THE Glucosinolates & Cyanogenic Glycosides

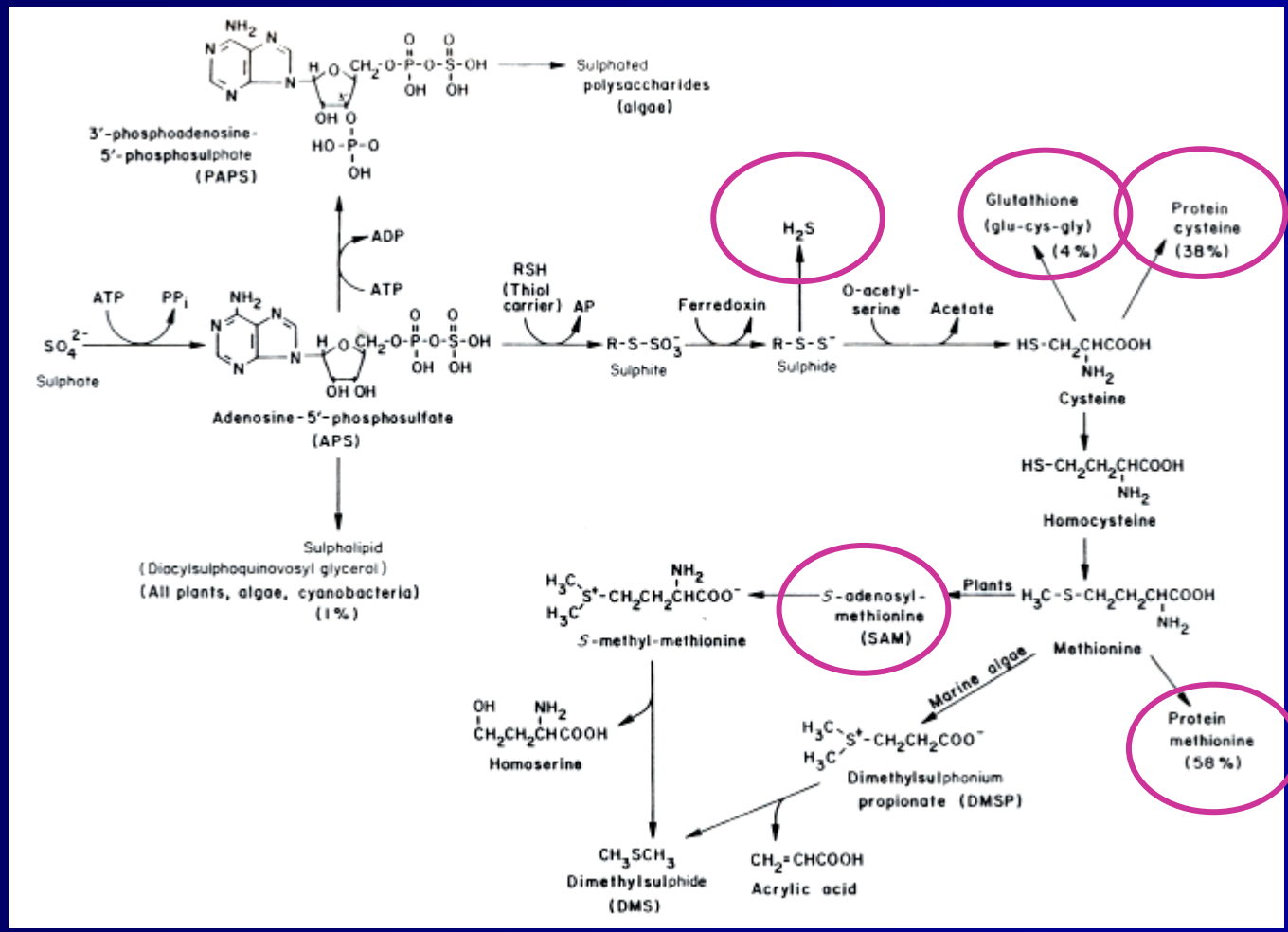
Assimilatory Sulphate Reduction

- Animals depend on organo-sulphur

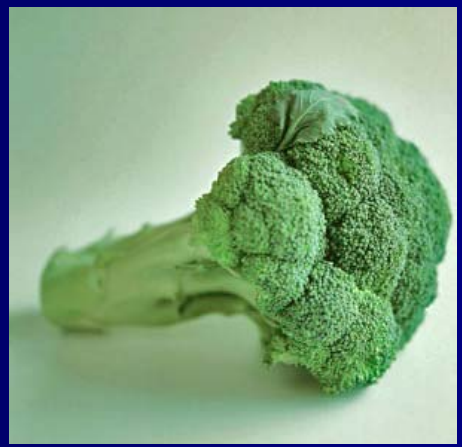
- In contrast, plants and other organisms (e.g. fungi, bacteria) can assimilate it

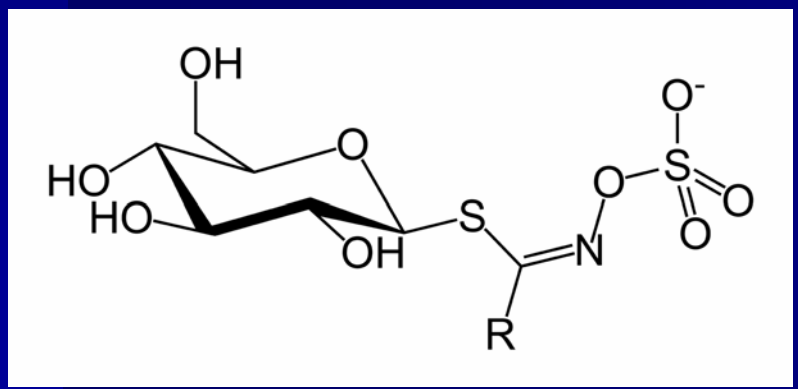
- Sulphate is assimilated from the environment, reduced inside the cell, and fixed to sulphur containing amino acids and other organic compounds

Assimilatory Sulphate Reduction



The Glucosinolates





The Glucosinolates

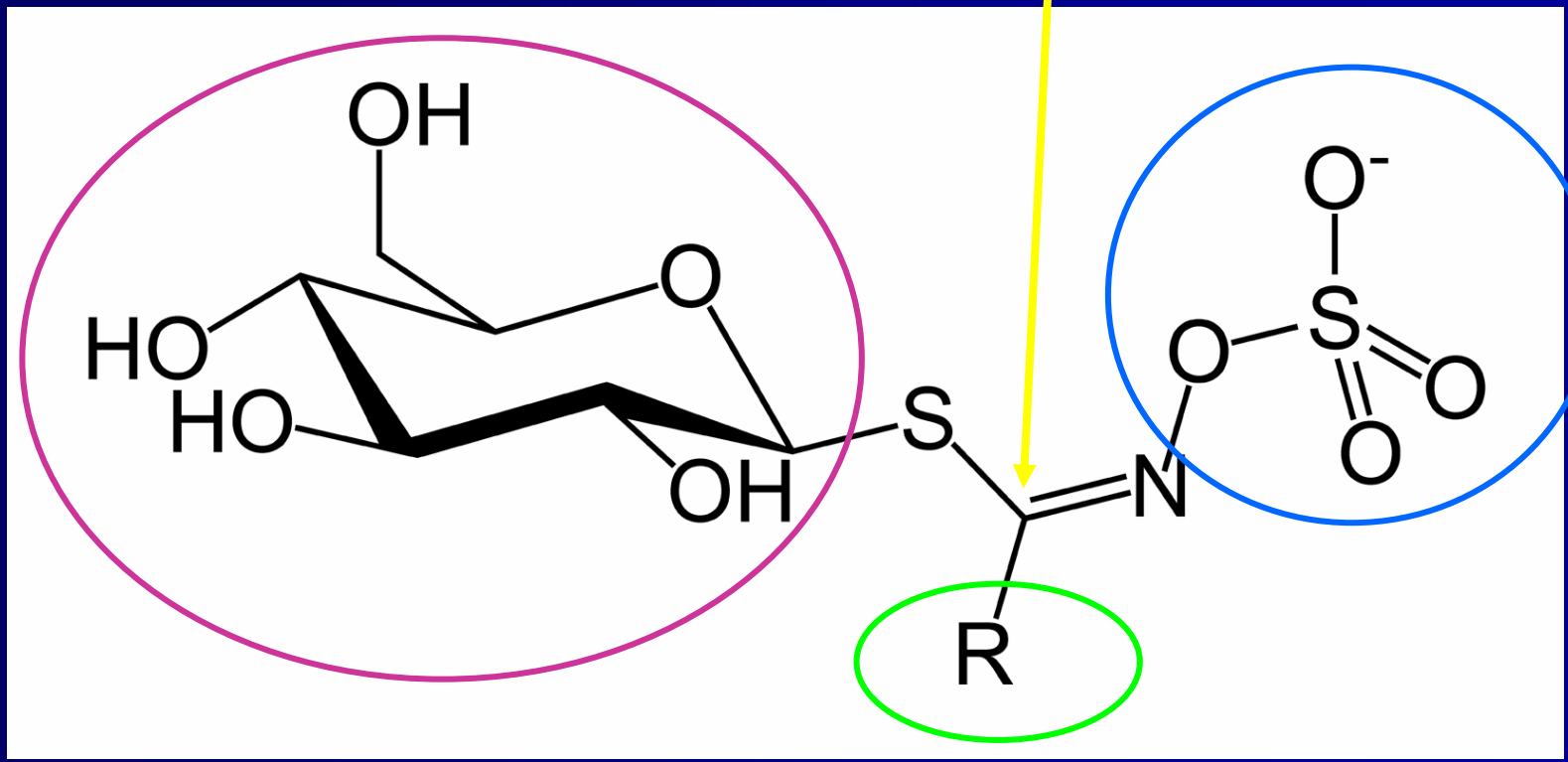
- Found in the Capparales order and are the main secondary metabolites in cruciferous crops

The Glucosinolates

- The glucosinolates are a class of organic compounds (water soluble anions) that contain sulfur, nitrogen and a group derived from glucose

- Every glucosinolate contains a central carbon atom which is bond via a sulfur atom to the glycone group, and via a nitrogen atom to a sulfonated oxime group. In addition, the central carbon is bond to a side group; different glucosinolates have different side groups

The Glucosinolates



The Glucosinolates

- About 120 different glucosinolates are known to occur naturally in plants.

- They are synthesized from certain amino acids: methionine, phenylalanine, tyrosine or tryptophan.

- The plants contain the enzyme myrosinase which, in the presence of water, cleaves off the glucose group from a glucosinolate

The Glucosinolates

- Post myrosinase activity the remaining molecule then quickly converts to a thiocyanate, an isothiocyanate or a nitrile; these are the active substances that serve as defense for the plant

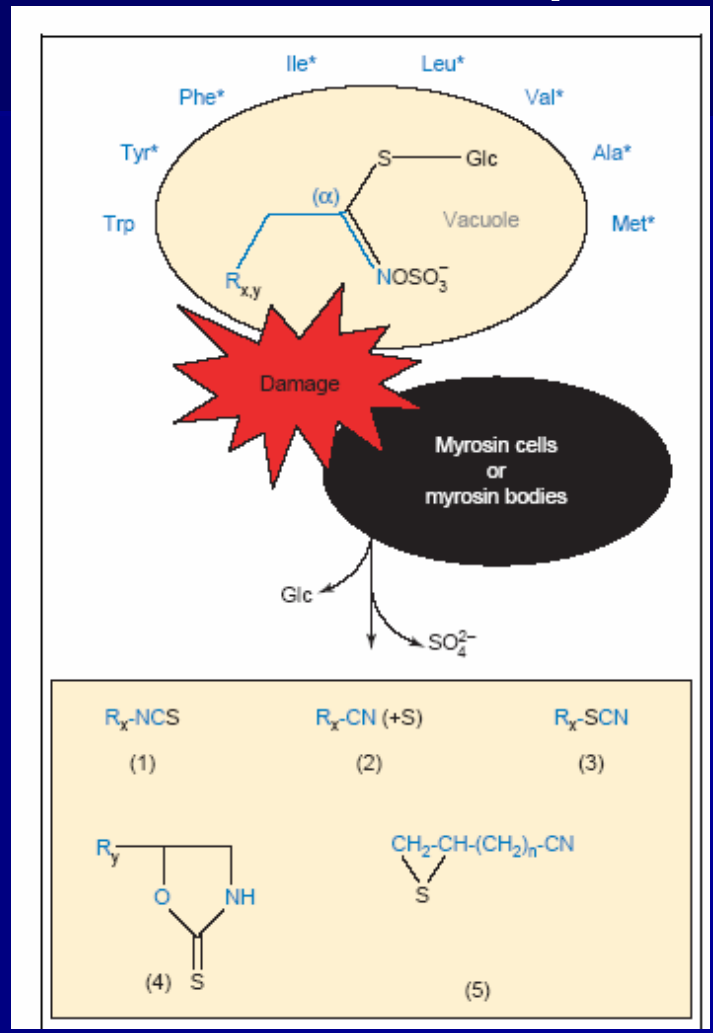
- To prevent damage to the plant itself, the myrosinase and glucosinolates are stored in separate compartments of the cell and come together only under conditions of stress or injury

- Gluocsinolates might also be beneficial to insects (signals for feeding or oviposition) that could overcome host toxicity

The "mustard oil bomb"-- A binary Glucosinolate-Myrosinase chemical defense system

Glucosinolates breakdown products

1- isothiocyanates
2- nitriles and elemental sulfur
3- thiocyanates
4- oxazolidine--thiones
5- epithionitriles



Grubb and Abel, TIPS, 2006

The Glucosinolates

- Due to negative effects, food crops have been developed that contain very low amounts of glucosinolates (e.g. canola).

- On the other hand, plants producing large amounts of glucosinolates are also desirable, because substances derived from these can serve as natural pesticides and are potent in the prevention of cancer
(e.g. sulforaphane in broccoli)

Glucosinolates are synonymous to 'Mustard oil glycosides' and 'Thioglucosides.'

The Glucosinolates

- Extensive research in Arabidopsis (at least 37 different GSs in all ecotypes, approx. 7 per ecotype)

- Side chains from mainly methionine (so called aliphatic glucosinolates, AGs) and Tryptophan (Indole Glucosinolates; IGs)

- GSs concentration and types vary significantly among organs and GSs are transported via the phloem to sink tissues, most probably intact

- Seeds have very distinctive GSs profile

The Glucosinolates

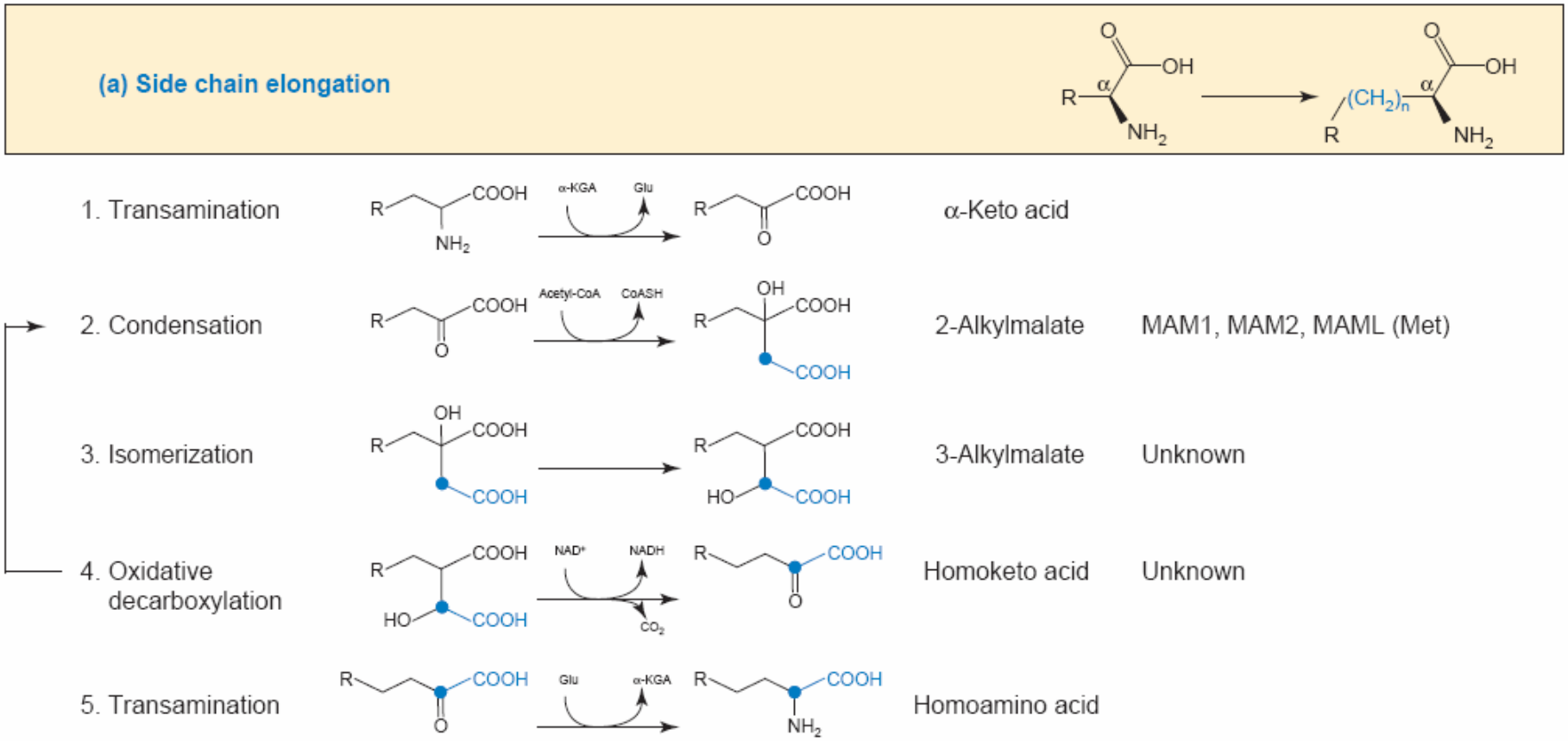
- Biosynthesis starts (Step i) with side chain elongation that could be further elongated or used to form the core structure (glucon)

- Glucon formation (Step ii) is a 5 steps pathway, starting with the formation of aldoxime through oxidation by P450's

- In the next step (iii), sulfation of desulfoglucosinolates occurs and thereafter secondary modification of the side chain takes place (various kinds of oxidation, esterification etc..)

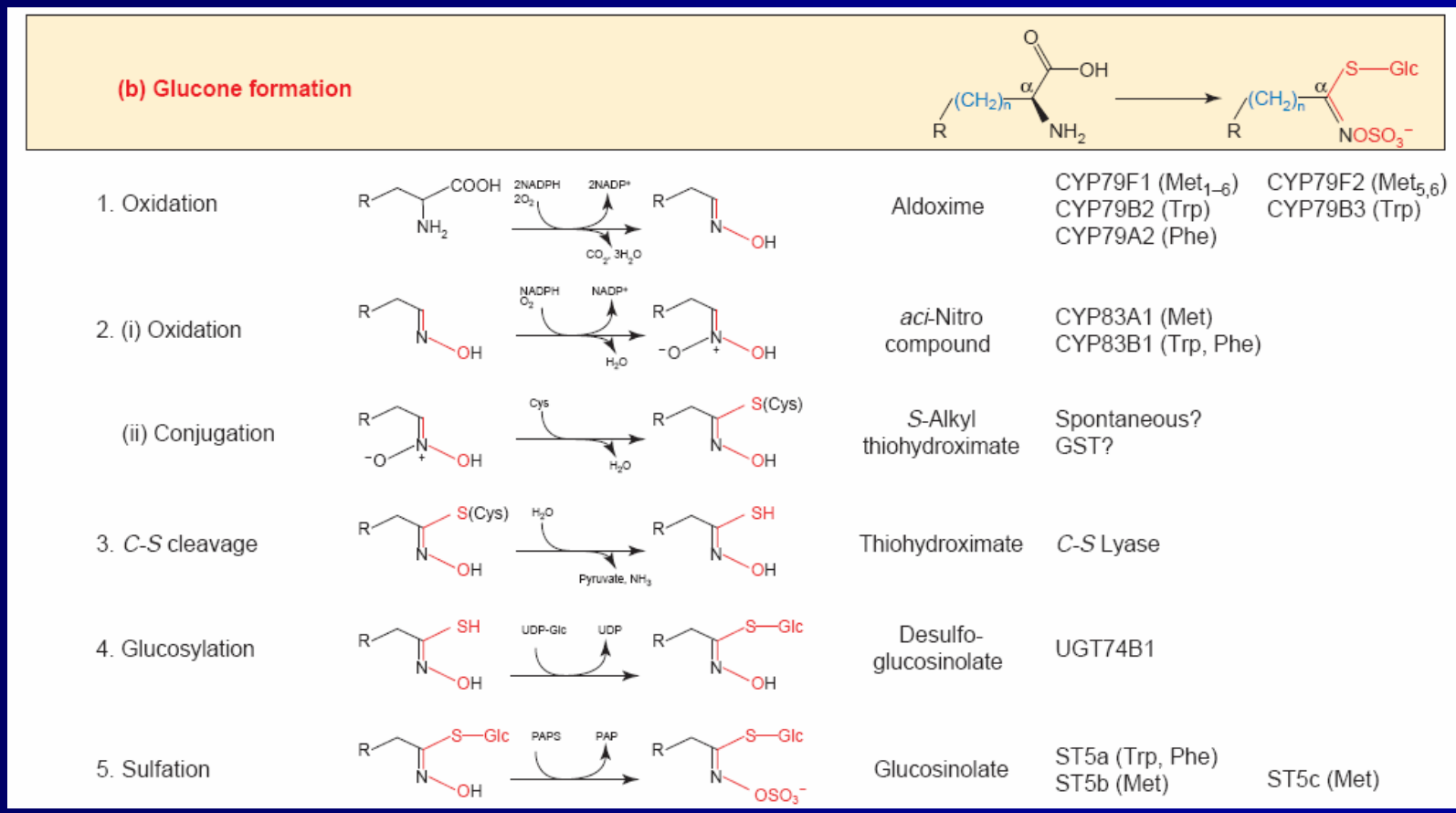
Grubb and Abel,

The Glucosinolates



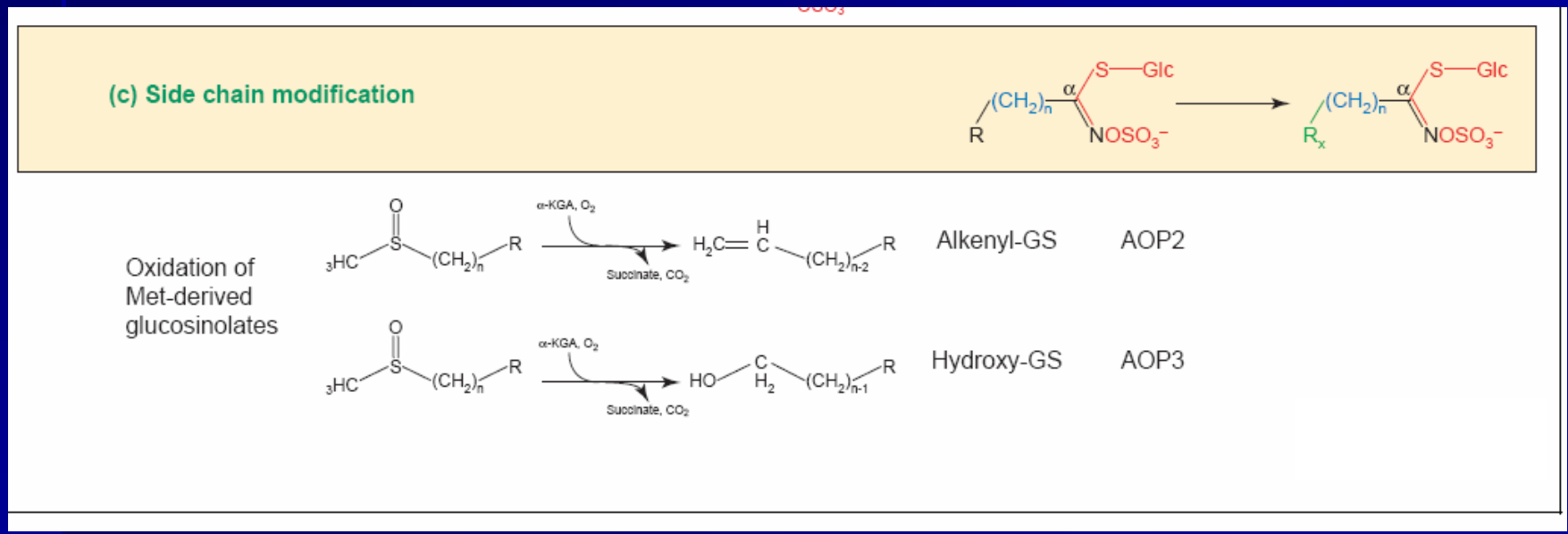
Grubb and Abel, 2006

The Glucosinolates



Grubb and Abel, 2006

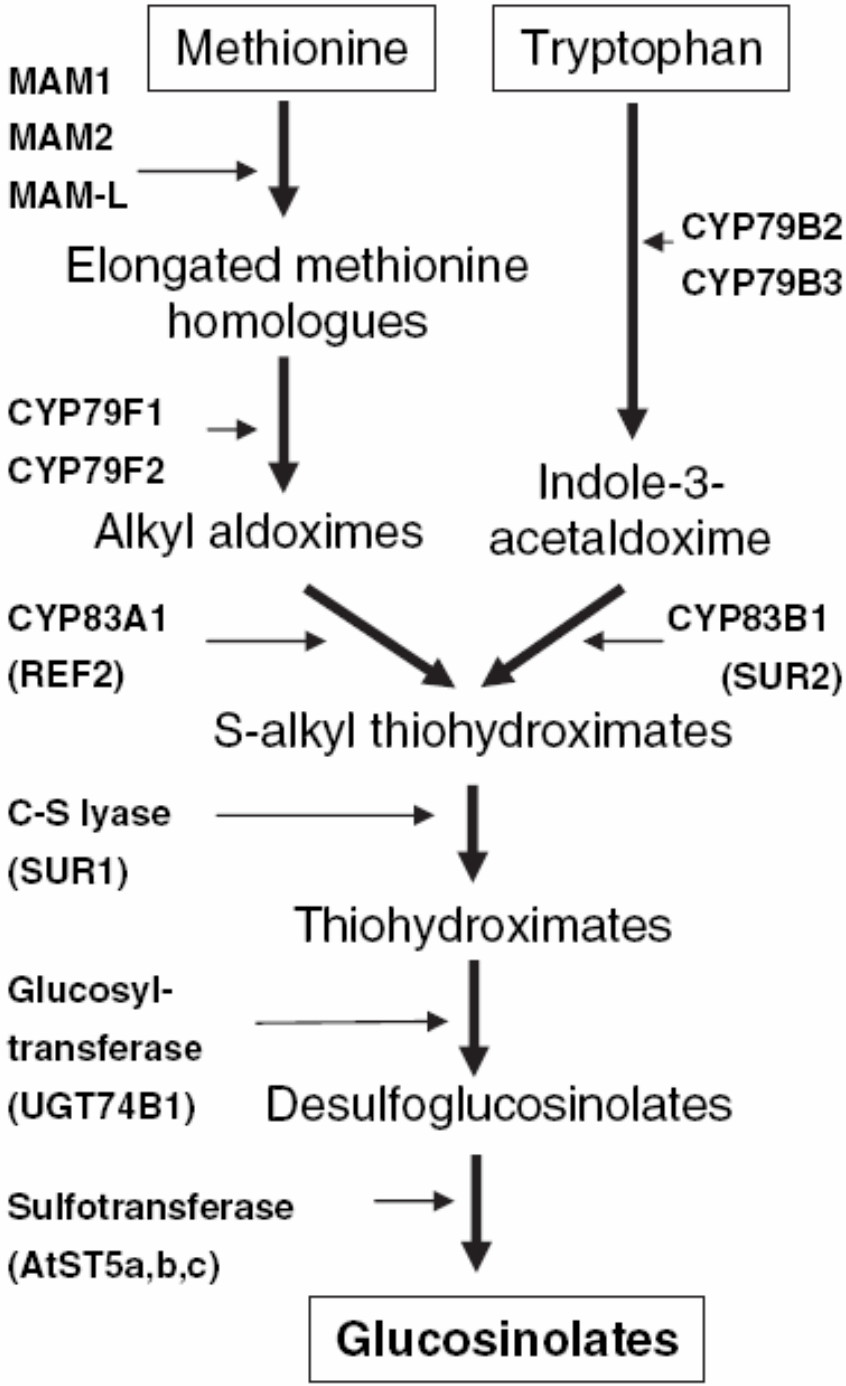
The Glucosinolates



Grubb and Abel, 2006

The Indole and Aliphatic Glucosinolates Metabolism

- The pathways share enzymes
- Genes clones marked by capital letters



Kim and Jander, 2007

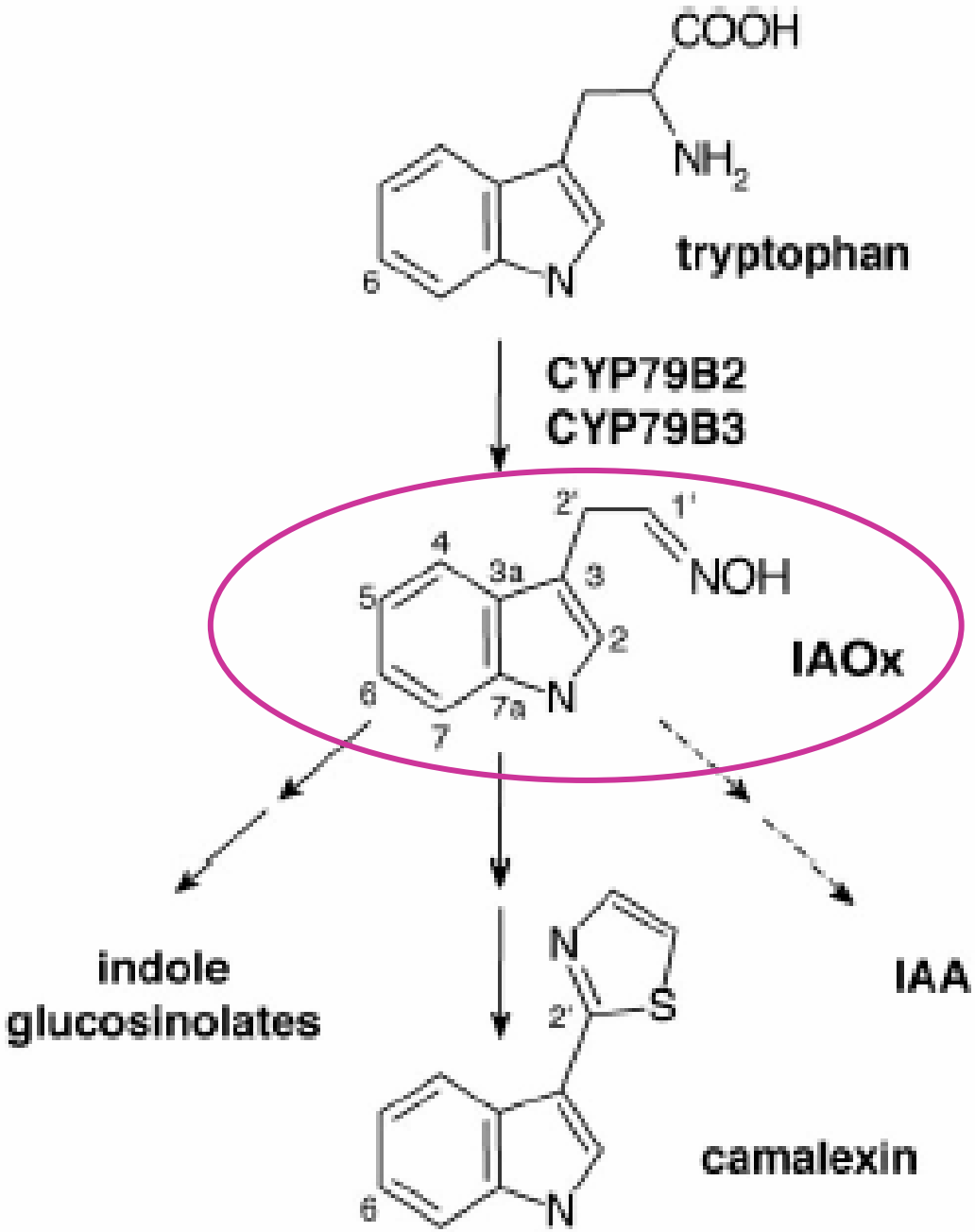
The Indole Glucosinolates (from Trp)

As in the case of other GSs types, the formation of IGs is inter- linked with metabolism of other key metabolites

- The Trp derived Indole Aldoxime (in the Glucon formation step) is an important branch point

- The aldoxime is a precursor for IGs, Auxin (IAA) and Camalexin biosynthesis

The Indole Aldoxime is a Majour Branch Point



The Indole Glucosinolates (from Trp)

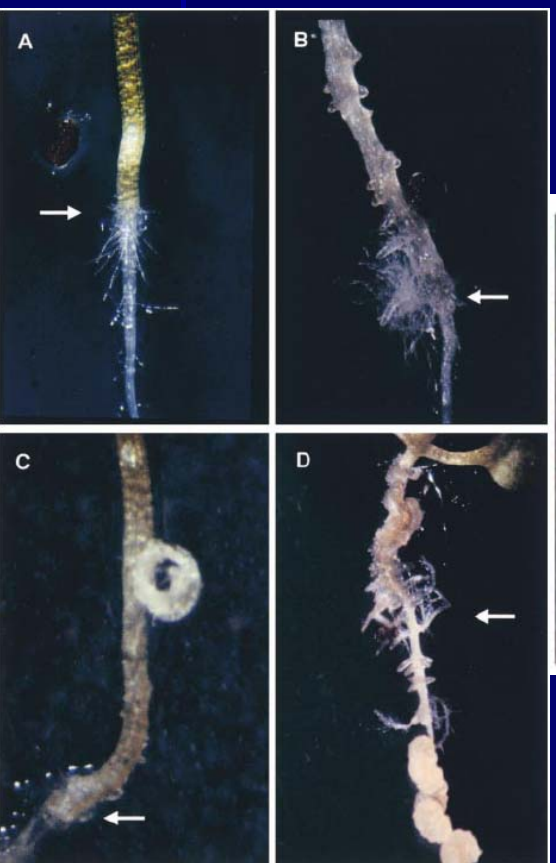
- Complex interplay between IGs biosynthesis and linked pathways

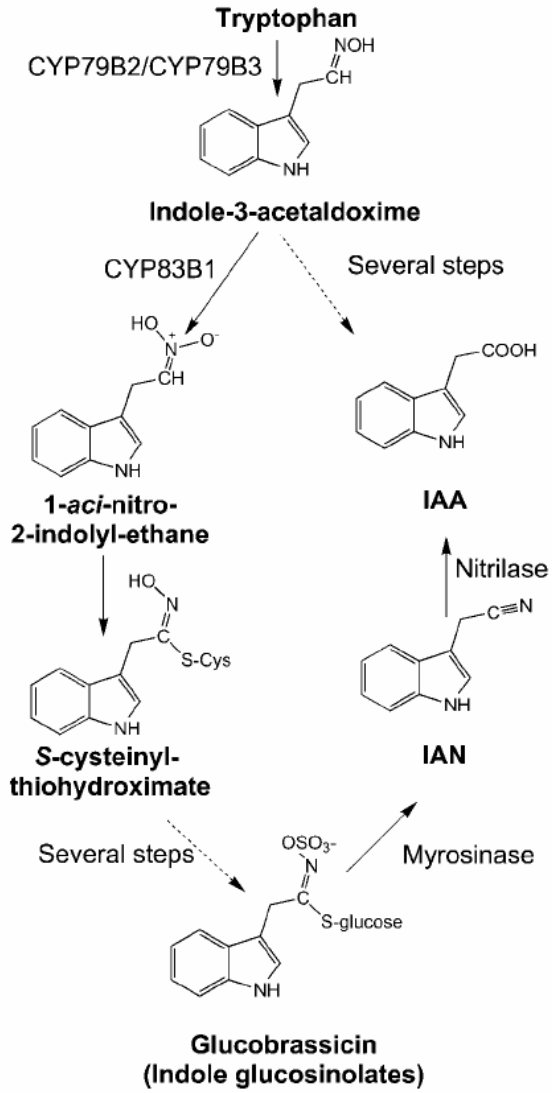
- Inhibition of flux between the aldoxime and the IGs results in increased levels of auxin

The Indole Glucosinolates (from Trp)

Bak et al., 2001

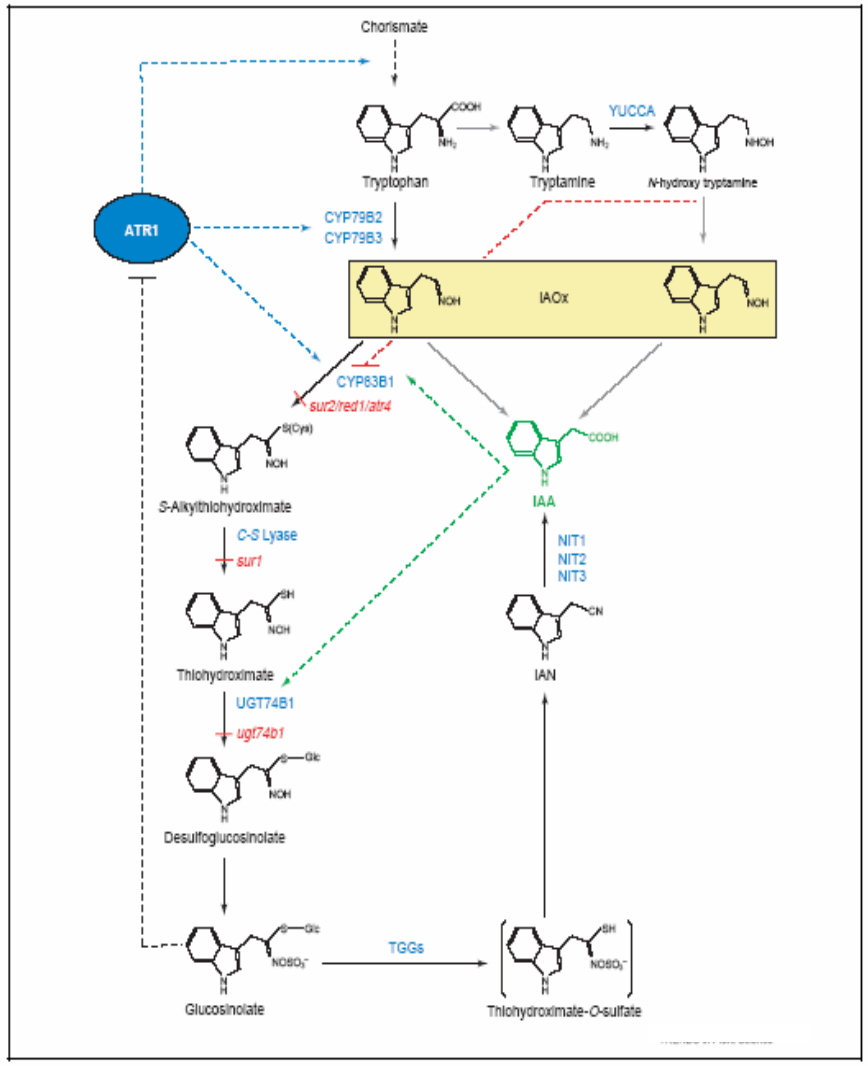
- CYP83B1 knockout (*rnt1*)





Regulation of the Indole Glucosinolates Pathway

Altered Trp Resistance (ATR1)- A dominant mutant



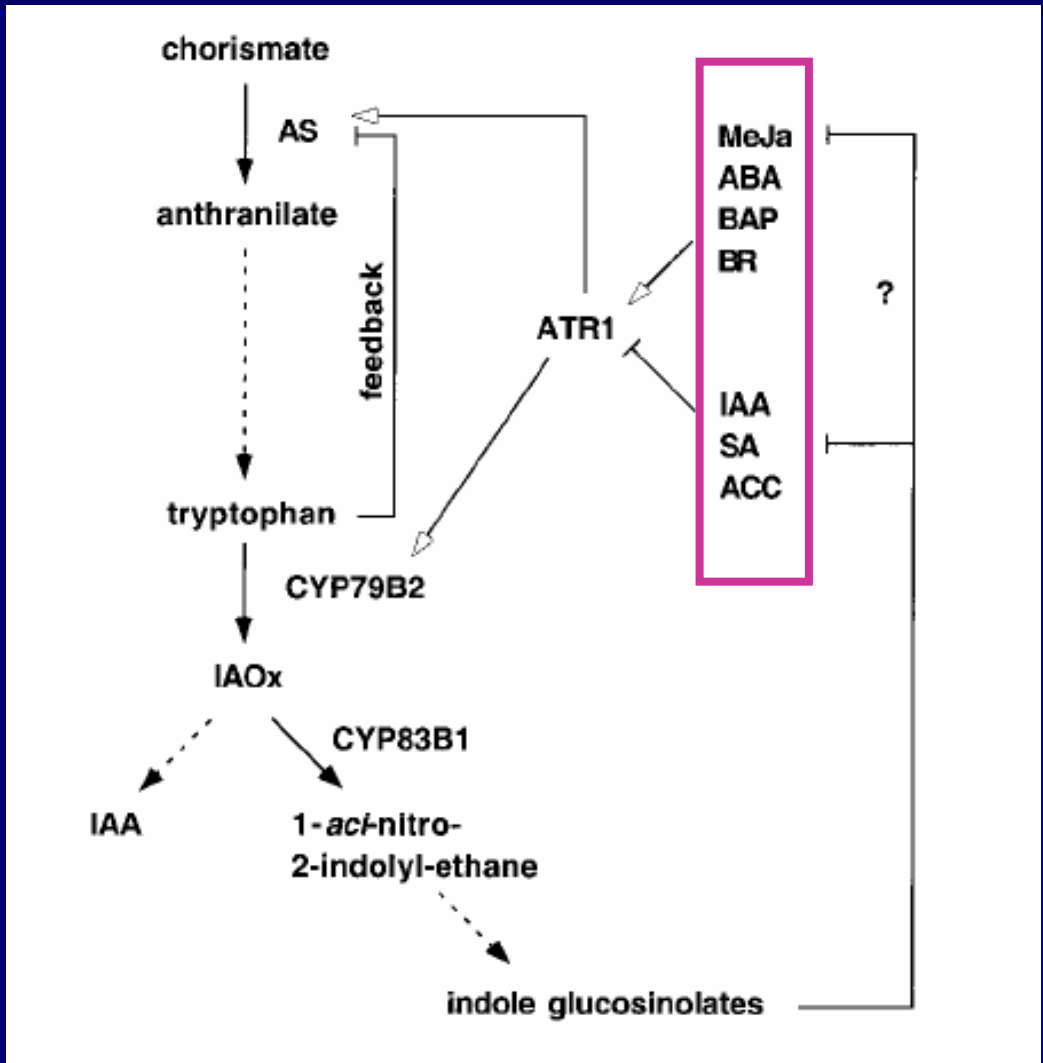
A Myb homologue, ATR1, activates tryptophan gene expression in *Arabidopsis*

(Myb transcription factor/translational control)

JUDITH BENDER* AND GERALD R. FINK

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 5655–5660, May 1998
Genetics

Regulation of the Indole Glucosinolates Pathway (ATR1)



Smolen and Bender, 2002

Regulation of Both Pathways by A Set of MYB Regulators

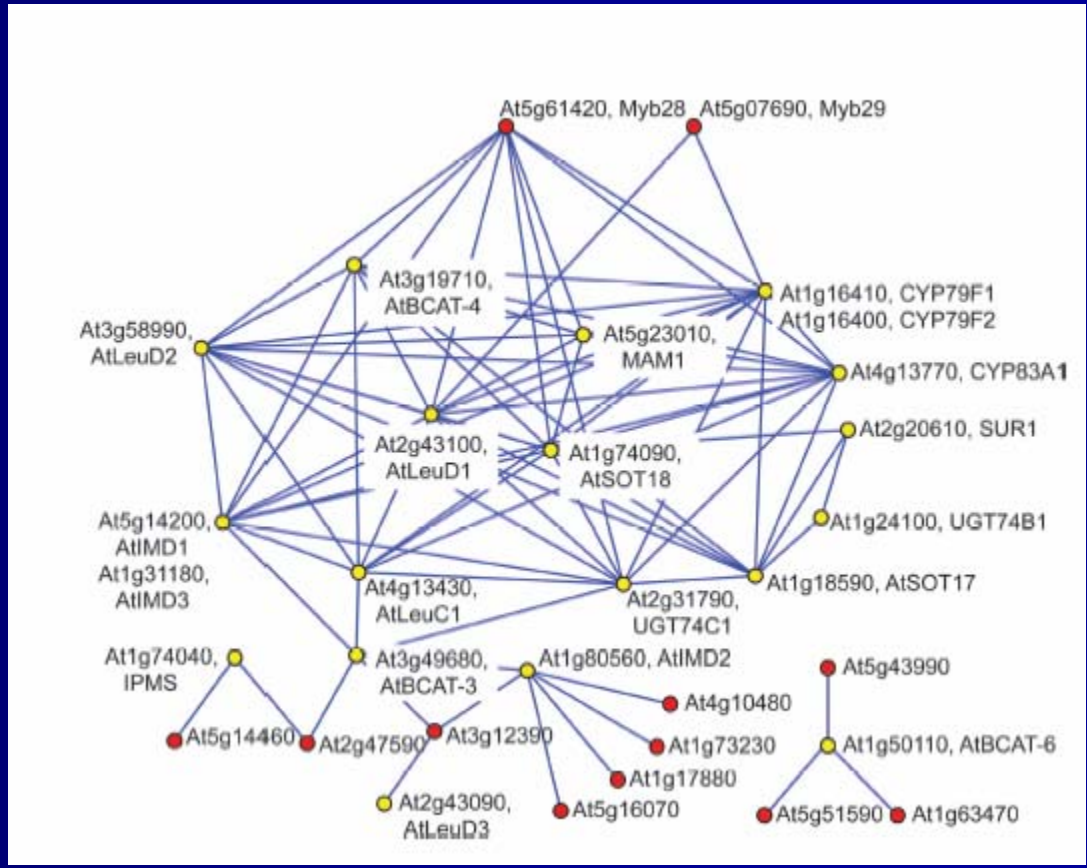
Omics-based identification of *Arabidopsis* Myb transcription factors regulating aliphatic glucosinolate biosynthesis

Masami Yokota Hirai*, Kenjiro Sugiyama†, Yuji Sawada*, Takayuki Tohge*, Takeshi Obayashi‡§, Akane Suzuki*, Ryoichi Araki*¶, Nozomu Sakurai†, Hideyuki Suzuki†, Koh Aoki†, Hideki Goda*, Osamu Ishizaki Nishizawa*¶, Daisuke Shibata†, and Kazuki Saito*‡‖

6478–6483 | PNAS | April 10, 2007 | vol. 104 | no. 15

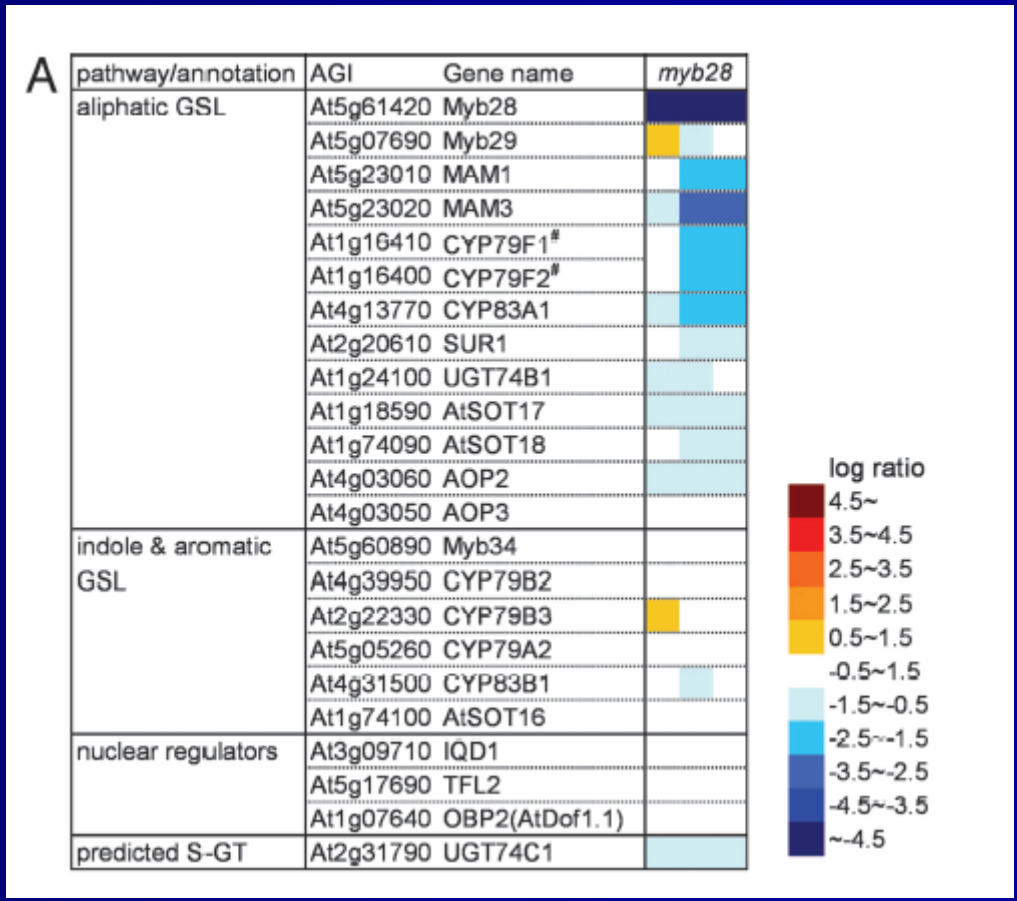
Regulation of Both Pathways by A Set of MYB Regulators

Identification of the Arabidopsis MYB28 and MYB29 by co-expression analysis (yellow-enzymes; red- transcription factors)

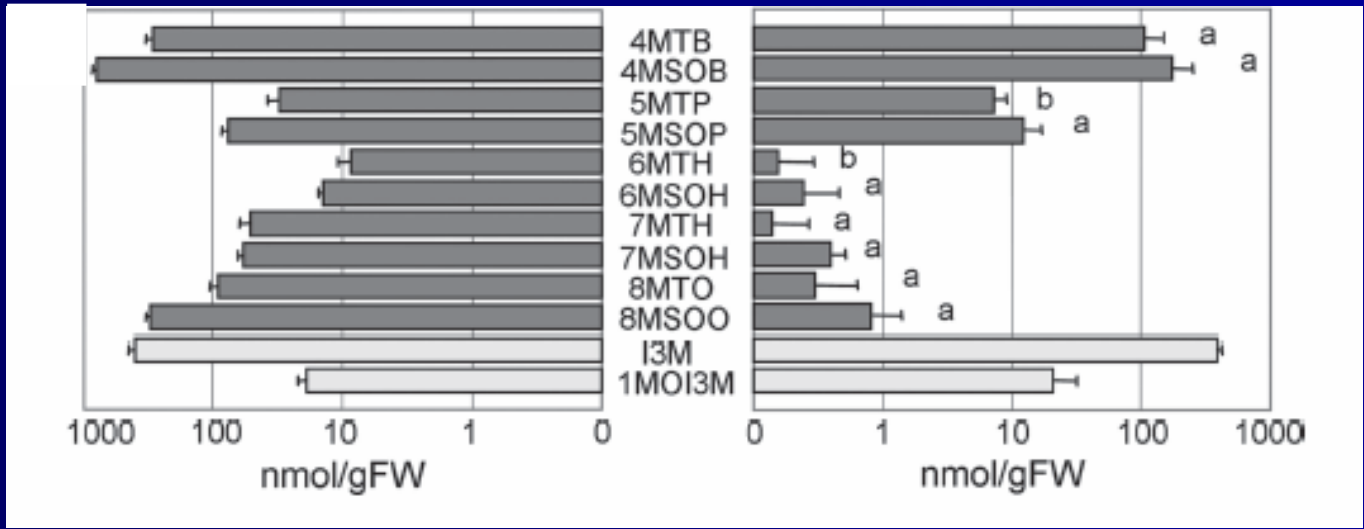


Regulation of Both Pathways by A Set of MYB Regulators

Downregualtion of genes encoding enzymes related to AGs metabolism in MYB28



Regulation of Both Pathways by A Set of MYB Regulators



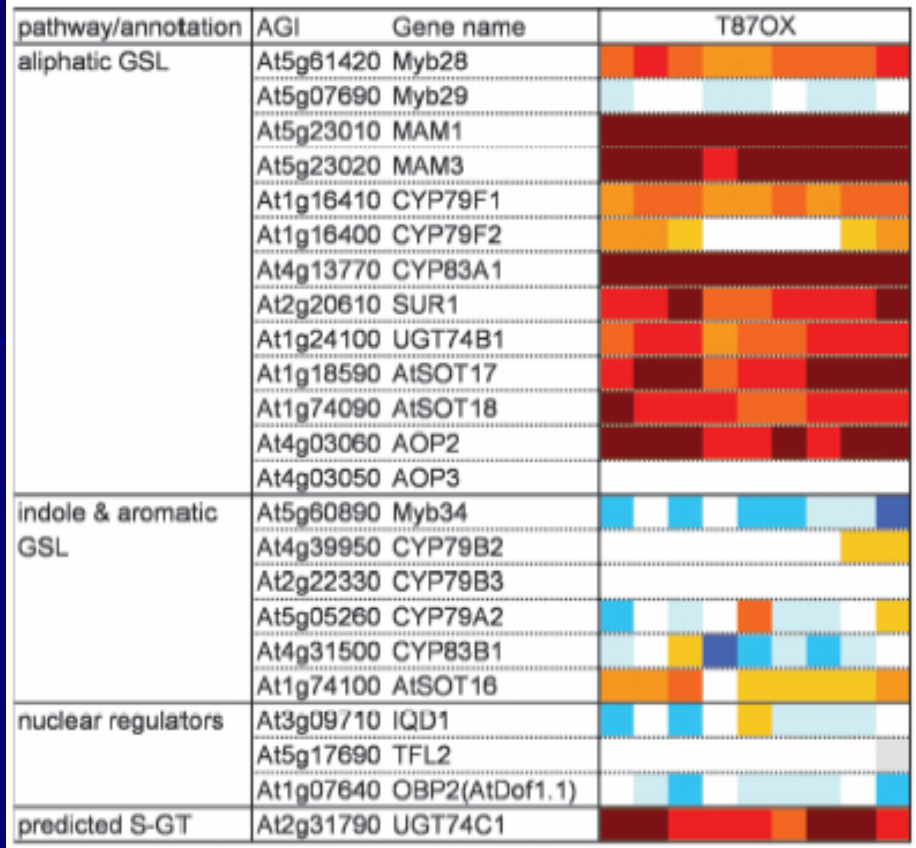
WT leaves

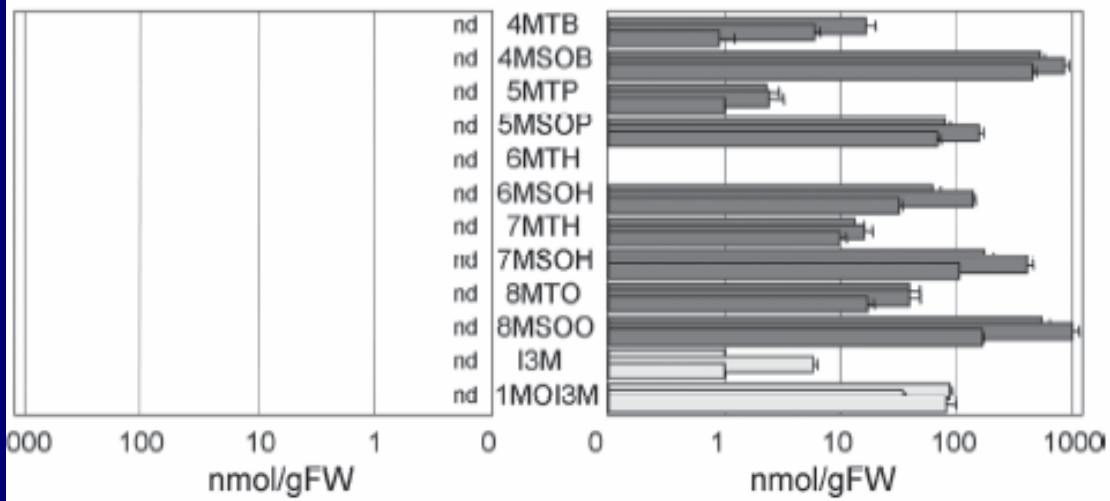
myb28 leaves

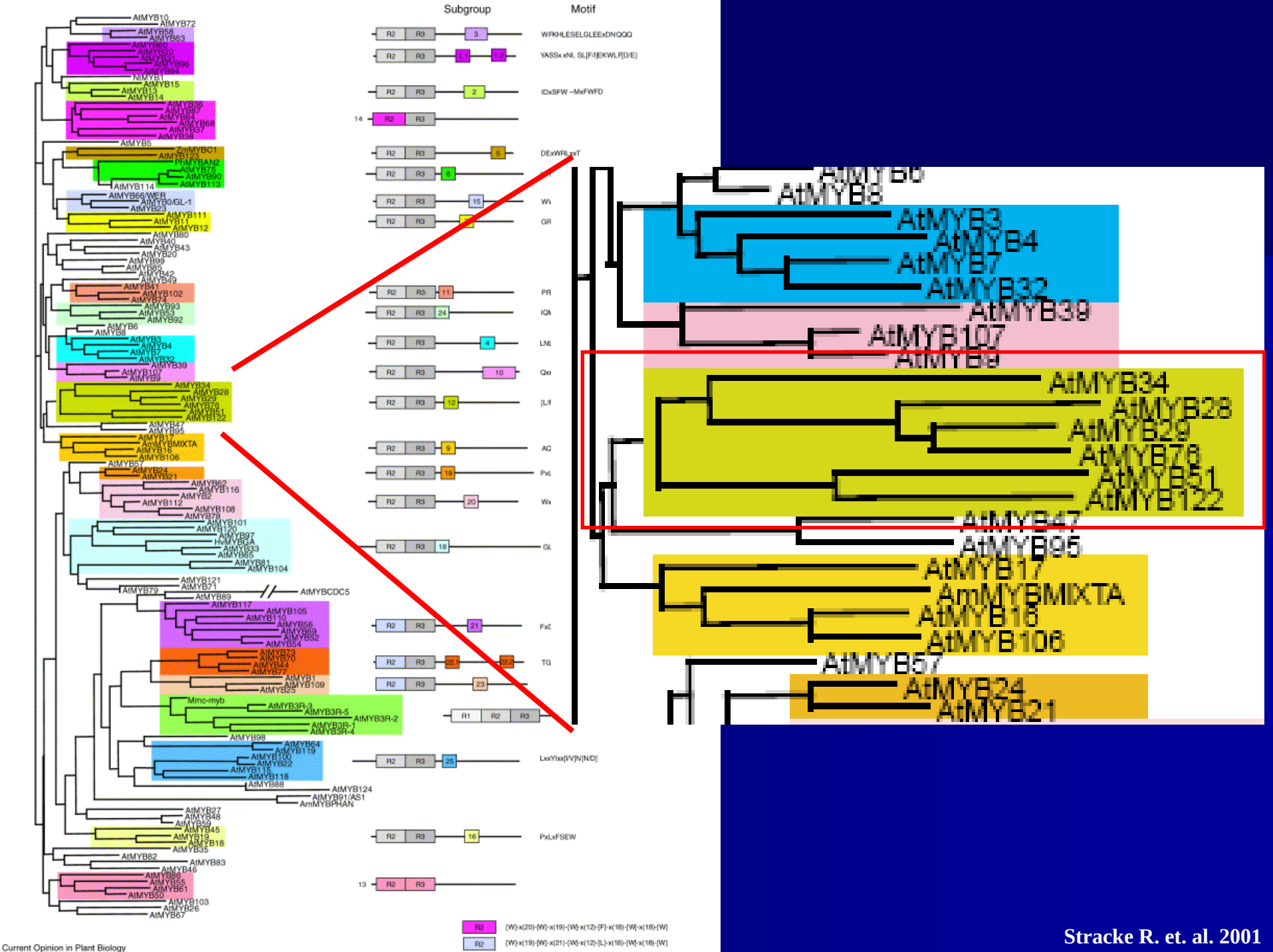
Down regulation of MYB28 results in reduced accumulation of AGs

Regulation of Both Pathways by A Set of MYB Regulators

Overexpression of *MYB28* in Arabidopsis cell cultures results in increased accumulation of AGs related transcripts and AGs






Subgroup
Motif
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AtMYB58
AtMYB63
AtMYB69
AtMYB30
AtMYB31
AtMYB96
AtMYB94
NtMYB1
AtMYB15
AtMYB13
AtMYB14
AtMYB38
AtMYB87
AtMYB84
AtMYB68
AtMYB37
AtMYB39
AtMYB5
ZmMYBC1
AtMYB123
PfMYBAN2
AtMYB75
AtMYB90
AtMYB113
AtMYB114
AtMYB66/WER
AtMYB0/GL-1
AtMYB23
AtMYB111
AtMYB21
AtMYB12
AtMYB80
AtMYB40
AtMYB43
AtMYB20
AtMYB99
AtMYB85
AtMYB42
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DExWRLxxT
PR
IQ
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Qx
Px
Wx
G
Fx
TG
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Current Opinion in Plant Biology
Stracke R. et. al. 2001

Regulation of Both Pathways by A Set of MYB Regulators

The Plant Journal (2007) **50**, 886–901 doi: 10.1111/j.1365-313X.2007.03099.x

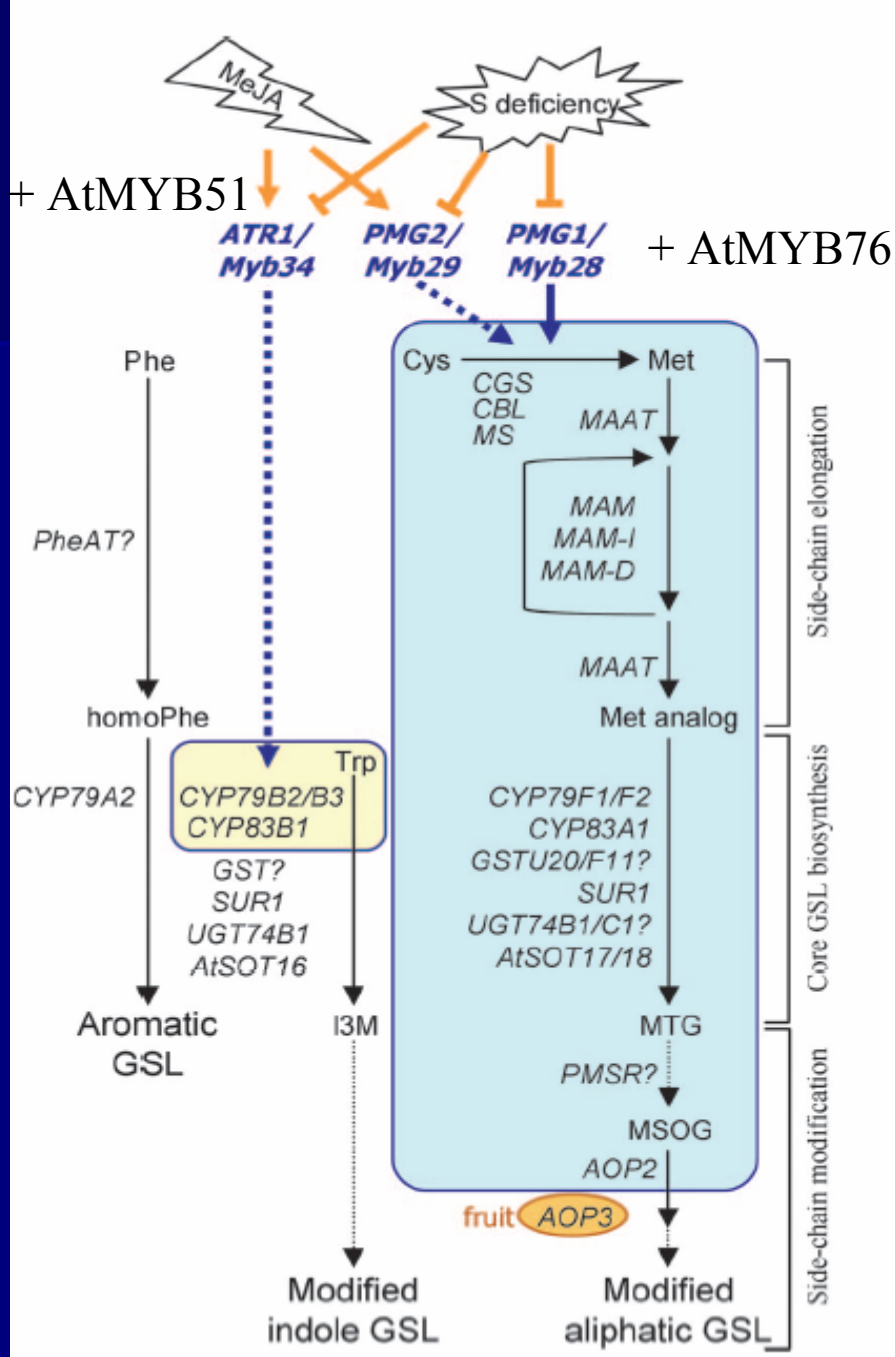
The transcription factor HIG1/MYB51 regulates indolic glucosinolate biosynthesis in *Arabidopsis thaliana*

Tamara Gigolashvili[1], Bettina Berger[1], Hans-Peter Mock[2], Caroline Müller[3], Bernd Weisshaar[4] and Ulf-Ingo Flügge[1,*]

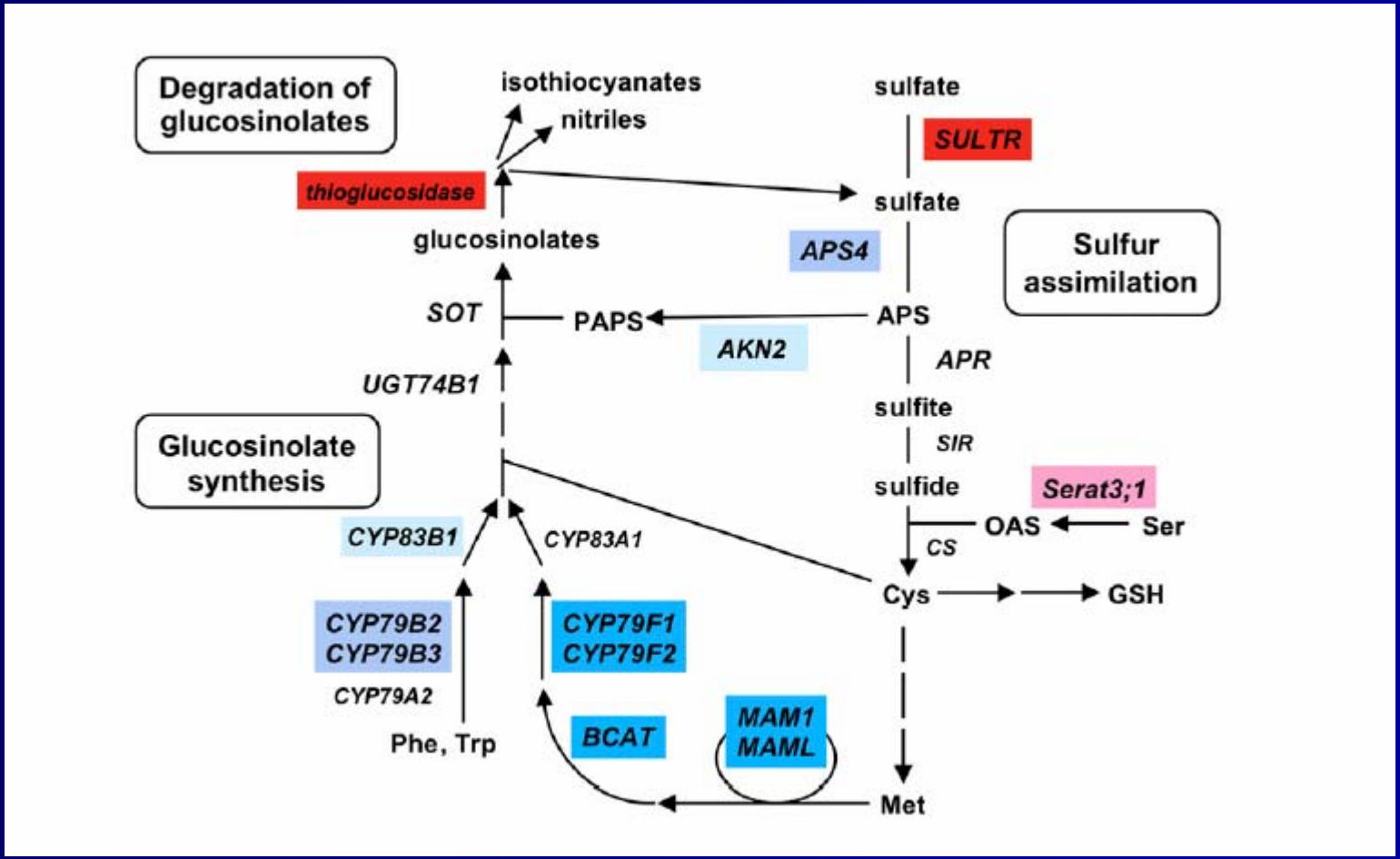
The Plant Journal (2007) doi: 10.1111/j.1365-313X.2007.03133.x

The R2R3-MYB transcription factor HAG1/MYB28 is a regulator of methionine-derived glucosinolate biosynthesis in *Arabidopsis thaliana*

Tamara Gigolashvili[1,†], Ruslan Yatusevich[1,†], Bettina Berger[1], Caroline Müller[2] and Ulf-Ingo Flügge[1,*]

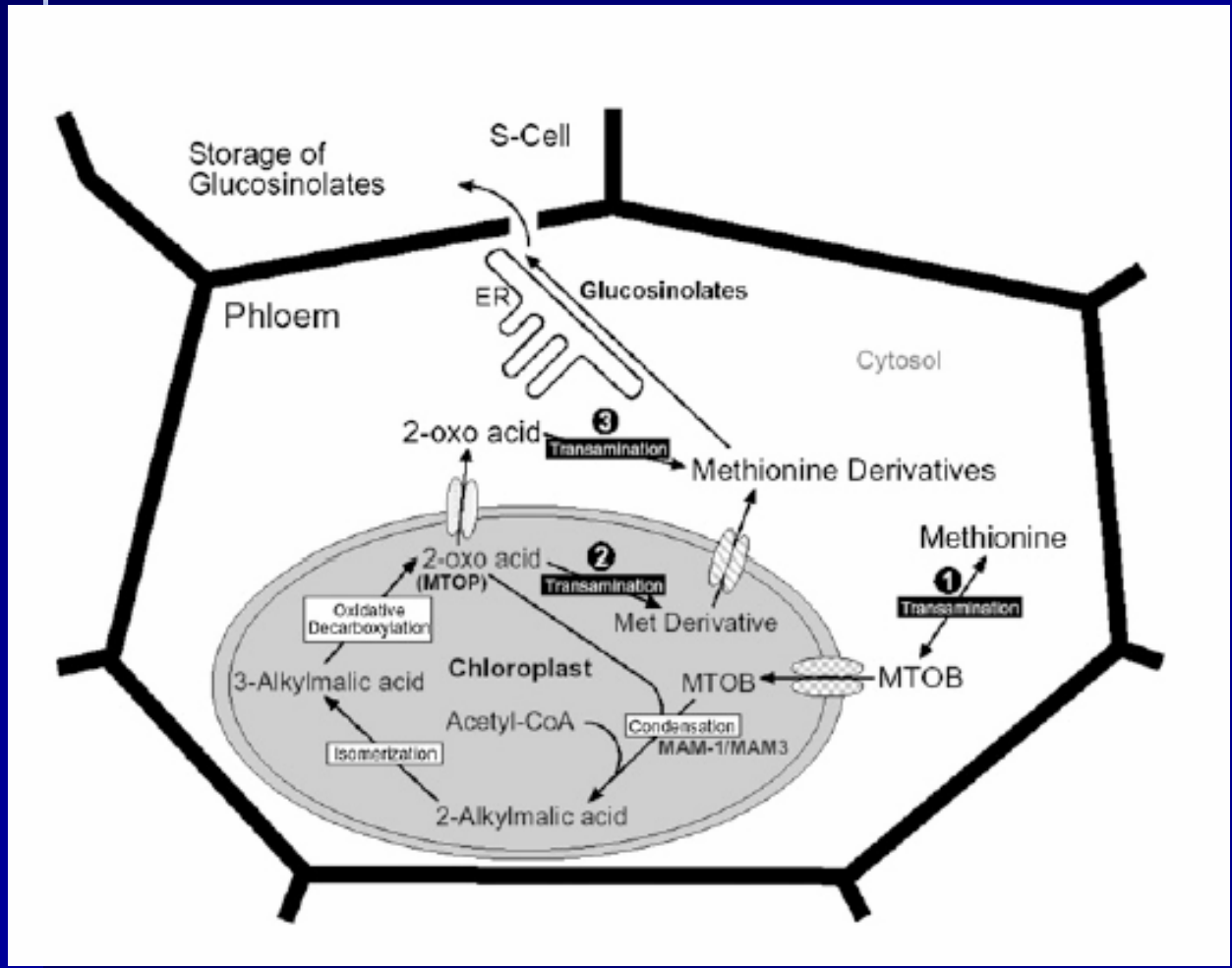


Regulation of the Glucosinolate and Sulfur Limitation (SLIM1 factor)



Marayuma-Nakashita et al, 2007

The Aliphatic Glucosinolates (from Met)- Subcellular Location of Enzymes



Schuster et al., 2007

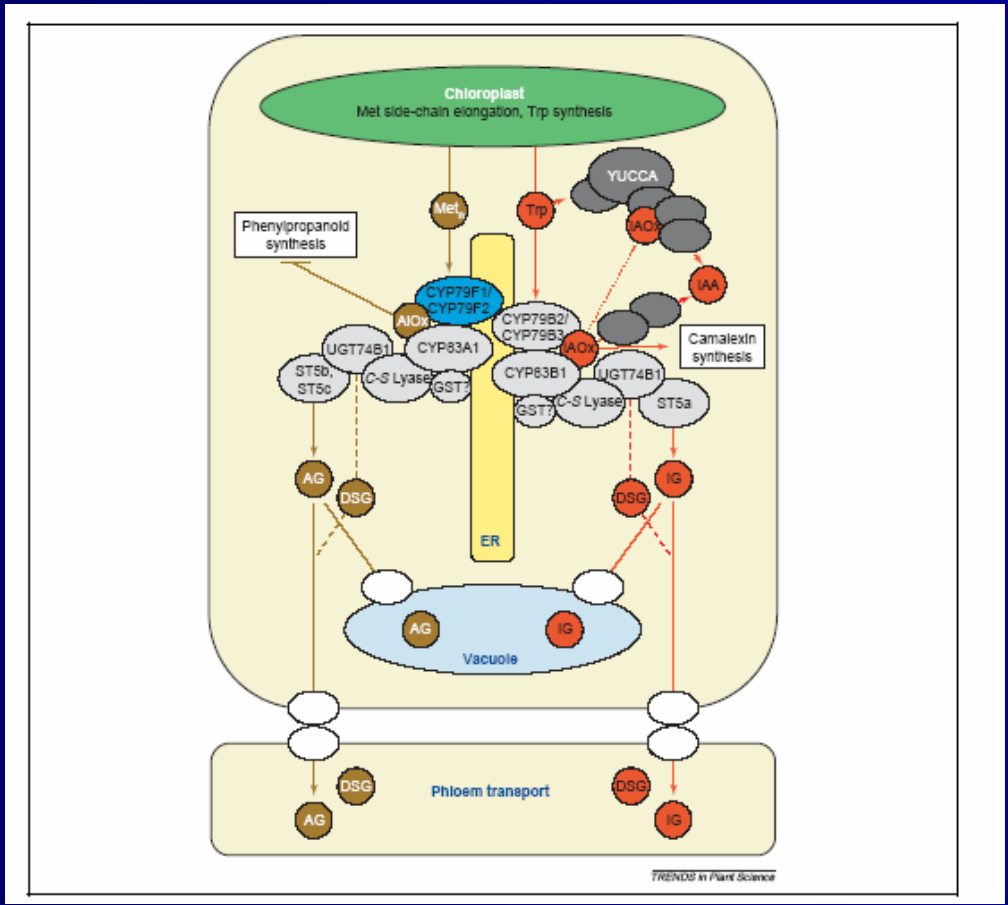
The Aliphatic Glucosinolates (from Met)- Subcellular Location of Enzymes

- BCAT (aminotransferase acting on Met) cytosol

- MAM and other chain elongation pathway enzymes are located in plastids

- Metabolism of Met derivatives in the ER and interface with cytosol

The Glucosinolates Metabolon



Grubb and Abel, 2006

Cyanogenic Compounds from Plants

Cyanogenic Compounds

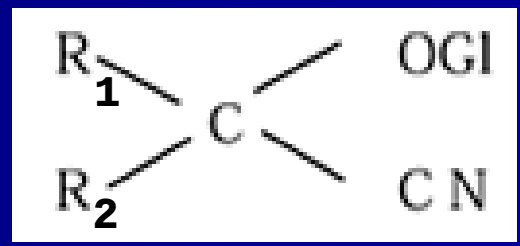
- Plants ability to produce cyanide (or cyanogenesis) is known for a long time

- Structure of only about 60 cyanogenic compounds have been published (in 2002)

- These (N-containing) compounds are either cyanogenic glycosides (glycosides of α-hydroxynitiles/ cyanohydrins)
Or: a few cyanogenic lipids

Cyanogenic Glycosides

- Based on the general formula:

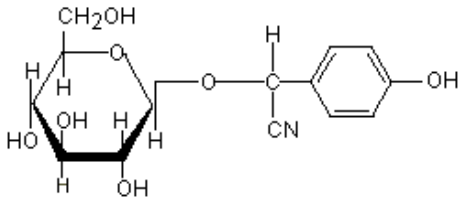


- The sugar residue is almost always D-glucose joined by an 0-beta-D-glucosyl linkage

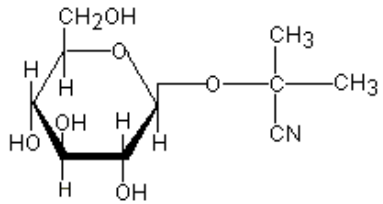
- R_1 is either an aliphatic or aromatic group and R_2 is mainly H

Cyanogenic Glycosides

Dhurrin

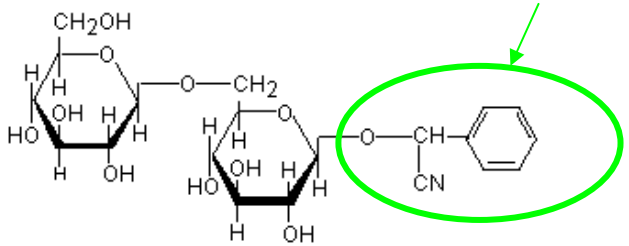


Linamarin

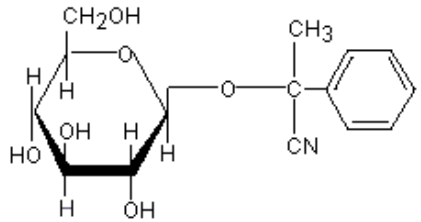


Amygdalin

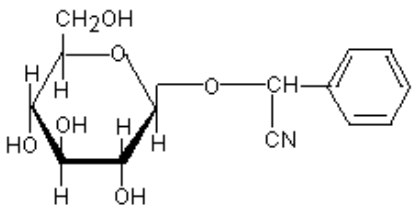
cyanohydrin



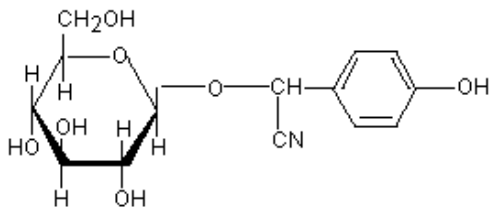
Lotaustralin



Prunasin



Taxiphyllin



Cyanogenic Glycosides

- Classified according to the AMINO ACID source of the R_1 group

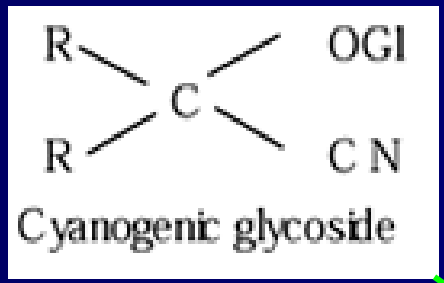
- Valine, Isoleucine, Leucine, Phenylaalanine, Tyrosine

Cyanogenic Glycosides Catabolism

- A beta-glycosidase will often produce the cyanohydrin (aglycon) and a sugar

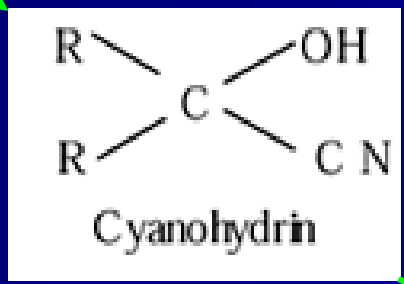
- A second type of enzyme (hydroxyynitrile lyase) will catalyse the dissociation of the cyanohydrin to a carbonyl compound and HCN (hydrogen cyanide)

Cyanogenic Glycosides Catabolism



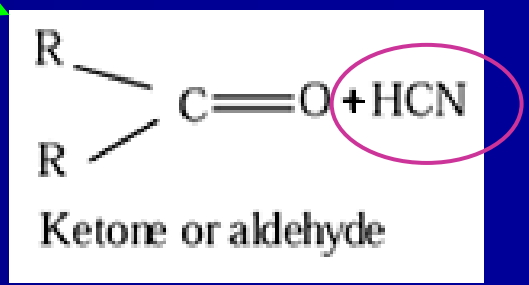
Cyanogenic glycoside

Beta-glycosidase



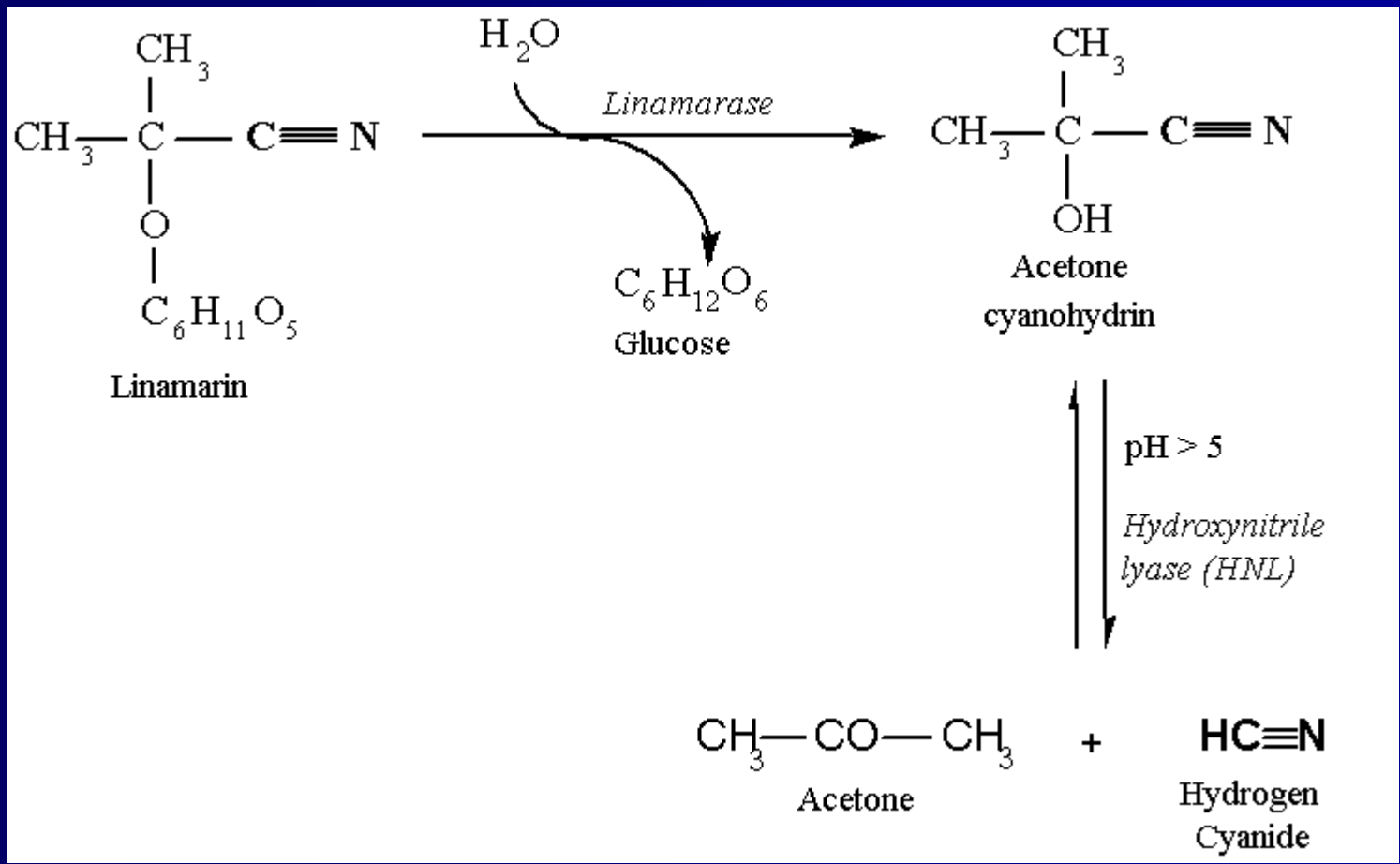
Cyanohydrin

Lyase



Ketone or aldehyde

Linamarin Catabolism (from Valine)



Cyanogenic Glycosides & HCN

- Hydrogen cyanide HCN, (cyanohydric acid or prussic acid)

- A powerful poison (to a wide spectrum of organisms), volatile colorless liquid with the odor of bitter almonds

- It is toxic due to its ability to link with metals (e.g. Fe++ and Mn++) that are functional groups of many enzymes

- It inhibits processes such as the reduction of oxygen in the cytochrome respiratory chain, electron transport in photosynthesis and the activity of enzymes such as catalase and oxidase

Cyanogenic Glycosides & HCN

- Prussic acid passes through the mucous membranes and the skin, but principally through the lungs, into the blood

- It blocks the process by which oxygen is released from red blood corpuscles

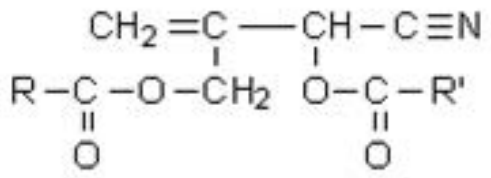
- Zyklon B- consisted of prussic acid

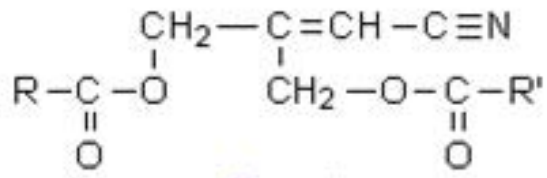
Cyanogenic Lipids

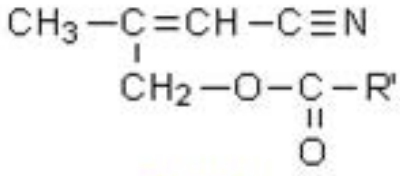
- Long-chain fatty acids (C_{18} or C_{20}) attached to the hydroxynitrile

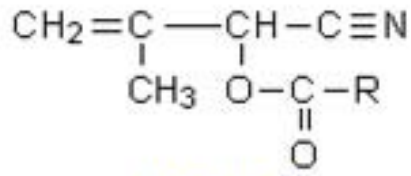
- In the seeds of the Sapindaceae (4 types)

Allophylus cobbe









Cyanogenic Plants

Prunus species
Sorghum
Lotus
Apples, peaches, cherries and apricots
Flax
Corn
Cassava
Almonds
Passion fruit

Cyanogenic Plants

- Cyanogenesis is a mechanism against predators such as herbivores (other functions are also N storage and production of antifungal compounds from their aglycons)

- Normally, substrates and enzymes are localized to different subcellular compartments

- Upon damage they are mixed

Cyanogenic Plants

- Bacteria, fungi and animals are also cyanogenic

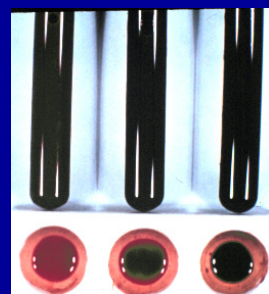
- Cyanogenicity is often associated with certain species but to different degree

- Certain structural types of cyanogenic glycosides are associated with specific groups of plants

Cyanogenic Plants

- The presence of cyanogenic compounds in food and forage plants is a problem (e.g. Cassava and Sorghum)

- Identification of cyanogenesis could be done by simple color tests (Guignard and Feigl-Anger)



Cyanogenic Crop Plants- Cassava

Staple Diet (second in Africa)

Cassava (*Manihot esculenta*, Crantz) roots (starchy food) are the primary source of calories for more than 500 million people, the majority of whom live in the developing countries of Africa

Cyanogenic Crop Plants- Cassava

- Plants with 20mg HCN /100 g fresh weight are considered toxic

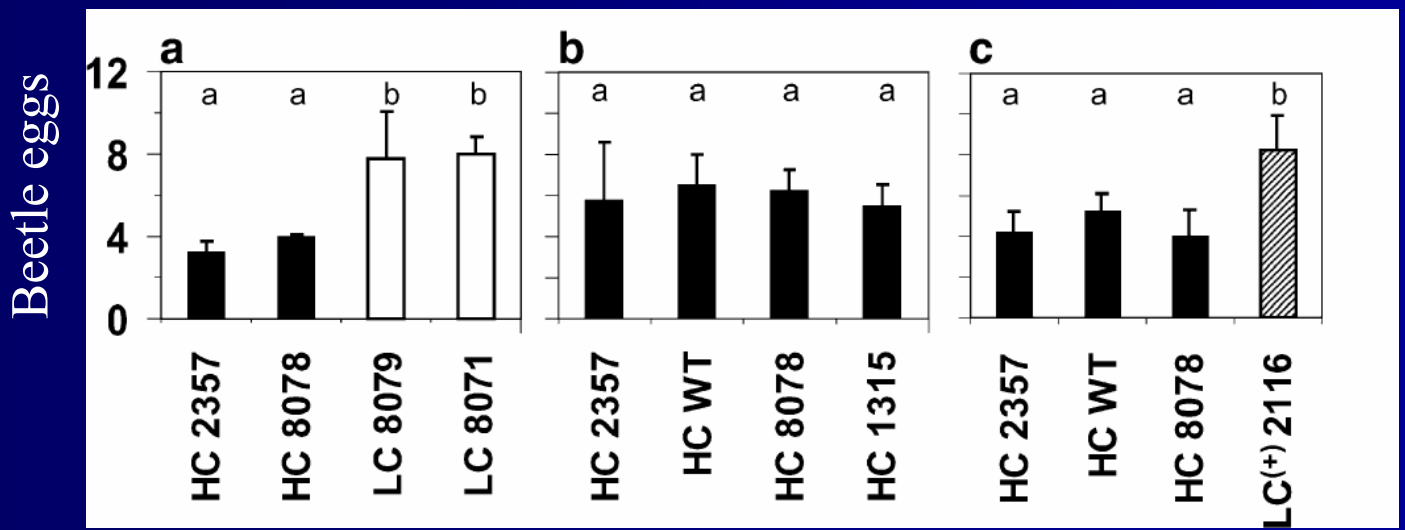
- Many cultivars exceed this value

- Detoxication methods developed by native Indians consuming the root:

grating or grinding the plant, pressing the ground material to remove liquid containing HCN (thus mixing the glucosidase with its substrate), storing the product and cooking)

Cyanogenic Plants- Activity in Plants

- Lima bean accessions with different cyanogenic capacity (black-high release/ open-low release/ strips-high precursors, low beta-gal, low release)



Ballhorn and Leiberei, 2006

Cyanogenic Plants- Activity in Plants

- Cyanogenic glycosides sometimes represent a substantial proportion of the plants nitrogen

- In some cases they are converted into other metabolites once moved from seeds to cotyledons

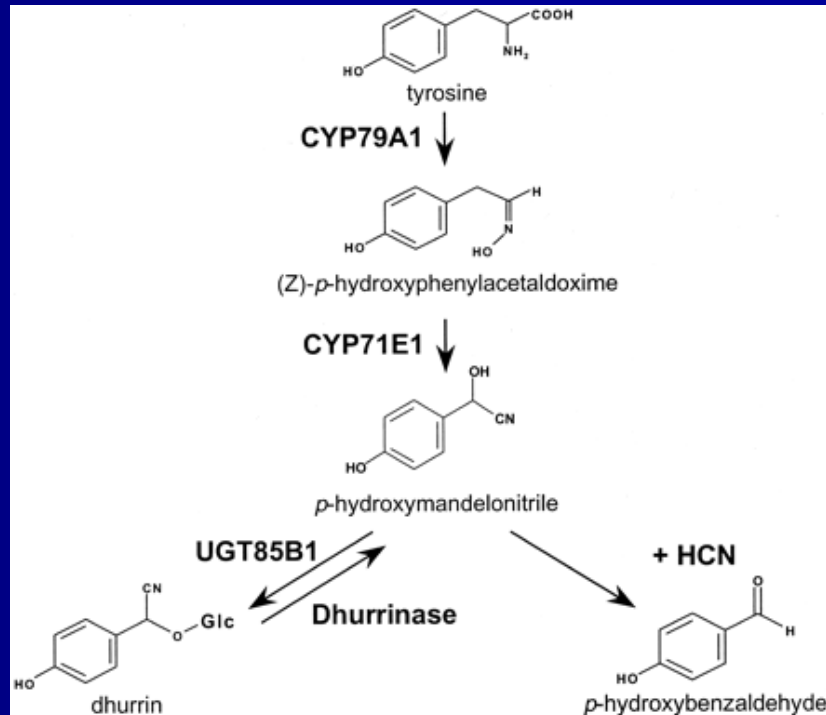
- Plants detoxify HCN by conversion into Asparagine and beta-cyanoalanine

Dhurrin in Sorghum (*Sorghum bicolour*)

- Dhurrin- highest accumulation in seedlings

- Content varies in plant age and growth conditions

- CYP79A1, CYP71E1 and the glucosyl transferase UGT85B1 catalyse its biosynthesis from Tyrosine (Metabolon formation?)



Busk and Muller, 2002

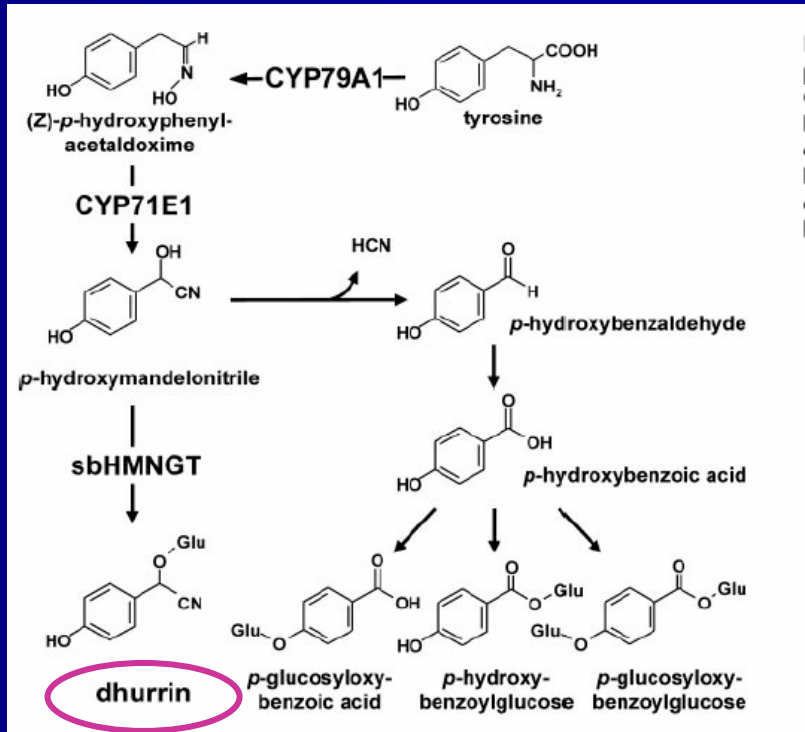
Engineering Dhurrin

- Introducing the entire pathway (CYP71E1/CYP79A1/sbHMNGT)

- Accumulation of 4 +/- 0.5 mg dhurrin per gram fw

- Similar to the concentration in sorghum bicolour seedlings

- Small reductions in growth



Resistance to an Herbivore Through Engineered Cyanogenic Glucoside Synthesis

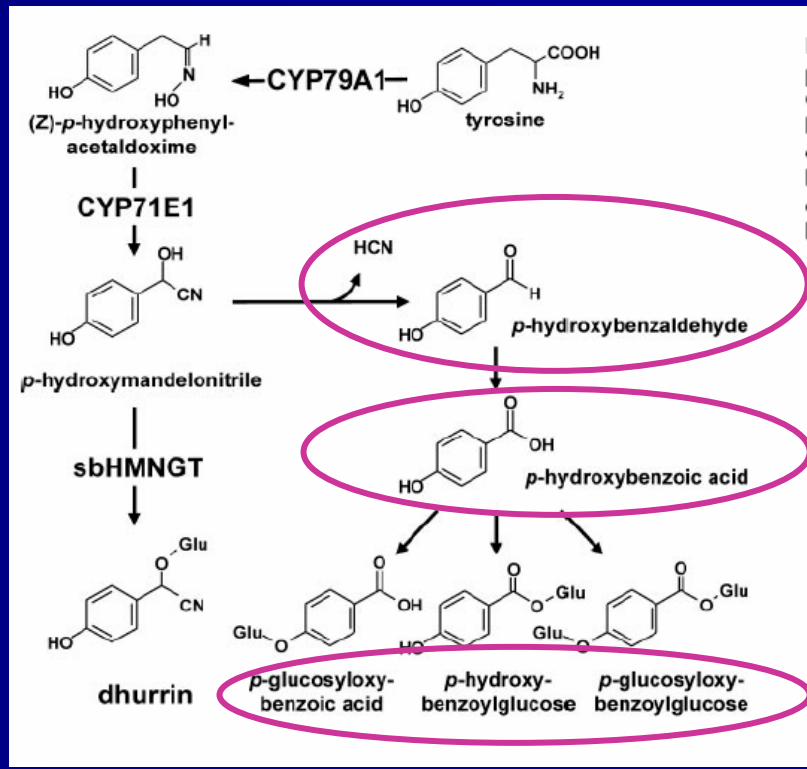
David B. Tattersall,[1,2] Søren Bak,[1,2*] Patrik R. Jones,[1,2,4*] Carl Erik Olsen,[2,3] Jens K. Nielsen,[3] Mads L. Hansen,[3] Peter B. Høj,[4,5] Birger Lindberg Møller[1,2†]

Engineering Dhurrin

- Introducing the P450's ONLY (CYP71E1/CYP79A1)

- Accumulation of p-hydroxybenzaldehyde and its derivatives

- More than 100 GTs of Arabidopsis cannot convert p-hydroxymandonitrile to dhurrin
- Severe phenotypes



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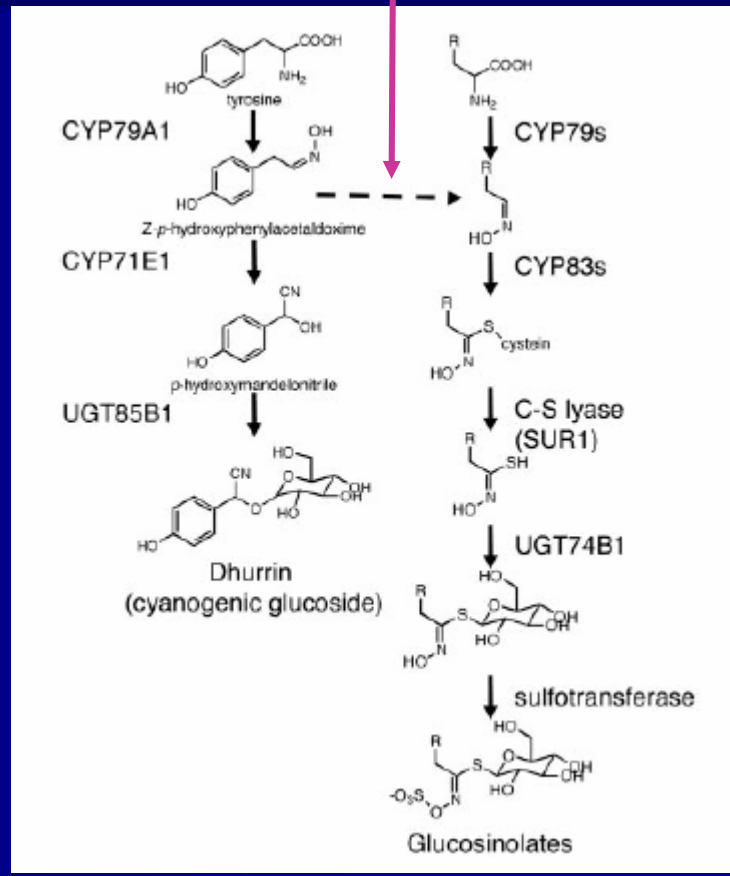
Engineering Dhurrin

- Introducing CYP79A1 ONLY

- The product of the CYP79A1 reaction could serve as substrate for CYP83 from the Glucosinolate pathway

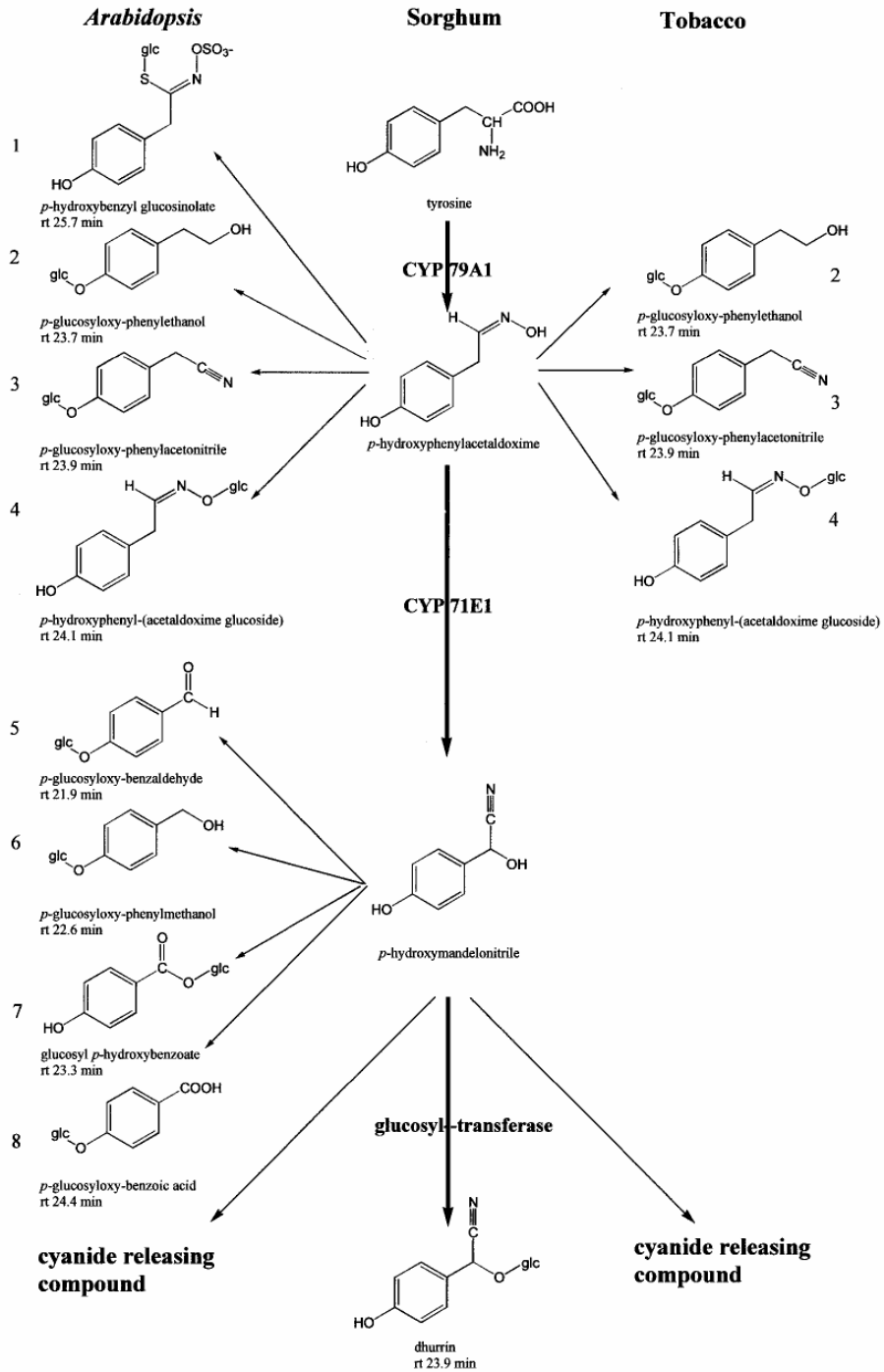
- Presence of CYP71E1 prevents this interaction



Engineering Dhurrin

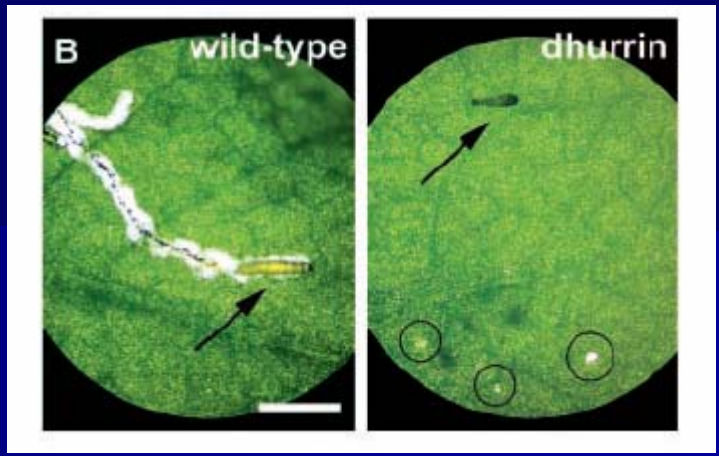
- Introducing the CYP's also to transgenic Tobacco

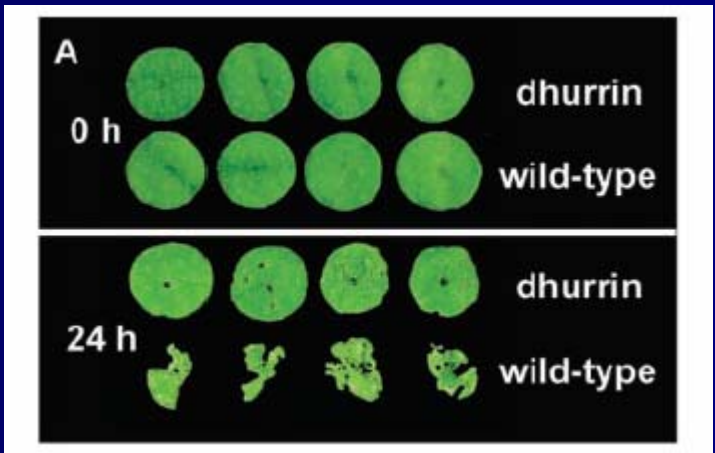
Bak et al., 2002

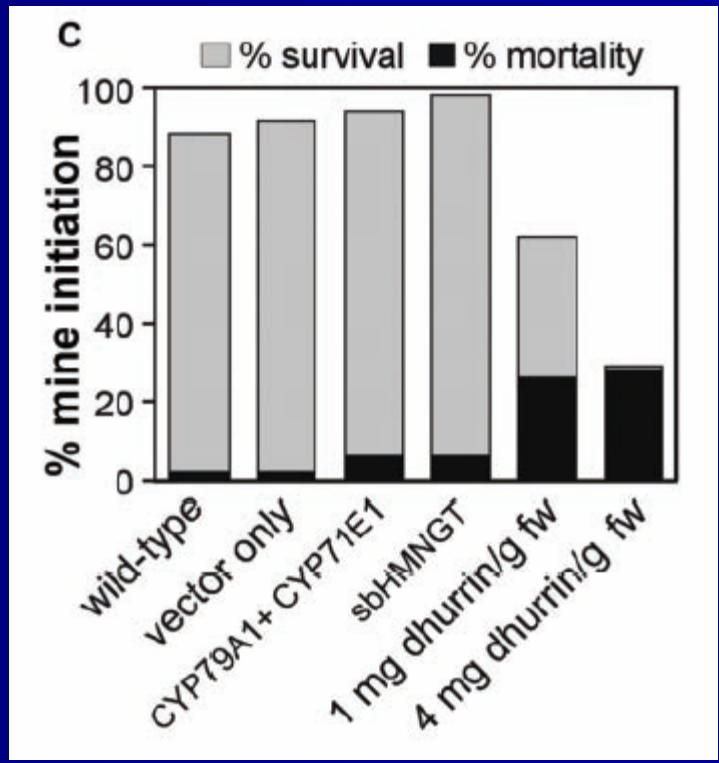


Engineering Dhurrin

- Flea beetle and larvae feeding







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Engineering Cyanogenic Glucosides levels in Cassava

- Traditional breeding could reduce levels of Cyanogenic Glucosides but not cassava devoid of these compounds

- Valine and Isoleucine are the precursors for Linamarin and Lotaustralin

- Major amount of Cyangonic glucosides are transported from the shoot to the tubers

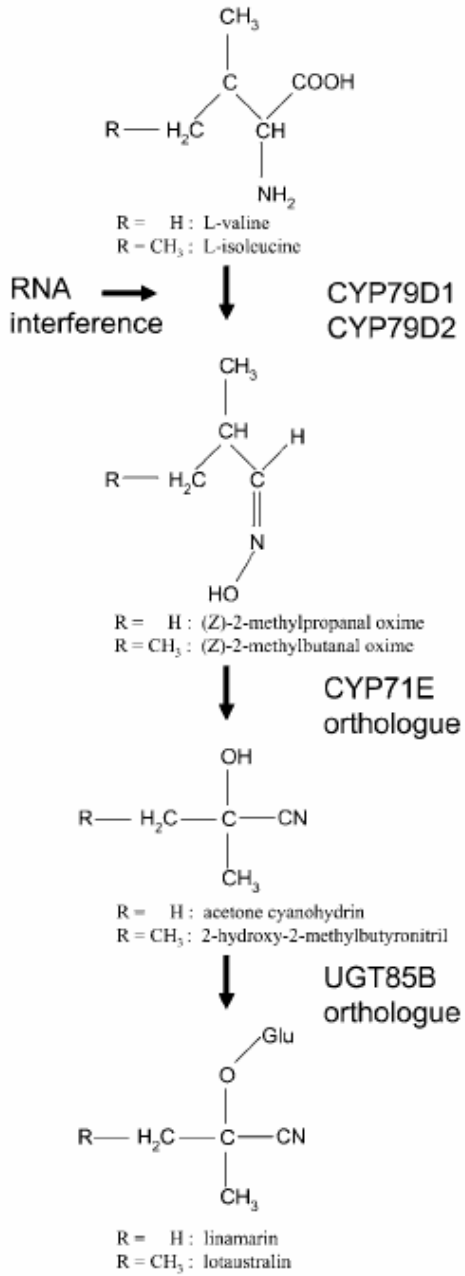


Figure 1. The biosynthetic pathway for the cyanogenic glucosides linamarin and lotaustralin in cassava. The enzymatic step blocked by RNAi technology is indicated.

Cassava Plants with a Depleted Cyanogenic Glucoside Content in Leaves and Tubers. Distribution of Cyanogenic Glucosides, Their Site of Synthesis and Transport, and Blockage of the Biosynthesis by RNA Interference Technology[1]

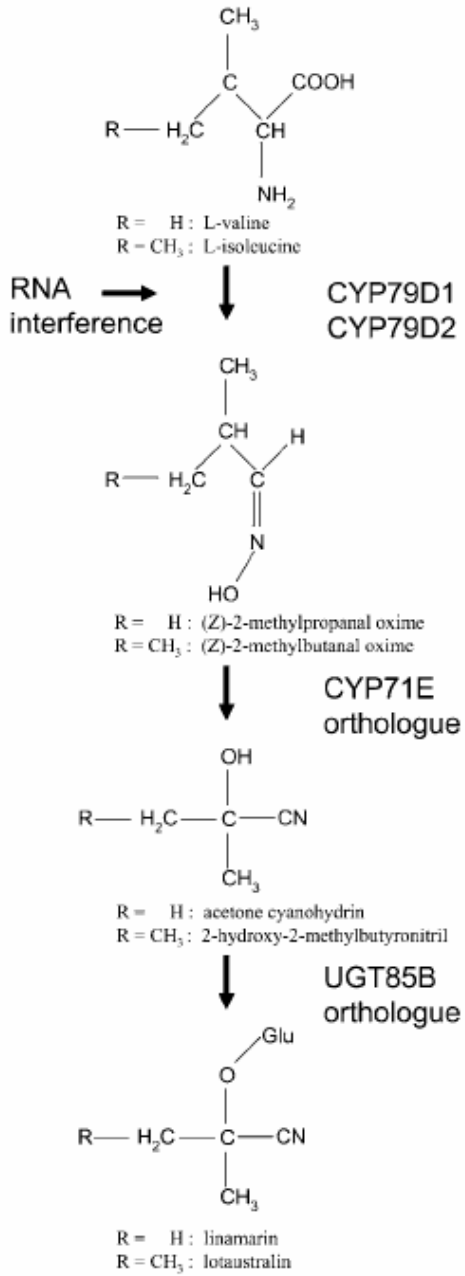
Kirsten Jørgensen, Søren Bak, Peter Kamp Busk[2], Charlotte Sørensen, Carl Erik Olsen, Johanna Puonti-Kaerlas[3], and Birger Lindberg Møller*

Engineering Cyanogenic Glucosides levels in Cassava

- RNAi silencing of the two redundant CYP79D genes (300 independent transgenic lines)

- 92% reduction in cyanogenic glucoside content (Linamarin and lotaustralin) in tubers

- Acyanogenic leaves (<1% of wild-type)



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Engineering Cyanogenic Glucosides Levels in Cassava

- RNAi Cassava lines with <25% of cyanogenic glucoside accumulation exhibited a morphological phenotype when grown in vitro (long internodes, barely any roots, slow growth)

- In soil the wild type phenotype was restored

- Nitrogen deficiency affected the transgenic lines more than wild-type (cyanogenic glucosides as N storage)

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END OF COURSE

- Up to the end of the month (July), a mini review (3-4 A4 pages) on "The secondary to primary metabolism interface"

- Pathways and Metabolites, your choice!