Engineering Plant C_1 Metabolism

- **Preface -** ME, genomics & pathway discovery
- **Background -** Why plant C_1 metabolism is crucial
- **Objectives & personnel -** 3 aims, 5 PIs & labs
- **Progress -** Emphasizing the unexpected
- **Project output -** Publications, workshops, websites

© 2000 University of Florida

Genomics & Pathway Discovery

"Pathway modification and discovery are part of the flux determination process"

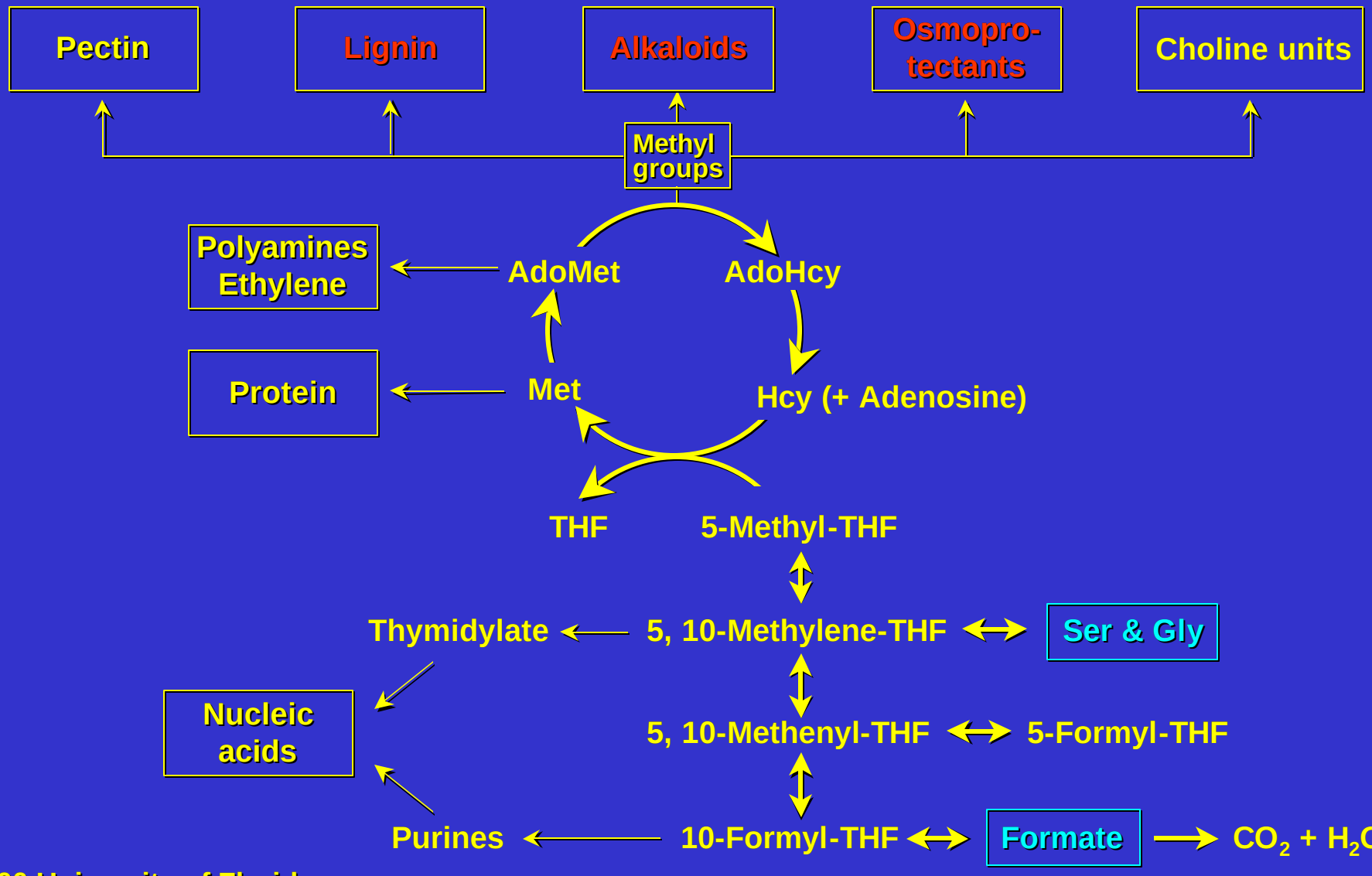
Gregory Stephanopoulos, *Metabolic Engineering* 1: 1(1999)

Background on Plant C_1 Metabolism

- Provides C_1 units (methyl, methylene, formyl) for biosyntheses - all are essential
- Engineering hi-methyl products (e.g. lignin, alkaloids, osmoprotectants) competes with other metabolism for C_1 units
- Massive photorespiratory C_1 fluxes

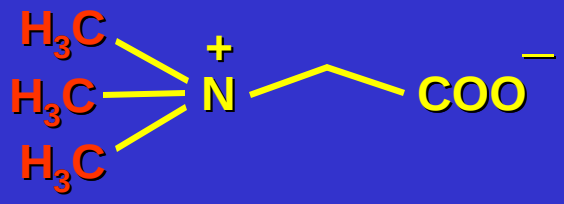
© 2000 University of Florida

Plant C_1 Metabolism

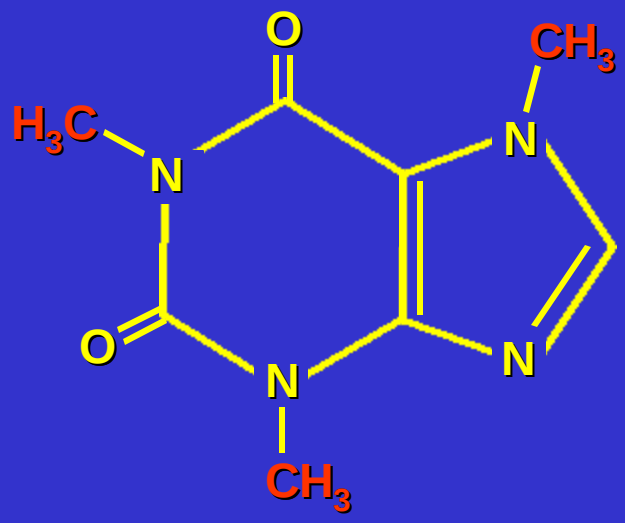


© 2000 University of Florida

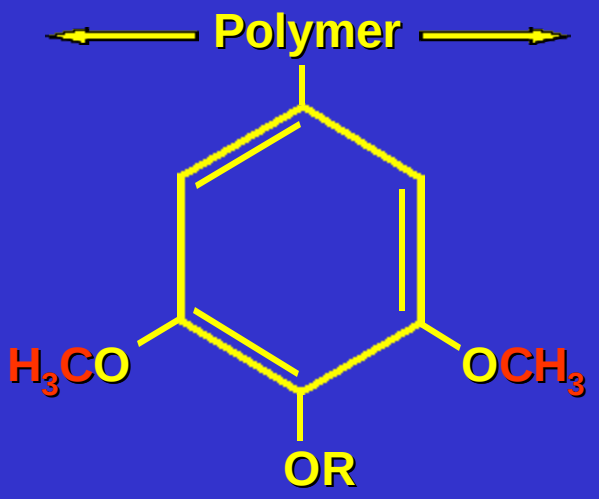
High-Methyl Engineered Products



Glycine betaine (osmoprotectant)



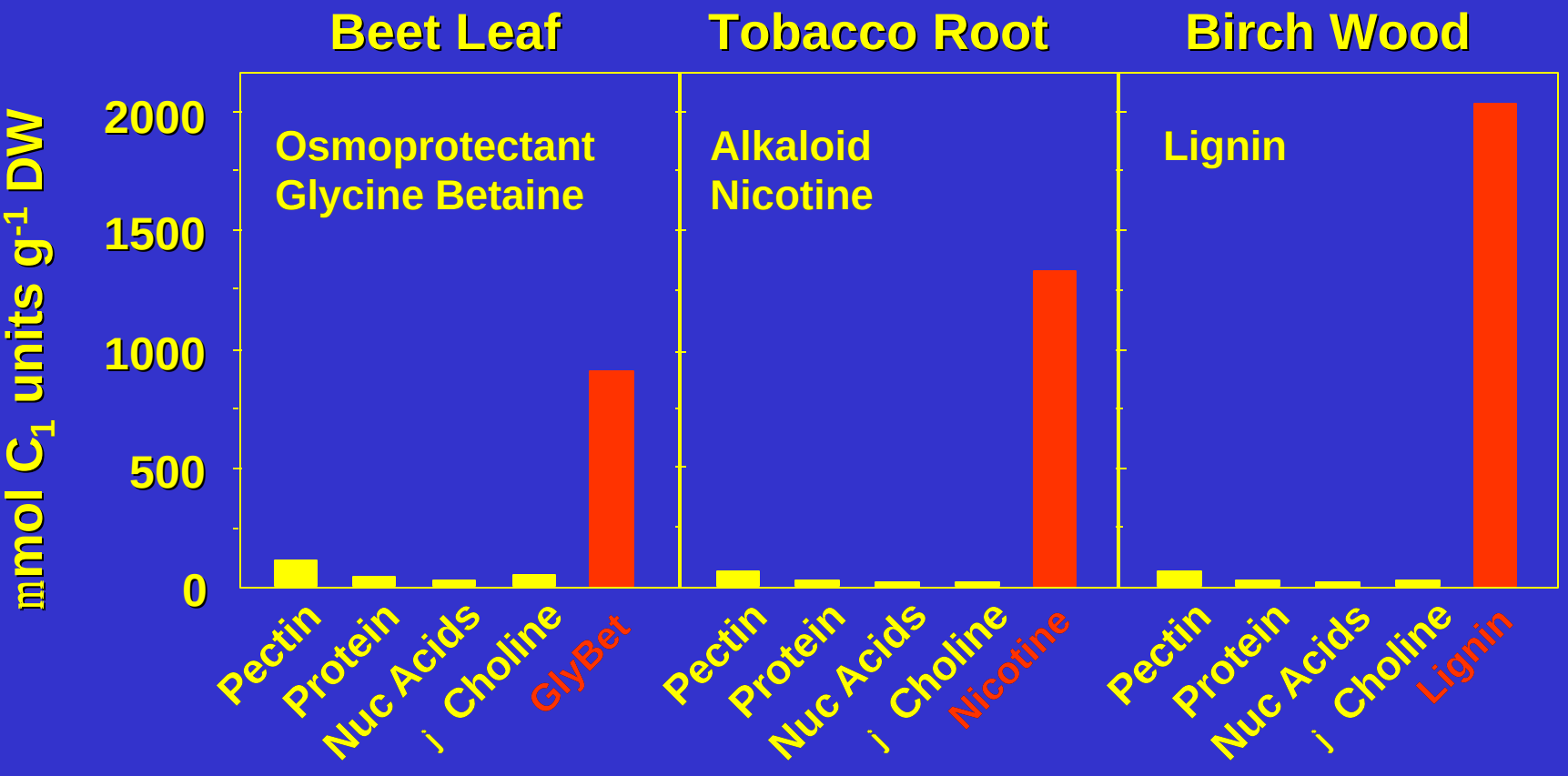
Caffeine (alkaloid)



Syringyl lignin

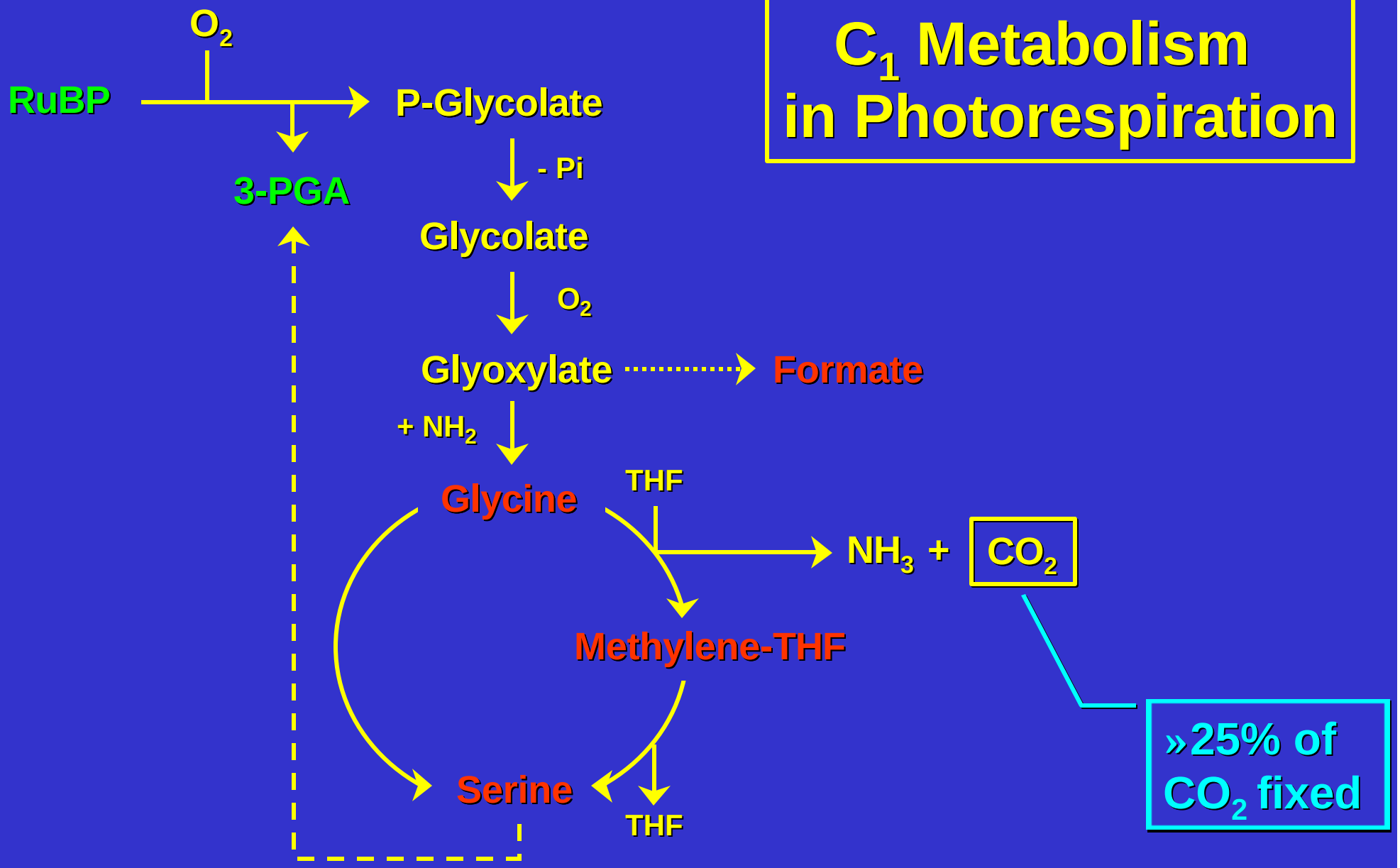
© 2000 University of Florida

The scale of C_1 demands in plants



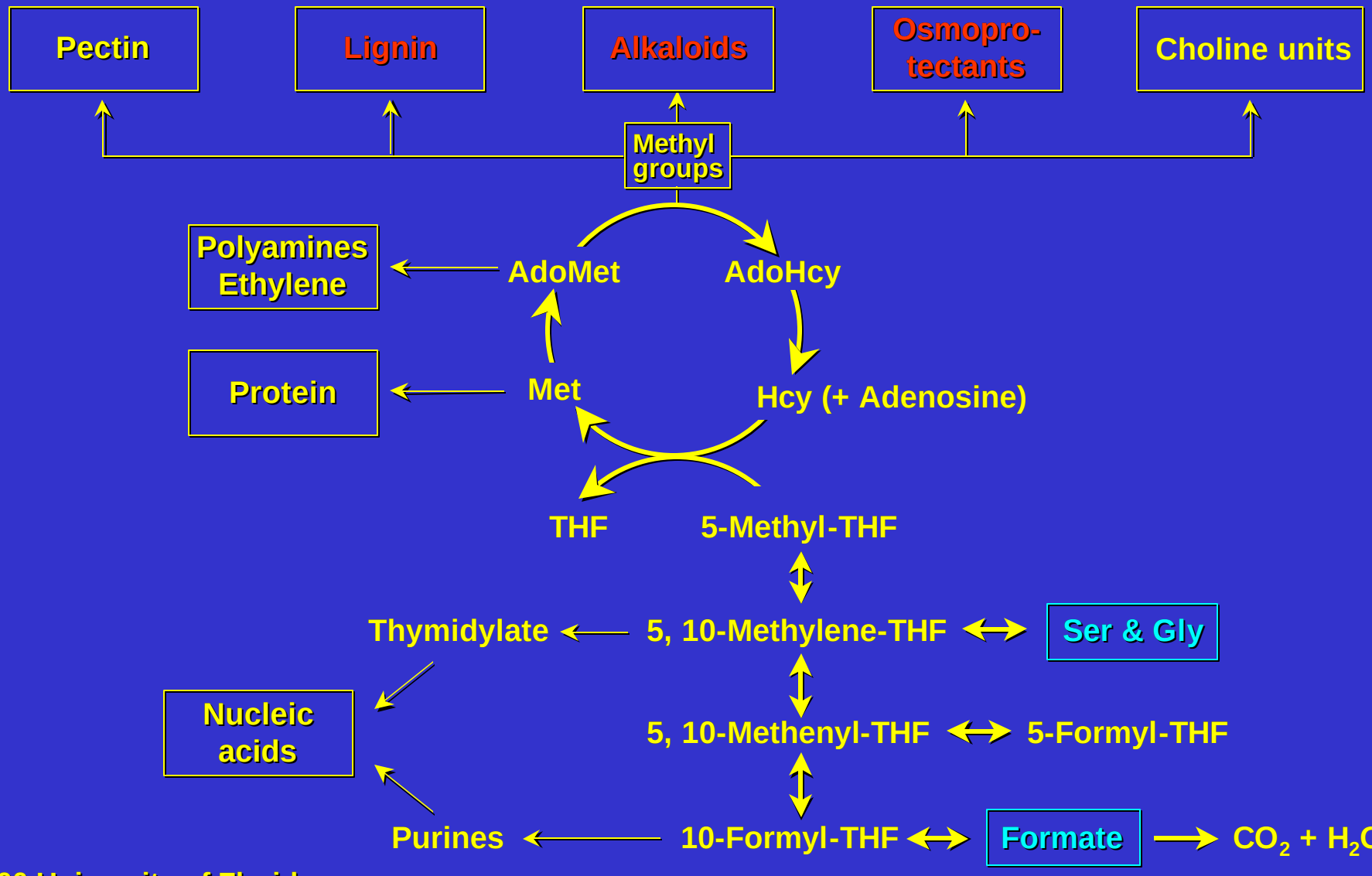
© 2000 University of Florida

C_1 Metabolism in Photorespiration



2000 Univ Florida

Plant C_1 Metabolism



© 2000 University of Florida

Engineering Questions

- System response to C_1 demand - or ¯ ?
  - Metabolic flux
  - Gene Expression
- Enzymes exerting major flux control ?
- Relative importance of C_1 sources ?
- More pathways ? Compartmentation ?

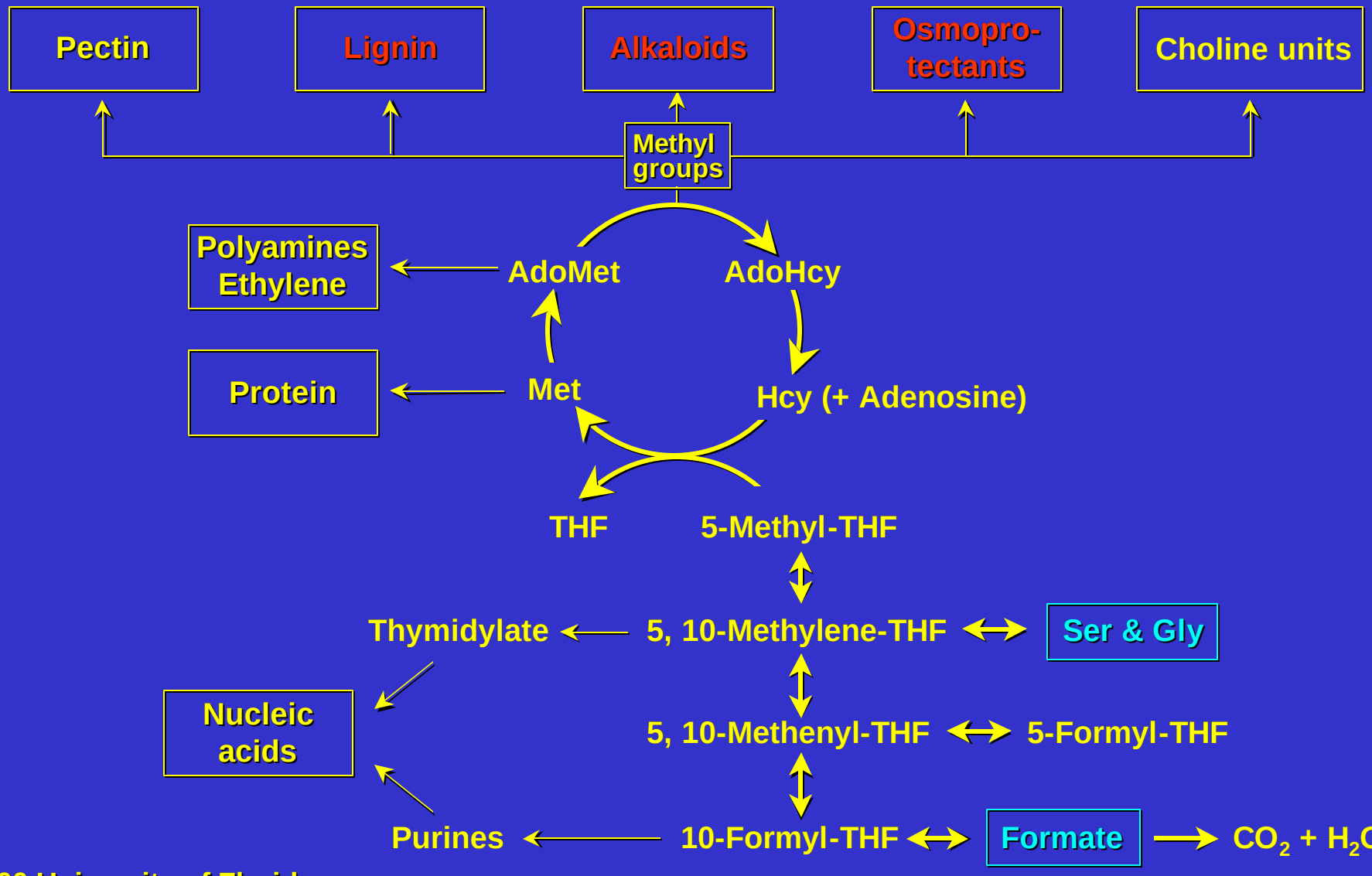
© 2000 University of Florida

Engineering Objectives
(Tobacco and maize)

- Essential preliminary cloning, build DNA arrays
  - MTHFR de novo
  - » 20 genes by homology
- Raise or lower C_1 demand and supply
  - Antisense/sense
  - Mutants
- Measure fluxes (MS, NMR, radiolabeling) & gene expression (DNA arrays), integrate by modeling

© 2000 University of Florida

Plant C_1 Metabolism



© 2000 University of Florida

Plant C_1 Metabolism Group

PI (Univ)	Expertise	GAs/PDFs
Hanson (UF)	Biochem/Mol Biol	1.5
Bohnert (UA)	Mol Biol	1
Rhodes (Purdue)	Models/Biochem	1
Gage (MSU)	An-Biochem/MS	1
Shachar-Hill (NMSU)	NMR/P-Biochem	1

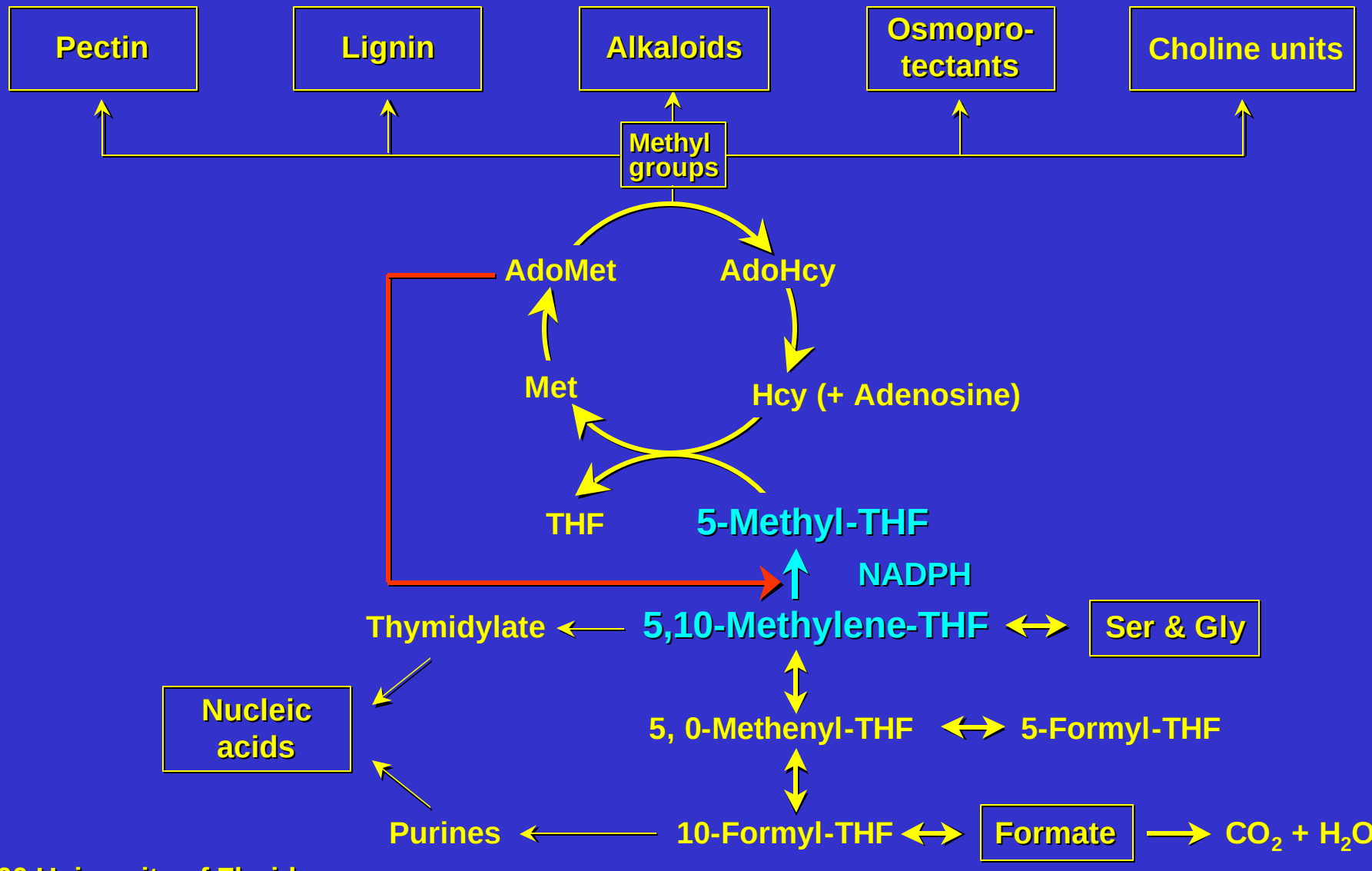
© 2000 University of Florida

Progress - Highlighting the Unexpected

- Methylene-THF reductases
- Maize DNA arrays
- Engineering glycine betaine synthesis
- Genomics & pathway discovery - PurU

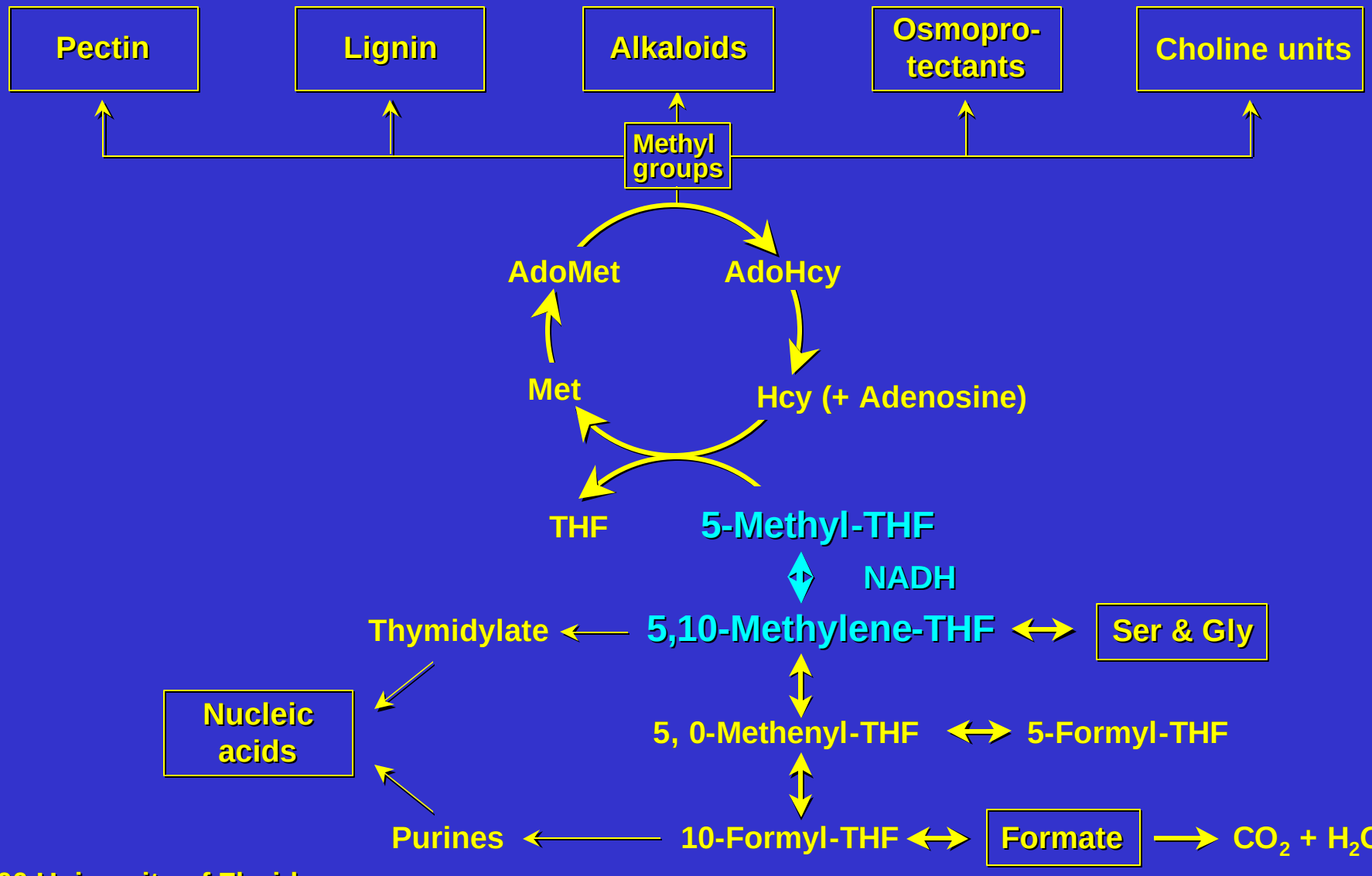
© 2000 University of Florida

MTHFR: What was surmised



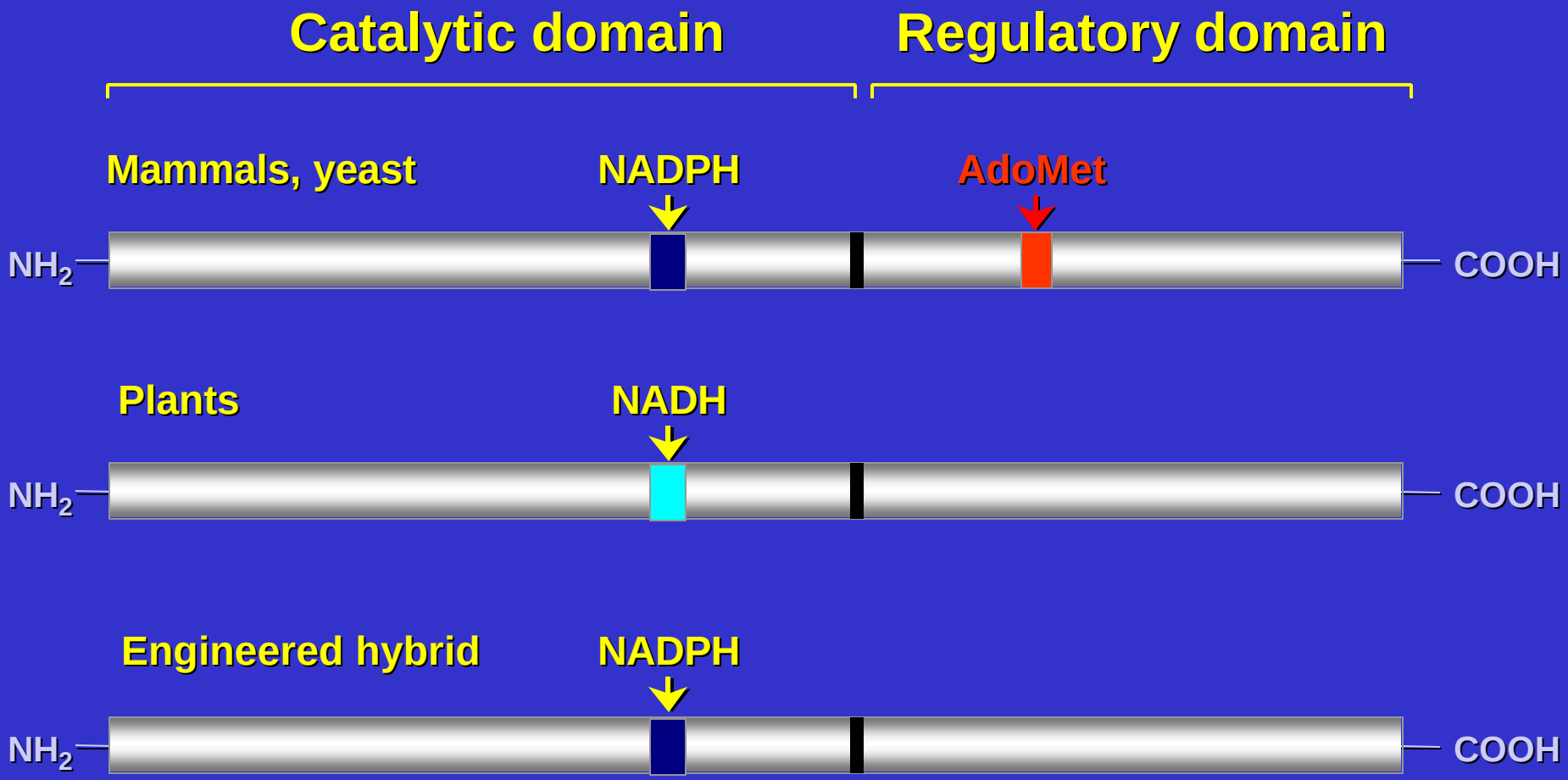
© 2000 University of Florida

MTHFR: What we observed



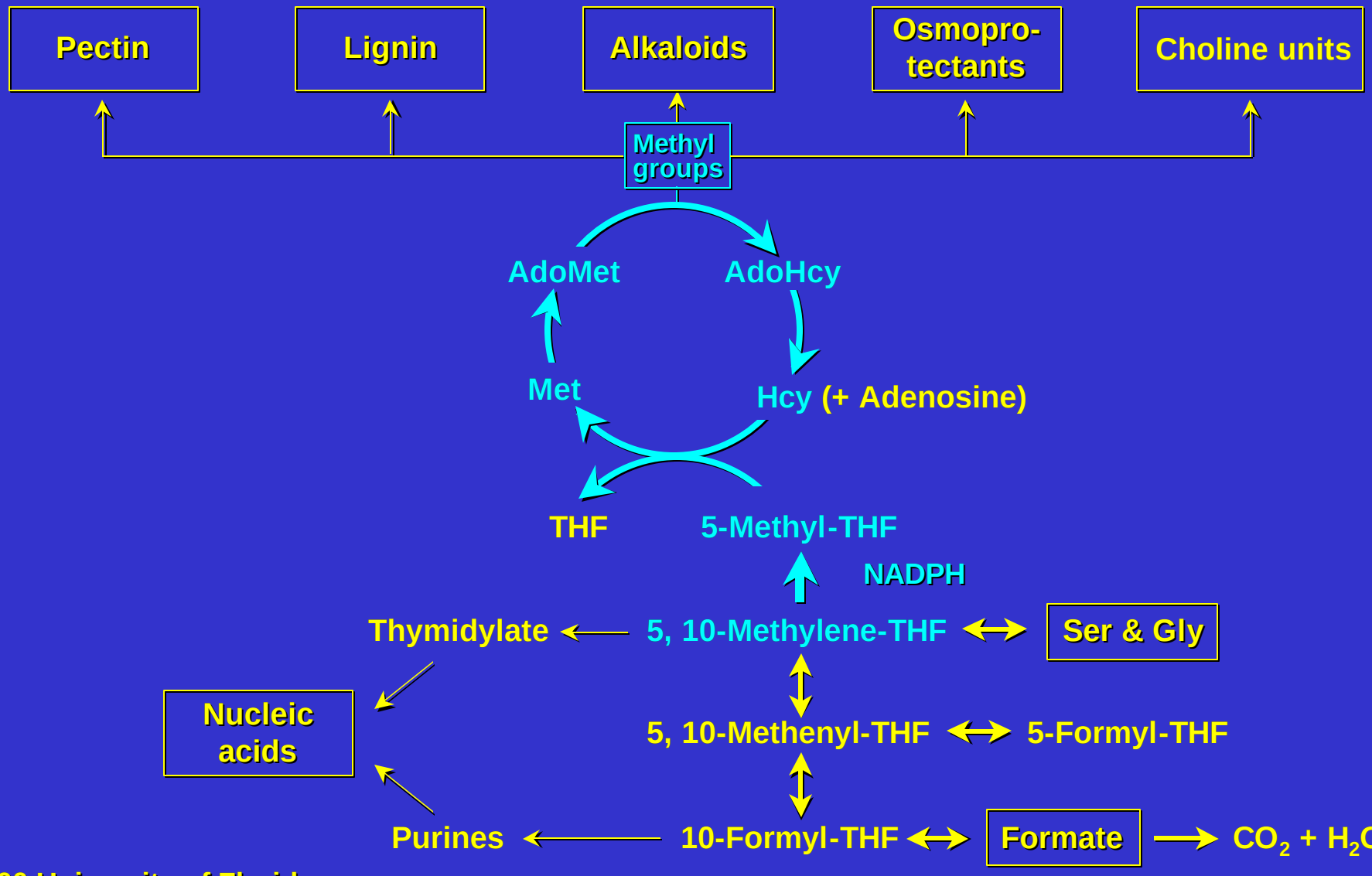
© 2000 University of Florida

Methylene-THF Reductases (»600 residues)



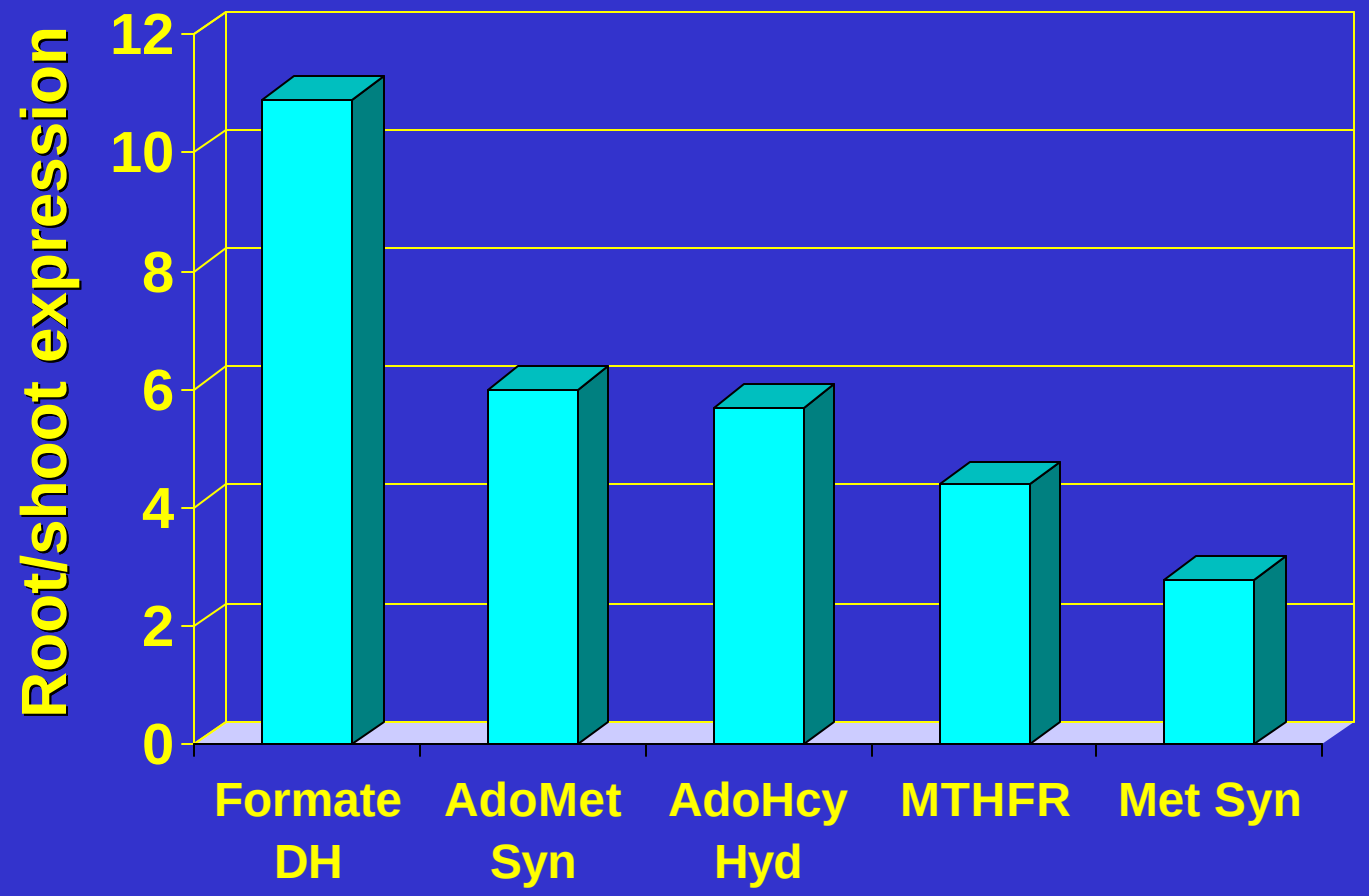
© 2000 University of Florida

Plant C_1 Metabolism



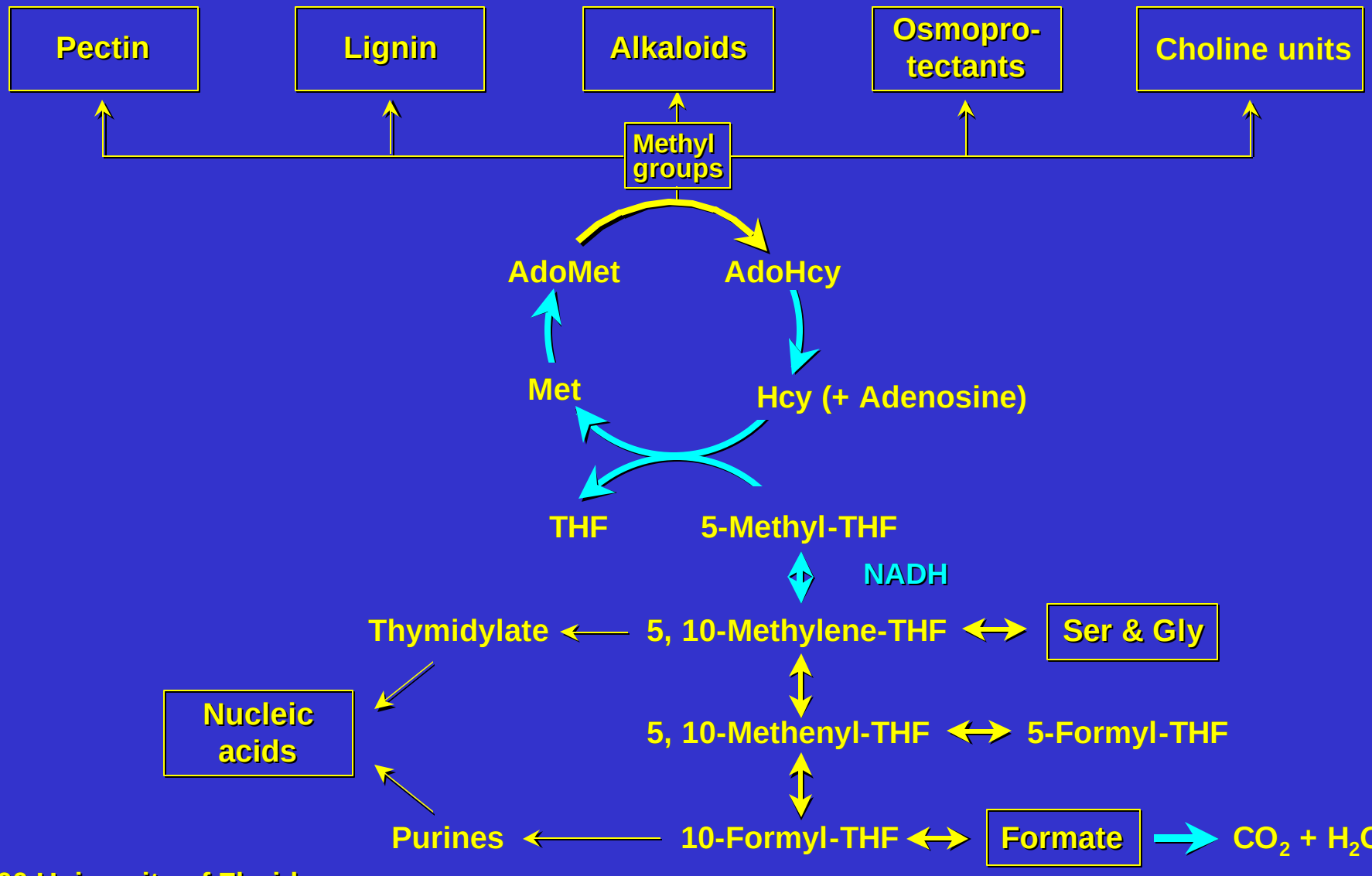
© 2000 University of Florida

First maize DNA array data



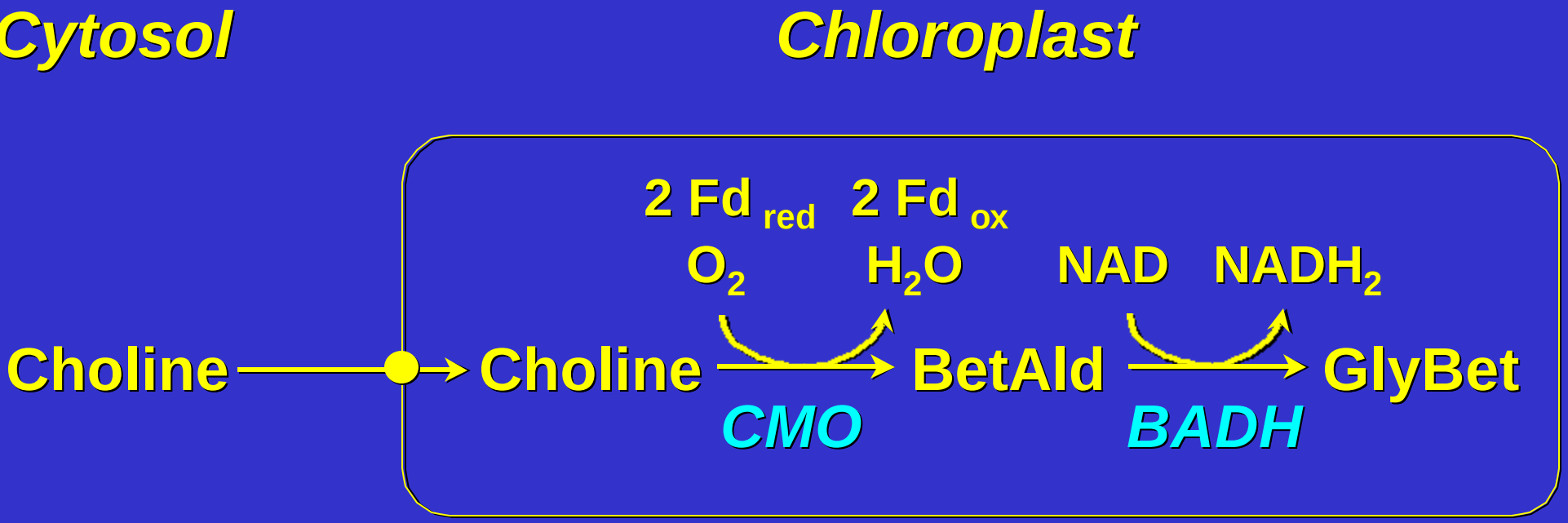
© 2000 University of Florida

Plant C_1 Metabolism



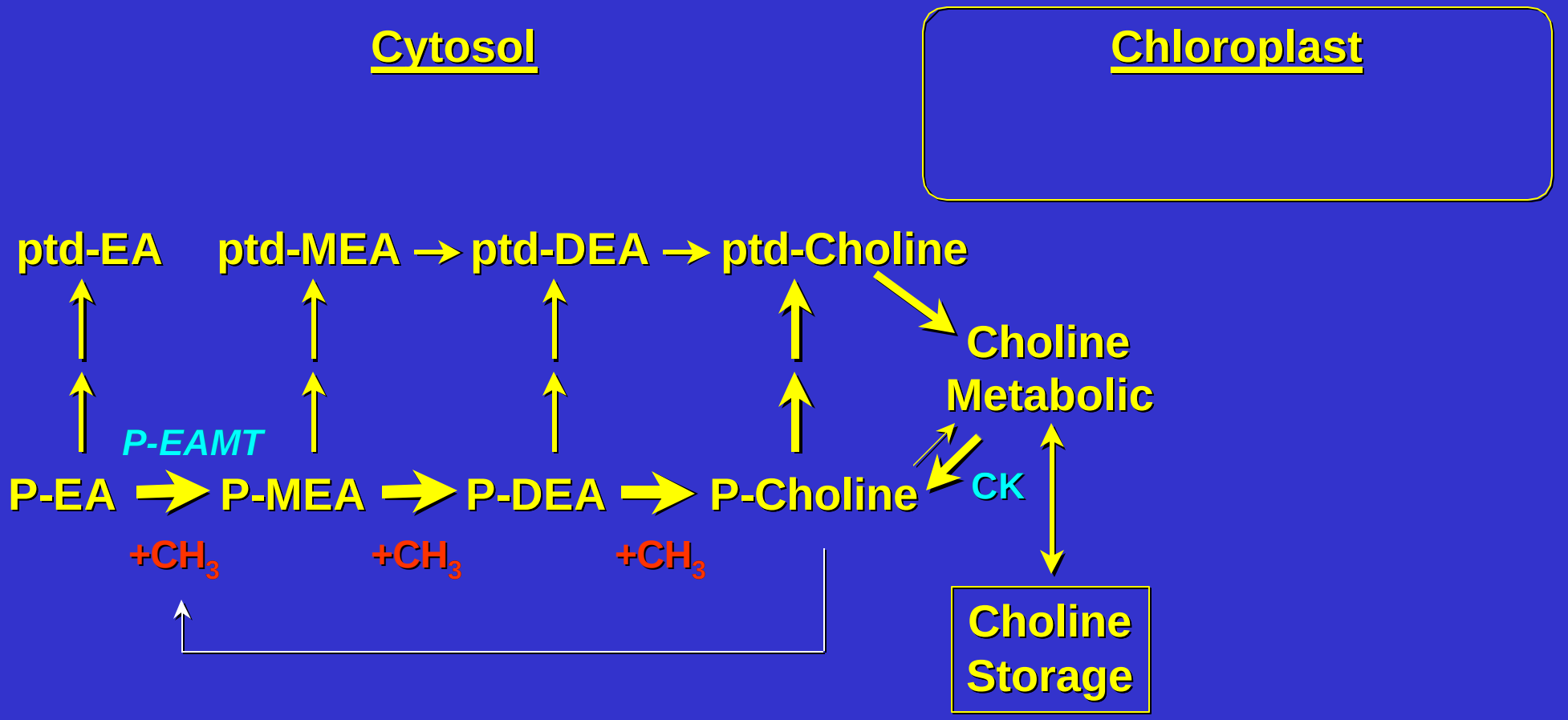
© 2000 University of Florida

Glycine betaine synthesis



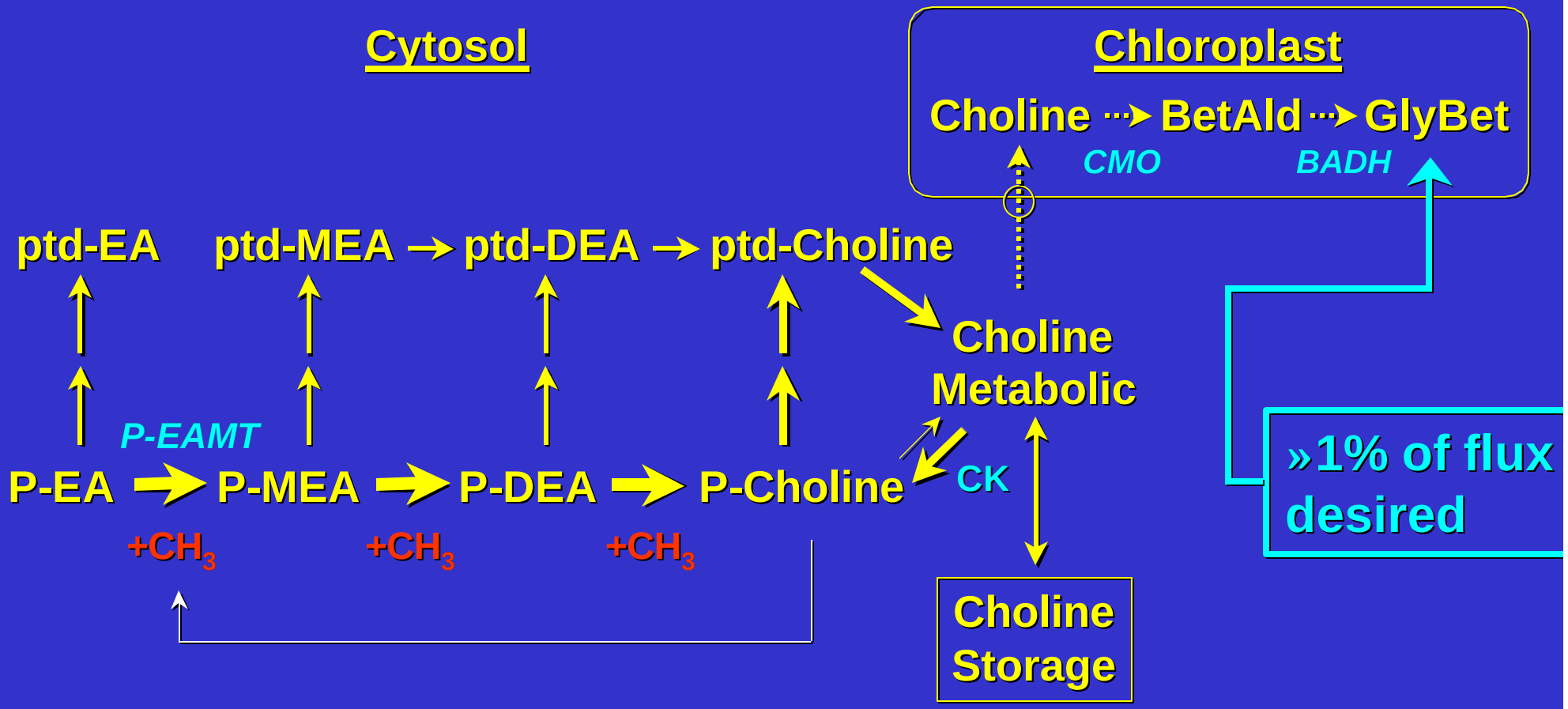
© 2000 University of Florida

Engineering glycine betaine synthesis
Wild -type tobacco



© 2000 University of Florida

Engineering glycine betaine synthesis
Transgenic tobacco

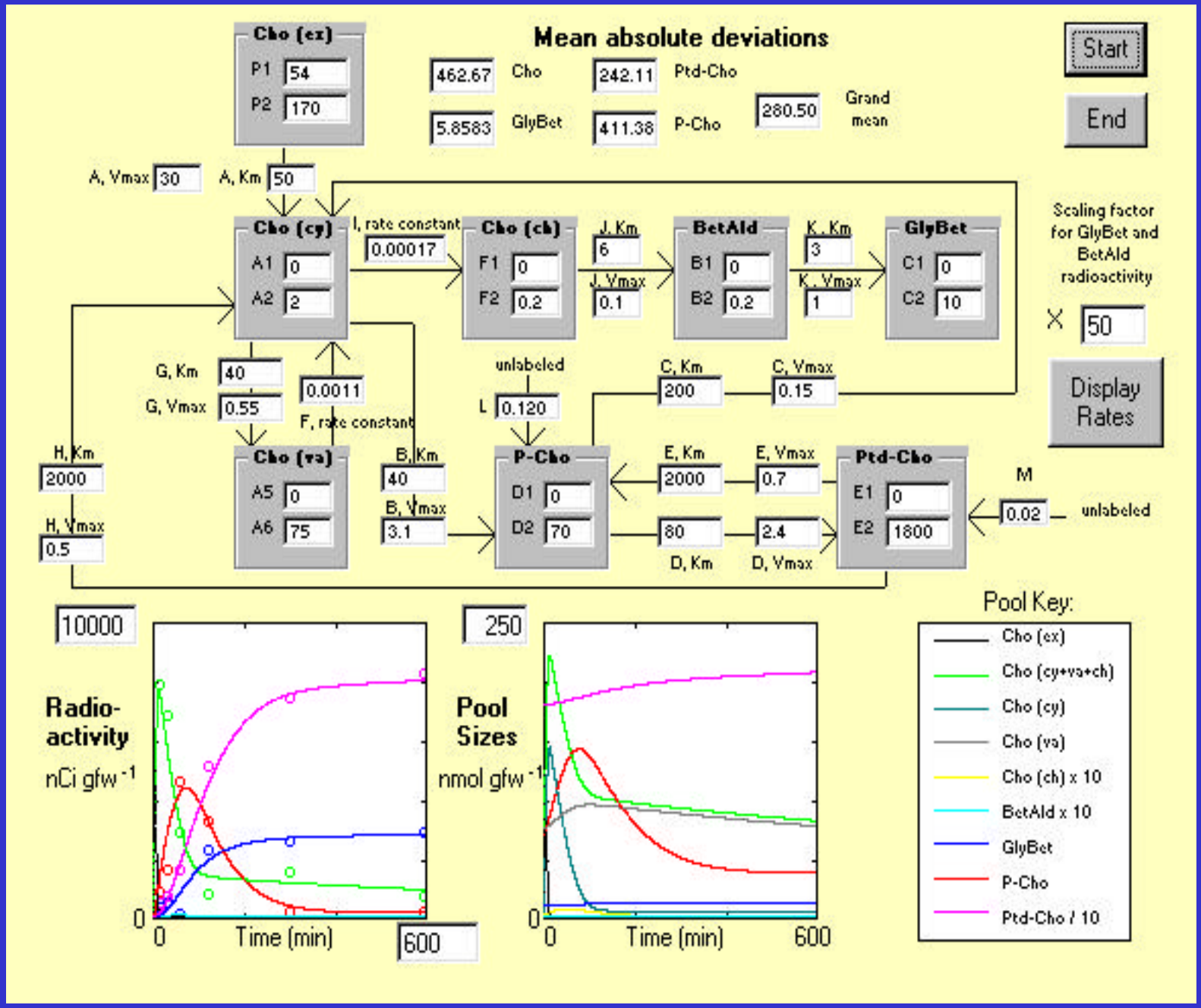


© 2000 University of Florida

Metabolic Models & Engineering

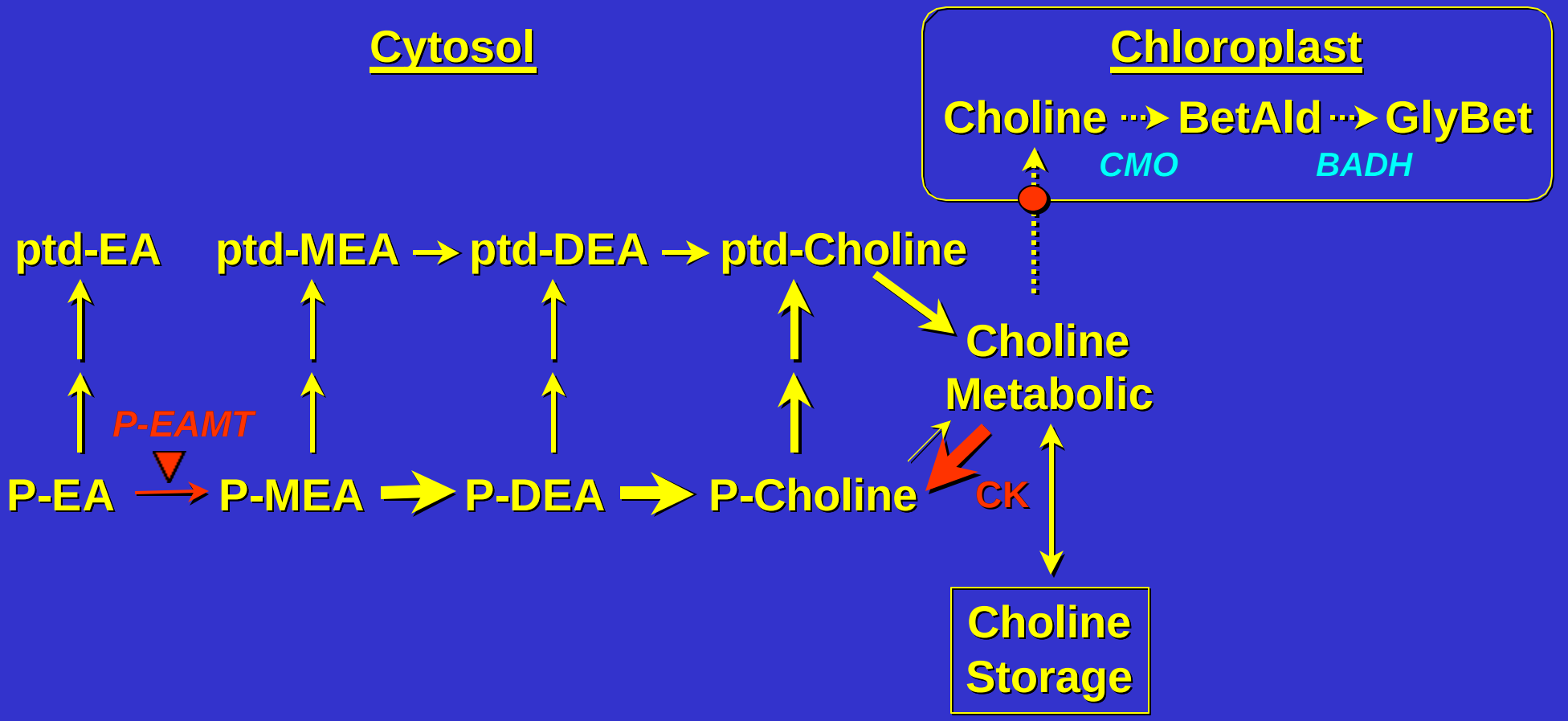
- David Rhodes modeling website
  - http://www.hort.purdue.edu/CFPESP/models/models.htm
- First lay out model network of reactions
- Isotope tracer (radiolabel, MS, NMR) and pool size measurements
- Fit model predictions to data by varying model parameters (fluxes, pool sizes)

© 2000 University of Florida



© 2000 University of Florida

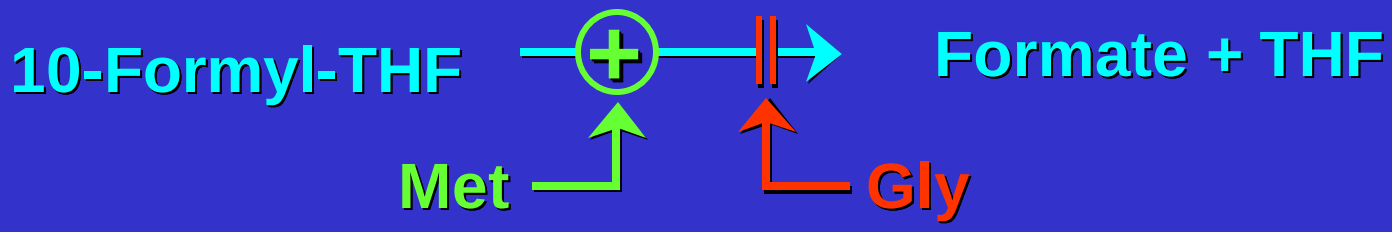
Engineering glycine betaine synthesis
Transgenic tobacco



© 2000 University of Florida

Genomics & pathway discovery - PurU

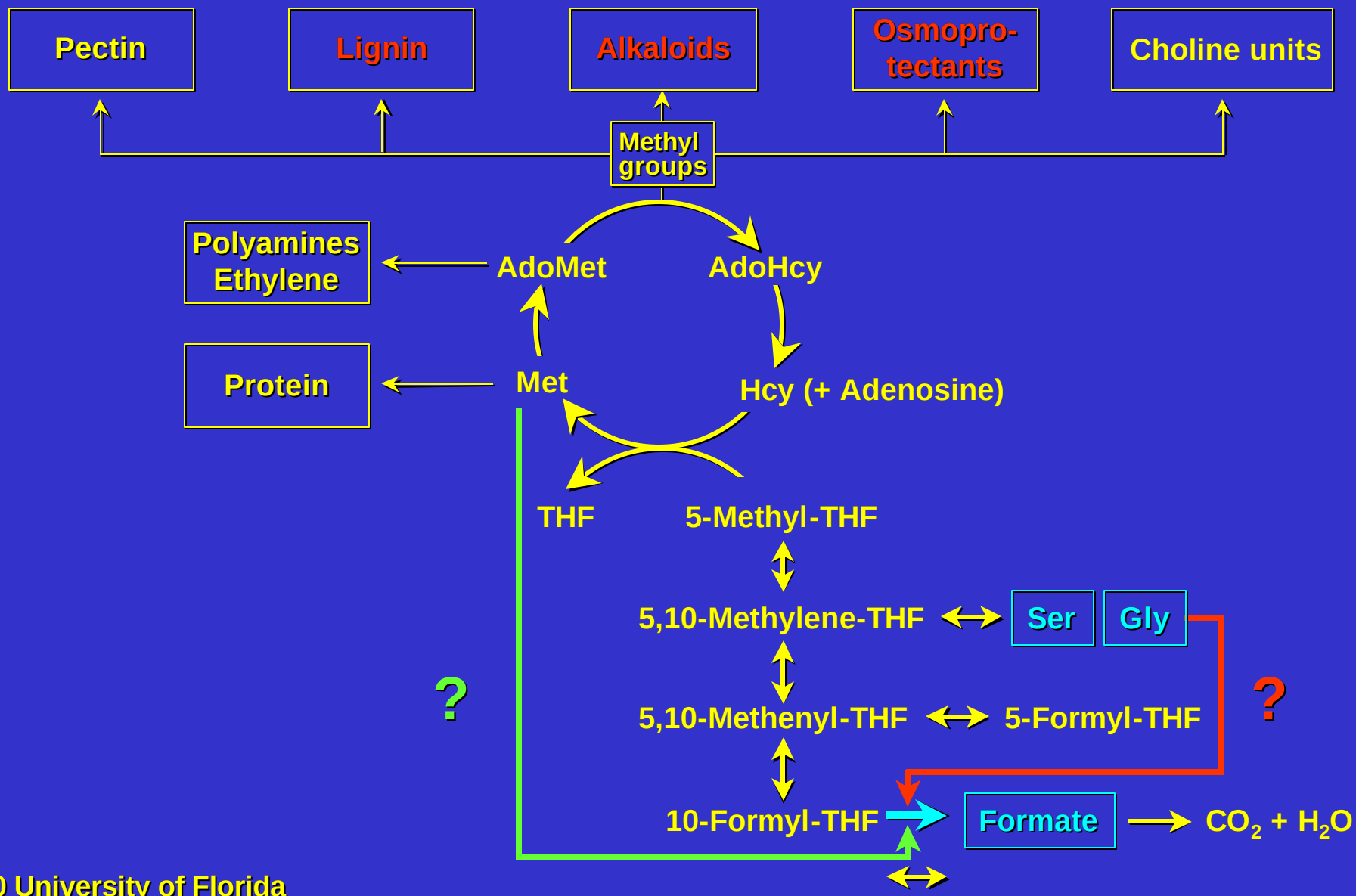
- *E. coli* PurU = 10-Formyl-THF hydrolase



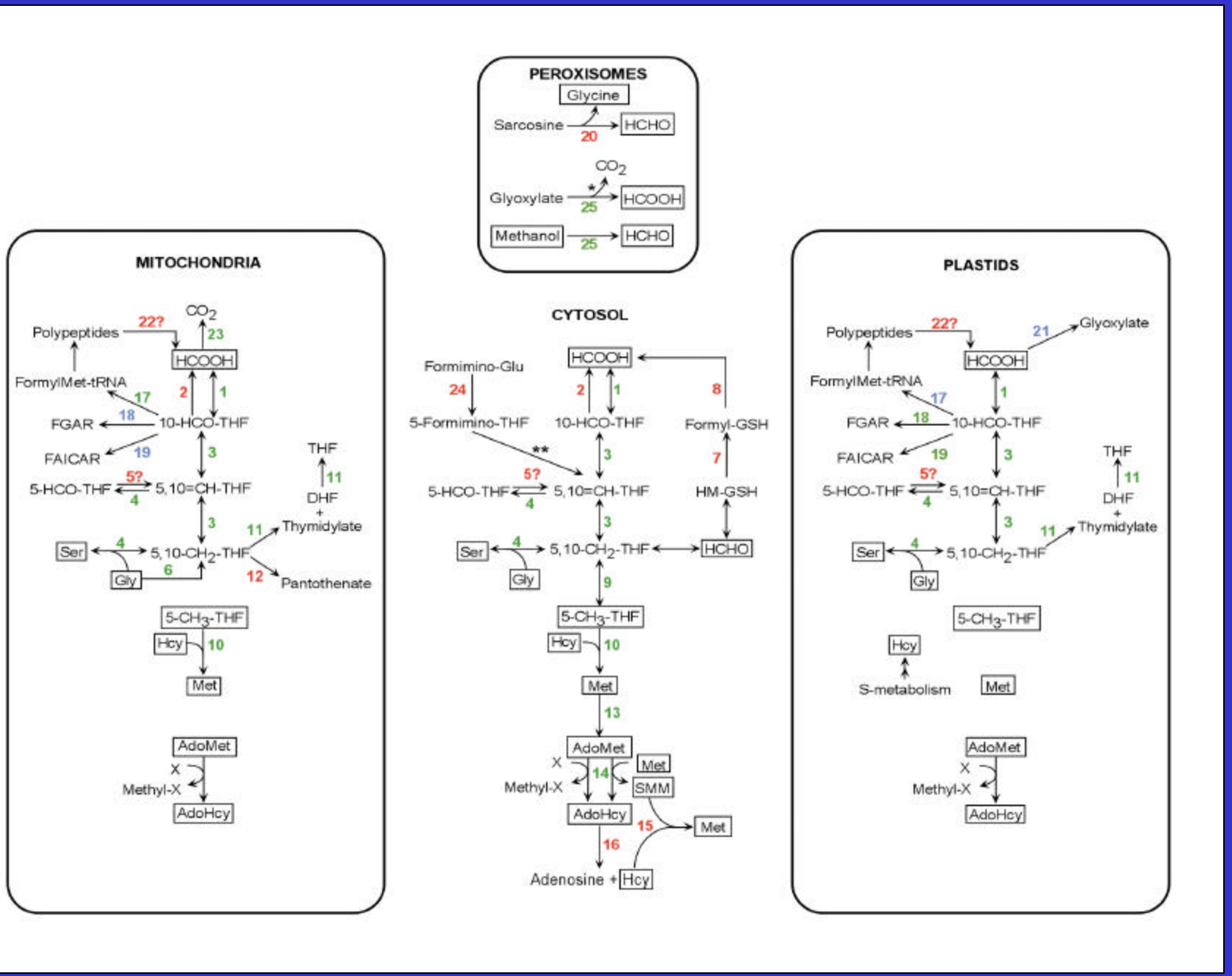
- Present in bacteria, not yeast or mammals
- Plants have ³ 2 PurU homolog genes & ESTs
- PurU homologs encode 10-formyl-THF hydrolase
- Plant enzymes appear mitochondrial (as is FDH)

© 2000 University of Florida

An unexpected source of formate



© 2000 University of Florida



© 2000 University of Florida

Outputs - Publications

- *J Biol Chem* - MTHFR cloning & characterization
- *TIPS* - Invited C_1 metab & engineering review (genomics)
- *J Biol Chem* - MS approach to S-methylMet cycle*
- *Plant Physiol* - Modeling of choline metabolism*
- *Plant Physiol* - Engineering/modeling GlyBet synthesis*
- *Metab Engin* - Engineering/modeling GlyBet synthesis*
- *Plant Cell* - Invited metabolic modeling review
- *Annu Rev Plant Physiol Plant Mol Biol* - Invited C_1 review

* MS or modeling supported by C_1 grant

© 2000 University of Florida

Outputs - Workshops, Websites

- Zia Symposium III - January 2000, NMSU

  Hands-on computer sessions, 60 participants

- ASPP Annual Meeting - July 2000, San Diego

  Minisymposium on models in metabolic research & eng

  Hands-on computer sessions, 60 participants

- David Rhodes metabolic modeling website

  http://www.hort.purdue.edu/CFPESP/models/models.htm

- C_1 Project website (data, materials, protocols)

  http://www.hos.ufl.edu/meteng/1Cpage1.html

© 2000 University of Florida

Funding Agencies

PI	Agency	Award dates
Hanson	NSF-IBN	Feb 99 - Jan 02
Bohnert	NSF-IBN	Feb 99 - Jan 02
Gage	NSF-BES	Feb 99 - Jan 02
Rhodes	DOE-BES	Sep 99 - Aug 02
Shachar-Hill	NIST	May 00 - Apr 03

© 2000 University of Florida