Engineering Plant C_1 Metabolism

- **Personnel** - 5 PIs & labs, funding
- **Background** - overview of plant C_1 metabolism
- **Objectives** - review of project aims
- **Progress** - methylene-THF reductase
  - genomics/pathway disc./compartmentation
  - development of transgenics/mutants
  - ME of glycine betaine (GB) synthesis in tobacco; labeling, metabolic modeling
  - comparison of wildtype & GB-deficient maize; labeling, C_1 DNA microarrays
- **Project outputs** - publications, workshops, websites

Plant C_1 Metabolism Group

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

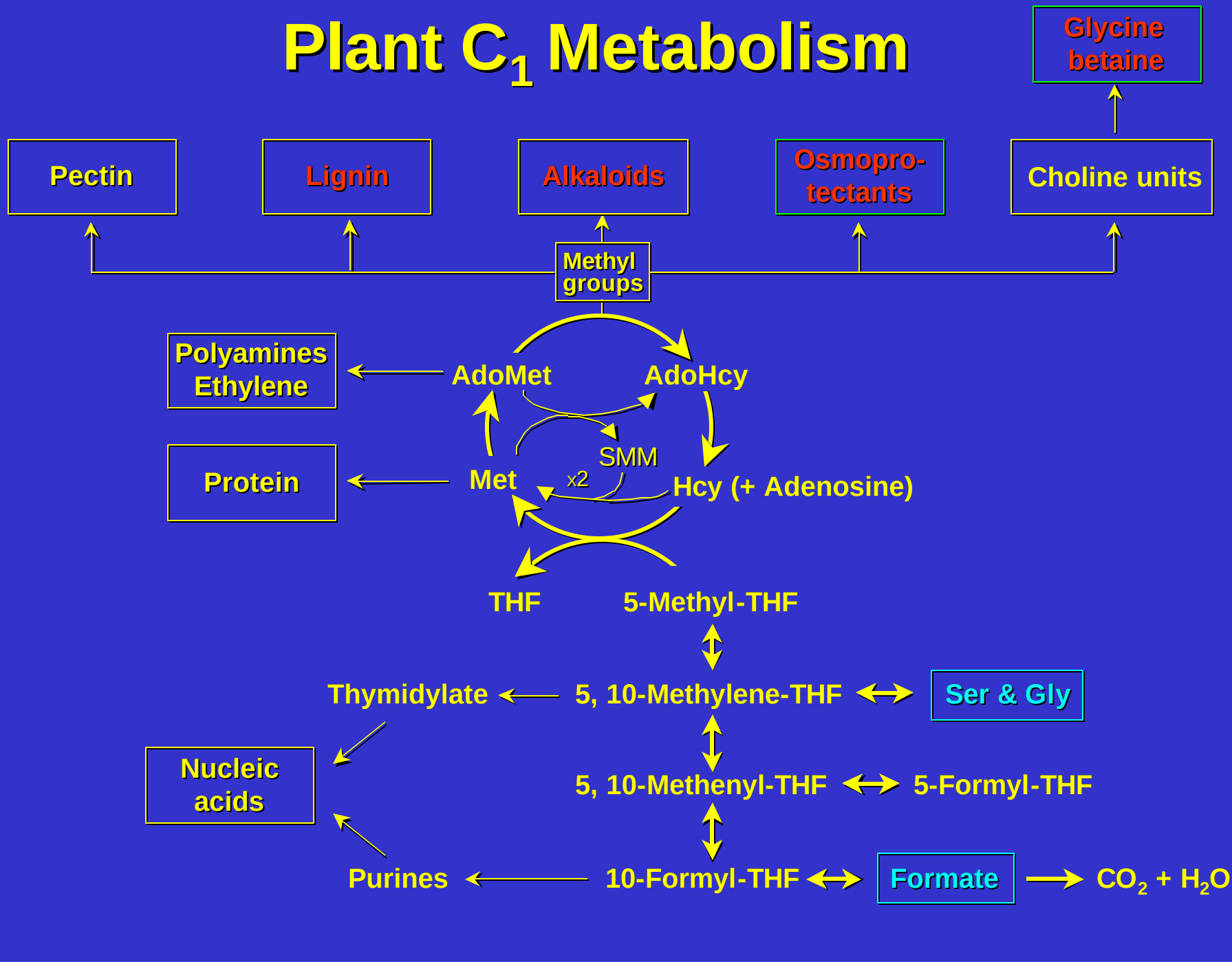
Funding Agencies

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 01

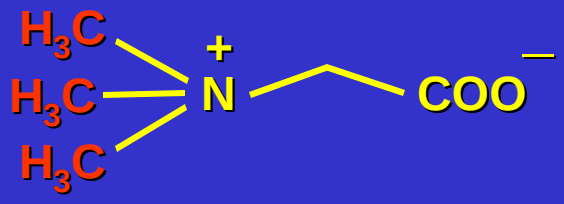
Background on Plant C_1 Metabolism

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

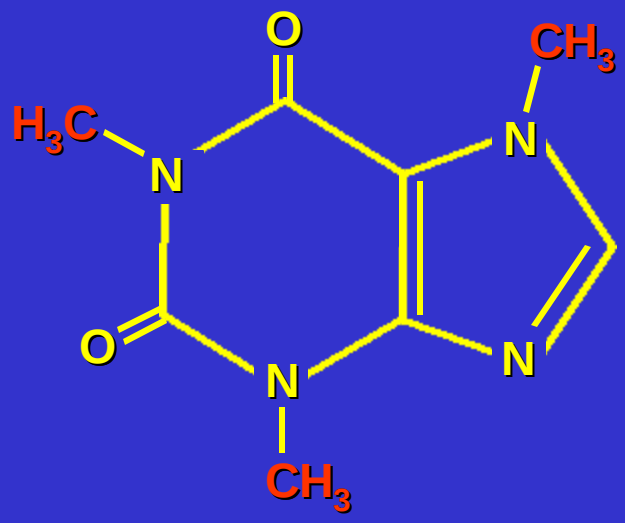
Plant C_1 Metabolism



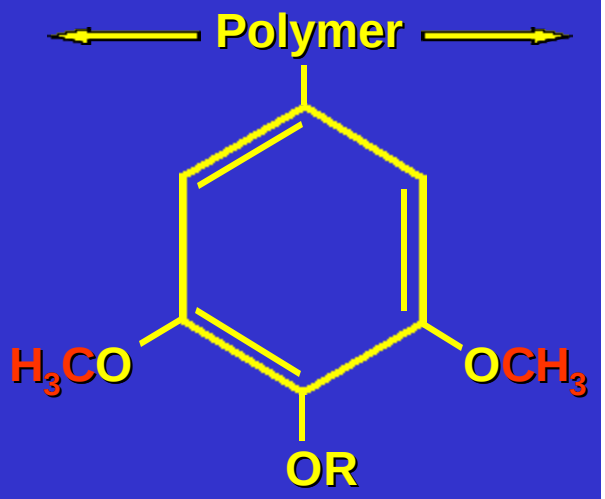
High-Methyl Engineered Products



Glycine betaine
(osmoprotectant)

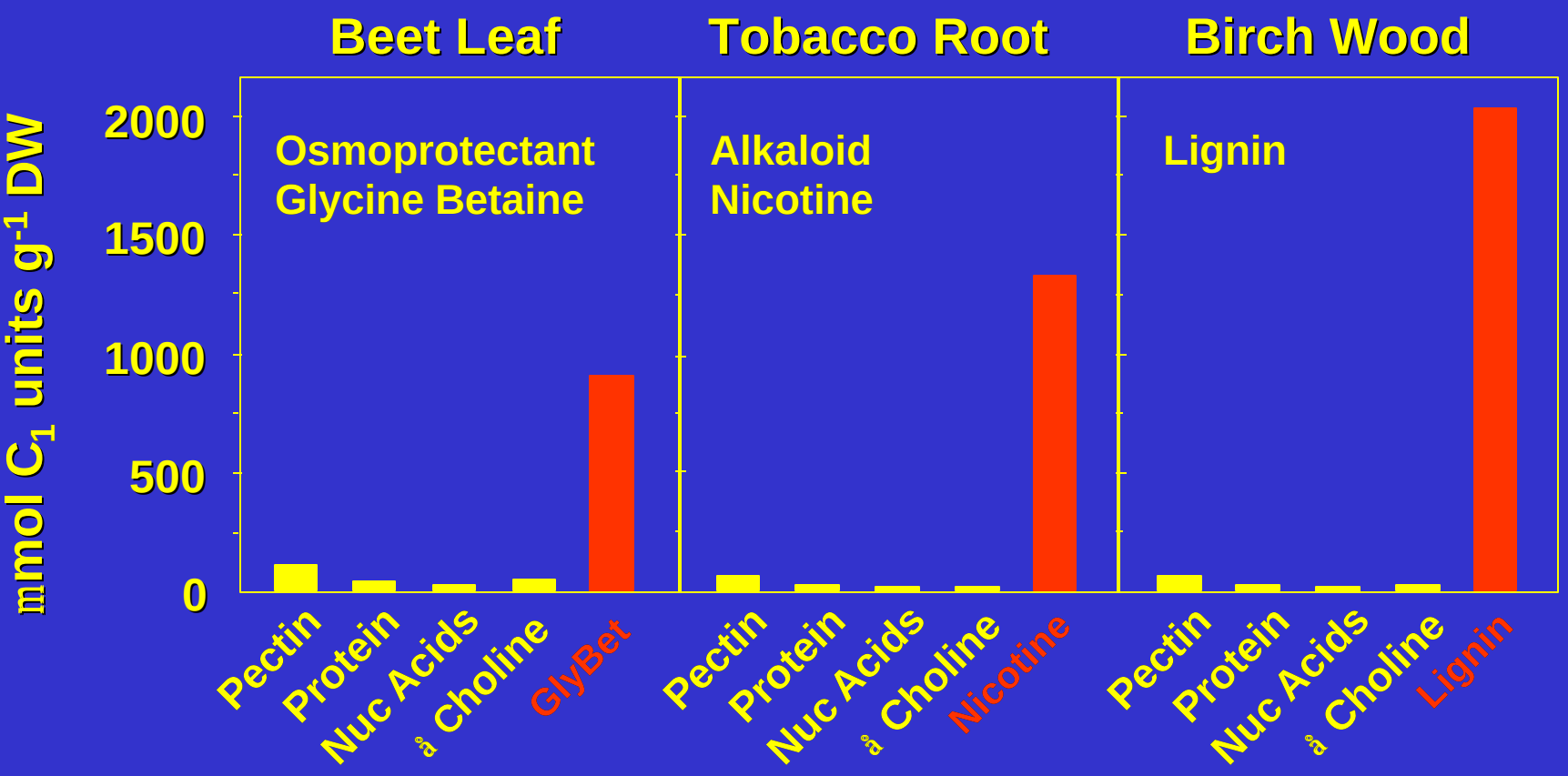


Caffeine
(alkaloid)



Syringyl lignin

The scale of C_1 demands in plants



Engineering Questions

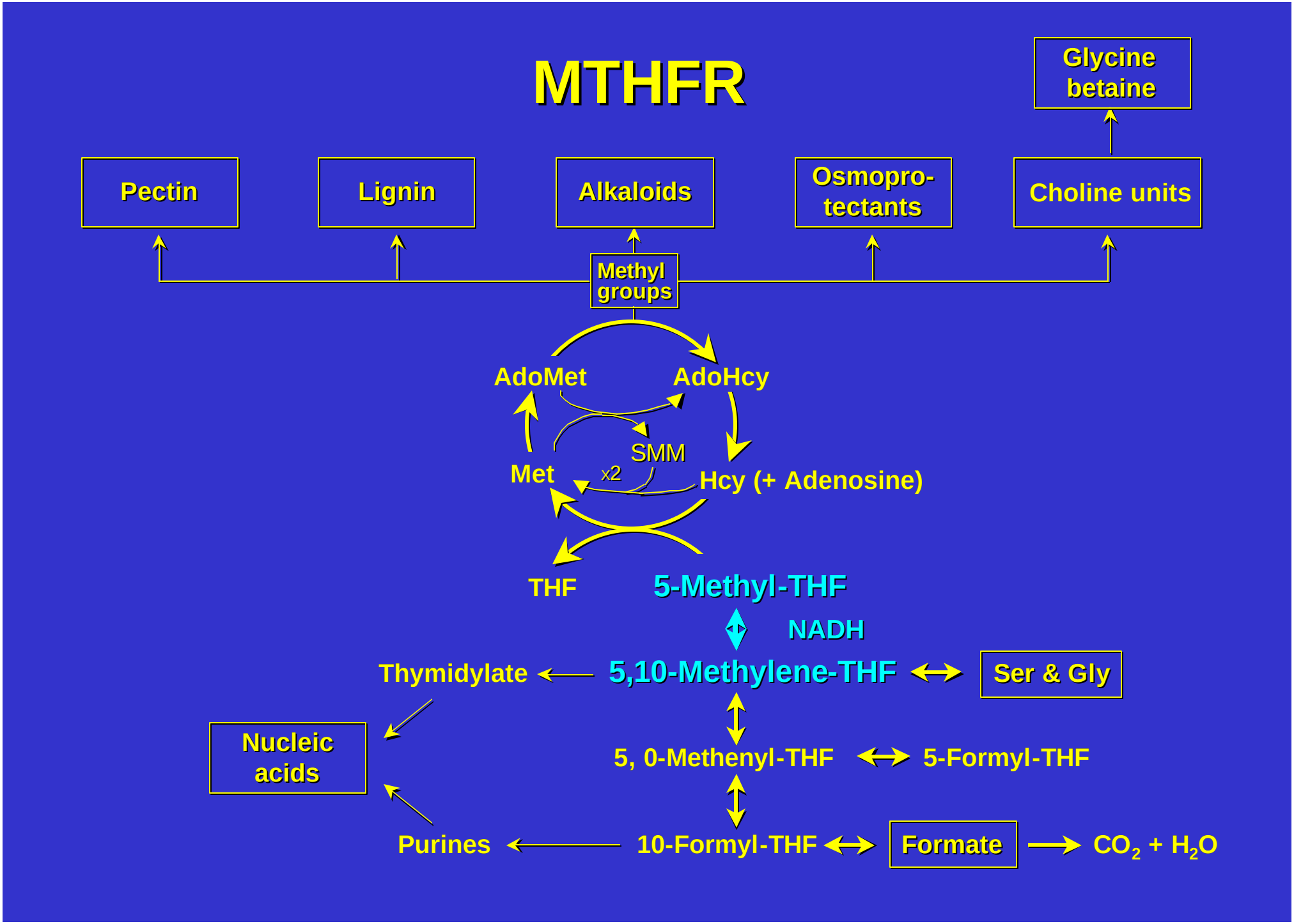
- System response to C_1 demand - or $\bar{}$?
  - Metabolic flux
  - Gene Expression
- Enzymes exerting major flux control ?
- Relative importance of C_1 sources ?
- More pathways ? Compartmentation ?

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, MFA/ modeling) & gene expression (DNA arrays)

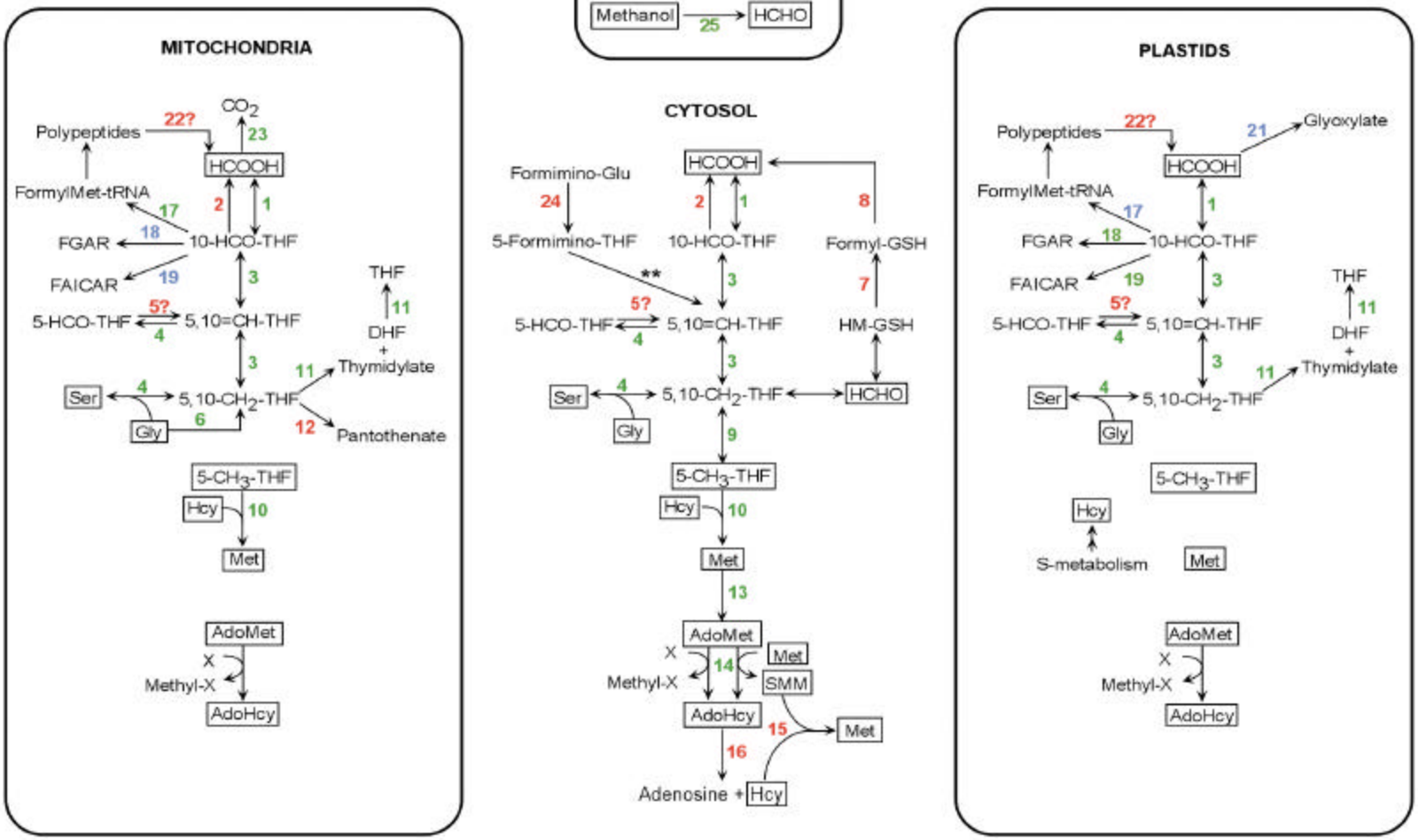
Progress (Years 1 & 2)

- Methylene-THF reductase
- Genomics & pathway discovery
- Development of transgenics/mutants
- Engineering glycine betaine synthesis; labeling & modeling (tobacco)
- Maize C_1 DNA arrays & labeling in wildtype & glycine betaine-deficient mutant lines

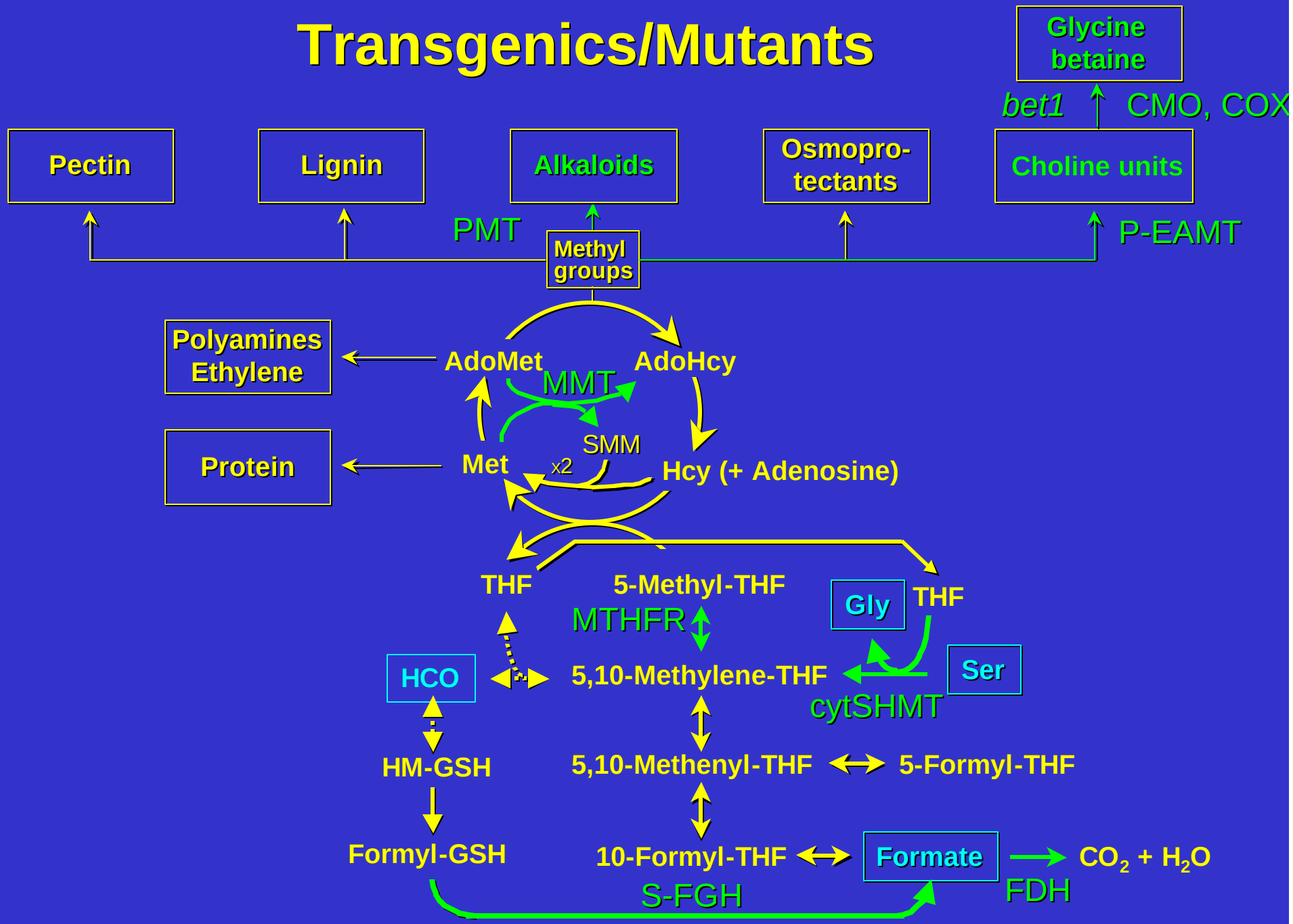

MTHFR
Glycine betaine
Pectin
Lignin
Alkaloids
Osmopro-tectants
Choline units
Methyl groups
AdoMet
AdoHcy
SMM
Met
x2
Hcy (+ Adenosine)
THF
5-Methyl-THF
NADH
Thymidylate
5,10-Methylene-THF
Ser & Gly
Nucleic acids
5, 0-Methenyl-THF
5-Formyl-THF
Purines
10-Formyl-THF
Formate
$CO_2 + H_2O$

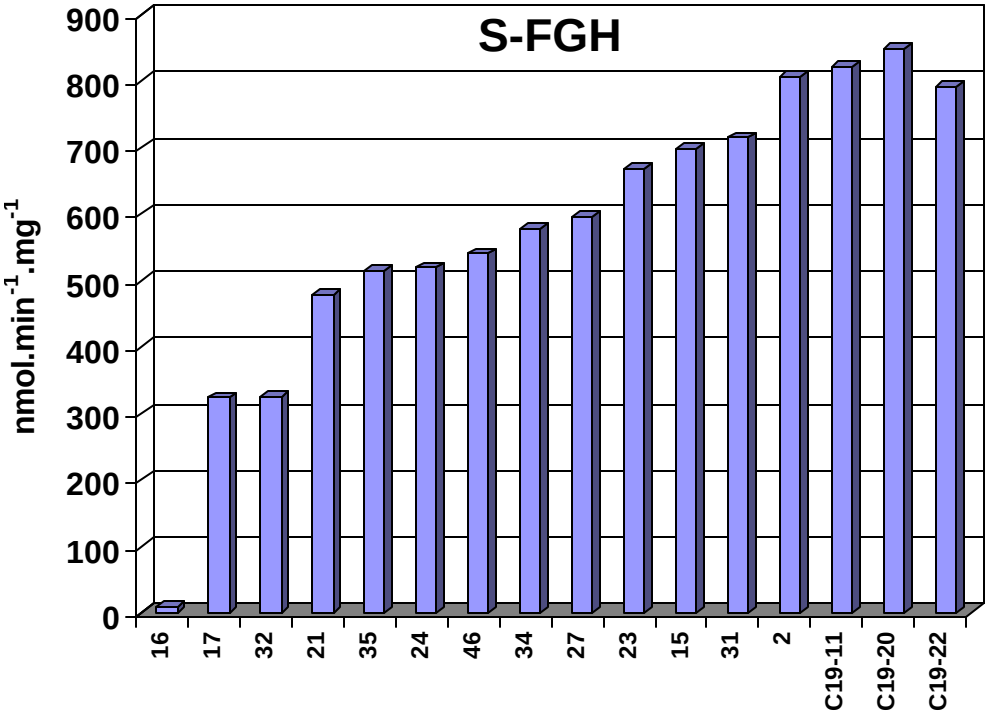
Genomics & pathway discovery; compartmentation

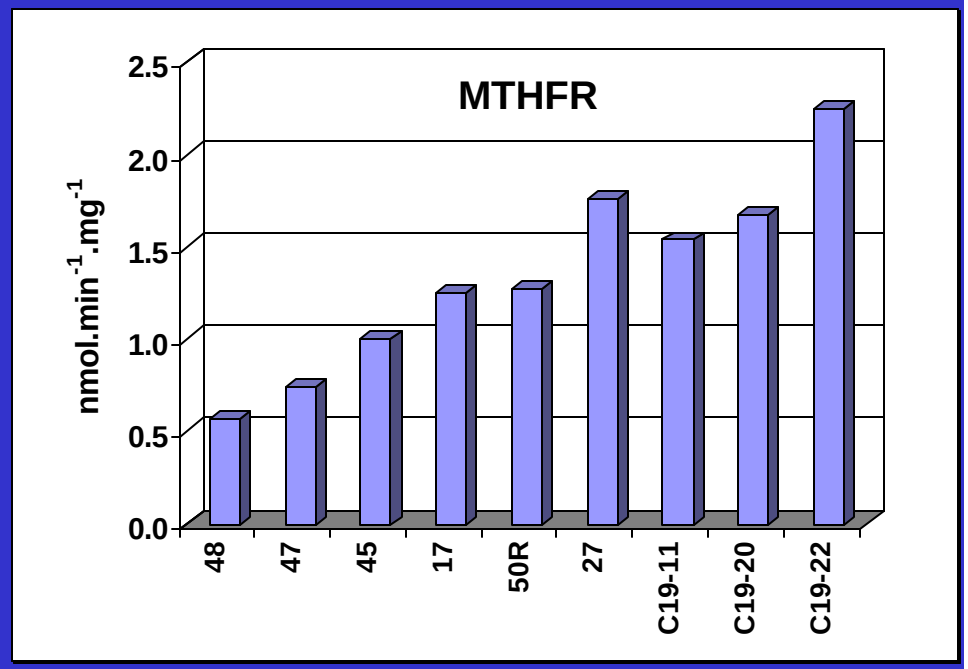
from *TIPS* Review (Hanson, Gage, Shachar-Hill)



Transgenics/Mutants

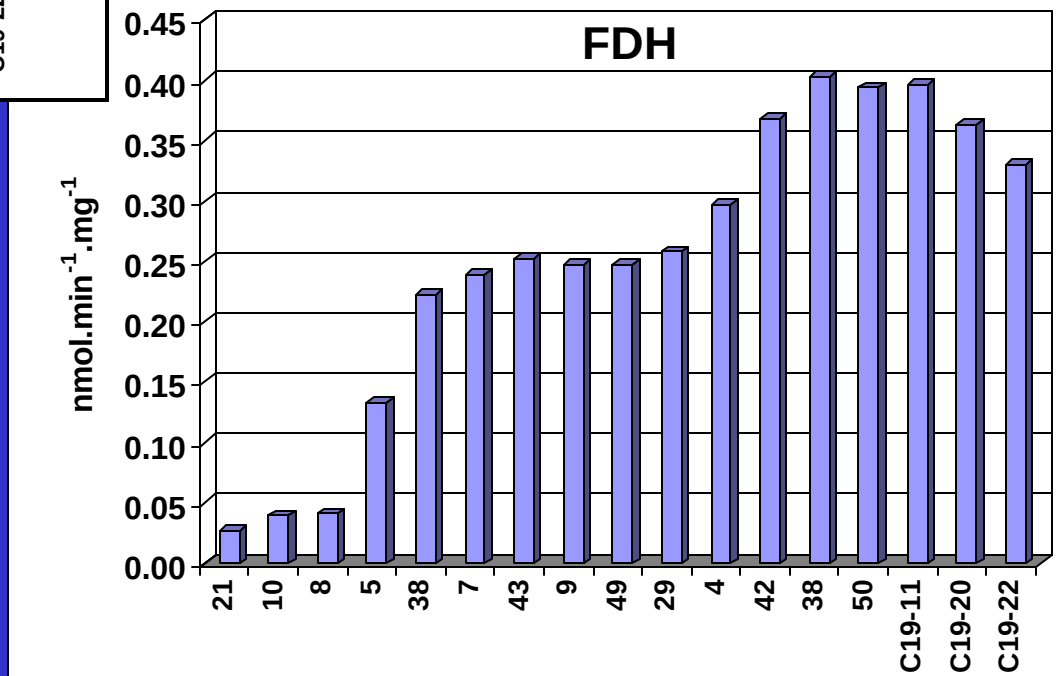




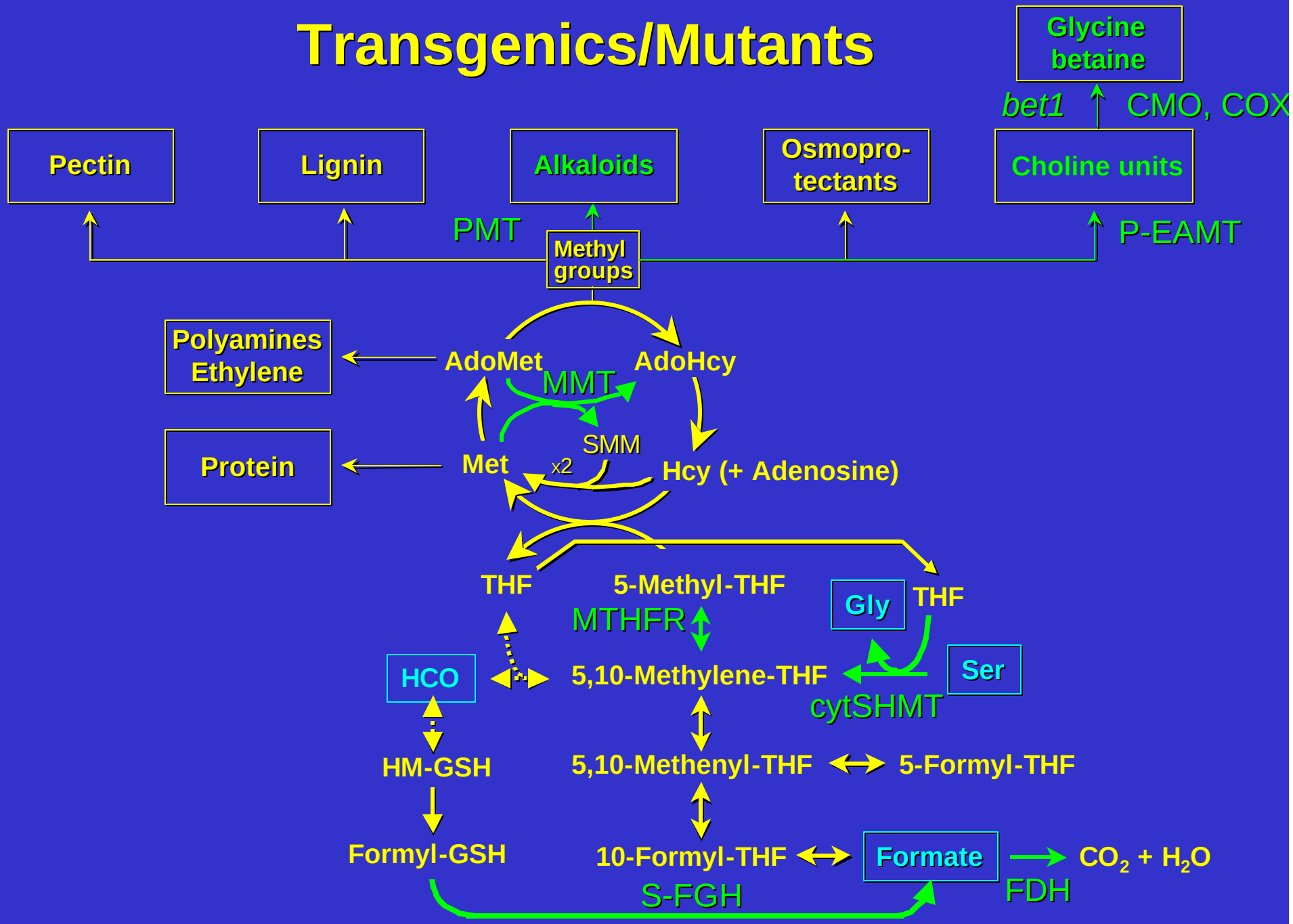


Mean enzyme activities of different tobacco clones expressing antisense FDH, MTHFR or S-FGH

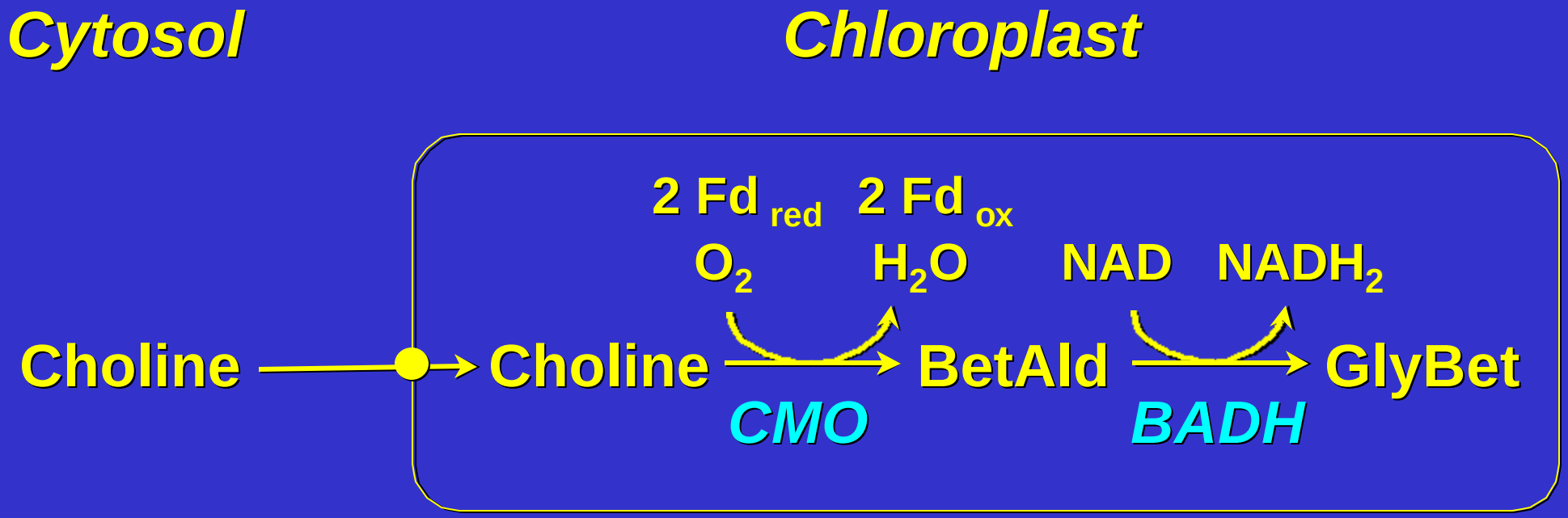
(Sanja Roje & A.D. Hanson, U. FL)



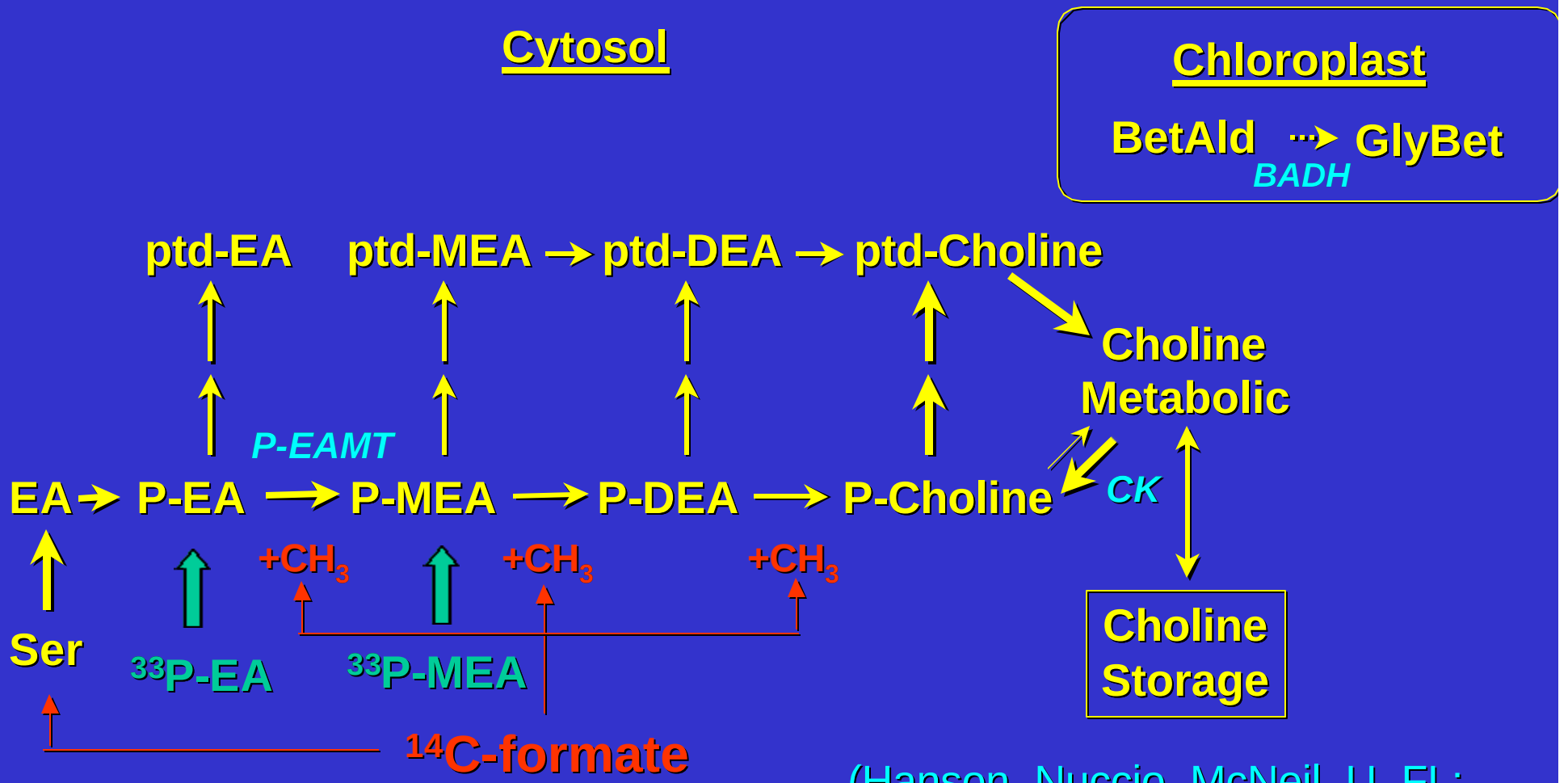
Transgenics/Mutants



Glycine betaine synthesis



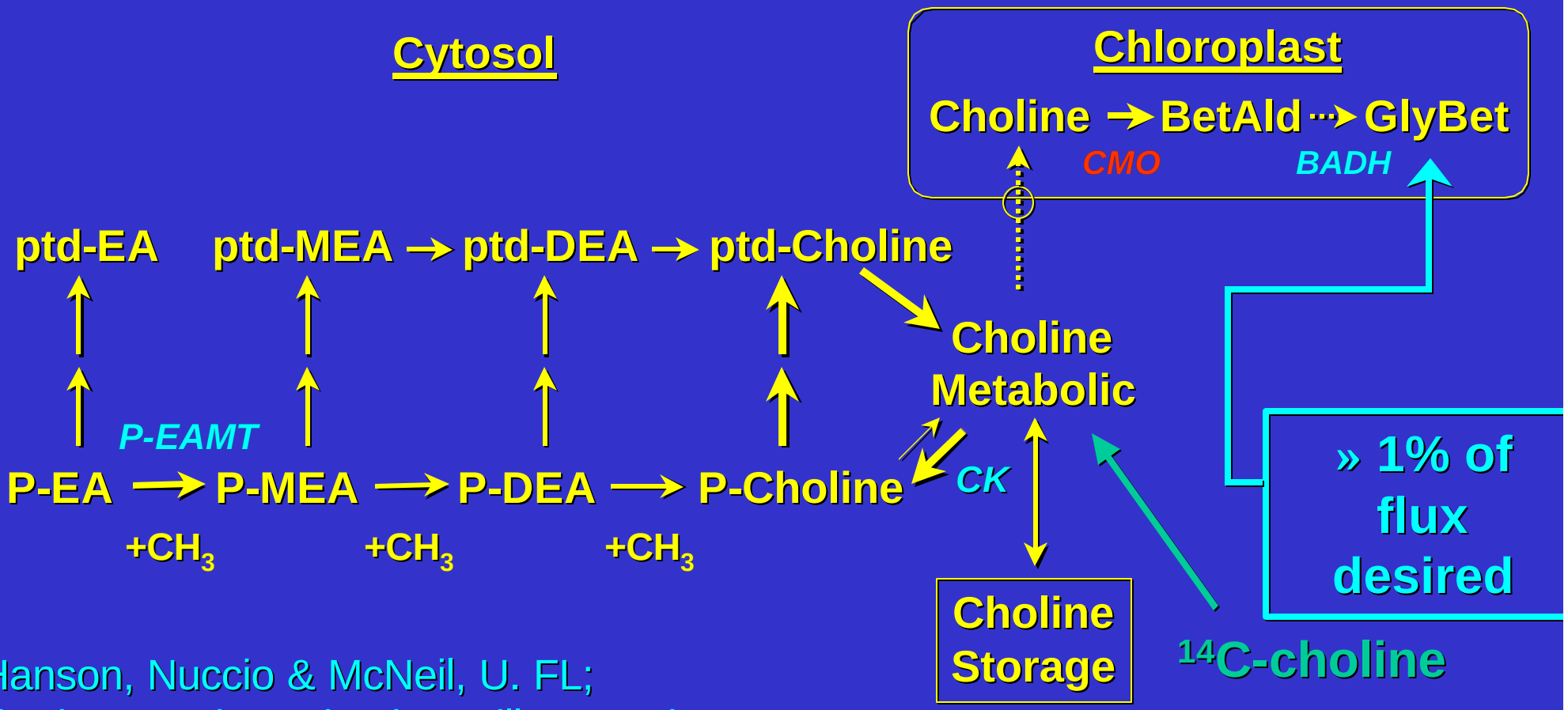
Engineering glycine betaine synthesis
Wild-type tobacco



(Hanson, Nuccio, McNeil, U. FL;
Rhodes, Purdue; Shachar-Hill, NMSU

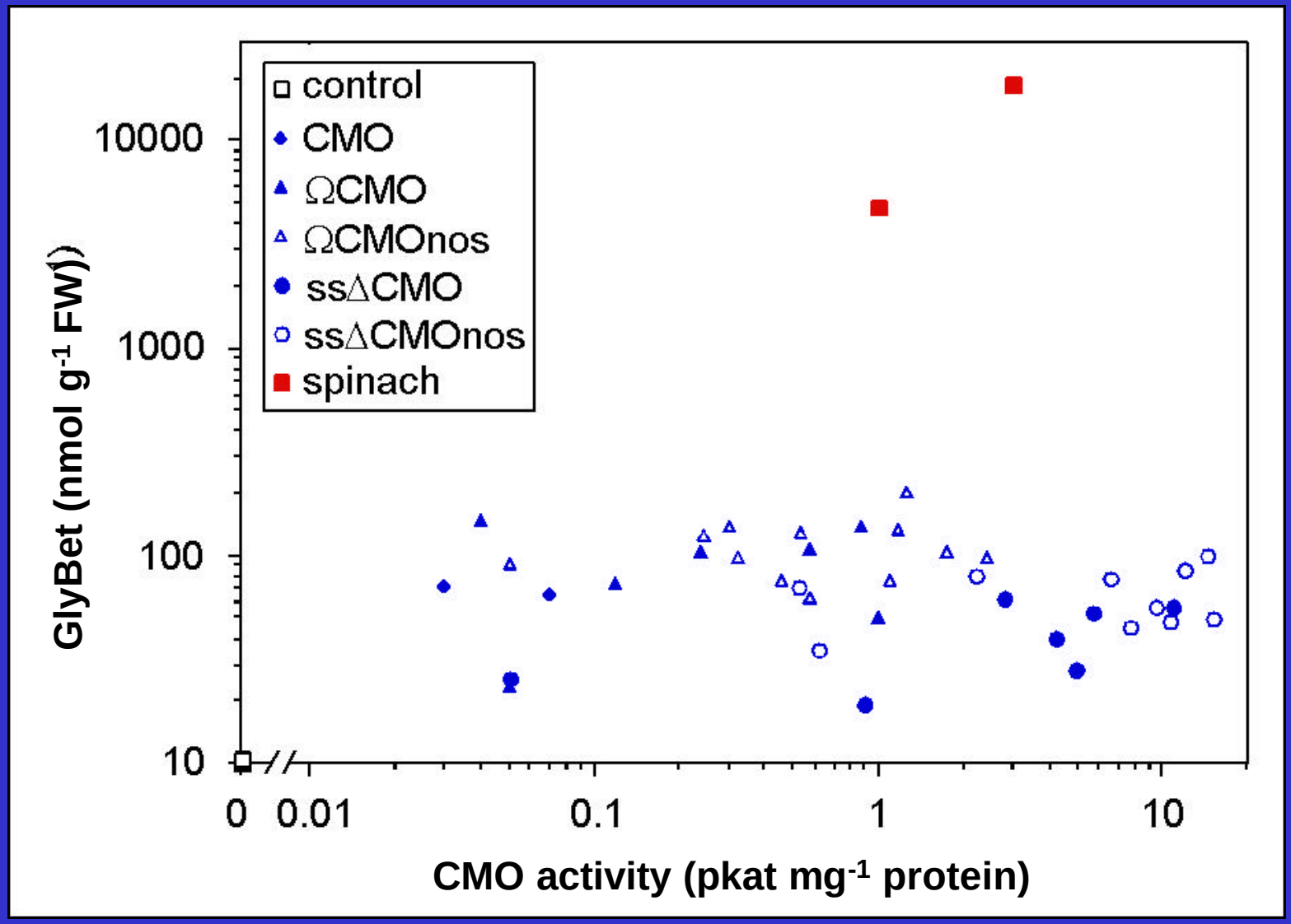
Engineering glycine betaine synthesis
Transgenic tobacco

Chloroplastic expression of CMO



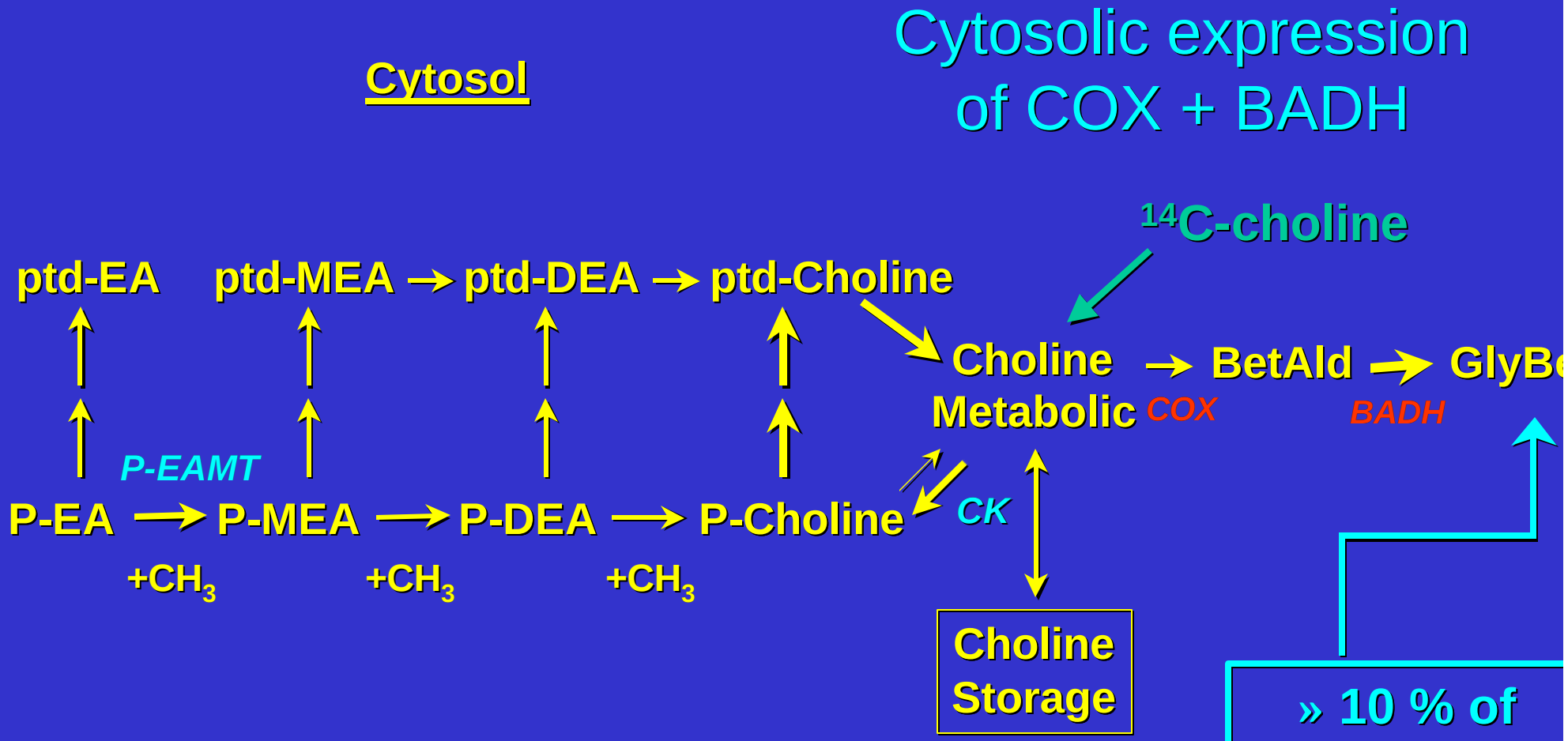
(Hanson, Nuccio & McNeil, U. FL;
Rhodes, Purdue; Shachar-Hill, NMSU)

CMO Expression & [GlyBet] in Tobacco



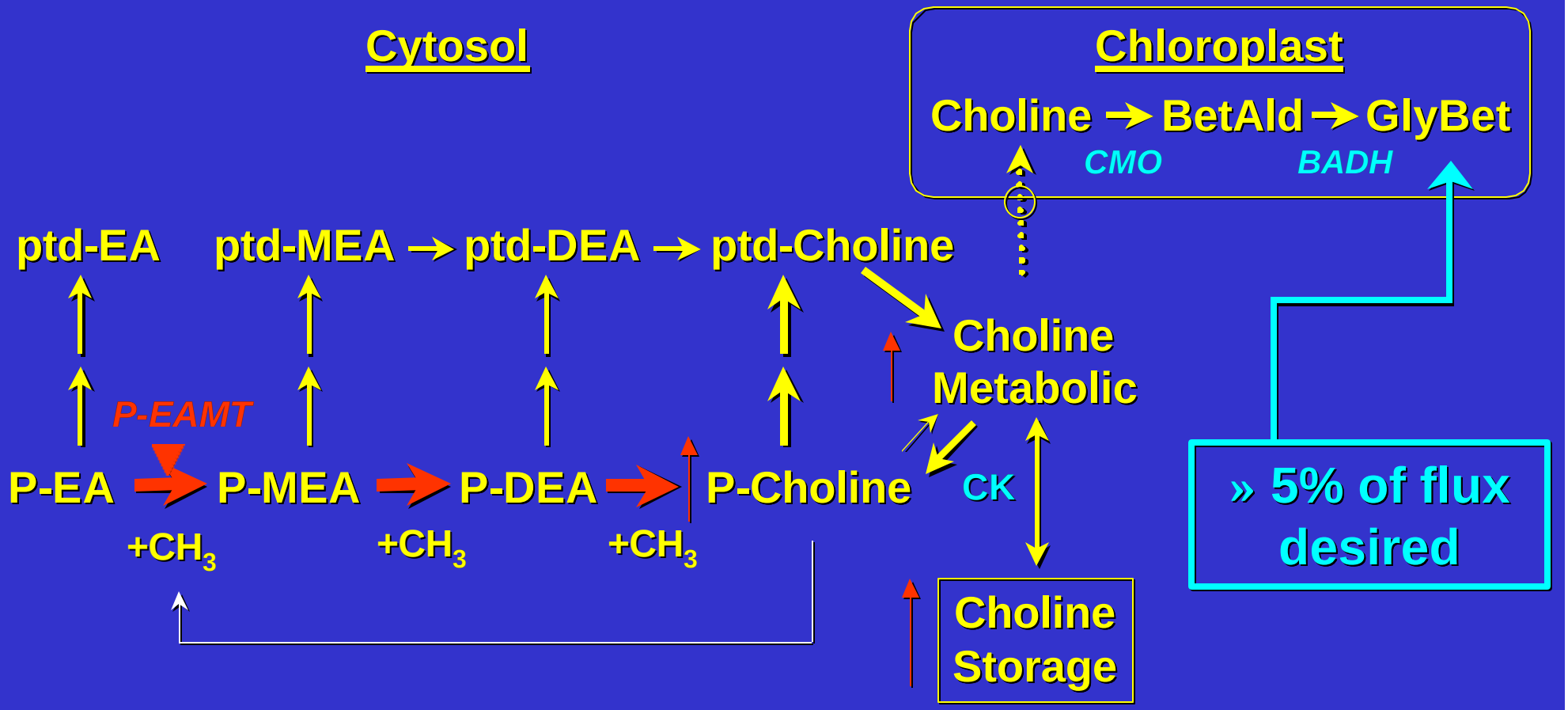
(Hanson, Nuccio & McNeil, U. F

Engineering glycine betaine synthesis
Transgenic tobacco



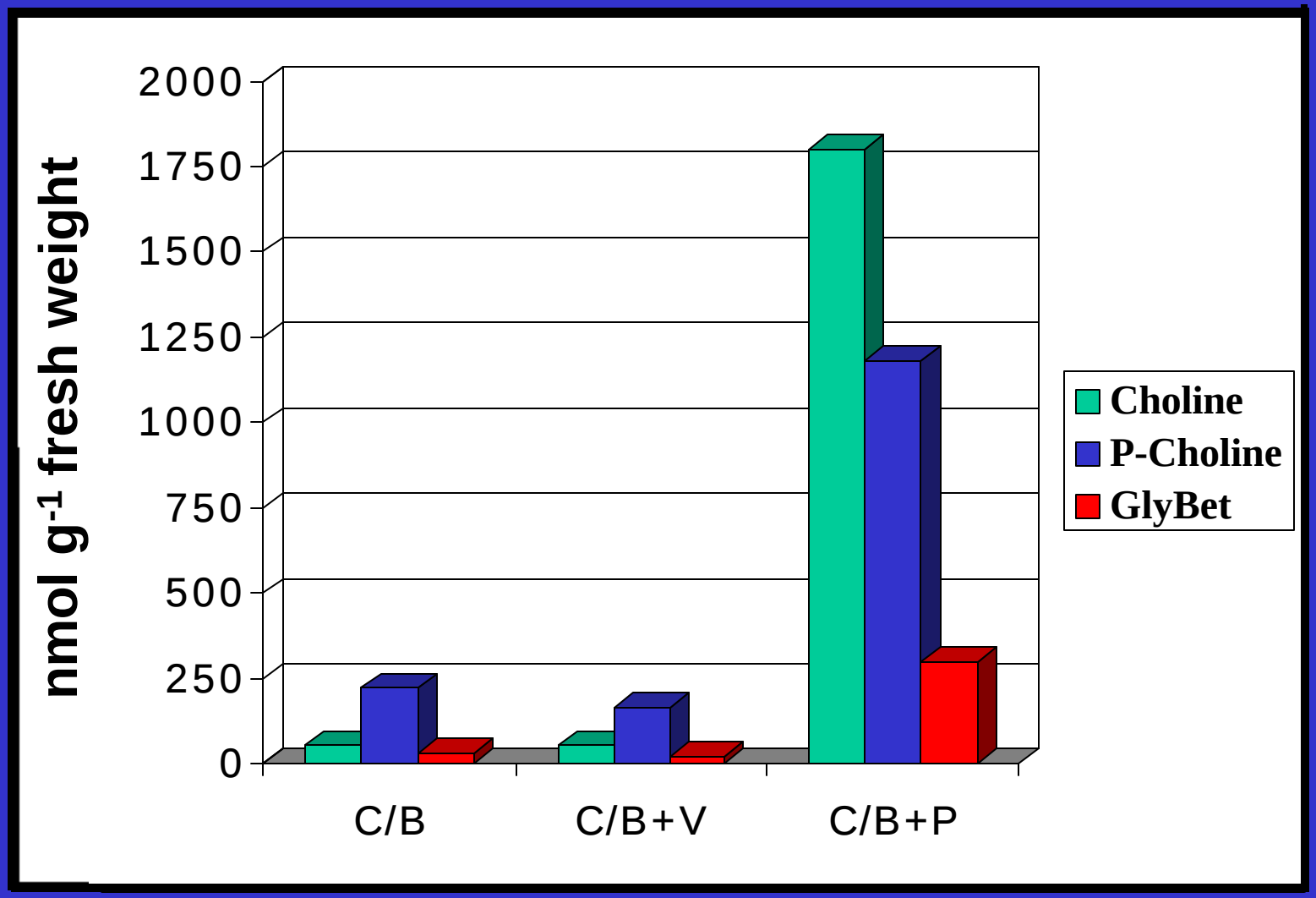
anson, Nuccio & McNeil, U. FL)

Engineering glycine betaine synthesis
Transgenic tobacco - overexpression of P-EAMT



(Hanson, Nuccio& McNeil, U. FL)

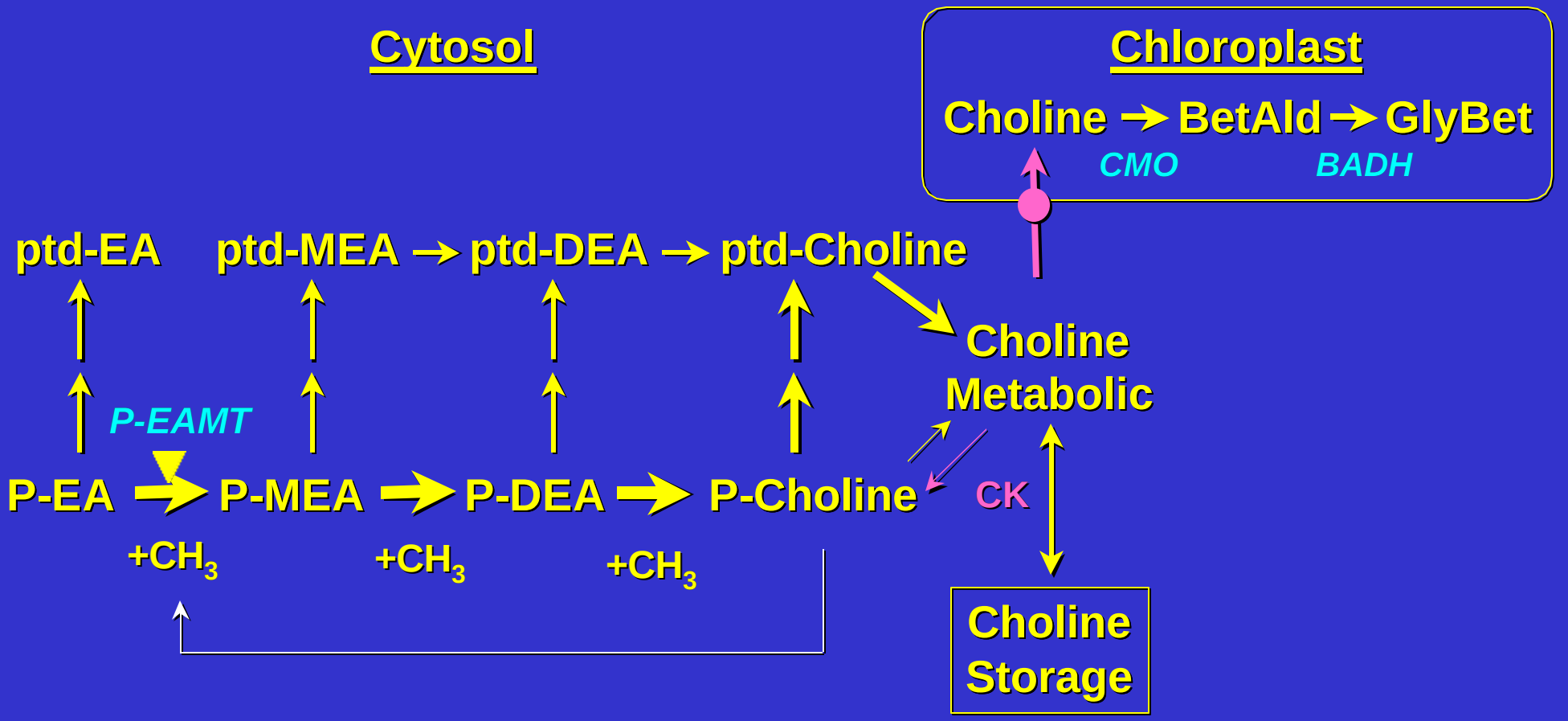
CMO+ BADH+ PEAMT+ Transgenics



(Hanson, Nuccio& McNeil, U. FL

Engineering glycine betaine synthesis

Transgenic tobacco - future targets



Maize betaine genes

Choline

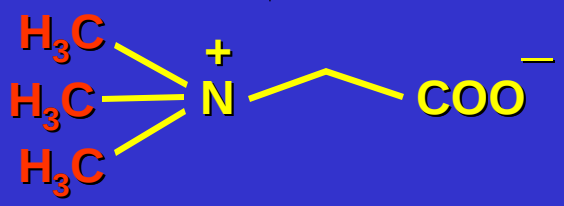
Cho ox. enzyme

bet1/bet1

chr3S

BetAld

BADH



Glycine betaine

M+H$^+$ = 118

Nicotinic acid

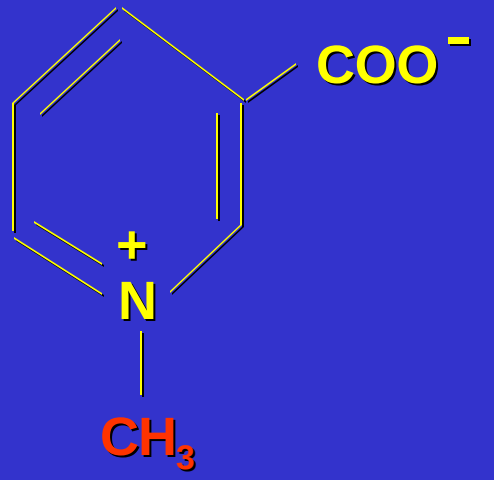
AdoMet

NAMT

AdoHCy

trg1/trg1

chr4L



Nicotinic acid betaine (trigonelline)

M+H$^+$ = 138

PD-MS of Maize Betaines

Bet1/Bet1
Trg1/Trg1
leaf

bet1/bet1
Trg1/Trg1
leaf

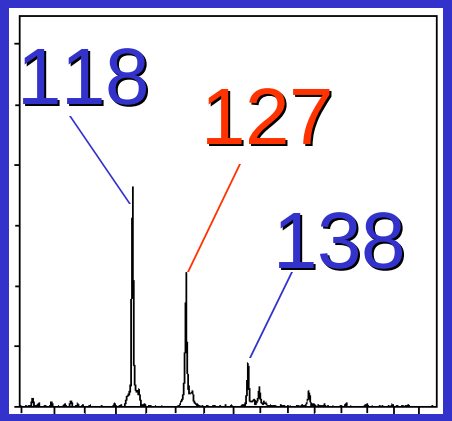
127 = d_9-GB internal std.

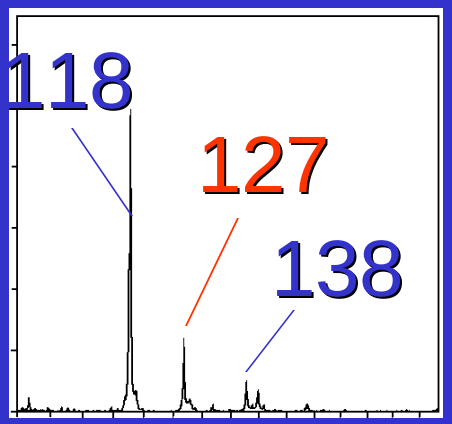
Positive ion

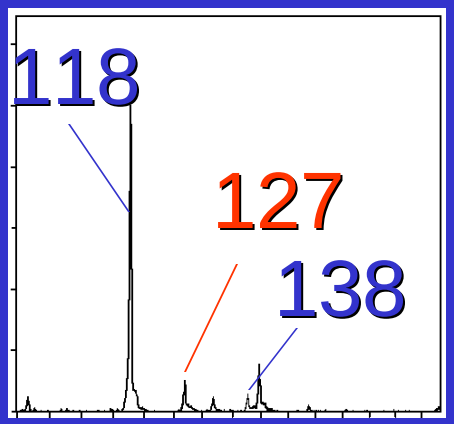
O mM NaCl

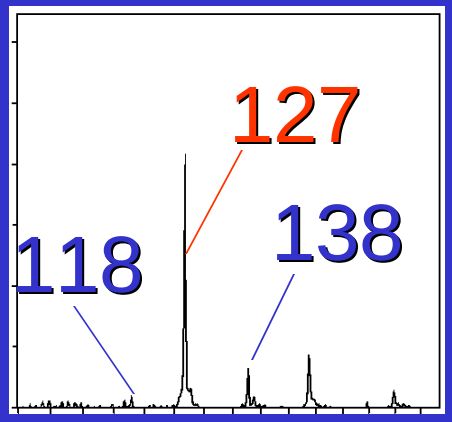
150 mM NaCl 3 days

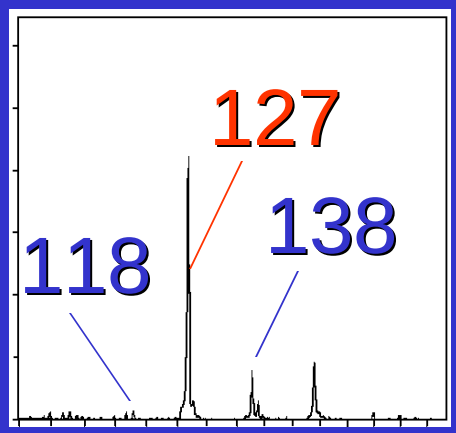
150 mM NaCl 7 days

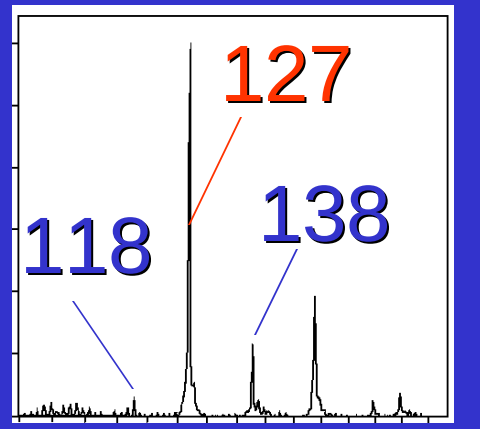












(Greg Peel & D. Rhodes, Purdu

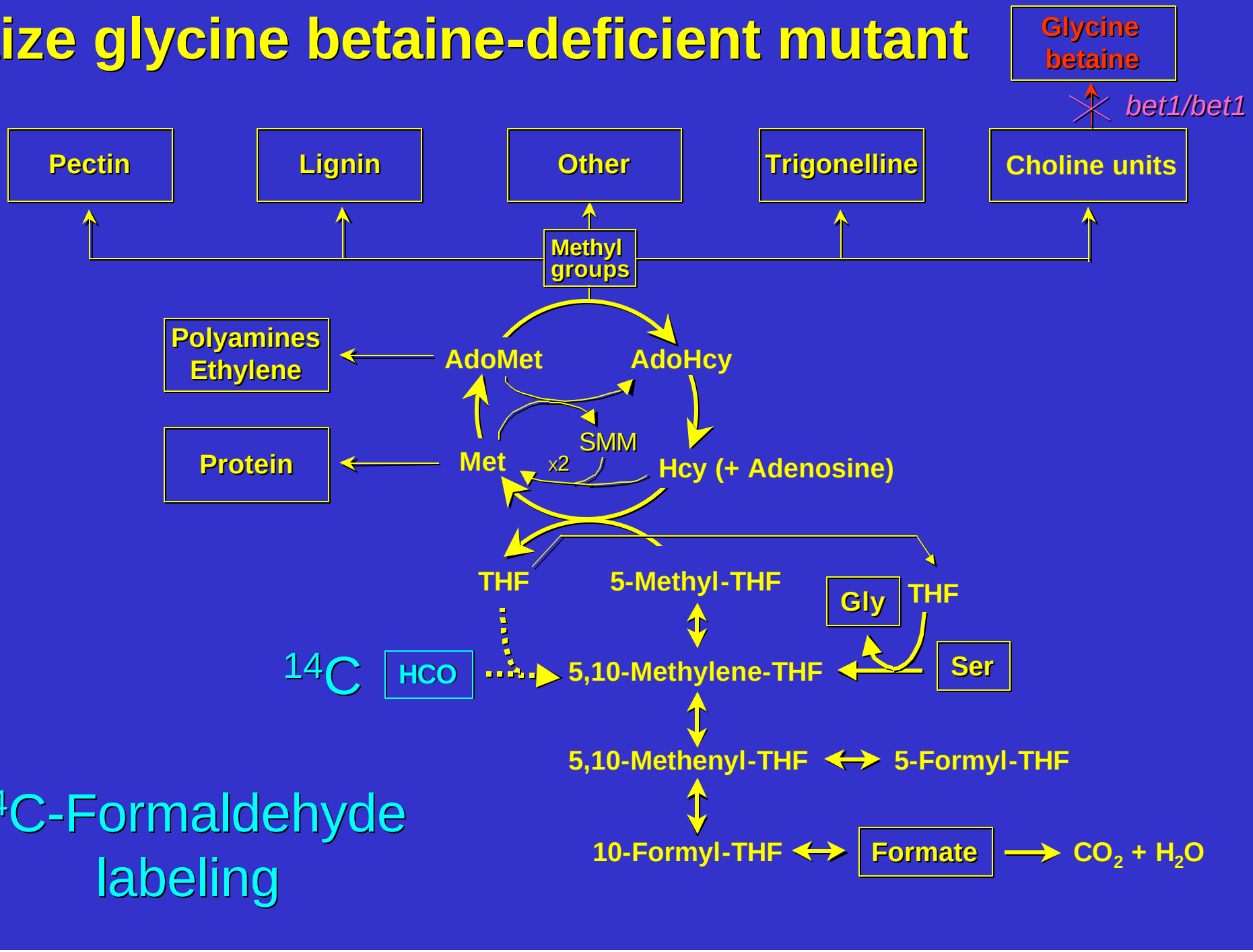
Maize metabolite pool sizes

			Glycine betaine nmol/gFw	Choline nmol/gFw	P-Choline nmol/gFw
Bet1/Bet1	L	Control	3047	754	100
		Salinized *	9518	988	48
	R	Control	9	271	61
		Salinized *	7	298	25
bet1/bet1	L	Control	6	1245	596
		Salinized *	6	1335	402
	R	Control	4	421	39
		Salinized *	2	315	53

Peel & Rhodes, Purdue)

** 3 days at 150 mM NaCl*

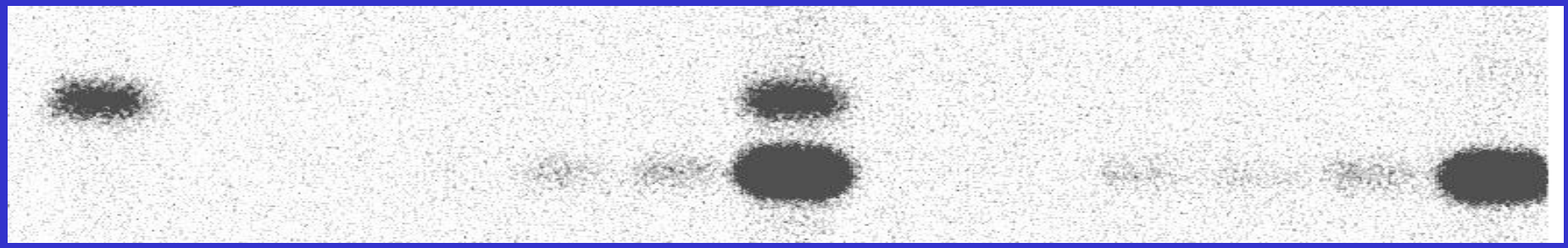
Maize glycine betaine-deficient mutant



^{14}C-Formaldehyde labeling

Maize leaf betaine fraction; ^{14}C-formaldehyde labeled

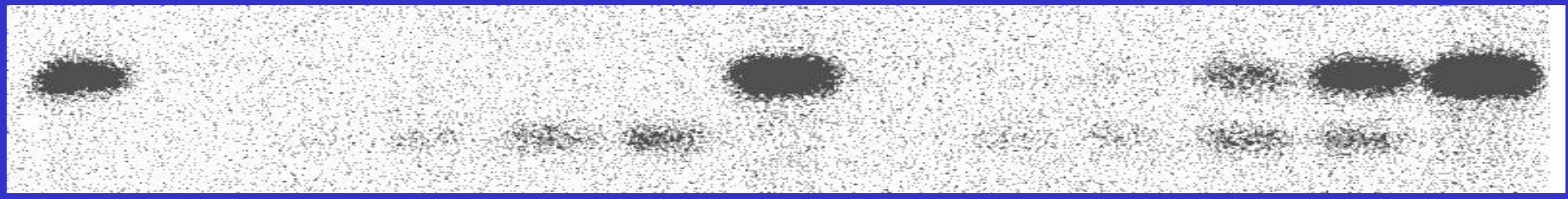
bet1/bet1 Control, Non-salinized *Bet1/Bet1*



GB std 0 30 60 120 240 GB + Trg std 0 30 60 120 240 Trg std

Time (min) Time (min)

bet1/bet1 Salinized (72h, 150 mM) *Bet1/Bet1*

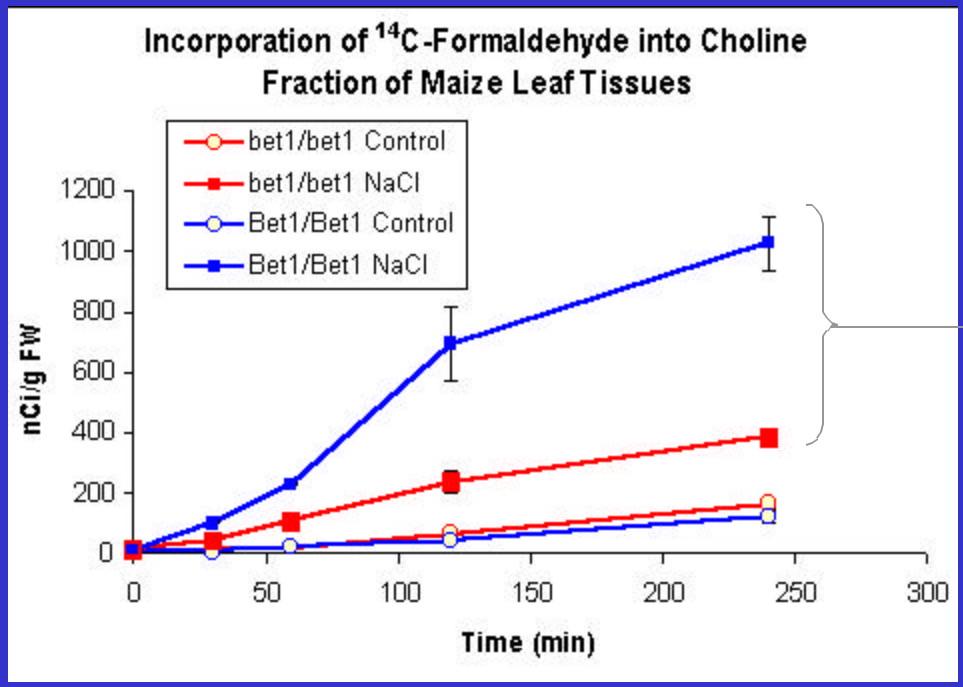


GB std 0 30 60 120 240 GB std 0 30 60 120 240 GB std

Time (min) Time (min)

Maize leaf base fraction; ^{14}C-formaldehyde labeled

(Greg Peel, Purdue)



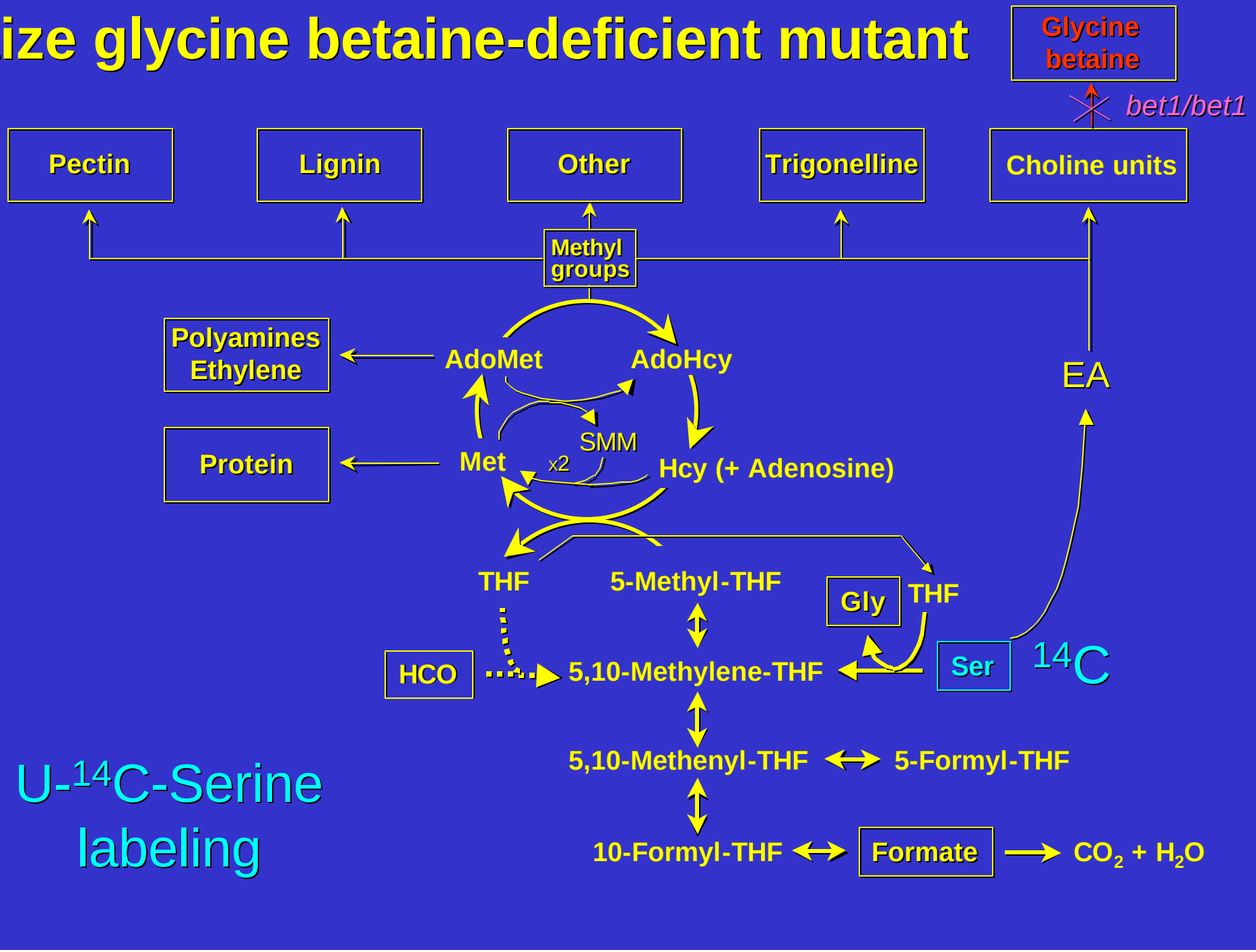
GB std 0 30 60 120 240 GB std

Time (min)

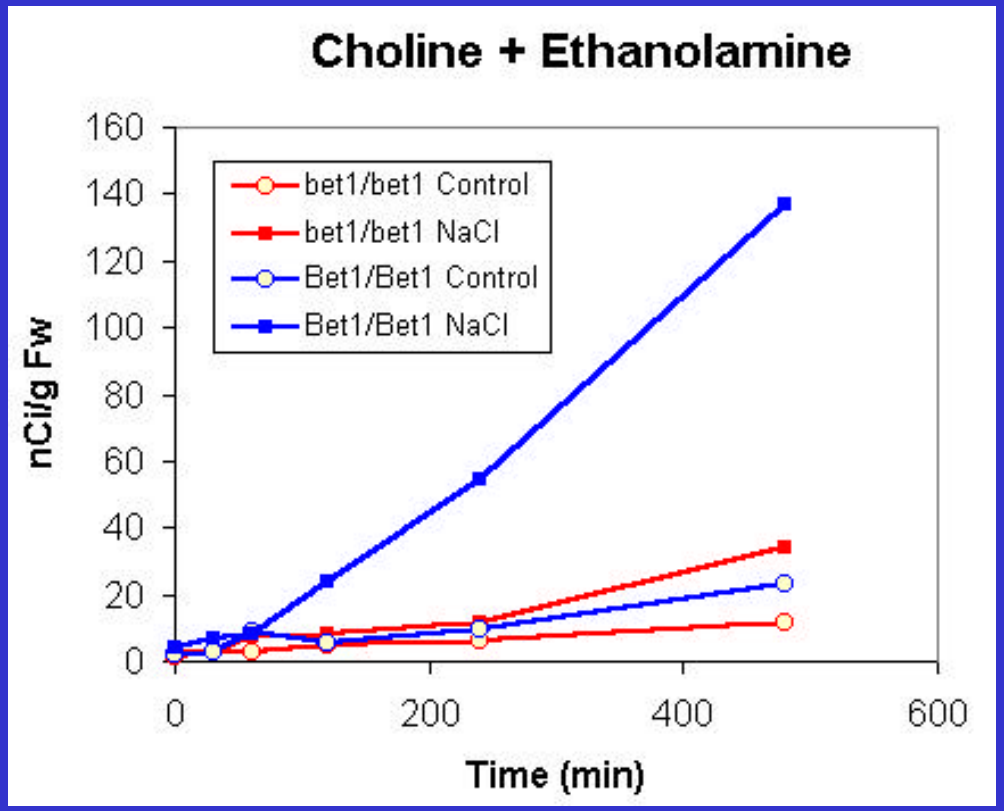
0 30 60 120 240 GB std

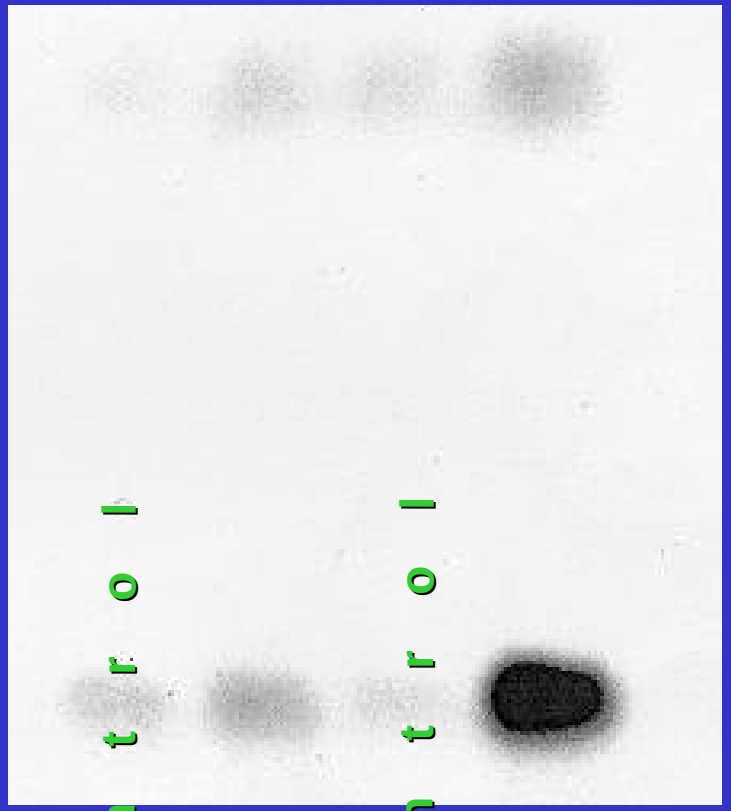
Time (min)

Maize glycine betaine-deficient mutant



U-^{14}C-Serine labeling





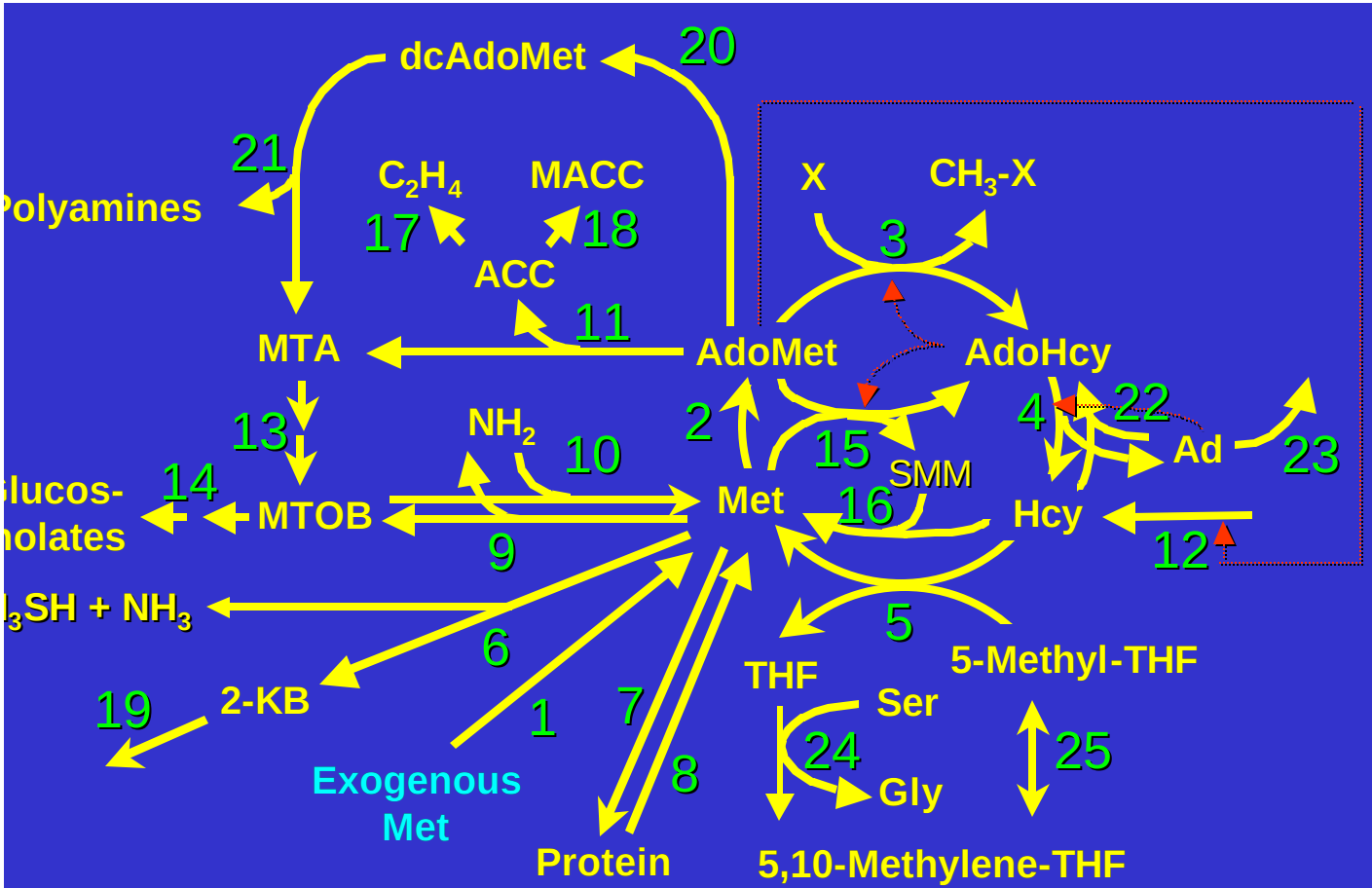
Maize leaf base fraction; U-^{14}C-serine labeled

(480 min)

(Greg Peel, Purdue)

Maize DNA arrays (Hong Wang, U. AZ)

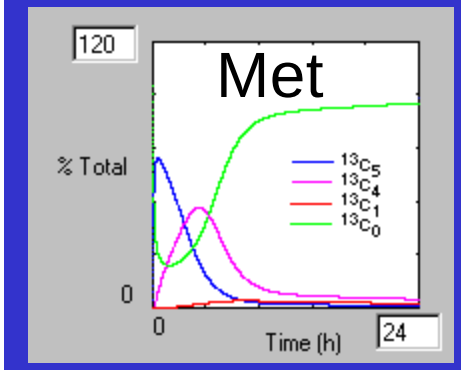
Bet1/Bet1 (GB) and *bet1/bet1* (gb) leaf comparison	GB/gb	GB/gb	GB/gb
Transcript	0hr	6hr	72hr
Methionine adenosyltransferase (cytosol)	1.07	1.01	1.13
S-Adenosyl-L-homocysteine hydrolase (cytosol)	1.07	0.71	0.94
Methionine synthase (cytosol)	1.10	0.94	1.01
Methylene-THF reductase (NADH) (cytosol)	1.07	0.89	1.35
Methylene-THF dehydrogenase/cyclohydrogenase 1 (cytosol)	1.05	1.07	0.87
Methylene-THF dehydrogenase/cyclohydrogenase 2 (cytosol)	0.97	1.22	0.96
Methylene-THF dehydrogenase/cyclohydrogenase 3 (mitochondrion)	0.93	1.10	1.03
Formate-tetrahydrofolate ligase (cytosol)	1.21	0.90	0.94
Serine hydroxymethyltransferase (mitochondrion)	1.15	1.00	0.88
Glycine cleavage system P-protein (mitochondrion)	1.25	1.17	1.32
Glycine cleavage system H-protein (mitochondrion)	1.00	1.06	1.00
Glycine cleavage system T-protein (mitochondrion)	1.01	1.21	1.08
Formate dehydrogenase (cytosol)	1.03	1.01	1.42
Methionine S-methyltransferase (cytosol)	1.27	1.19	0.86
S-Adenosylmethionine decarboxylase (cytosol)	1.11	1.51	1.63
ACC synthase (cytosol)	0.95	1.21	0.86
Cystathionine gamma-synthase (cytosol)	1.10	0.89	0.95
Adenosine kinase (cytosol)	0.89	1.31	1.08
Betaine aldehyde dehydrogenase (BADH) (plastid)	1.13	0.98	1.13
Glutathione-dependent formaldehyde dehydrogenase (cytosol)	0.99	0.86	0.90
S-Formyl glutathione hydrolase (cytosol)	1.01	0.93	0.93
5,10-Methenyl-THF synthetase (cytosol)	1.14	0.86	0.88



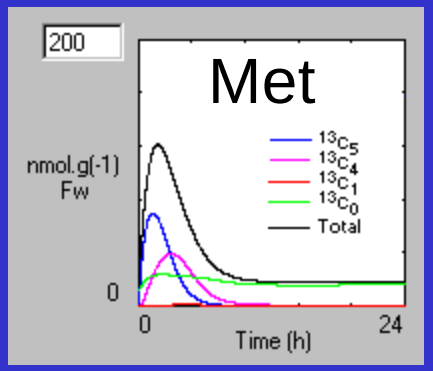
Kinetic Modeling

e.g. simulation of metabolism of a pulse of $^{13}C_5$-Met

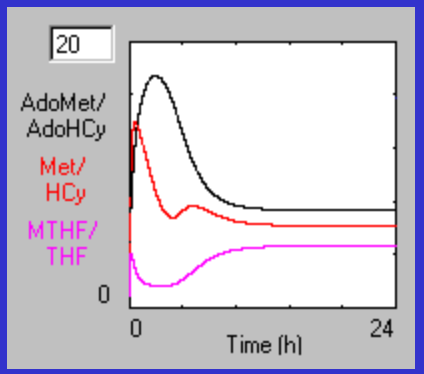
(Rhodes, Purdue)



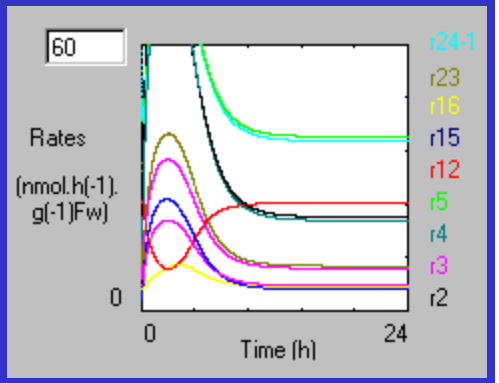
Isotopomer %



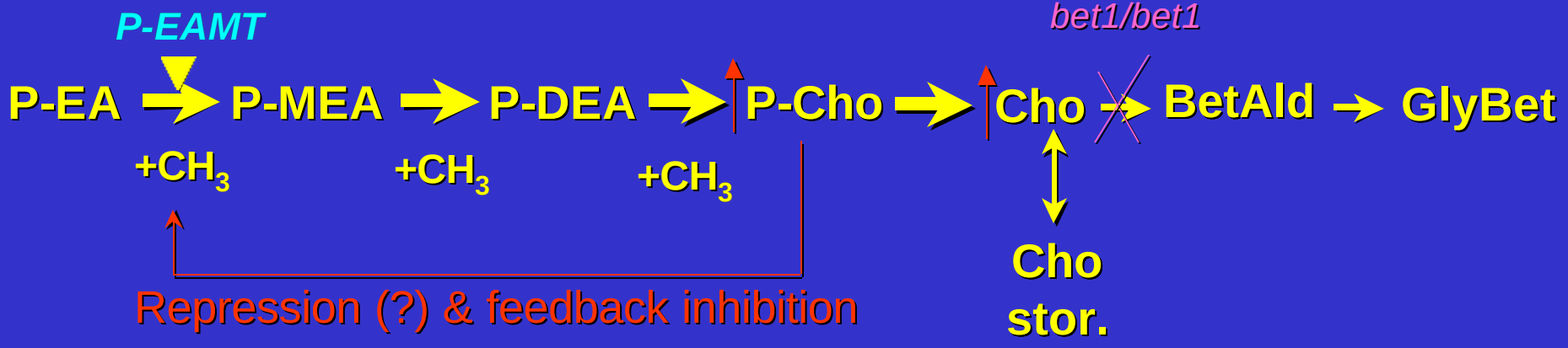
Pool sizes

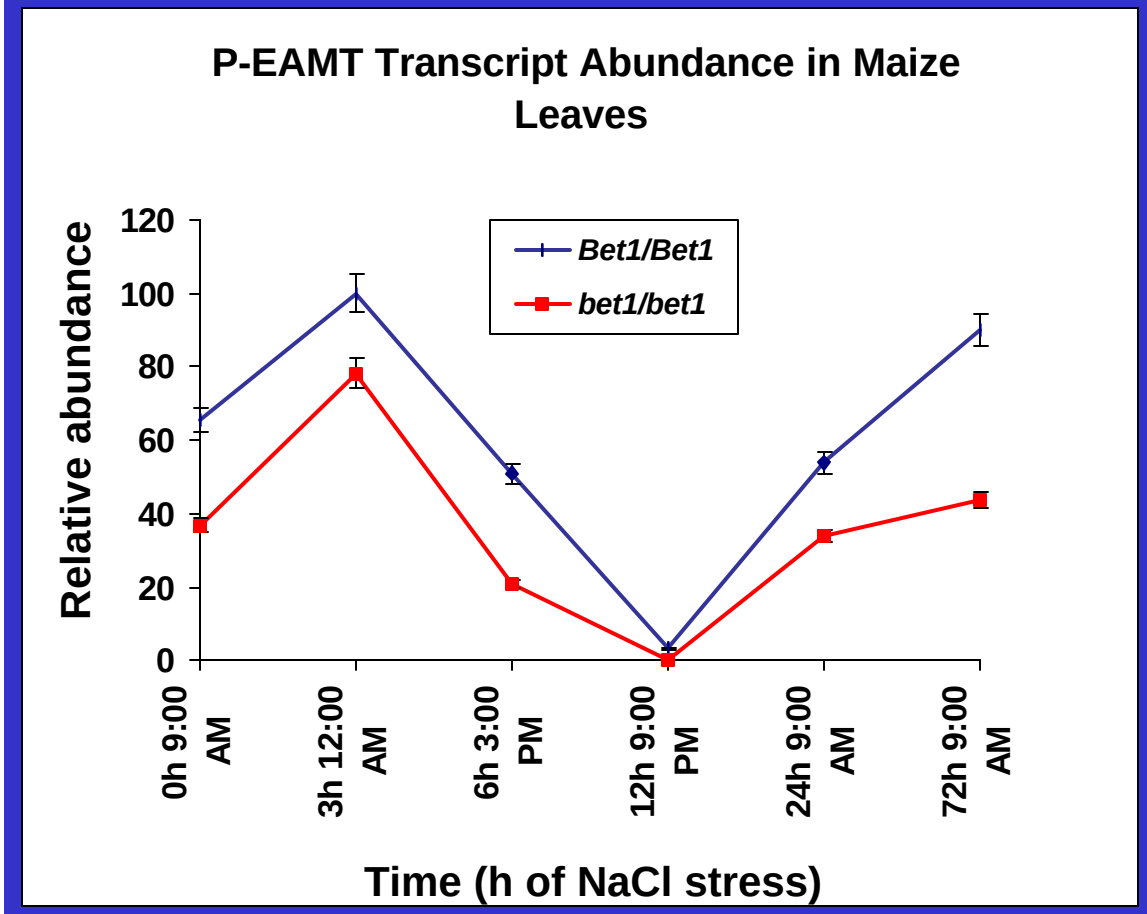


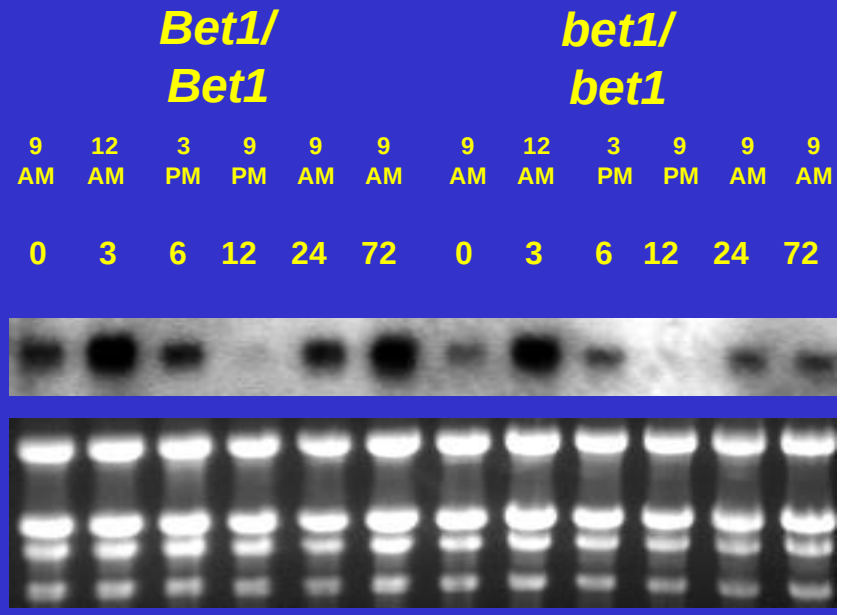
Metabolite ratios



Rates







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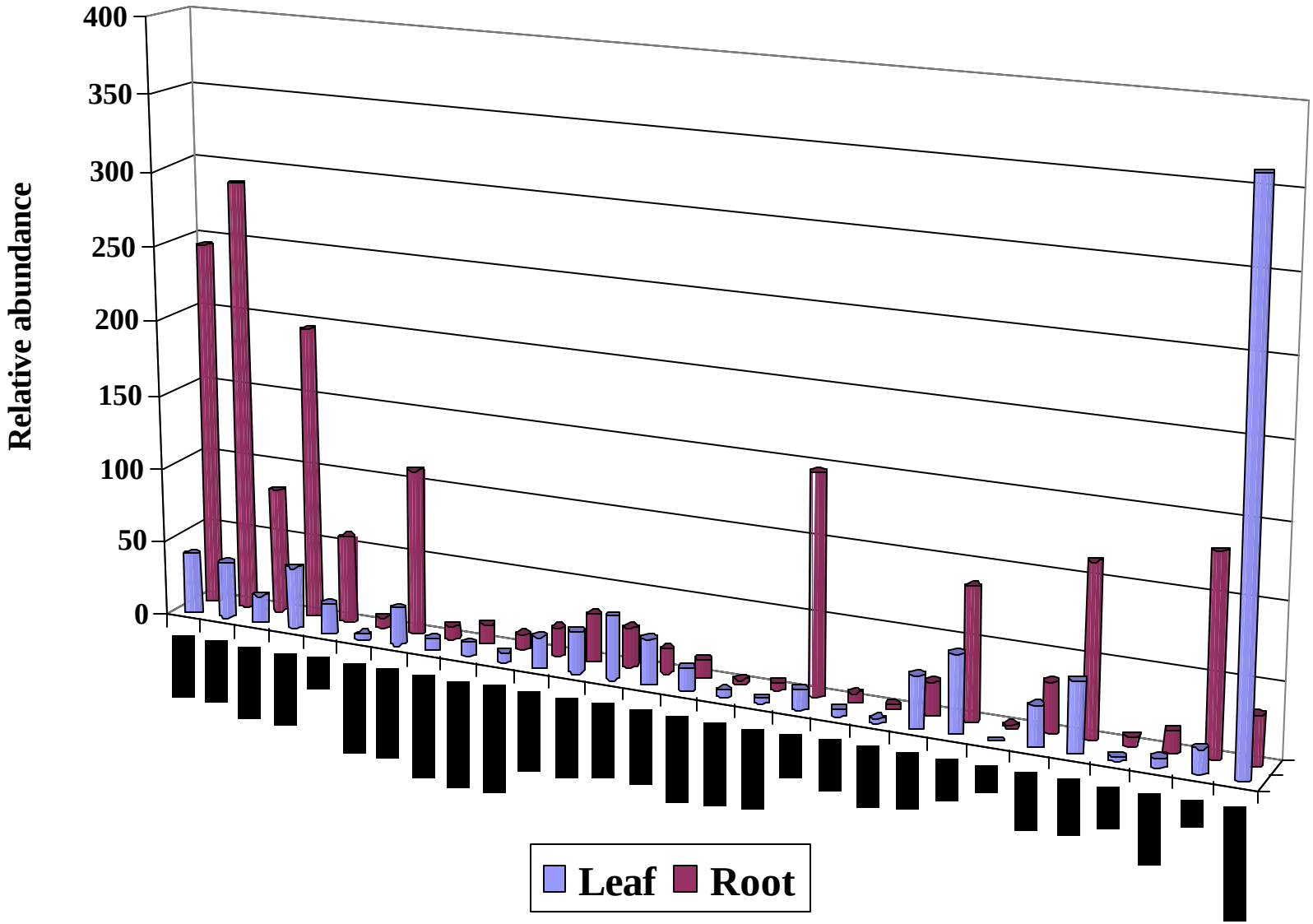
Maize DNA arrays



(Hong Wang, U. AZ)

Publications

- *J Biol Chem* - MTHFR cloning & characterization
- *TIPS* - Invited C_1 metab & engineering review (genomics)
- *J Biol Chem* - MS approach to SMM cycle * *
- *Plant Physiol* - Modeling of choline metabolism * *
- *Plant Physiol* - Engineering/modeling GlyBet synth. (CMO) * *
- *Metab Engin* - Engineering/modeling GlyBet synth. (CMO, COX + BADH) * *
- *Annu Rev Plant Physiol Plant Mol Biol* - Invited C_1 review
- *Plant J* - Fluxes via transmethylation & SMM cycles in Arabidopsis * *
- *Annu Rev Plant Physiol Plant Mol Biol* - Invited NMR review
- *Metab Engin* - Invited review on modeling of plant pathways
- *Metab Engin* - Invited review on ME of plant osmolytes
- *Plant Physiol* - Microarray & labeling data for maize; *Bet1/Bet1 cf. bet1/bet1*

* MS or modeling supported by C_1 grant; * NSF; * USDA

Outreach - 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

- Plant Biochemistry Summer Course - July 2001, Pullman

- 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