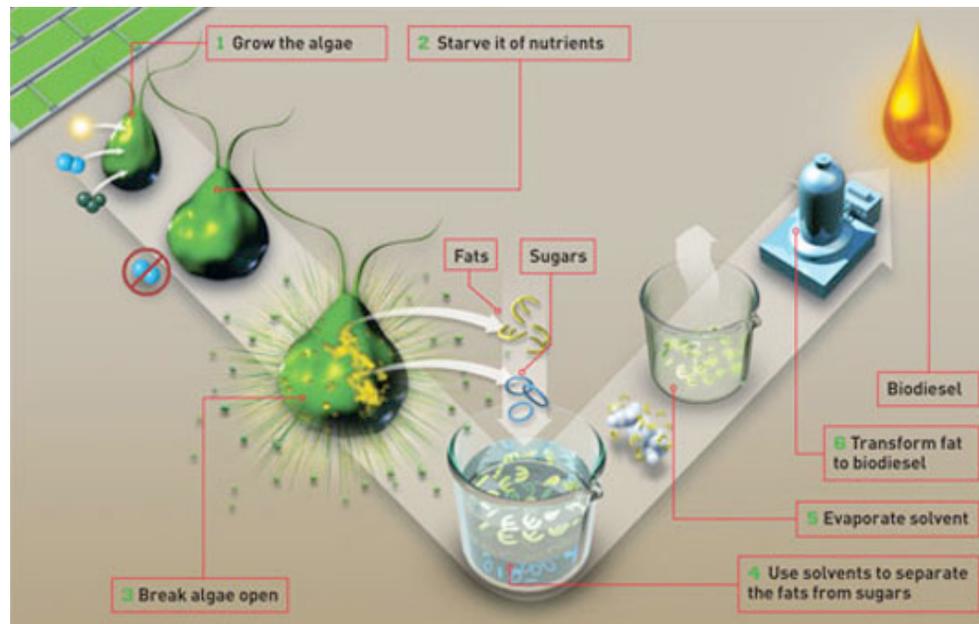
Katie R. Is

# 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 sequestration



SYNTHETIC GENOMICS™



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# Algae Yield

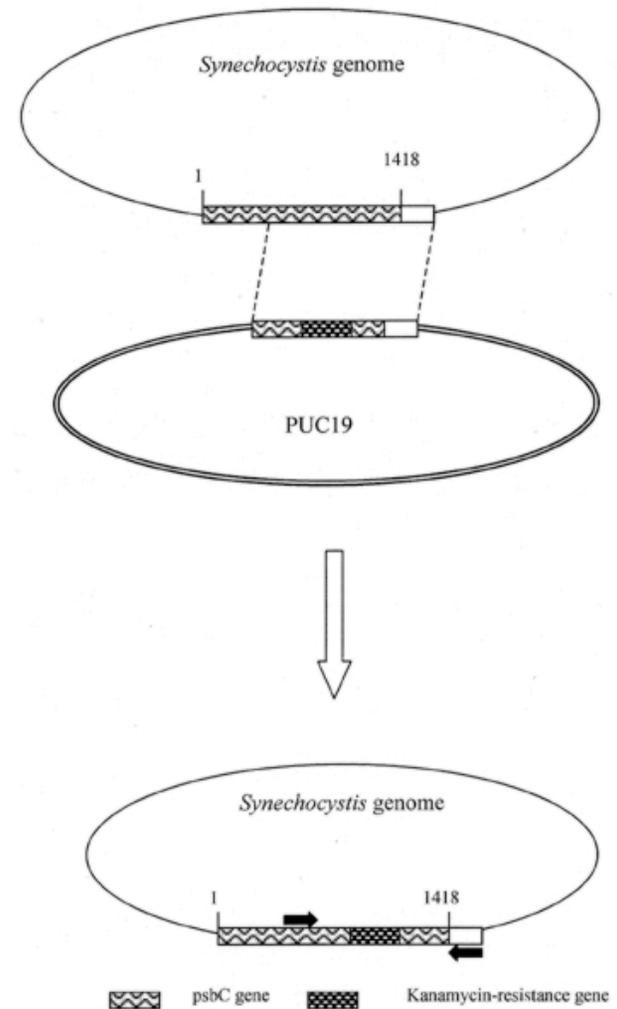
<u>Crop</u>	<u>US gal / acre-yr</u>	
Corn (Yellow #2)	18	
Soybeans	48	
Mustard seed	61*	
Corn (Mavera™ Hybrid)	66*	
Sunflowers	102	
Peanuts	113	
Rapeseed (canola)	127	
Jatropha	202*	
Oil palm	635	
Micro-algae	>1,500*	<i>Source data: Consultant Consensus</i>

# 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



# 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**

	<b>Nuclear</b>	<b>Chloroplastic</b>
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

# Considerations

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