Metabolic Engineering

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What is metabolic engineering (ME)?

- Metabolic engineering is a redirection of enzymatically-catalyzed reactions for the production of a new compound or the degradation of a compound
  - genetic modification of a single organism
  - engineering a consortium of organisms

Why do metabolic engineering?

- Introduction of new chemistries into the cell
- Production of novel compounds
  - new biopolymers
  - antibiotics
- Production of existing compounds in better ways
- Bioremediation of recalcitrant compounds
  - pesticides/nerve agents
  - PCBs

ME is not new

- Metabolic engineering started many decades ago with production of
  - Amino acids
  - Vitamins
  - Solvents
  - Antibiotics
- ME was done by chemical mutagenesis and selection techniques

Recombinant DNA and ME

- The advent of recombinant DNA opened a whole new dimension
- Precise manipulation of specific enzymatic reactions
- Directed pathway modification

ME is like other fields of engineering

- Synthesis
  - expression of new genes in various host cells
  - amplification of endogenous enzymes
  - Deletion of genes
- Analysis
  - Identification of important parameters that affect physiology
  - Elucidation of control architecture of a network
  - Biochemical impact of genetic and enzymatic manipulations

More synthesis in ME

- Focus on integrated metabolic pathways, not single genes
- Examination of complete biochemical reaction networks
- Pathway synthesis and thermodynamics feasibility
- Metabolic network is central

ME is interdisciplinary

- Biochemistry
- Genetics and molecular biology
- Chemistry
- Chemical engineering

Metabolic pathway

- A metabolic pathway is any sequence of feasible and observable biochemical reaction steps connecting a specified set of input and output metabolites

Metabolic fluxes

- Metabolic flux is defined as the rate at which input metabolites are processed to form output metabolites
- The determination of fluxes *in vivo* has been termed metabolic flux analysis (MFA).

Flux is a fundamental determinant of cell physiology

- Along with intracellular metabolite concentrations, fluxes define a minimum set of information needed to describe metabolism and cell physiology
- Fluxes determine the degree of engagement of various enzymes in a conversion process
- Fluxes are necessary to elucidate metabolic flux control
- Fluxes are also useful for
  - Calculating theoretical yields
  - Determining non-measured metabolic rates
  - Observing pathway function *in vivo*

Metabolic fluxes

- Metabolic engineering focuses on controlling metabolic fluxes
- The combination of analytical methods to quantify fluxes and their control with molecular biological techniques to implement suggested genetic modifications is the essence of metabolic engineering.

Flux

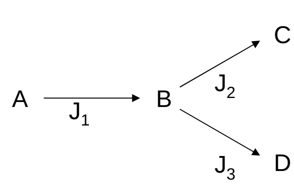
- Flux is a fundamental determinant of cell physiology and the most critical parameter of a metabolic pathway
- For the following pathway

$$A \xrightarrow{v_1} \xrightarrow{v_2} \xrightarrow{v_3} \xrightarrow{v_4} \xrightarrow{v_5} B$$

- its flux is equal to the rates of the individual reactions at steady state

Flux (continued)

- During a transient, the individual reaction rates are not equal and pathway flux is variable
- For the branched reaction:



- We have two additional fluxes: $J_1 = J_2 + J_3$

Investigating fluxes and their control

1. Develop the means to observe as many pathways as possible and measure their fluxes.
2. Introduce well defined perturbations to the bioreaction network and determine fluxes after the system relaxes to steady state.
3. Analysis of perturbation results.

Research Goals

- To develop methods for *in vivo* flux determination
  - Fundamental determinant of cell physiology
  - Critical for understanding the control of flux
  - Uncorrelated to activity assays
- To understand the response of metabolic networks
  - Flux distributions at branch points
  - Amplification of flux through metabolic networks
- System: steady-state chemostat. Measurements include metabolic rates, ^{13}C label enrichment in metabolites, etc.

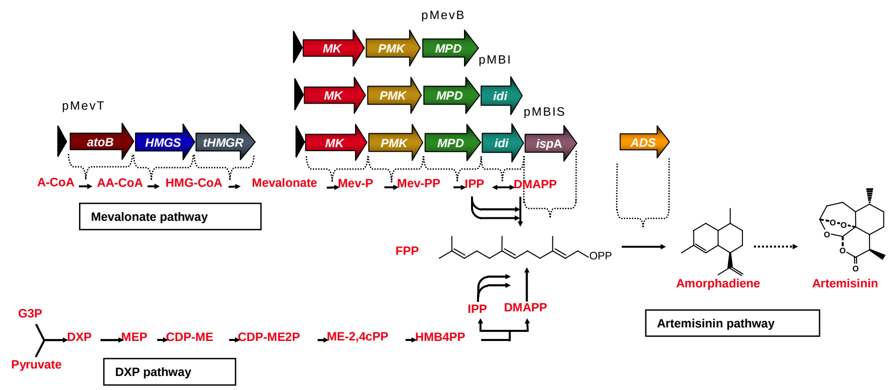
Metabolic control analysis

- MCA = metabolic control analysis
- Developed in 1970’s for the quantitative representation of the degree of flux control exercised by the pathway enzymatic activities, metabolites, effectors, and other parameters.

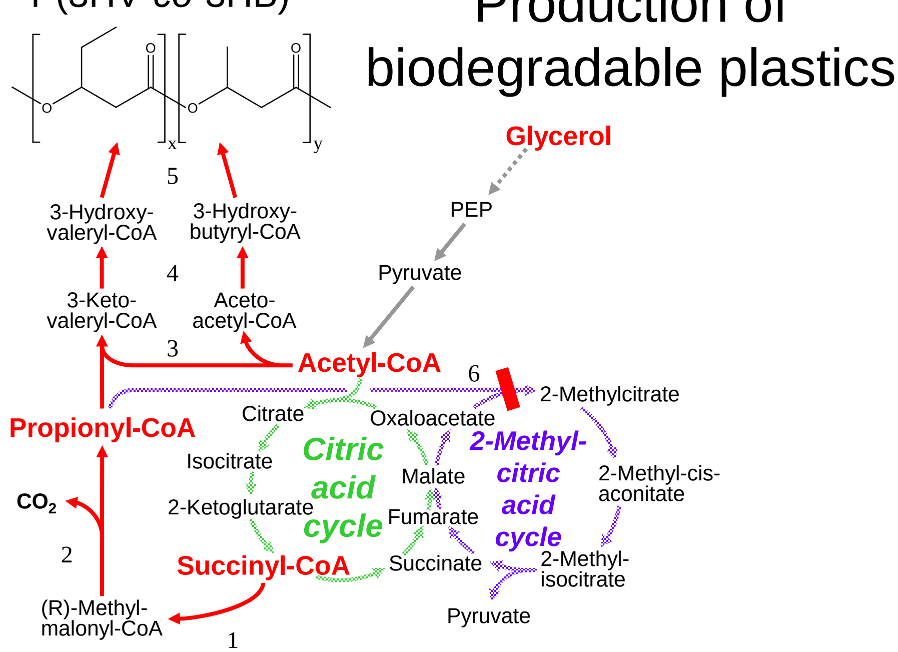
Applications of ME

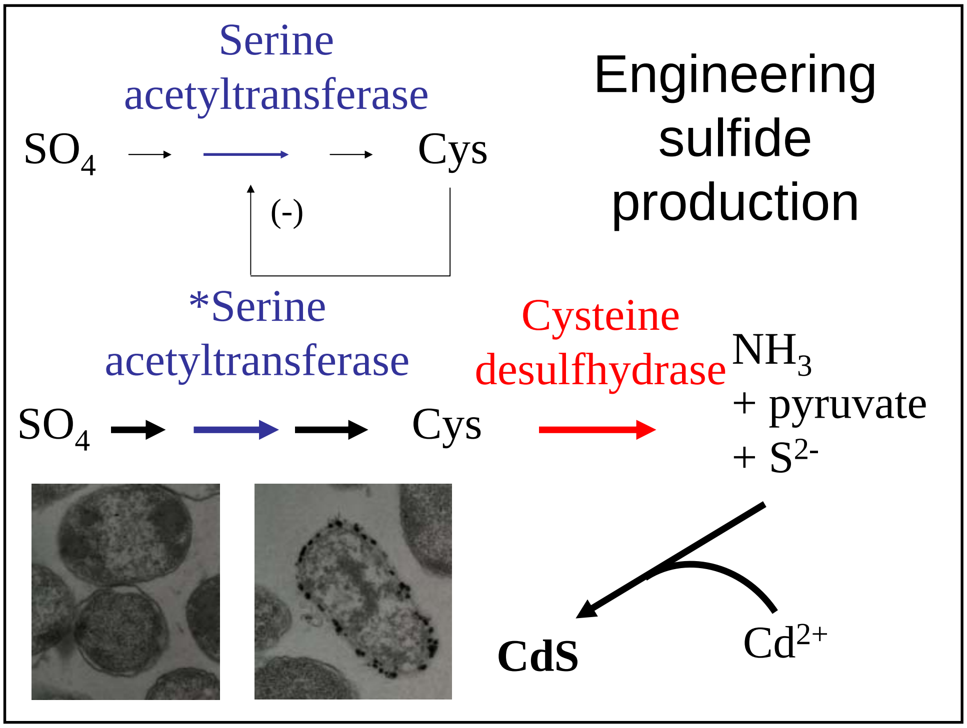
- Improve product yield and productivity
- Extend range of substrate utilization
- Produce new (to the cell) or novel products
- Improve general cellular properties
- Manufacture of chiral compounds
- Metabolism of whole organs and tissues

Production of drugs



Production of biodegradable plastics




Serine
acetyltransferase
Engineering
sulfide
production
SO_4
Cys
(-)
*Serine
acetyltransferase
Cysteine
desulfhydrase
NH_3
+ pyruvate
+ S^{2-}
SO_4
Cys
CdS
Cd^{2+}