Exploiting the synthetic capacity of microbes for the production of novel value-added biochemicals

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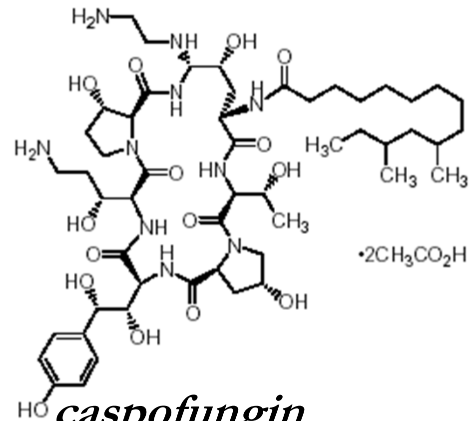
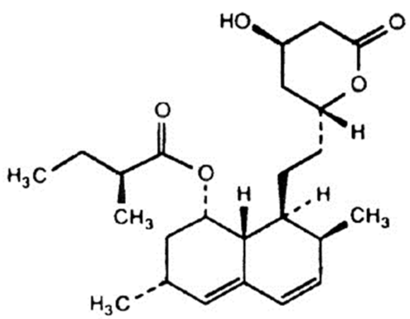
Microbes as Chemical Factories

- Antibiotics/Antimicrobials



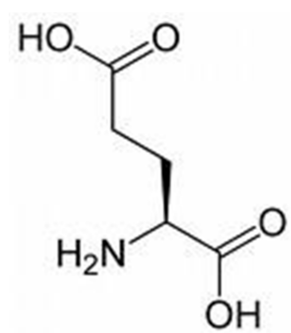
caspofungin

- Other therapeutics

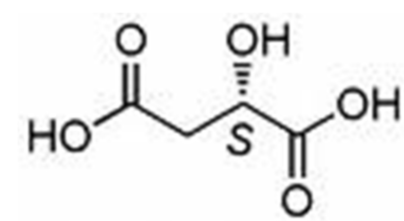


lovastatin

- Organic Acids
- Amino Acids



glutamic acid



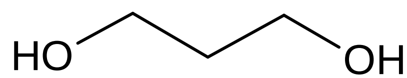
malic acid

Improvement of natural producers

Microbes as Chemical Factories

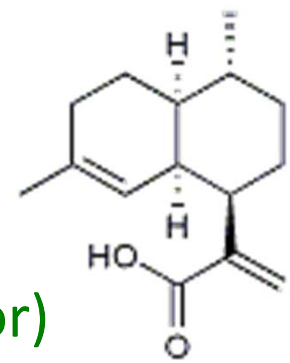
- Antibiotics/Antimicrobials
- Other therapeutics (lovastatin)
- Amino Acids
- Organic Acids

- 1,3-Propanediol



(industrial chemical, materials)

- Artemisinic Acid



(anti-malarial precursor)

Re-constitution of natural pathways in heterologous hosts

Changing the paradigm* – from "CH_2" to "CH_2O"

Crude Oil

↓

Petroleum Refinery

Fuels

Aviation Fuel
Kerosene
Gasoline
Fuel Oil

Petrochemicals

Olefins and **Aromatics** for polymers, resins, adhesives, detergents, fibers, lubricants

Biomass (Glucose & other simple sugars)

↓

Bio- Refinery

Bio-fuels

Ethanol, Butanol
branched chain alcohols

Value added Bio-chemicals

**Prof. Bradley D. Olsen, MIT Chemical Engineering*

"Retro-biosynthetic" Pathway Design*

- Integration of Biocatalysis ("Parts" selection) and Metabolic Engineering ("Systems" assembly, analysis)

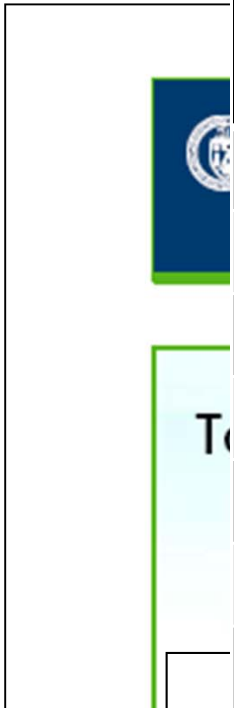
- (Others) On-going work on algorithms for biosynthetic pathway design

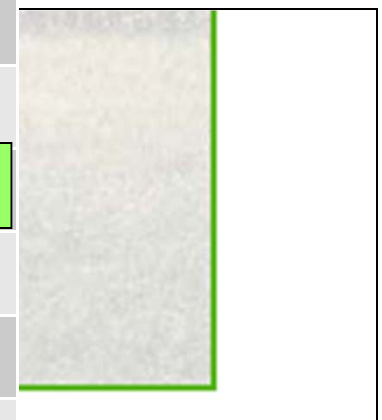
- Elucidation of Design Principles

- Development of Design and Assembly Tools ("Devices")

* *Curr. Opin. Biotechnol.* **2008. 19:468-474**

Candidates for Target Molecules

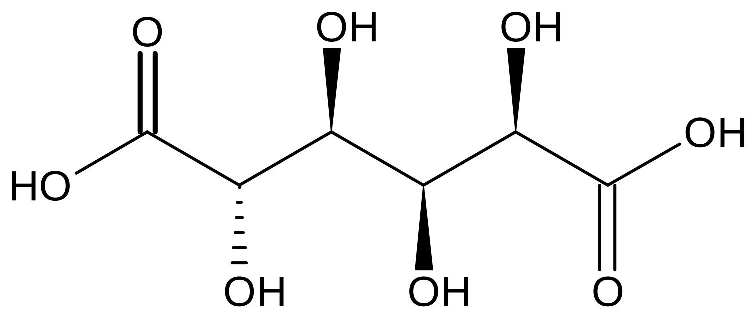




Building Blocks
1,4-diacids (succinic, fumaric and malic)
2,5-furan dicarboxylic acid
3-hydroxypropionic acid
aspartic acid
Glucaric Acid
glutamic acid
itaconic acid
levulinic acid
3-Hydroxybutyrolactone
glycerol
sorbitol
xylitol/arabinitol

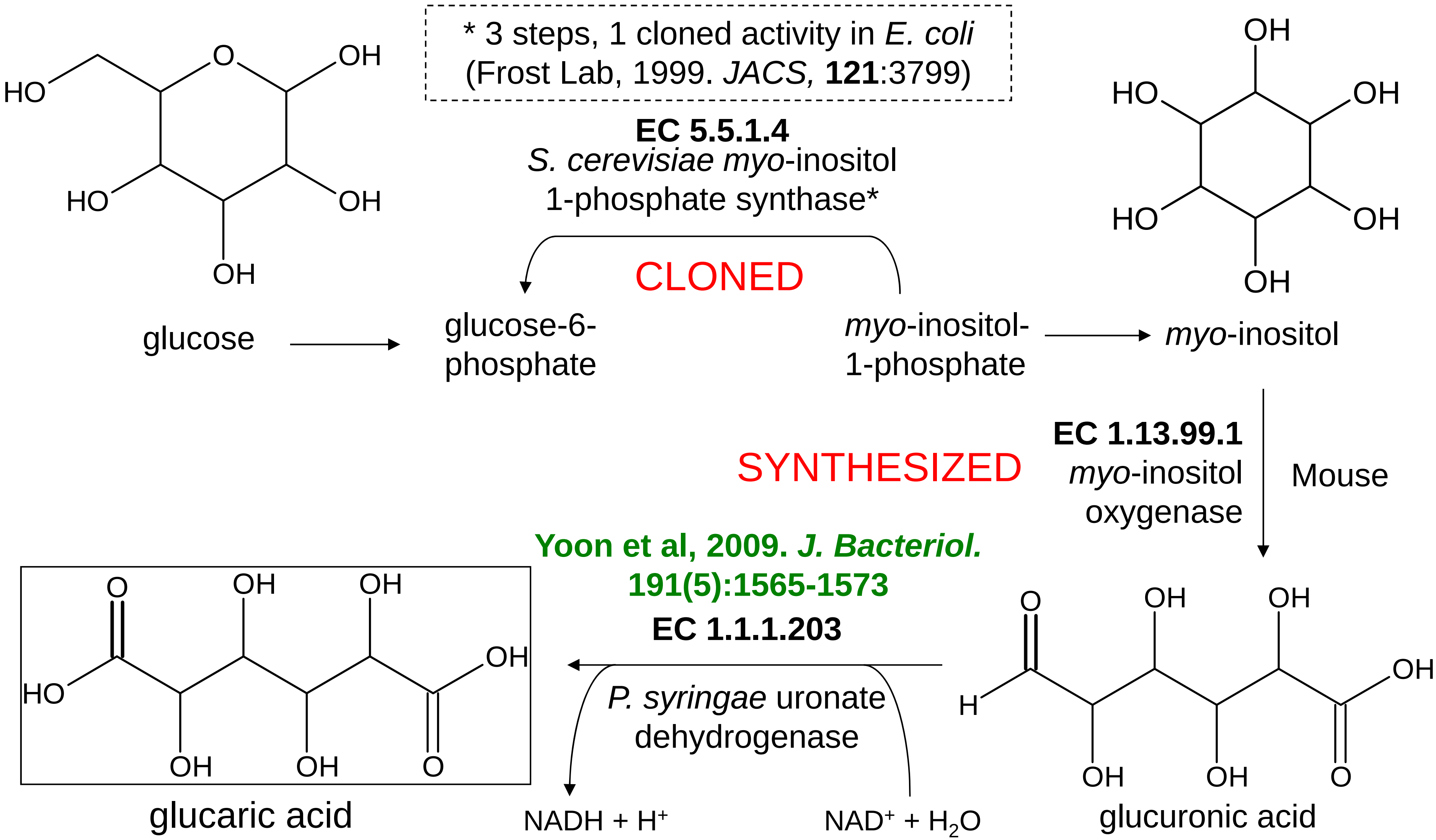
August 2004

Glucaric Acid

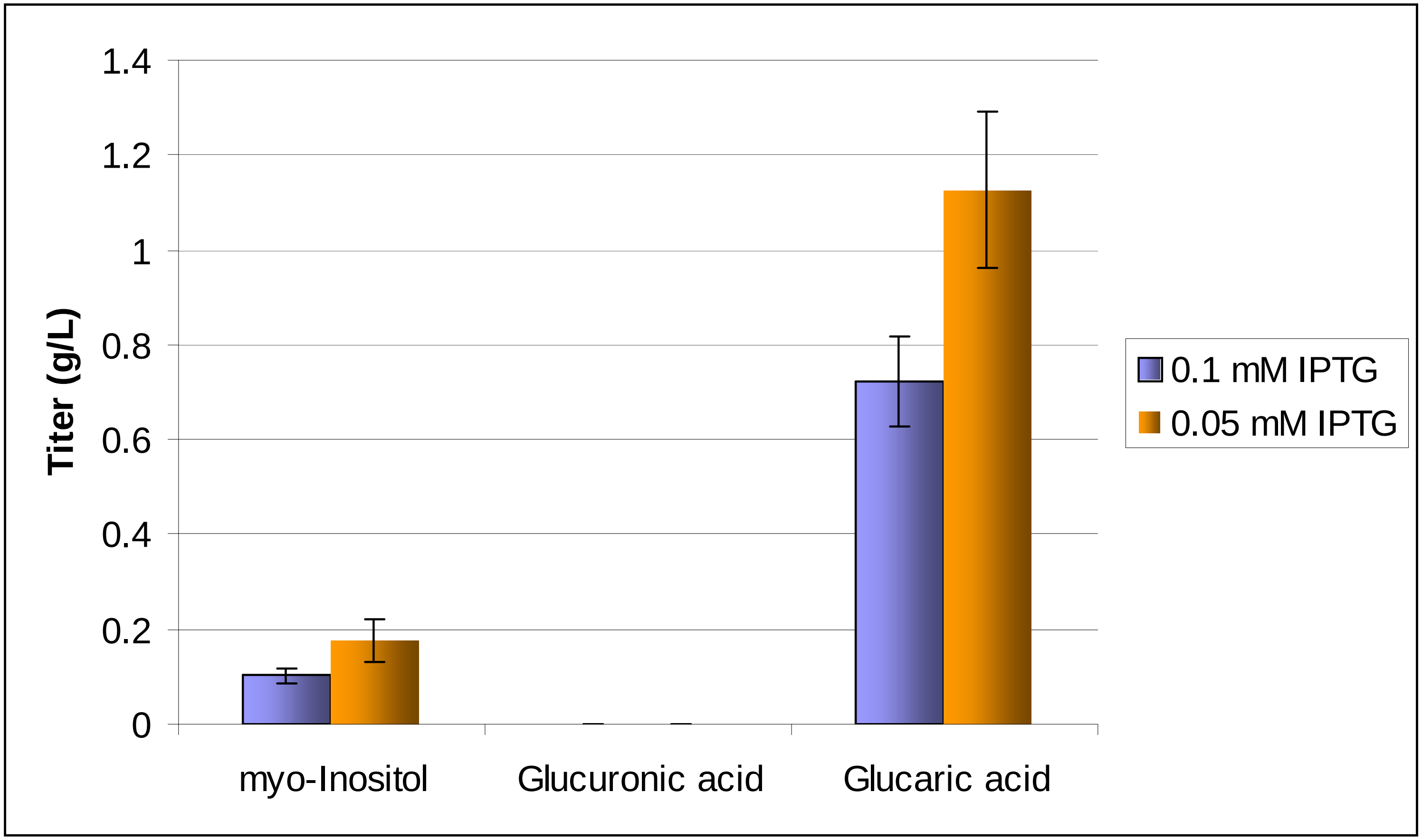


- Found in fruits and vegetables, mammals
- No known microbial pathway
- Previously studied for cholesterol-reducing, chemotherapeutic effects
- Potential use as building block for polymeric materials (nylons), detergents
- Produced chemically through acid-catalyzed oxidation of glucose

Novel pathway using naturally occurring enzymes (*Bioprospecting*)



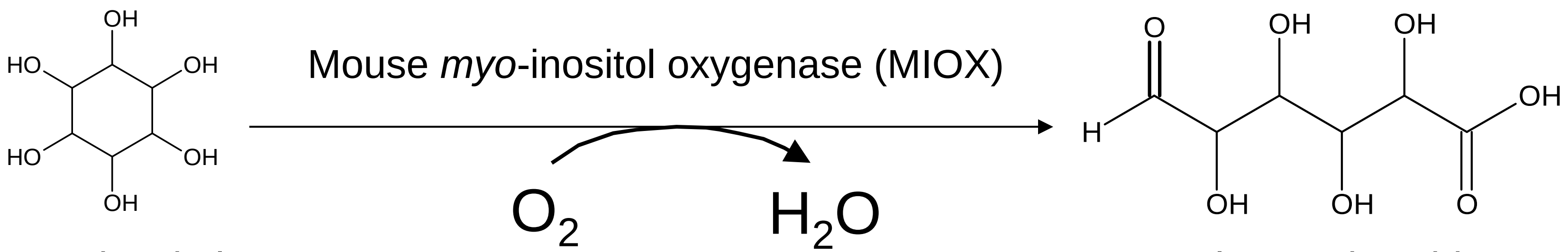
Co-expression of 3 genes in *E. coli*



Build-up of 1st intermediate indicates a limitation with the 2nd enzyme (MIOX)

Moon et al, 2009. *Appl. Environ. Microbiol.* 75(3):589-595

A closer look at MIOX…



myo-inositol **(MI)**

glucuronic acid

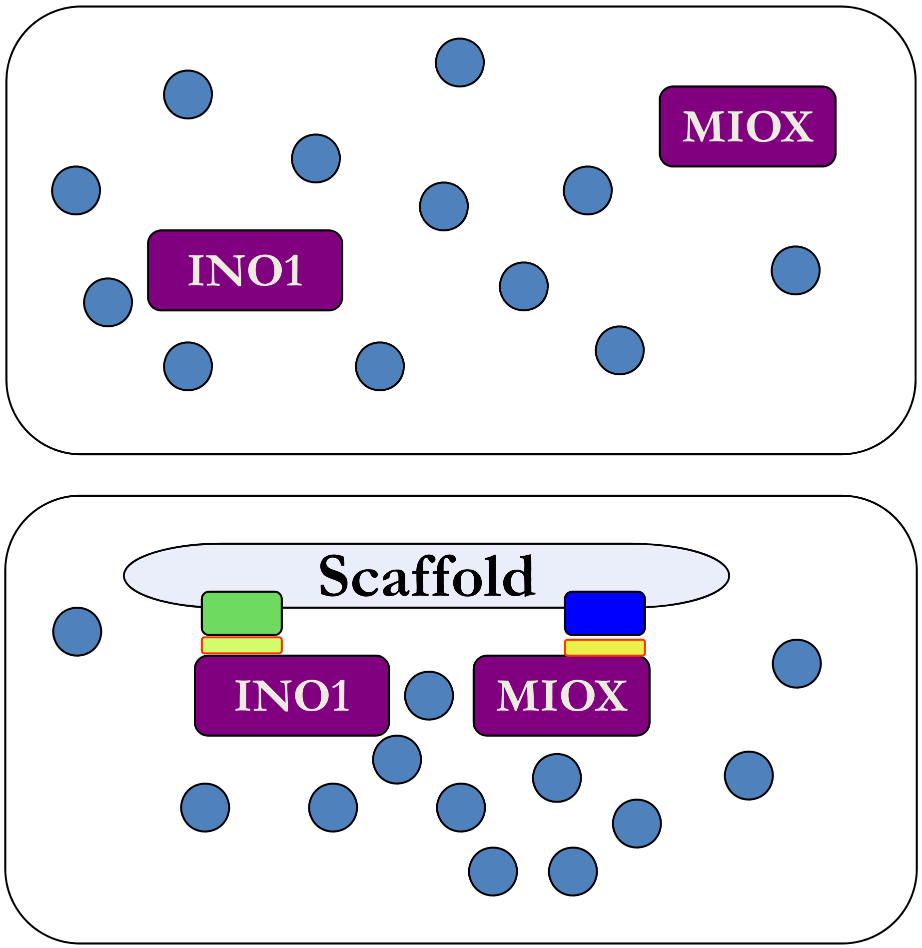
Culture Conditions	Activity at 6 hr (nmol/min/mg)
w/ 60 mM MI	430
w/o MI	28

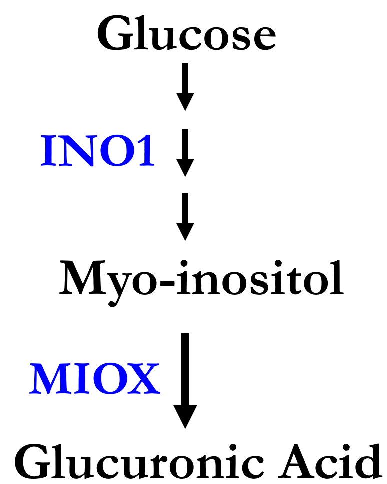
➔High MI production by 1st enzyme (Ino1) is desired.

➔Easier said than done…

Moon et al, 2009. *Appl. Environ. Microbiol.* 75(3):589-595

Enzyme Co-localization
(Collaboration with Dr. John Dueber, SynBERC*)





→ Higher $[MI]_{local}$?

→ Better activation of MIOX?

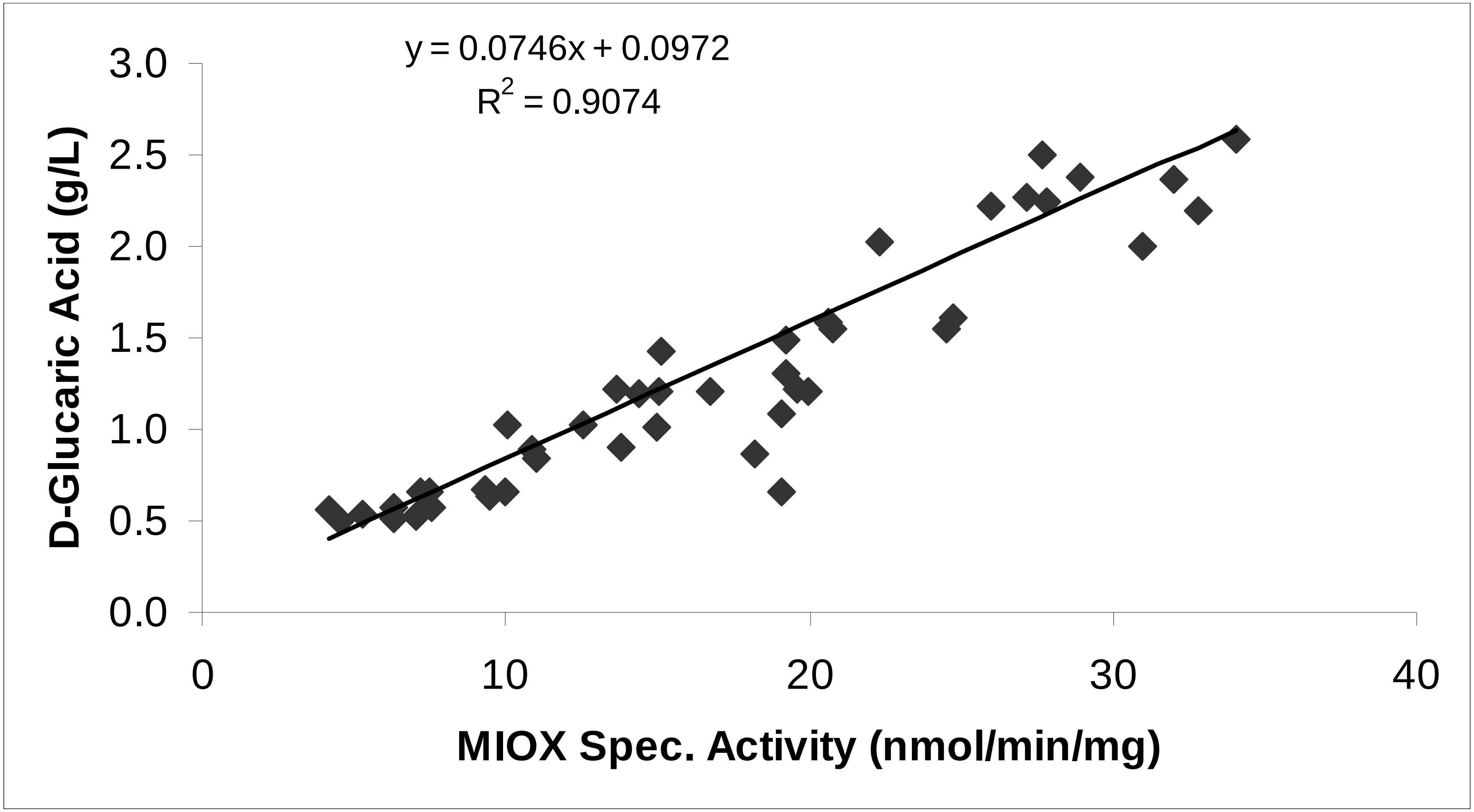
→ Faster conversion?

MI = *myo*-Inositol

* QB3 Fellow, University of California, Berkeley, USA
Synthetic Biology Engineering Research Center (SynBERC)

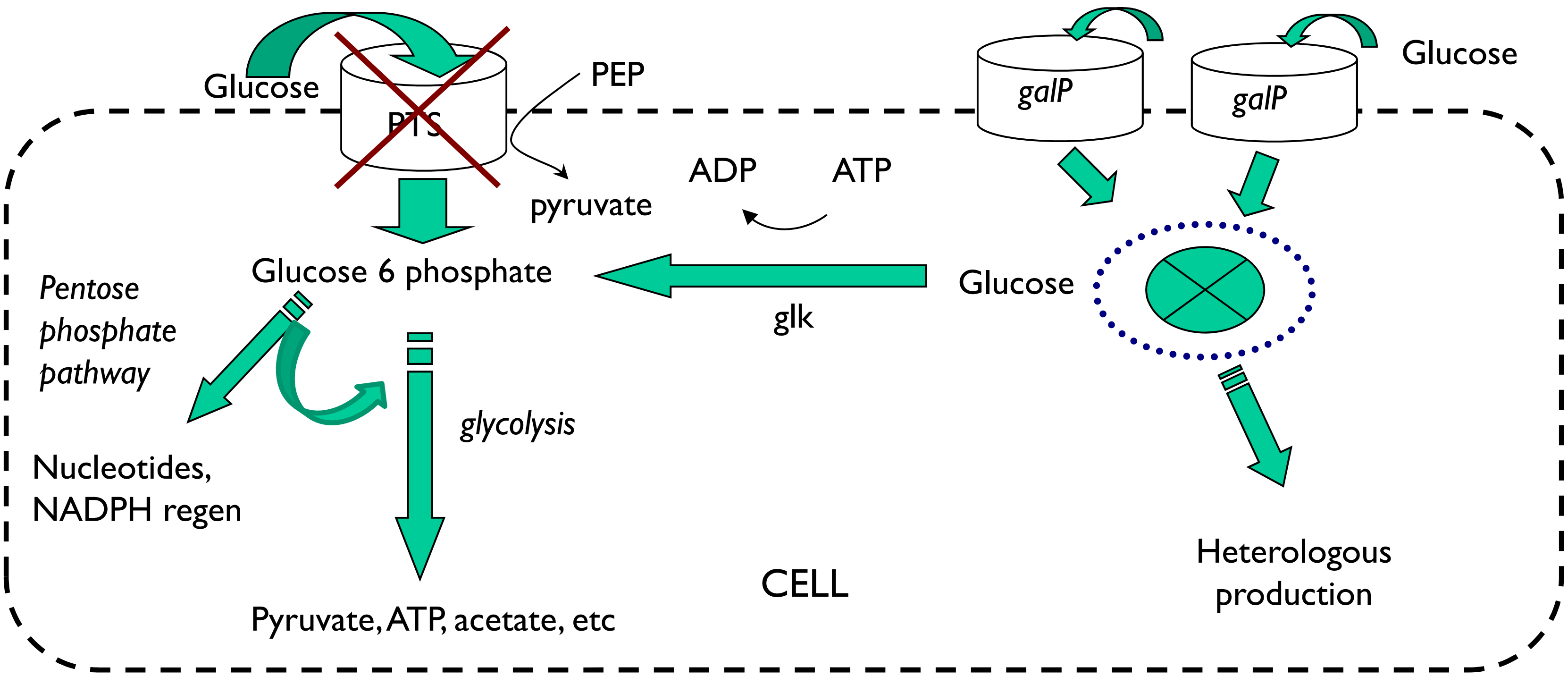
Effect of Co-Recruitment on Enzyme Activity

Fixed enzyme induction level, variable scaffold induction levels



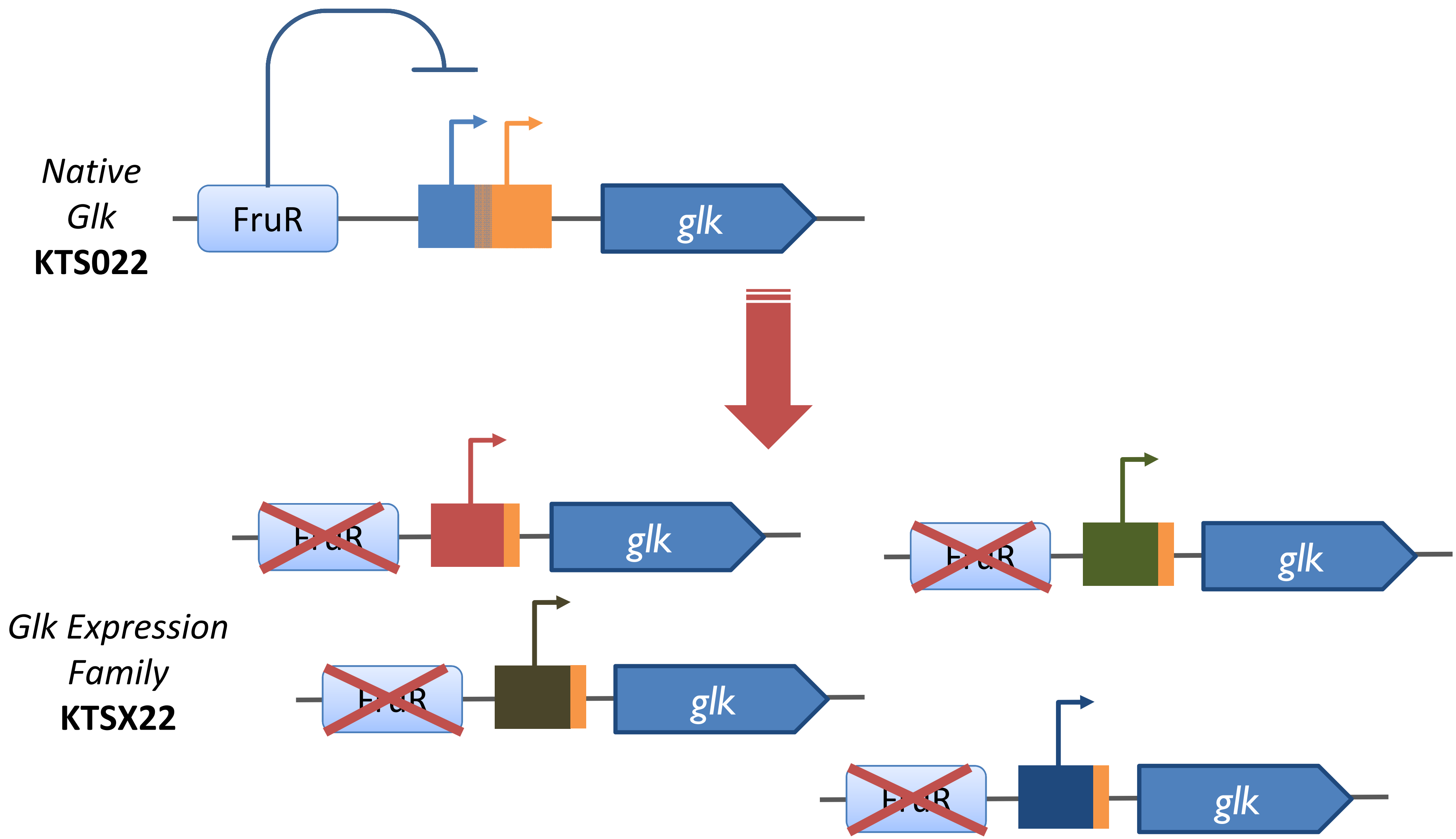
Moon et al, 2010. *Metabolic Engineering,* 12, 298-305

Manipulating Glucose Metabolism



Modulation of glucokinase (glk) may be used to redirect glucose and increase pathway productivity with glucose as sole carbon source

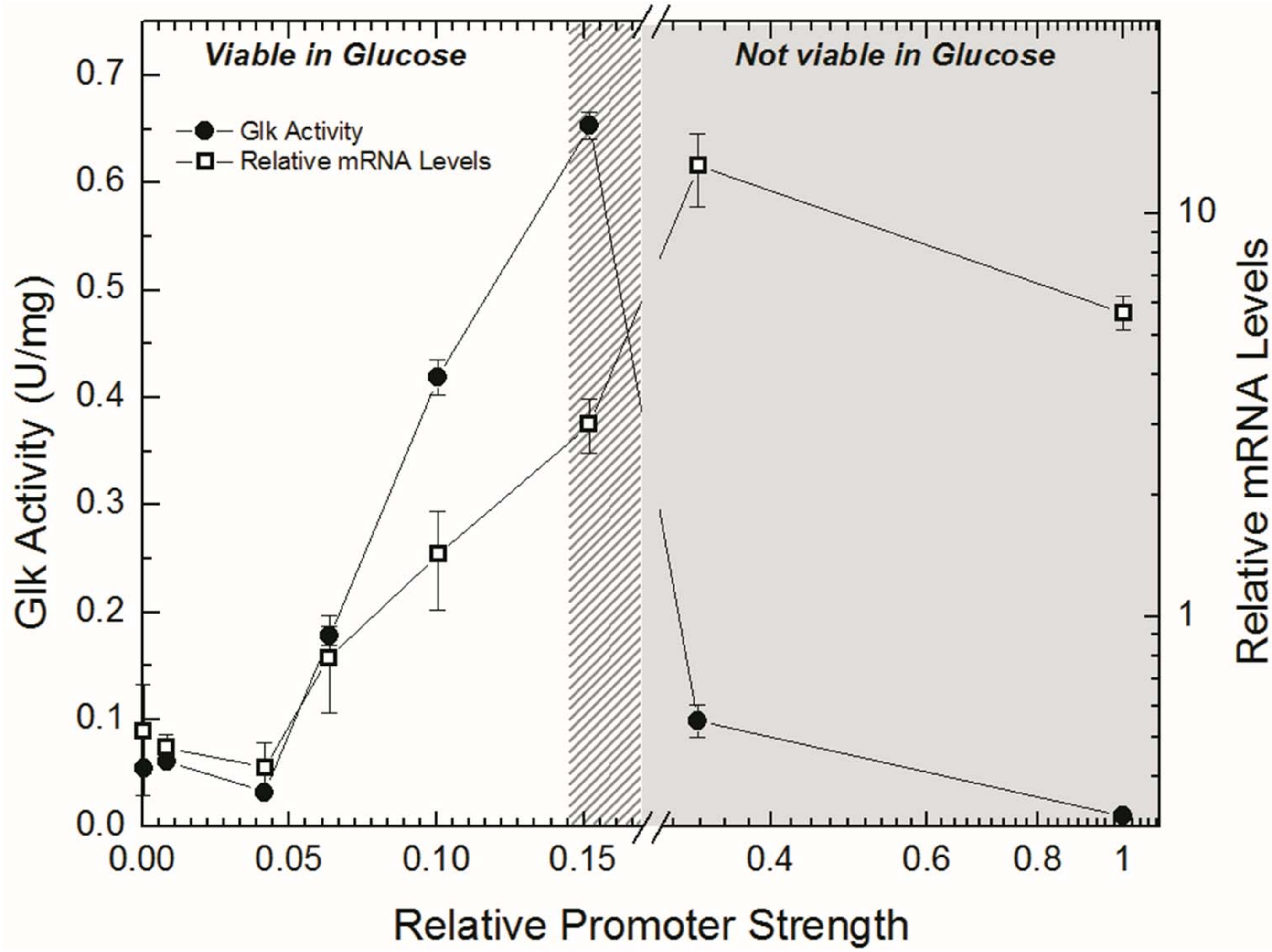
Glk Expression Library



Promoter part #: J231xx

Anderson Promoter Library, http://partsregistry.org

Glk Expression Library



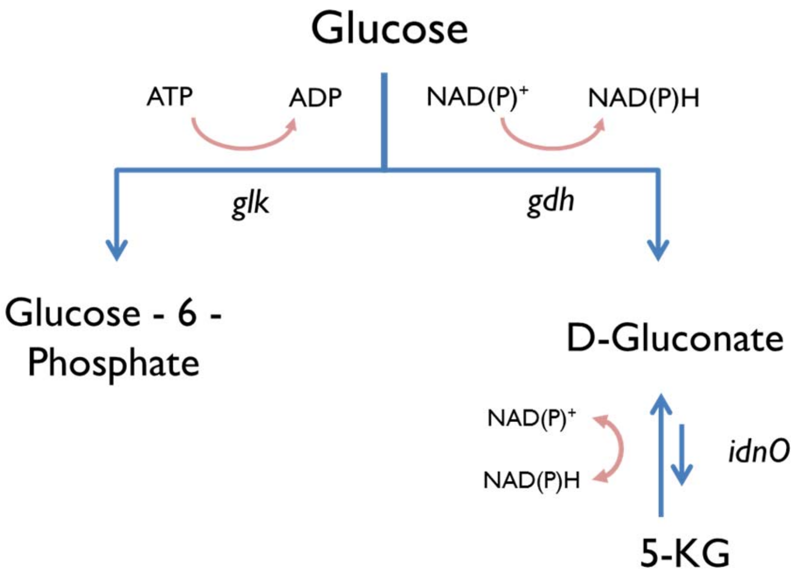
Glk activity measured during growth on glycerol to decouple from glycolysis

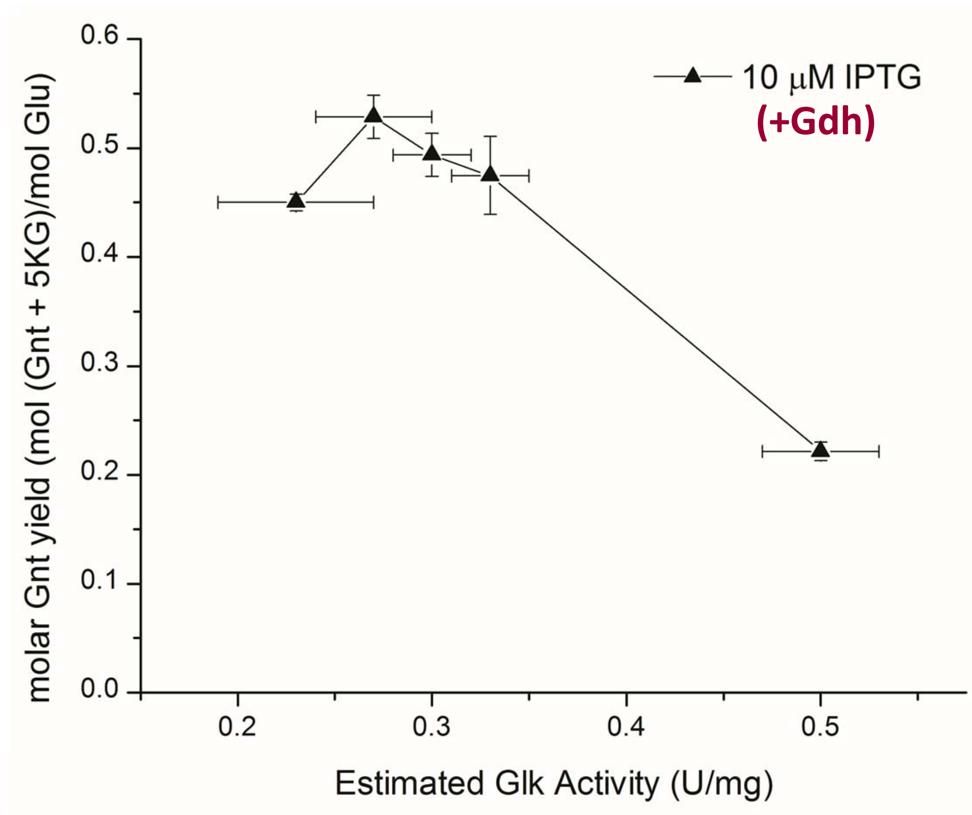
On glucose, growth rate varies with Glk activity

Solomon et al, 2013. *ACS Synthetic Biology,* 2:126-135.

Gluconate Productivity as a Function of Glk Activity

Target Compound:
Gluconate

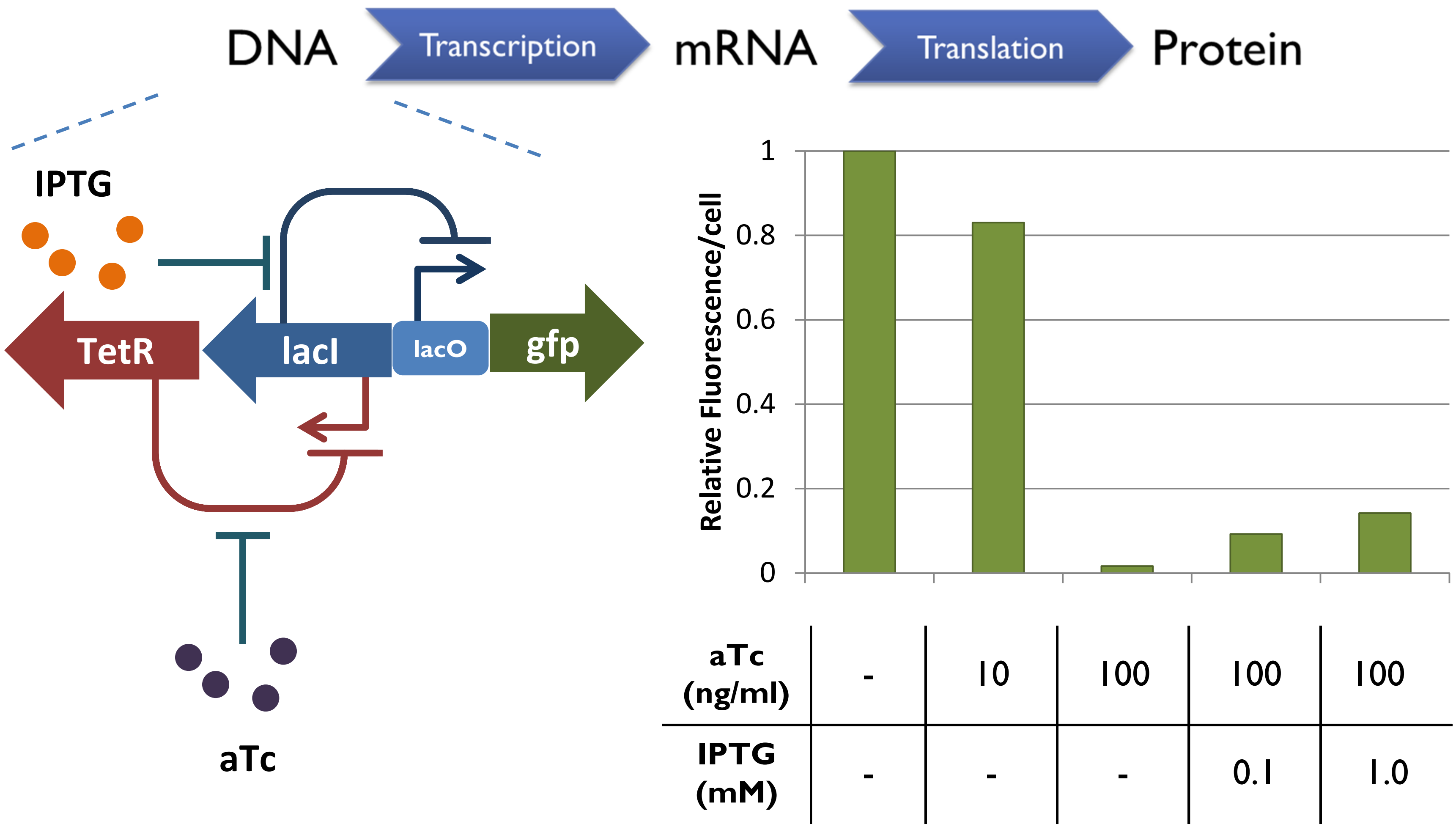




Reductions in Glk activity lead to increases in molar yield but only when endogenous needs are met

Solomon et al, 2013. *ACS Synthetic Biology,* 2:126-135.

Inverters as a glucose valve

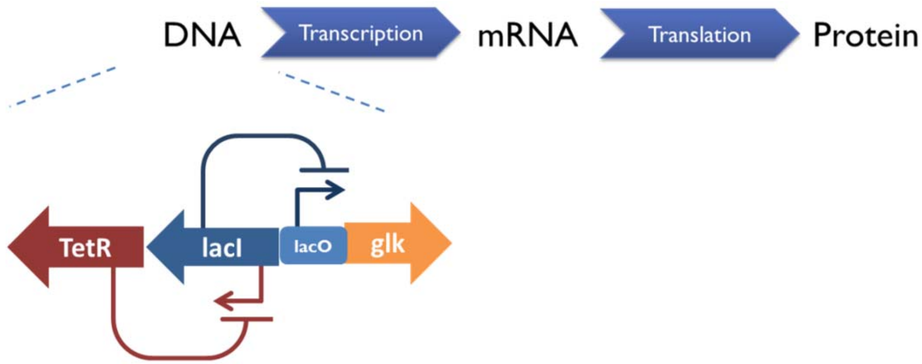


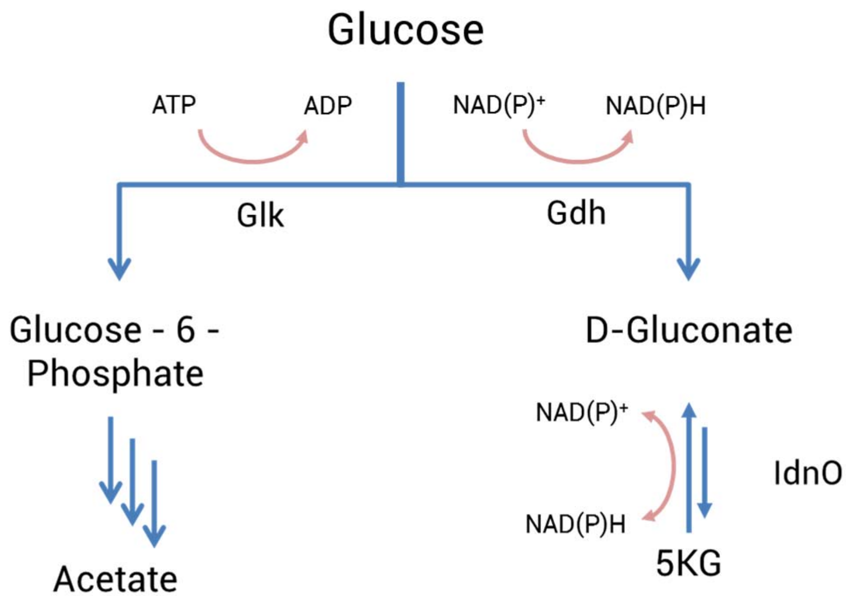
aTc (ng/ml)	-	10	100	100	100
IPTG (mM)	-	-	-	0.1	1.0

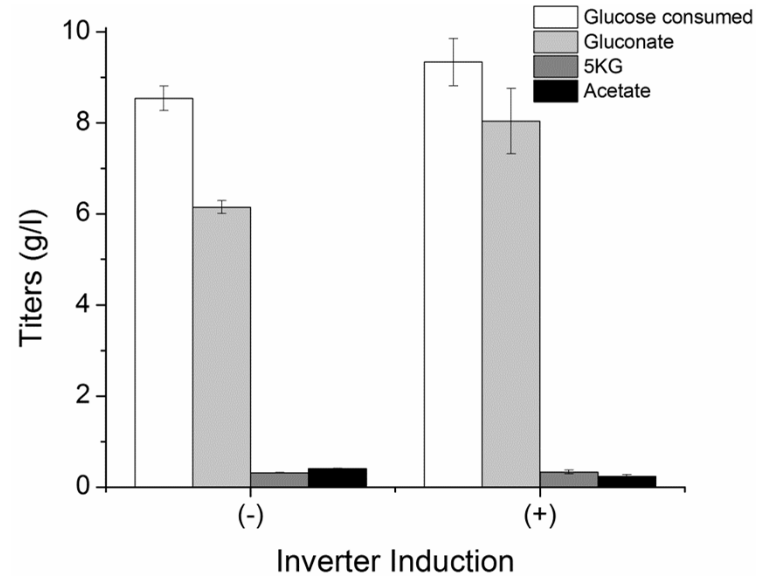
Biological inverters show a dose dependent response

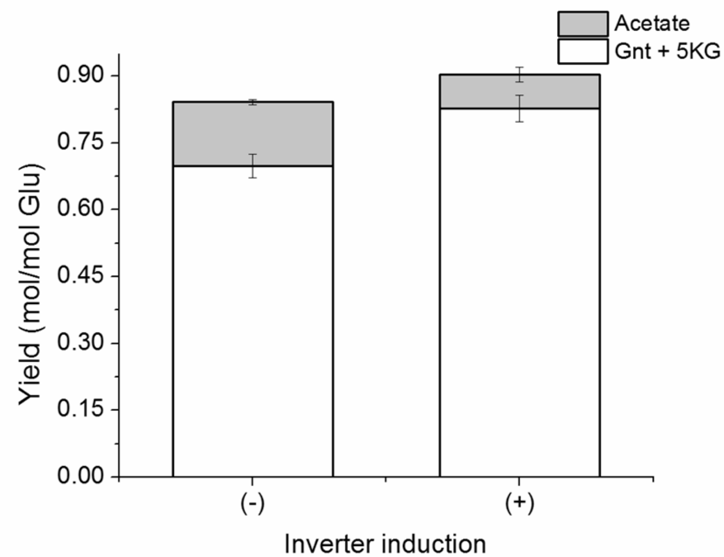
Solomon et al, 2012. *Metabolic Engineering,* 14:661-671.

Inverter Effect on Productivity





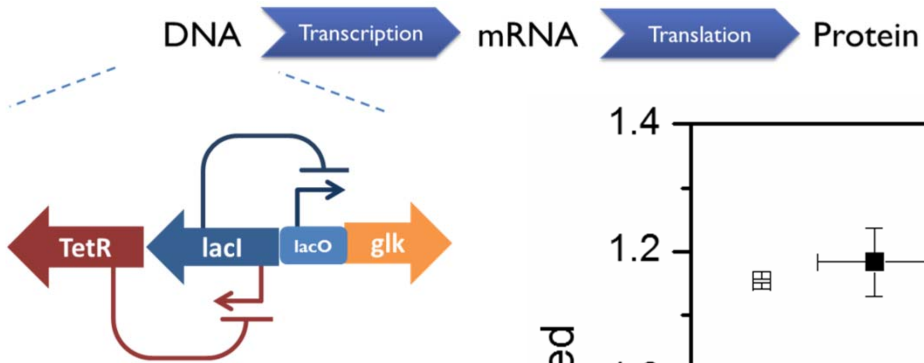




Dynamic control → + 18% gluconate yield, – 46% acetate waste (molar)

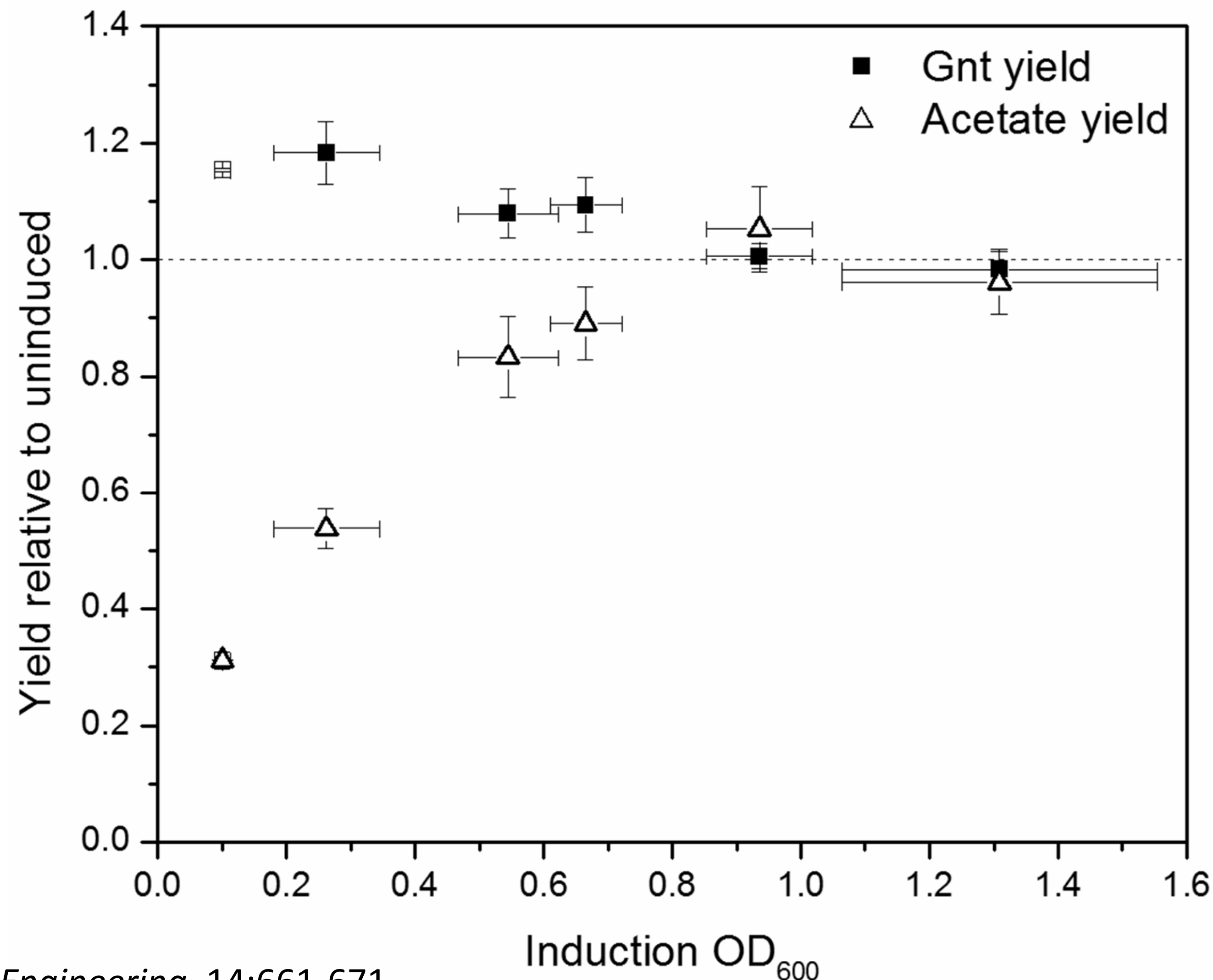
Solomon et al, 2012. *Metabolic Engineering,* 14:661-671.

Effect of Timing of Induction



Inverter controls gluconate and acetate yields

- Yield improvements highest at early induction



Solomon et al, 2012. *Metabolic Engineering,* 14:661-671.

Summary of Glucaric Acid Production

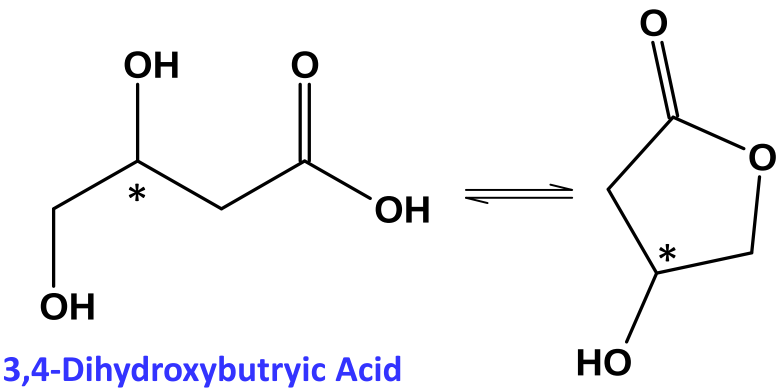
- Bioprospecting for Part Selection resulted in identification of enzymes necessary to create a novel pathway.
- Use of Synthetic Biology Devices led to increases in productivity.
- Host engineering may provide a means to further improve flux and titers.

3-Hydroxybutyrolactone (3-HBL)

- Key Intermediate in Higher, Chiral Synthesis of Solvents (e.g. Furan Derivatives) and Pharmaceuticals (e.g. Statins)

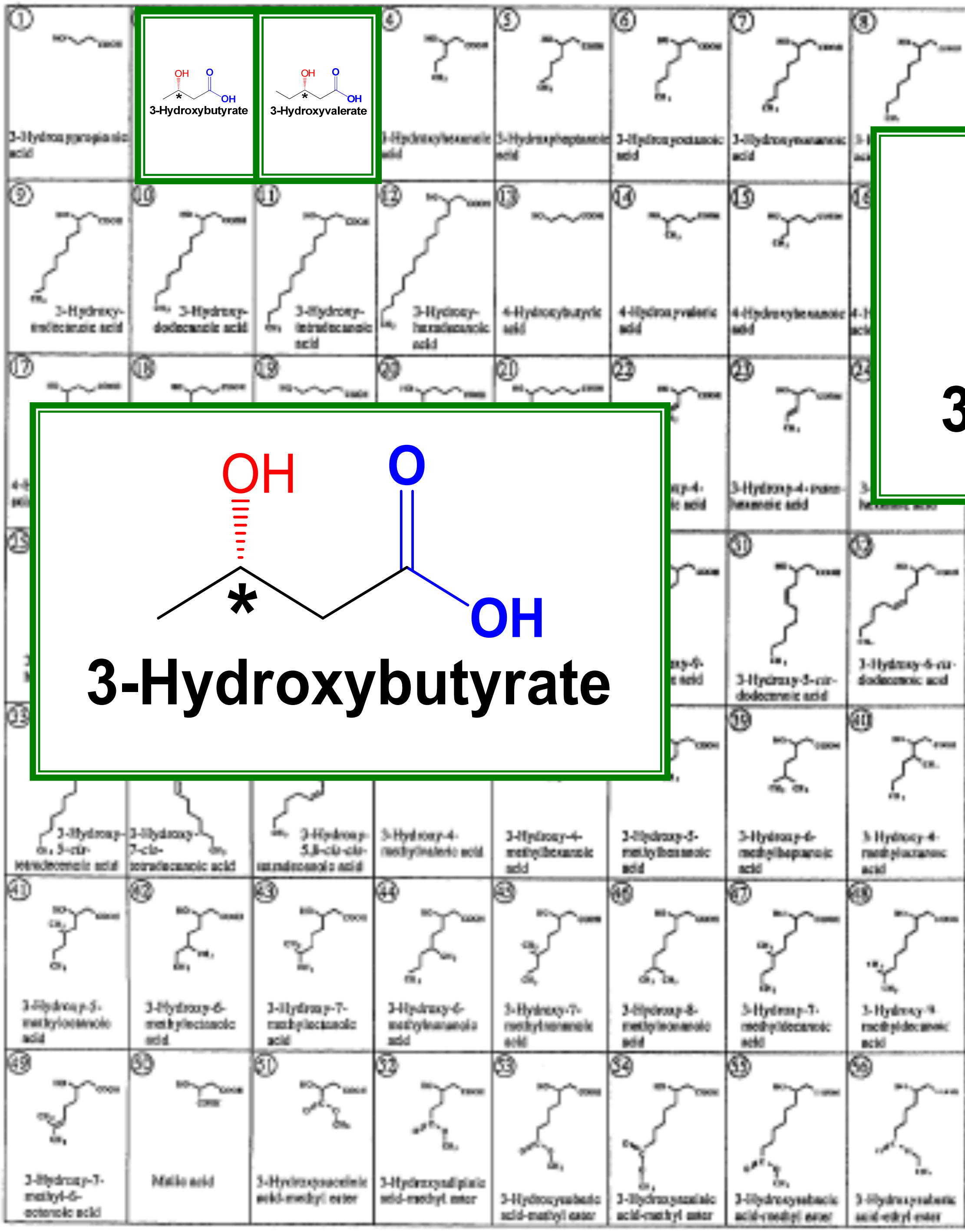
- Wholesale Cost ~ $450/kg ($20-50/gram for lab-scale quantities)

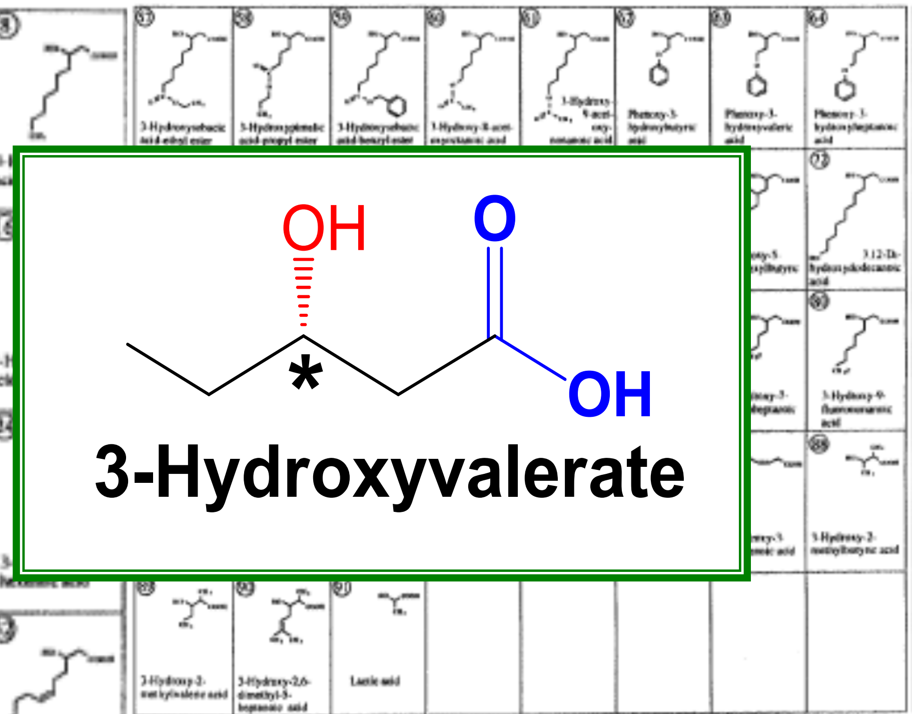
- **No Known Biological Routes towards DHBA or 3-HBL.**



3,4-Dihydroxybutryic Acid (DHBA)

3-Hydroxybutyrolactone (3-HBL)

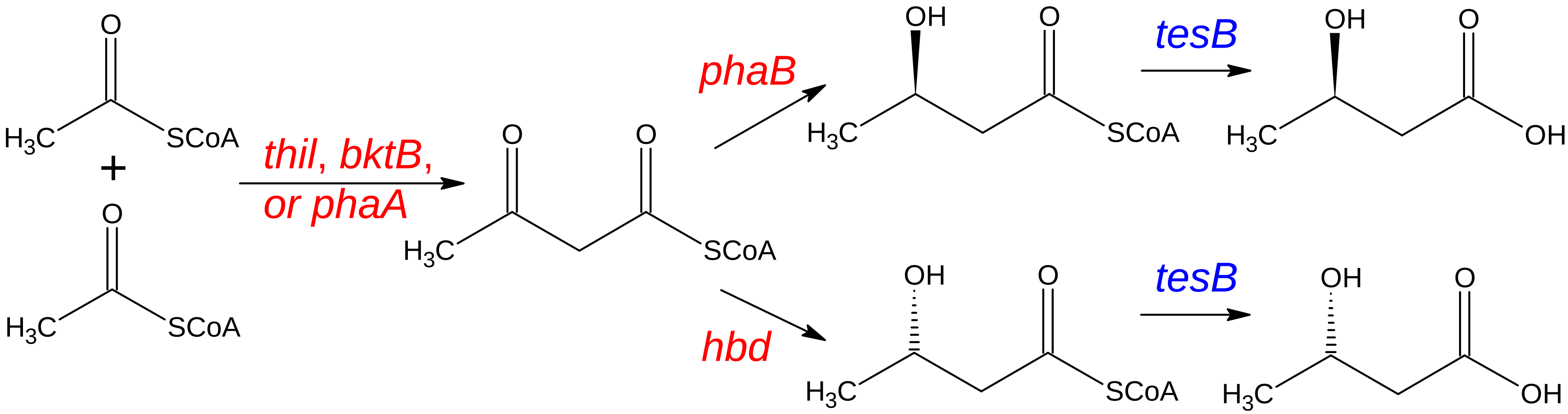




- Many hydroxyacids have been made as constituents of intracellular PHAs (biopolymers).
- Very few can be produced economically as free monomers.

Steinbüchel *et al*, FEMS Microbiol. Lett., 1995, 128:219-228.

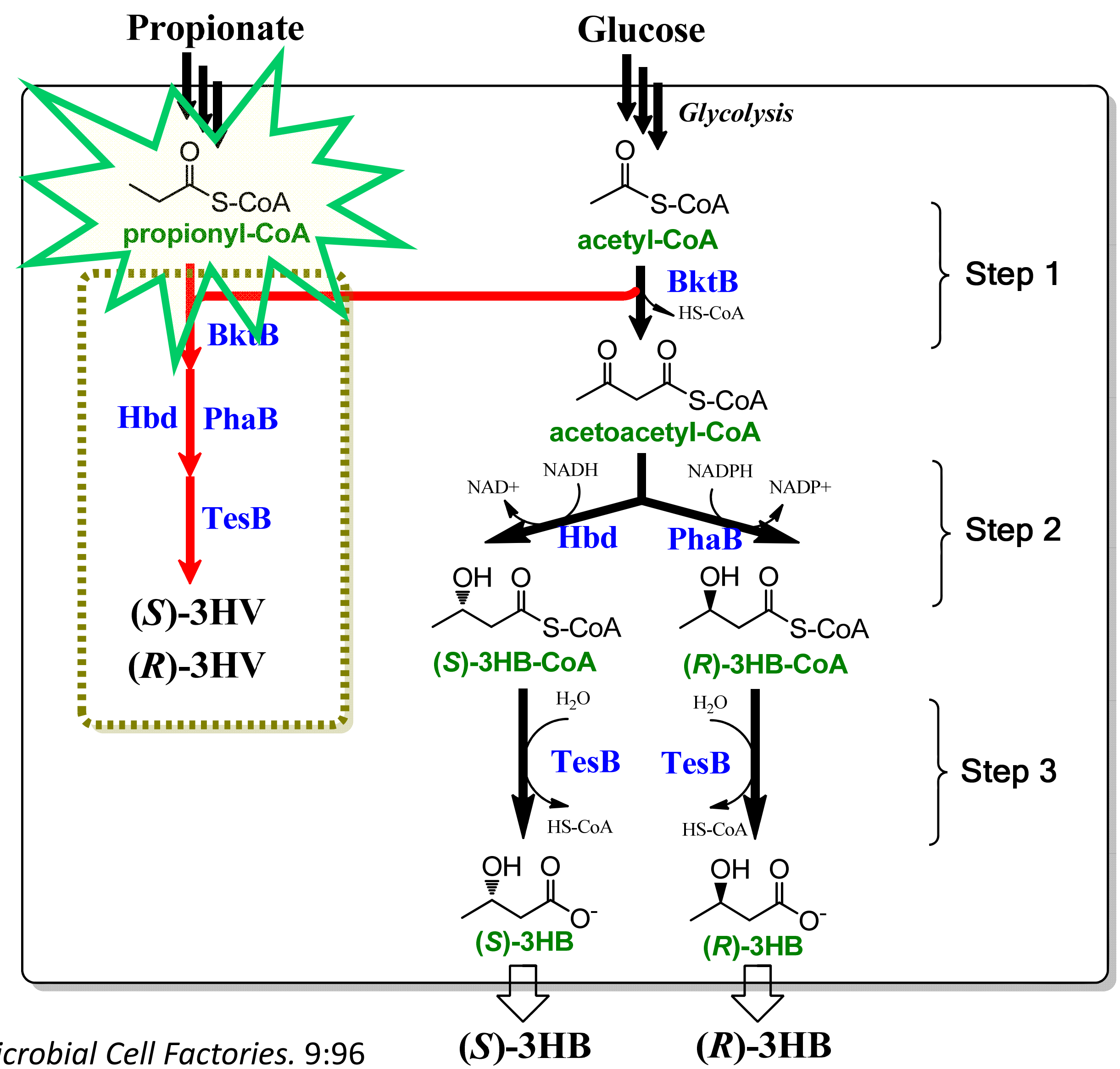
The 3-Hydroxybutyrate Pathway



Enzyme	Organism of Origin	Properties	Reference
bktB	*R. eutropha* H16	Acetyltransferase, broad substrate range (C_4-C_6)	Slater, 1998
hbd	*C. acetobutylicum* 824	Dehydrogenase, forms S stereoisomer product	Boynton, 1996
phaA	*R. eutropha* H16	Acetyltransferase, used in biopolymer synthesis	Schubert, 1988
phaB	*R. eutropha* H16	Dehydrogenase, forms R stereoisomer product	Schubert, 1988
tesB	*E. coli* K12	Thioesterase, Very broad substrate range (C_4-C_{16})	Huisman, 1991
thil	*C. acetobutylicum* 824	Acetyltransferase, high activity	Stim-Herndon, 1995

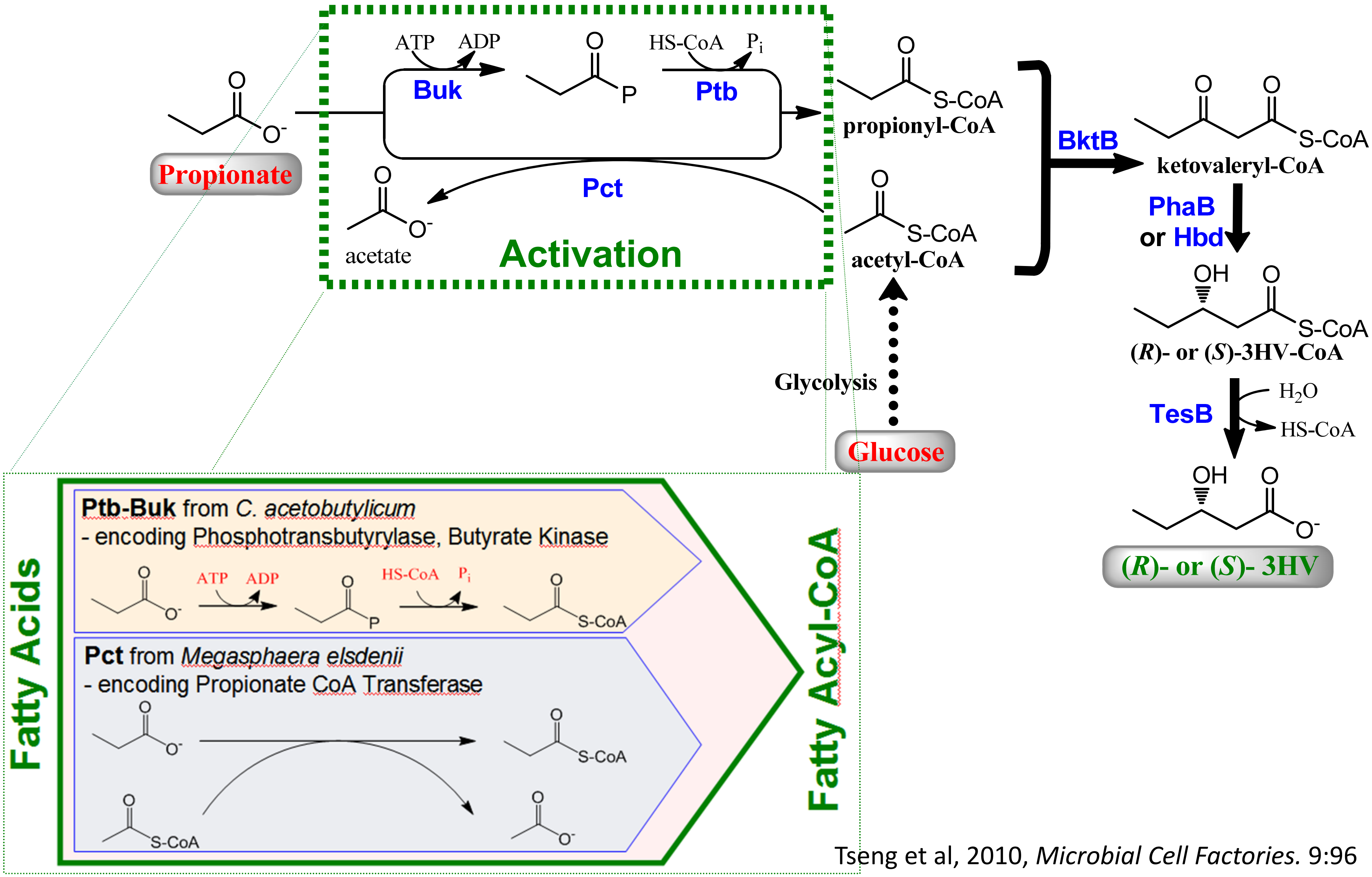
Tseng et al, 2009. *Appl. Environ. Microbiol.* 75(10):3137-3145. **>2 g/L *R*- or *S*- 3HB**

3-Hydroxyvalerate Synthesis



Tseng et al, 2010, *Microbial Cell Factories.* 9:96

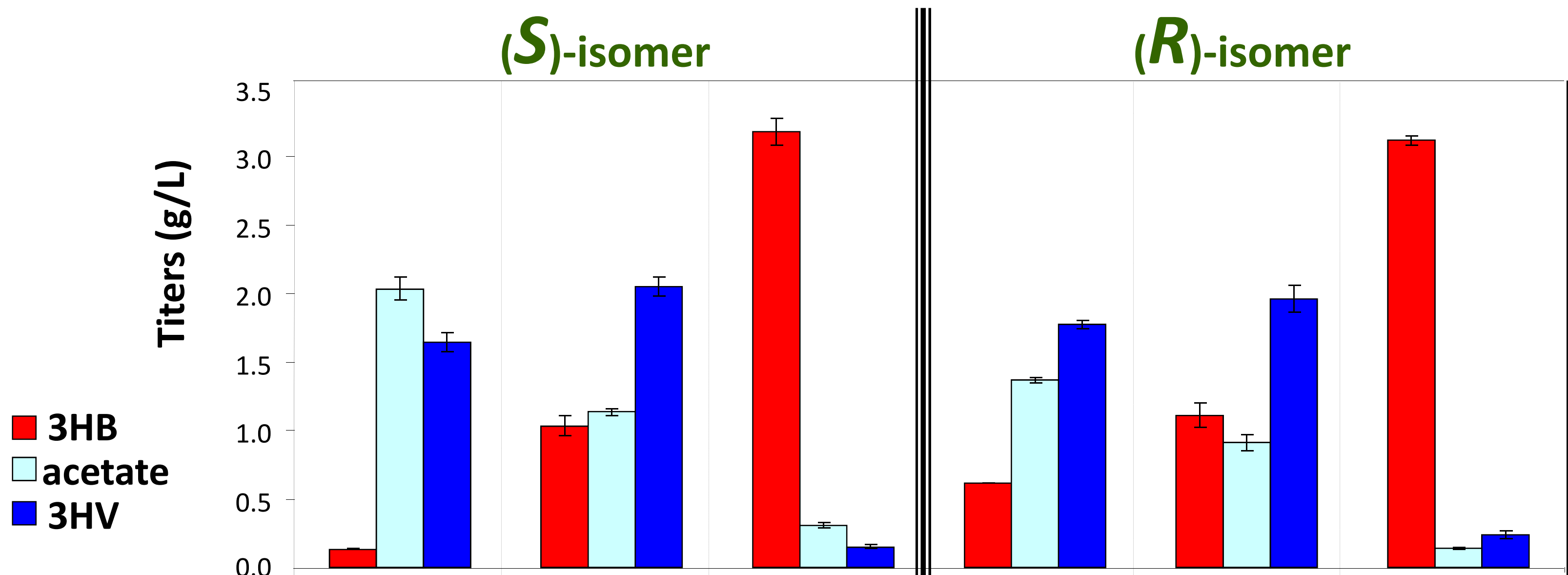
CoA-activation Enzymes



Tseng et al, 2010, *Microbial Cell Factories.* 9:96

Chiral 3HV Production

- ✓ **Activation of propionate is crucial**
- ✓ **Activation mechanism determines the product distribution**

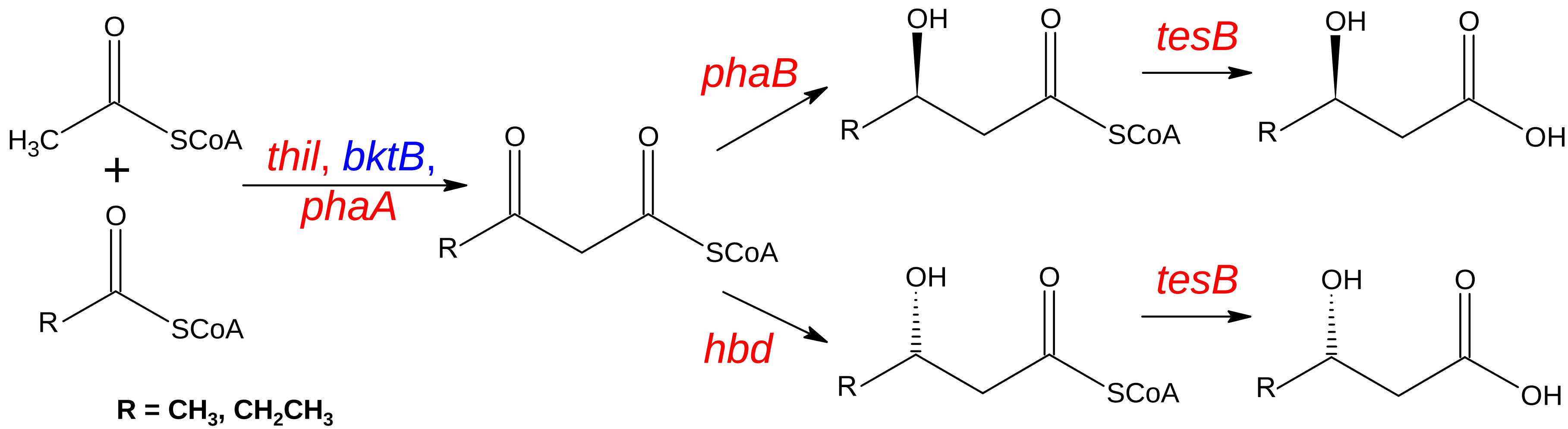


Activation Enzyme	Pct	Ptb-Buk	None	Pct	Ptb-Buk	None
3HV/3HB ratio	12.6	1.97	0.05	2.88	1.77	0.08

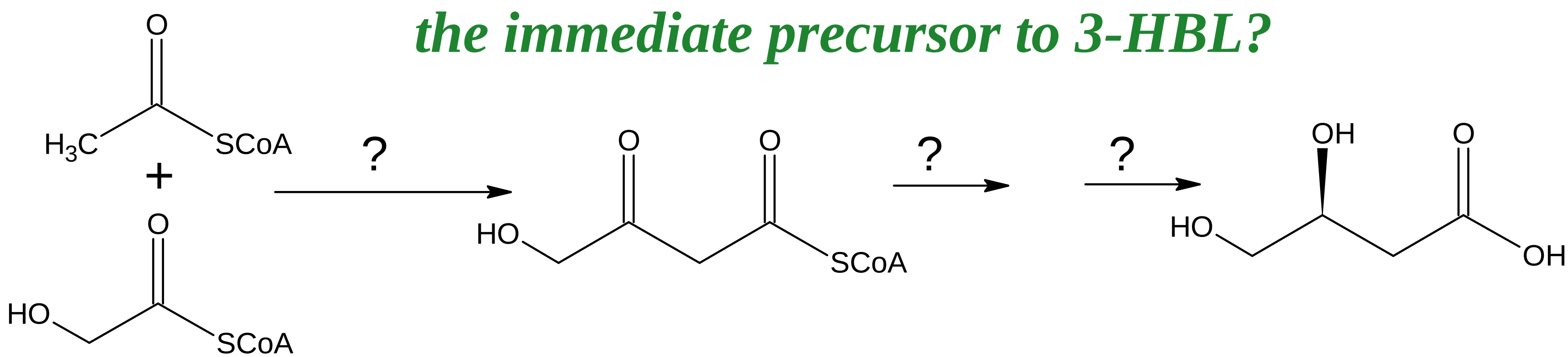
Tseng et al, 2010, *Microbial Cell Factories.* 9:96

From 3HB/3HV to DHBA?

3-hydroxybutyrate & 3-hydroxyvalerate biosynthetic pathway

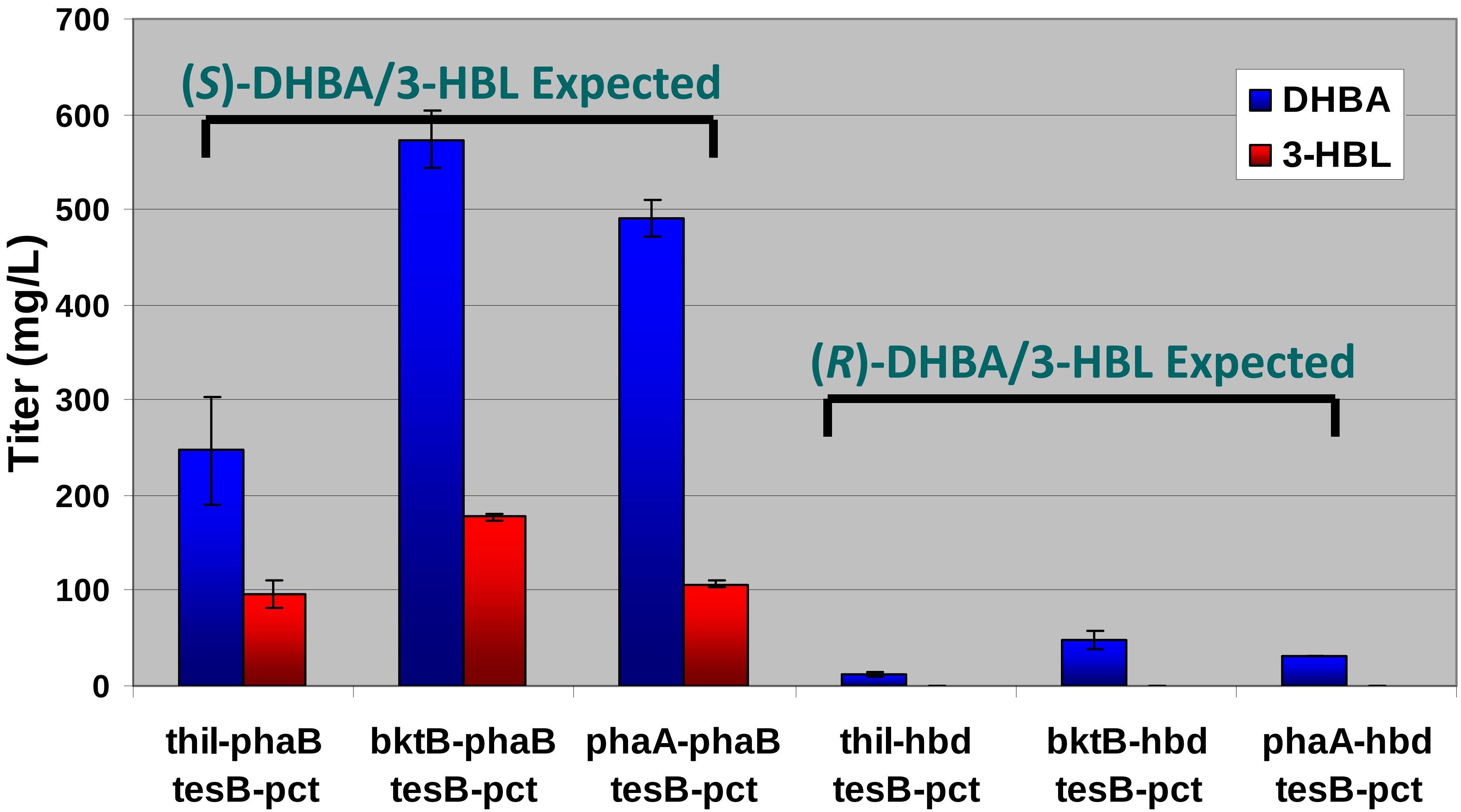


Can this pathway be adapted to make DHBA, the immediate precursor to 3-HBL?



Production of 3,4-Dihydroxybutyrate (DHBA)

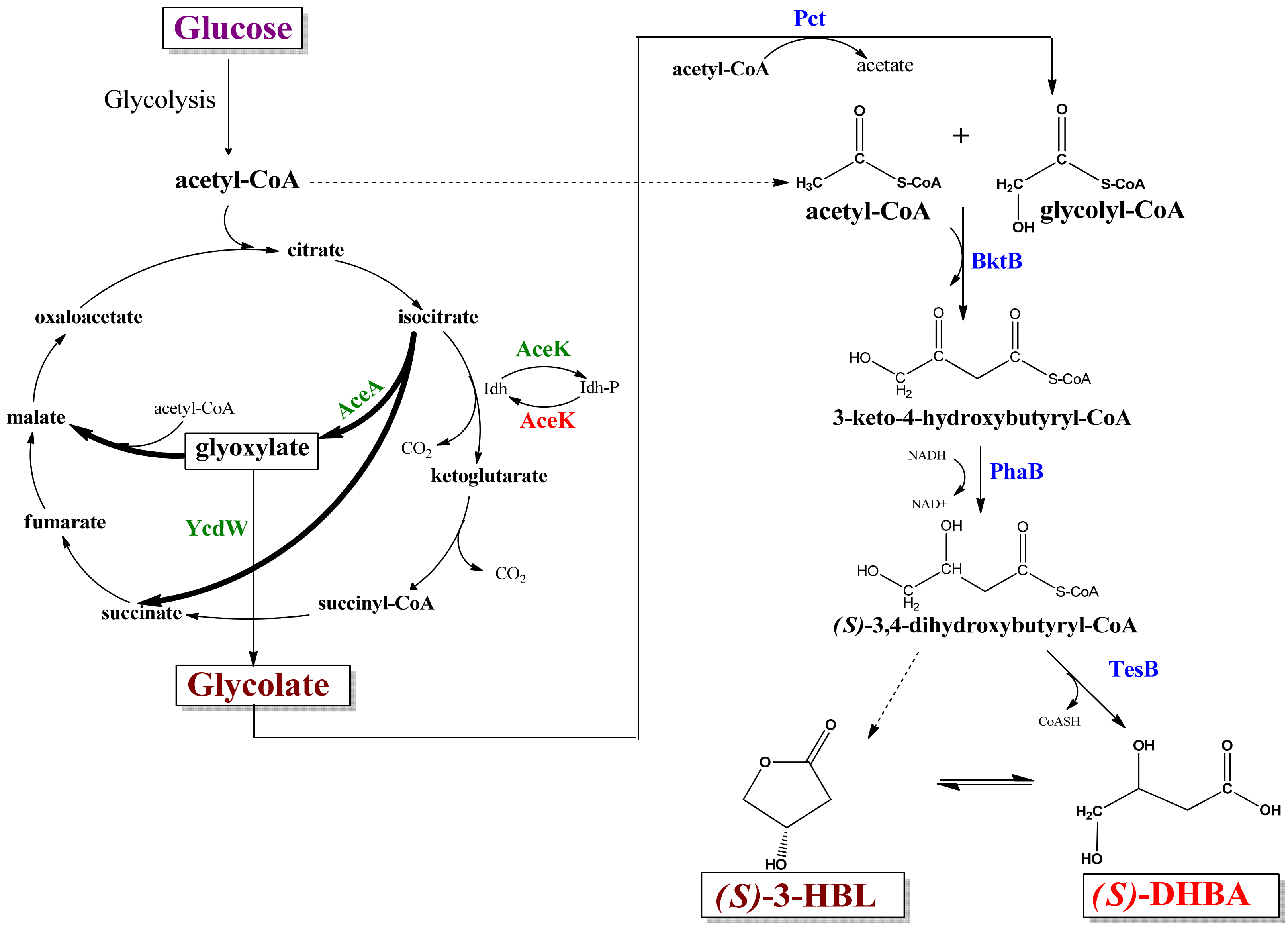
Glucose + Glycolate



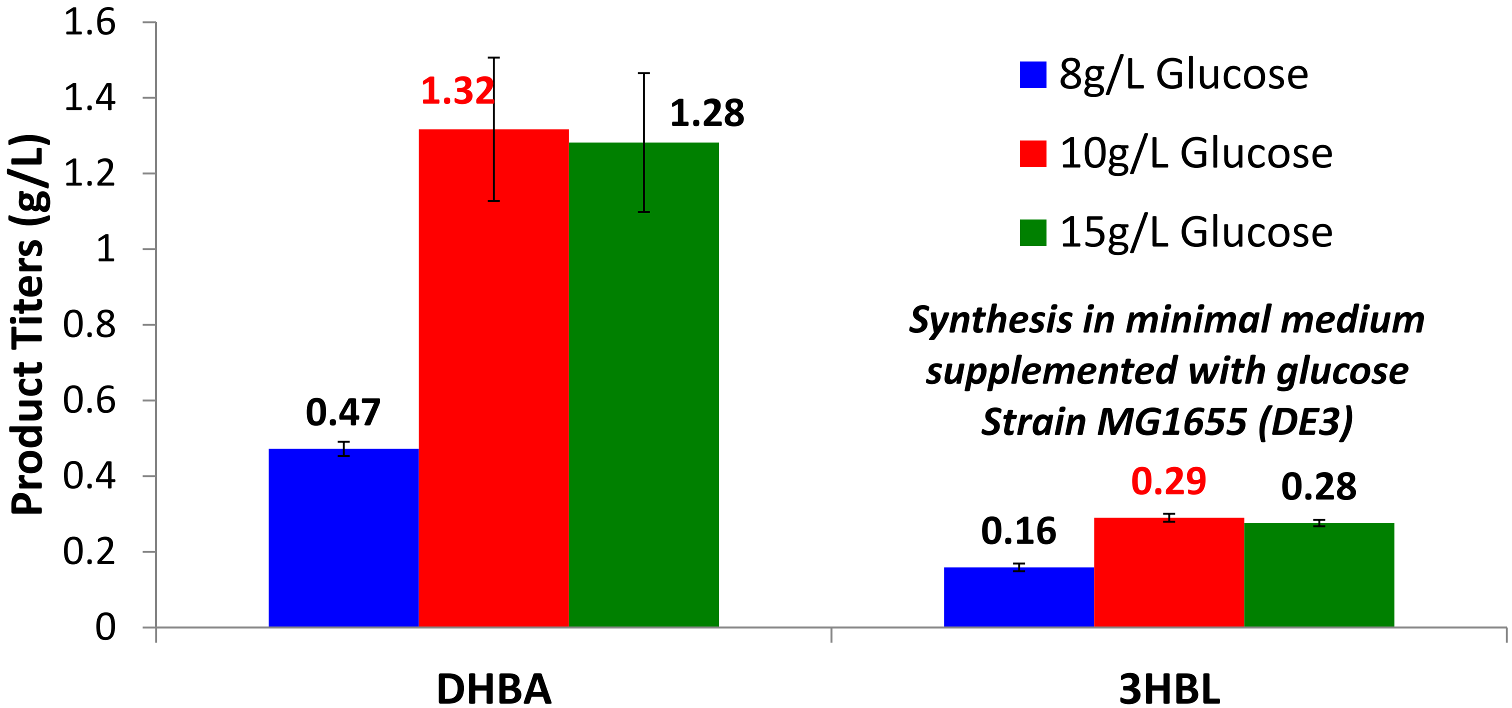
All cultures were *E. coli* MG1655(DE3) *end*A⁻ recA⁻ grown in LB for 72 hours and were supplied with glycolate.

Martin et al, 2013. *Nat. Commun.* 4, 1414

Direct Synthesis of Glycolate, DHBA and 3-HBL from Glucose



Effect of Glucose Concentration on DHBA

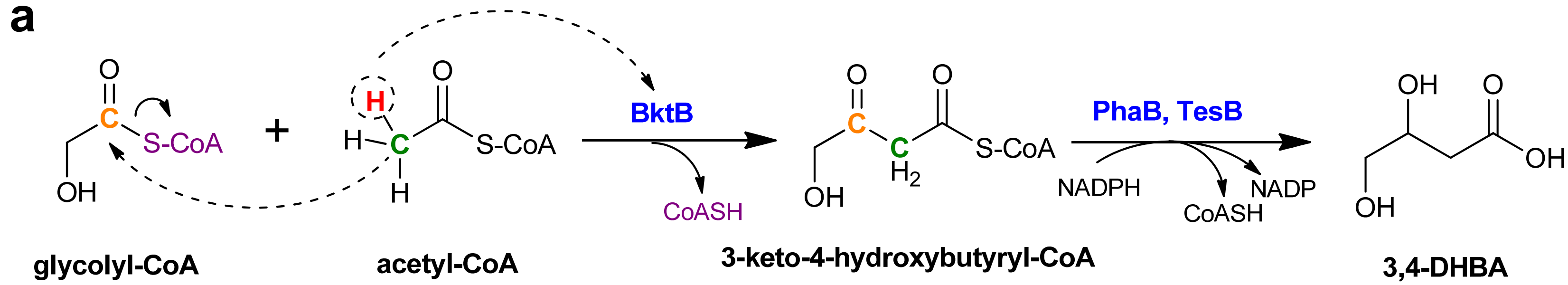


Synthesis in minimal medium supplemented with glucose
Strain MG1655 (DE3)

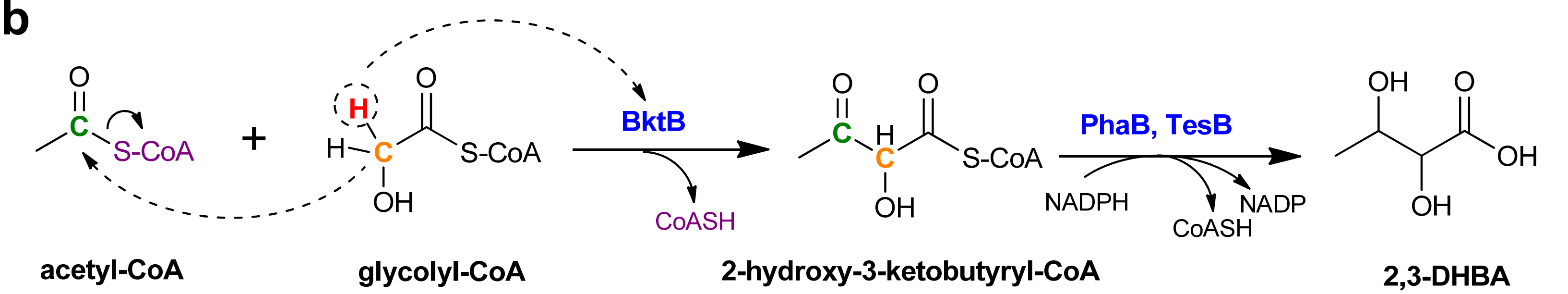
Glucose Feed	Molar Yield on Glucose	% of Theoretical Yield	([3,4-DHBA]+[3HBL]) / [3HB]
0.8%	0.095	14.4	3.514
1.0%	**0.141**	**21.3**	2.557
1.5%	0.136	20.6	2.242

An Unexpected Product

a



b



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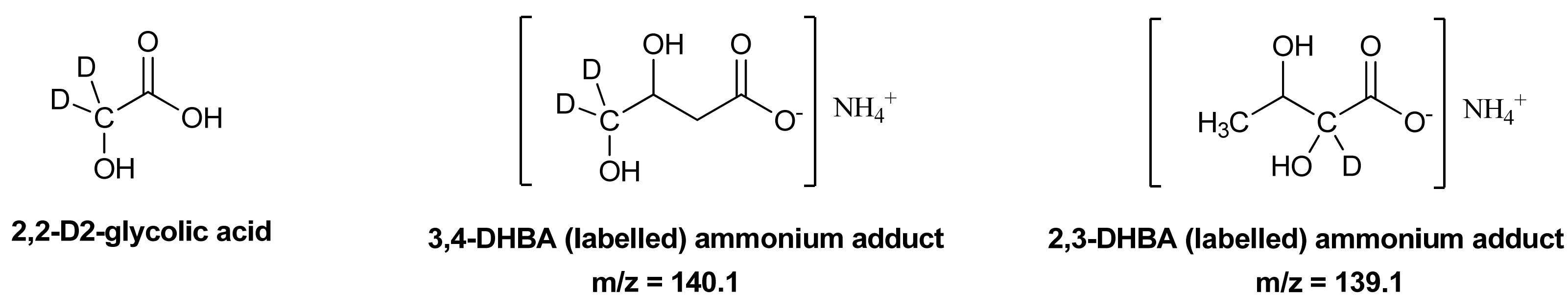
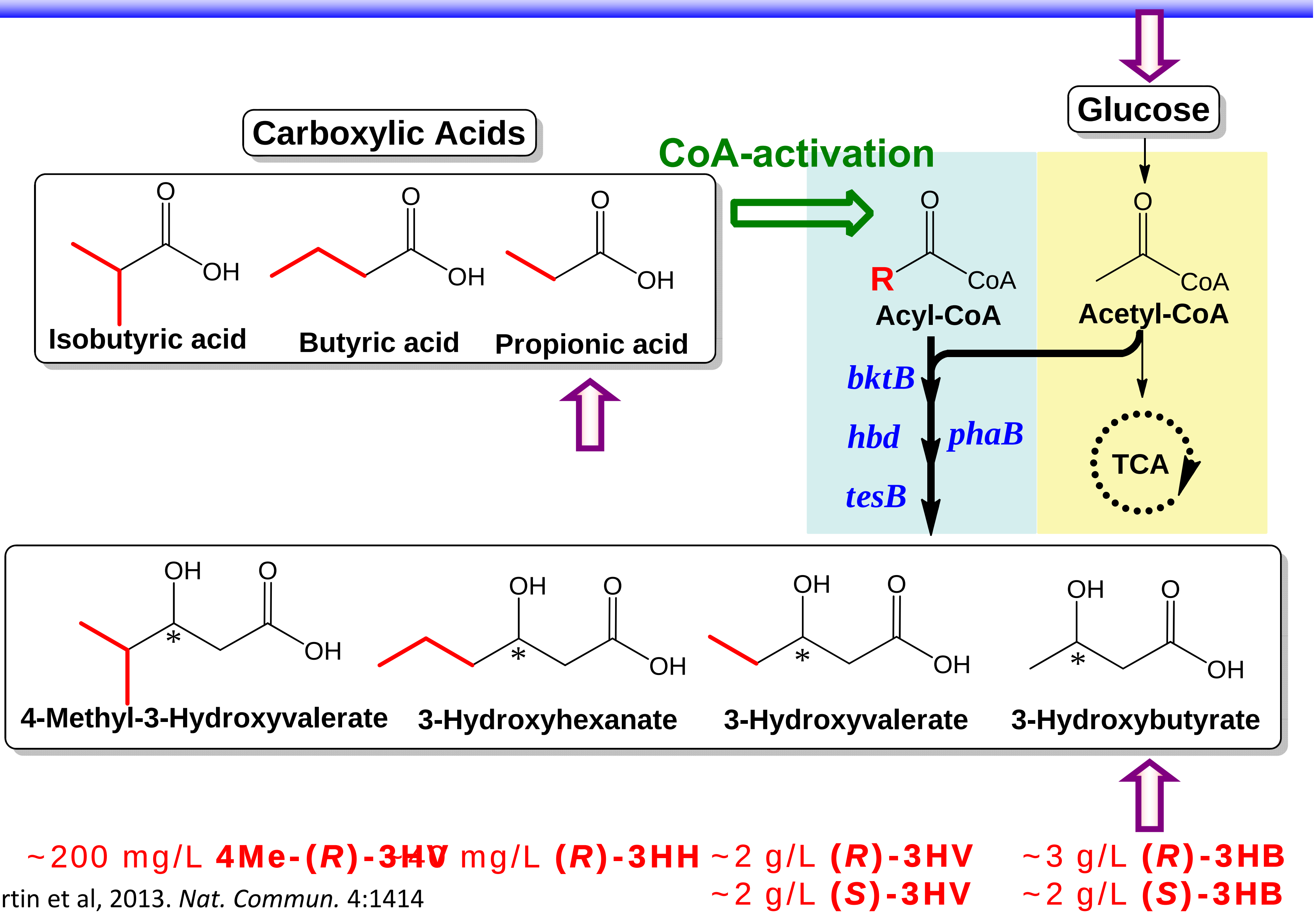


Pharma

Materials

Extending the Hydroxyacid Pathway



Martin et al, 2013. *Nat. Commun.* 4:1414

Summary of Hydroxyacids Production

- Established a versatile platform for biological synthesis of chiral hydroxyacids.
- ***Demonstrated 1st pathway for biological production of 3-hydroxybutyrolactone from simple (and sole) carbohydrate substrates.***
- Reliance on promiscuity of enzymes results in wide range of productivities for novel substrates.
  - **Bioprospecting and Protein Engineering**

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Current Group

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