Frontiers in METABOLOME Sciences

Asaph Aharoni, Department of Plant Sciences
Ulmann Building, room 105
Tel: 3643
E.mail: asaph.aharoni@weizmann.ac.il

WEIZMANN INSTITUTE OF SCIENCE
מכון ויצמן למדע

Frontiers in METABOLOME Sciences

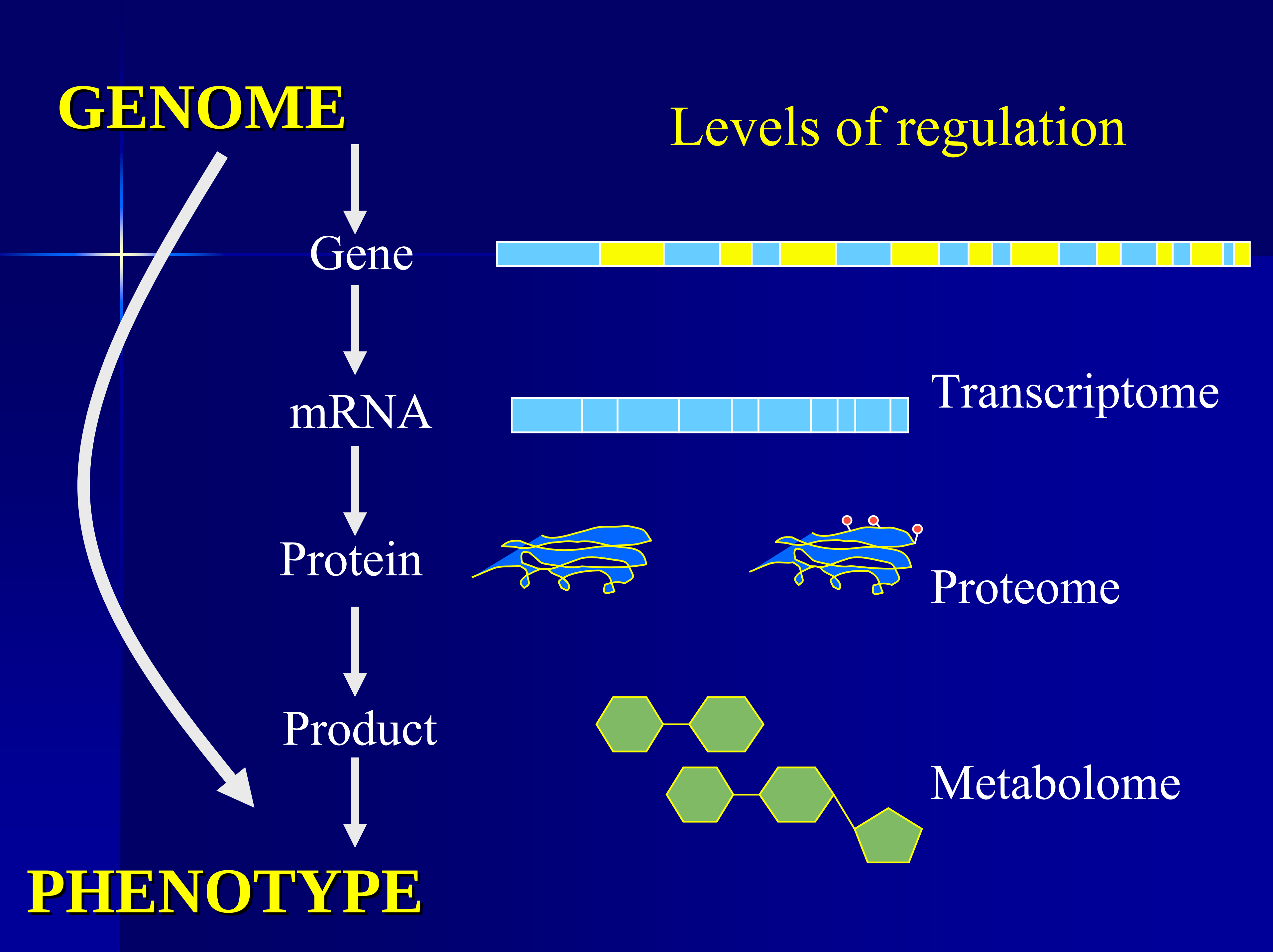
Code: 20063212
Credits: 1.5
Thursday 14:00-16:00, Wolfson Room 18

- Every class, seminar on a topic- 40-45 min. followed by questions and discussion
- End of semester, minireview 3-6 pages on the topic

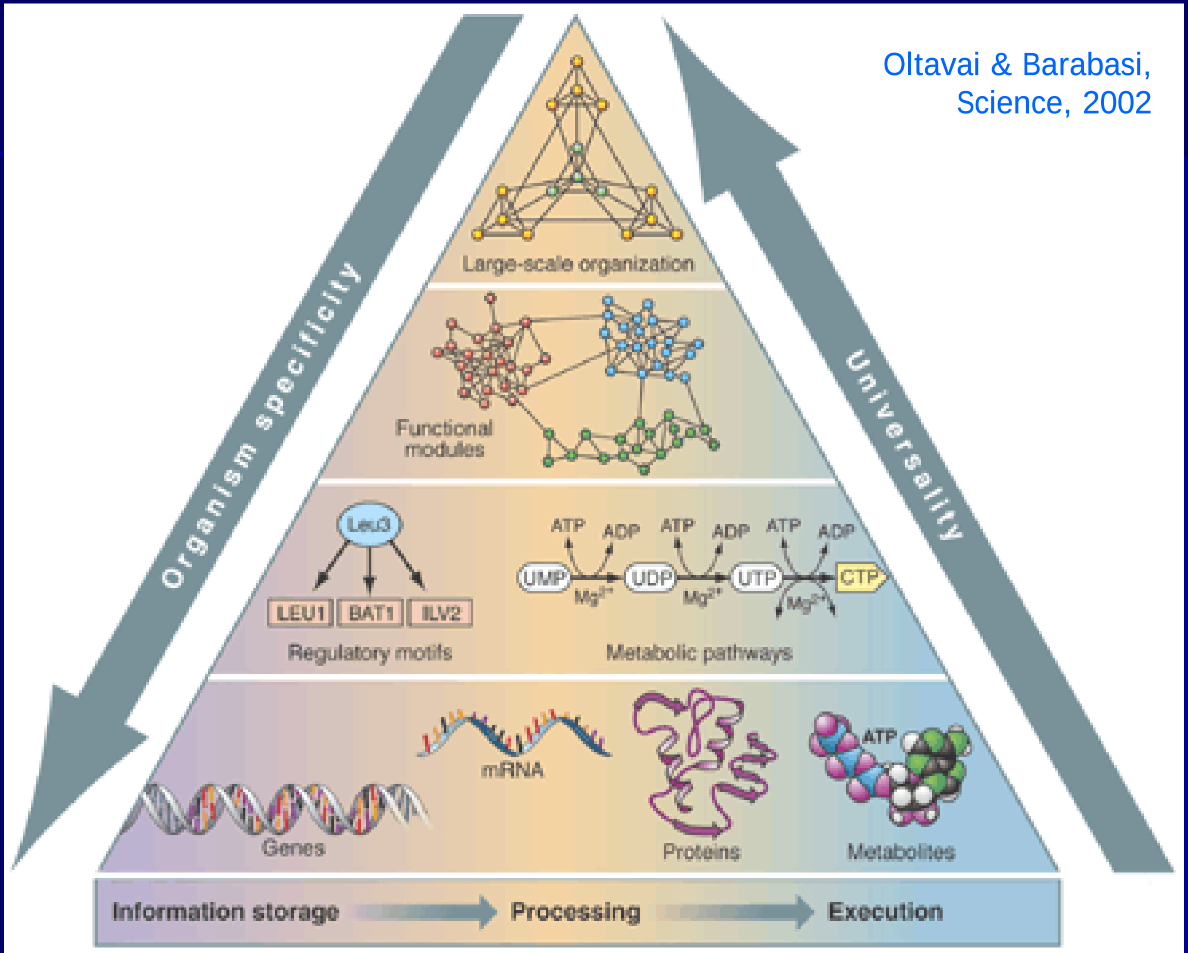
The Metabolome:

Is a comprehensive profile of all the low molecular weight compounds of an organism, a tissue, or a cell

Oliver et al., (1998)


GENOME
Levels of regulation
Gene
mRNA
Transcriptome
Protein
Proteome
Product
Metabolome
PHENOTYPE

Life's Complexity Pyramid



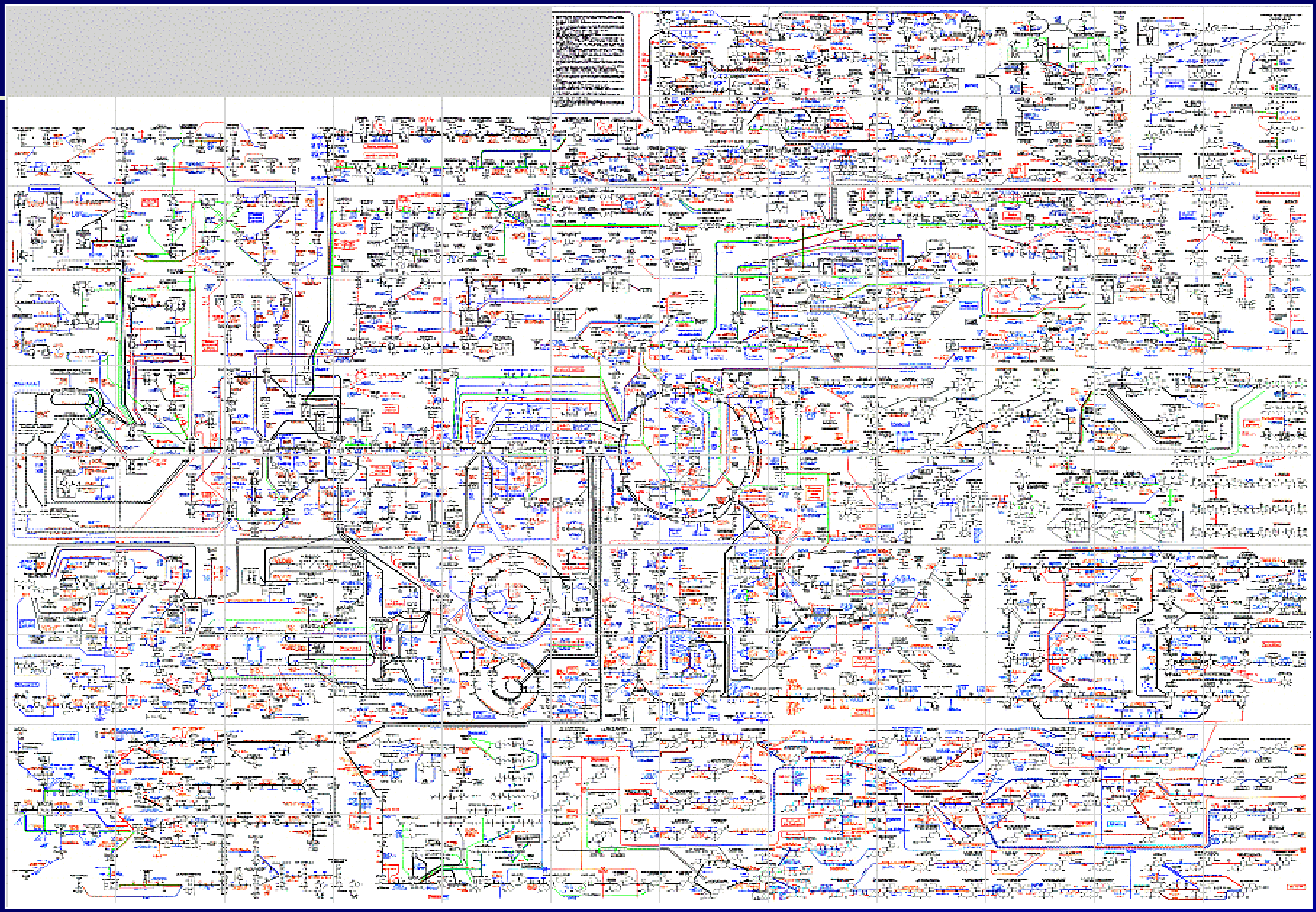
The cell is a complex network in which the components are connected by functional links

What will we hear and talk about?

Recent advances in Metabolome:

- ANALYSIS / MEASURMNET
- REGULATION
- EVOLUTION
- APPLICATIONS
- ENGINEERING

Network of Metabolic Pathways in a Single Cell



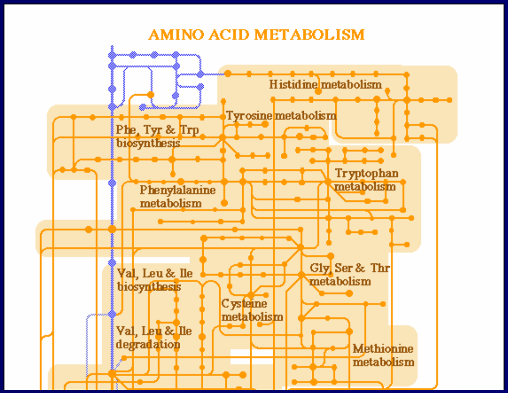
Estimated Size of the Metabolome

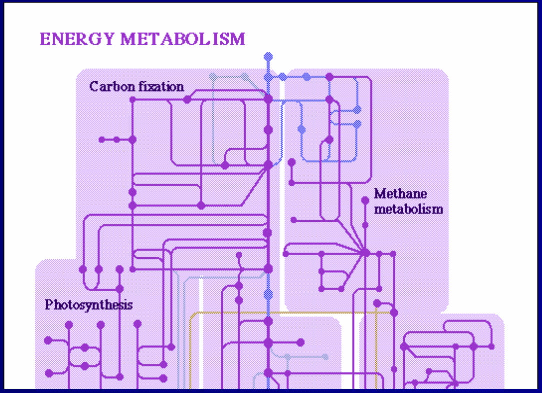
- In yeast 550 compounds in a cell

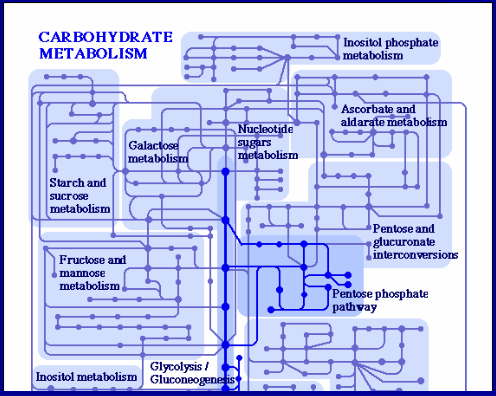
- In plants 4,000 to 20,000 compounds in a cell

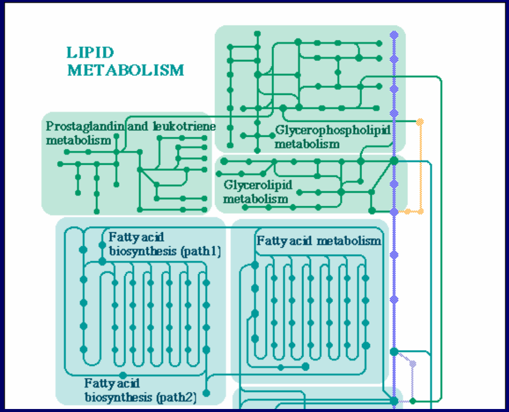
- To date, a total of 200,000 structures identified from plants

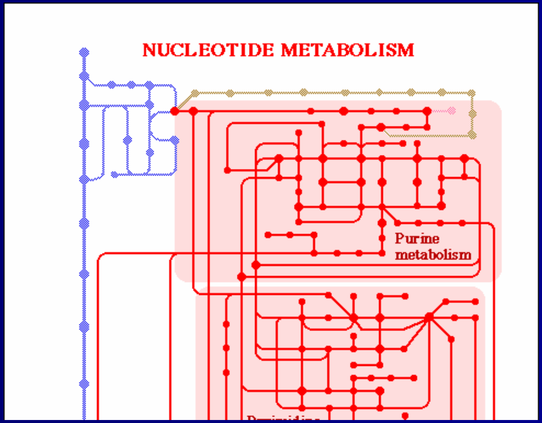
KEGG: 13,803 compounds, 6579 reactions

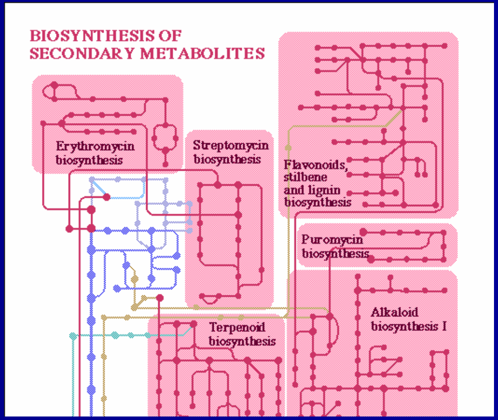








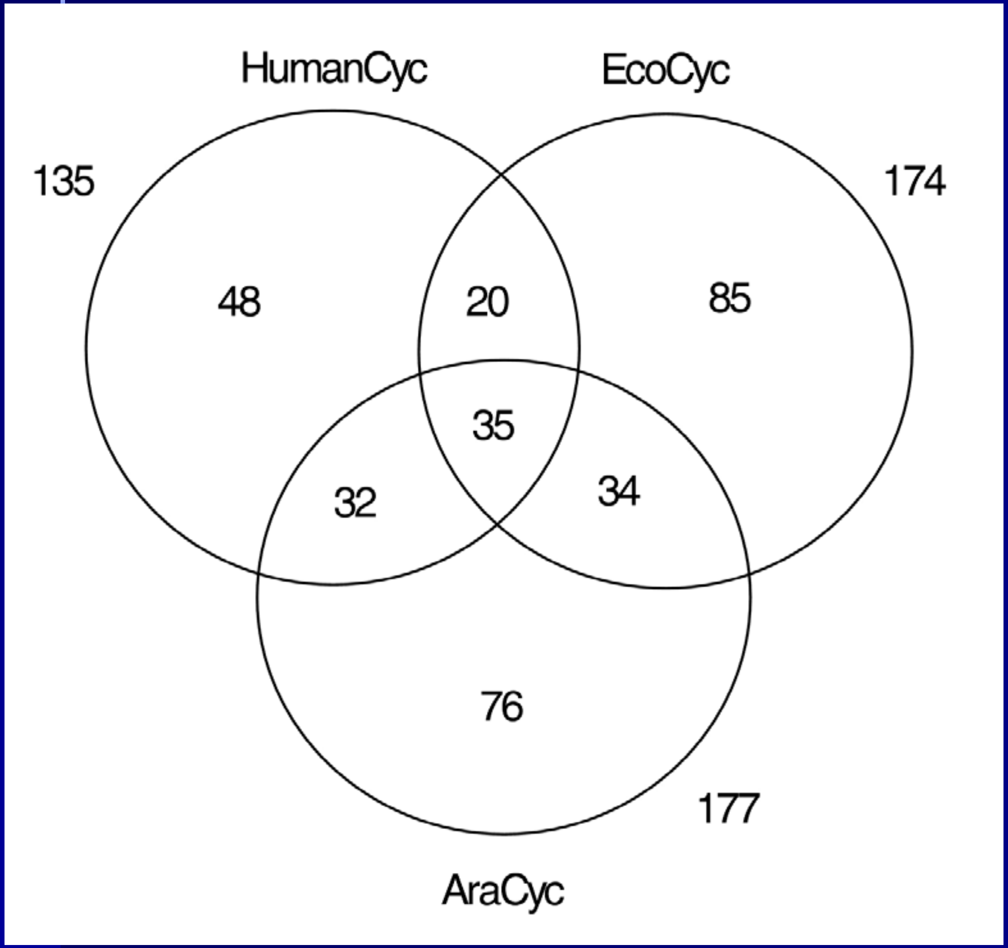




The Metabolome

1. The Metabolome is organism, tissue and cell specific
2. Influenced by the environment

The Metabolome of Human, E.coli & Arabidopsis



According to HumanCyc (http://HumanCyc.org/):

35 metabolic pathways are common to all three organisms

ANALYSING the METABOLOME, WHY?

- Assessing gene function and relationship to phenotypes

- Understanding metabolism and predicting novel pathways

- To increase metabolite fluxes into valuable biochemical pathways using metabolic engineering

- To compare genetically modified organisms to non-modified

- To measure flux of carbon under varying conditions

- To asses the effect of environmental changes

ANALYSING the METABOLOME

- More complicated compared to protein and nucleic acids:

- Unknown pathways
- Often difficult to purify
- Can be impossible to synthesize
- No amplification

ANALYSING the METABOLOME

Different metabolites, different characteristics:

- volatile
- non volatile: polar, semi polar and apolar

ANALYSING the METABOLOME

Making the difference:

Elemental composition

Order of the atoms

Type of bonds

Stereochemical orientation

(+)-Carvone (-)-Carvone

ANALYSING the METABOLOME

- Ideally: The comprehensive, quantitative and qualitative analysis of all metabolites within a cell, tissue or an organism

- Far from reality in any system

- Multiple technologies are required

Analysing the METABOLOME

1. Metabolite Extraction
2. Metabolite (separation not always) detection
3. Data analysis:

- From raw data to information which is ready for mining
- Extraction of biological relevance
- Data visualization (maps, tables, charts etc..)

Analysing the METABOLOME

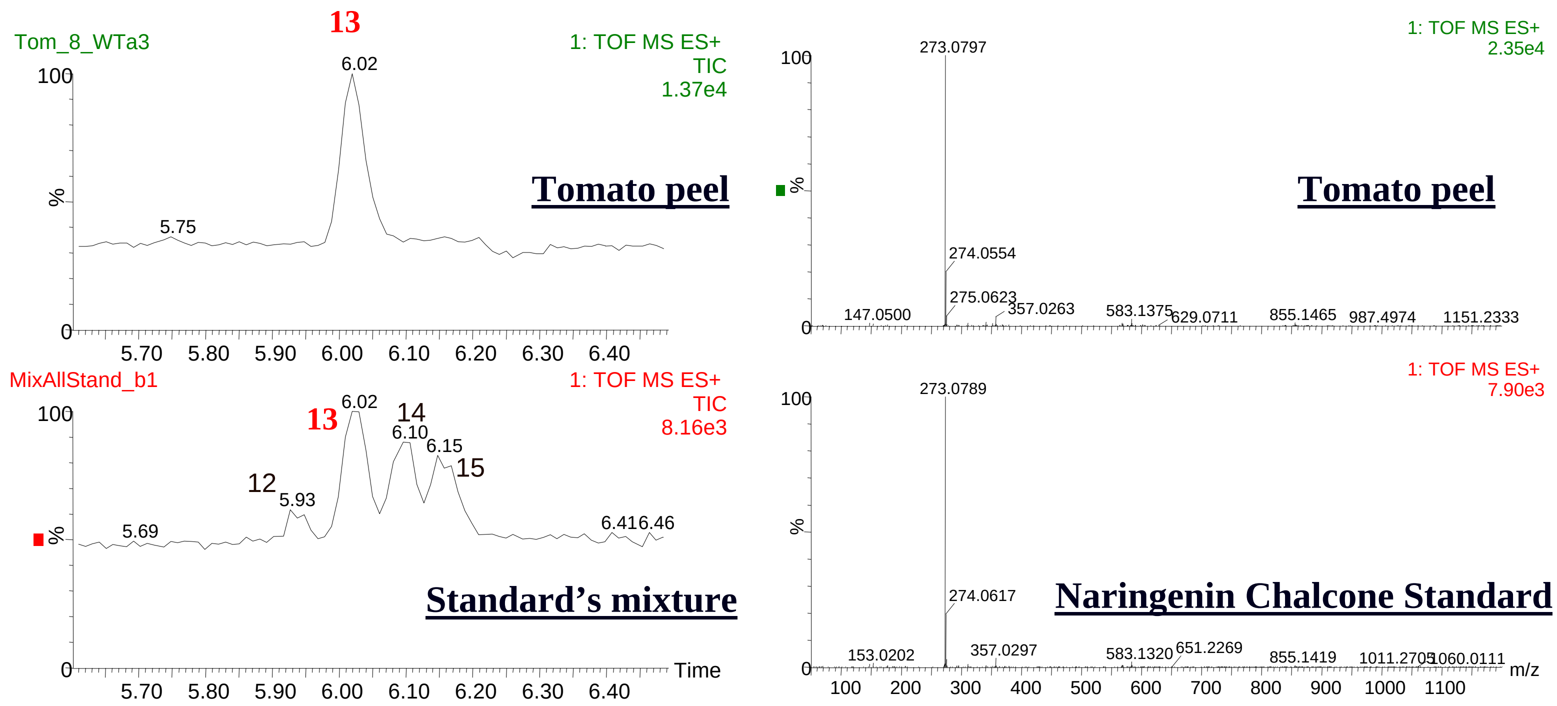
- Metabolite target analysis: just a few specific compounds
- Metabolic profiling: classes of compounds
- Metabolic fingerprinting: with pattern recognition
- Metabolomics: as comprehensive as possible ⁂

Fiehn, Comp. Func. Genomics 2,155-168 (2001)

Metabolome Analyses Technologies

- Infrared spectroscopy (IR)
- Nuclear magnetic resonance (NMR)
- Thin layer chromatography (TLC)
- High performance liquid chromatography (UV & photodiode array)(LC/UV/PDA)
- Capillary electrophoresis coupled to UV absorbance detection (CE/UV)
- Capillary electrophoresis coupled to laser induced fluoresence detection(CE/LIF)
- Capillary electrophoresis coupled to mass spectrometry (CE/MS)
- Gas chromatography mass spectrometry (GC/MS)
- Liquid chromatography mass spectrometry (LC/MS)
- Liquid chromatography tandem mass spectrometry (LC/MS/MS)
- Fourier transform ion cyclotron mass spectrometry (FTMS)
- HPLC coupled with MS and NMR detection (LC/NMR/MS)
- LC/NMR/MS/MS

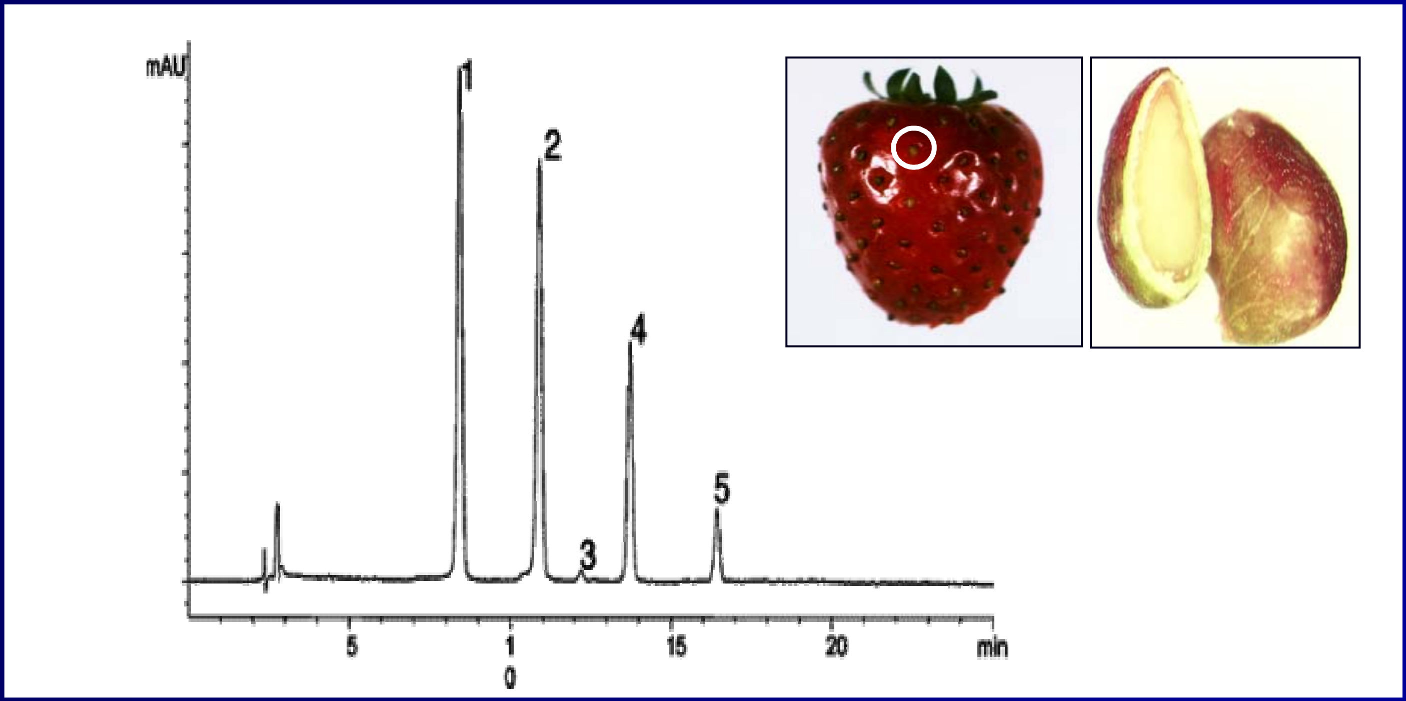
Metabolite Target Analysis



12 – Cinnamic acid
13 – Naringenin Chalcone
14 – Naringenin
15 – Kaempferol

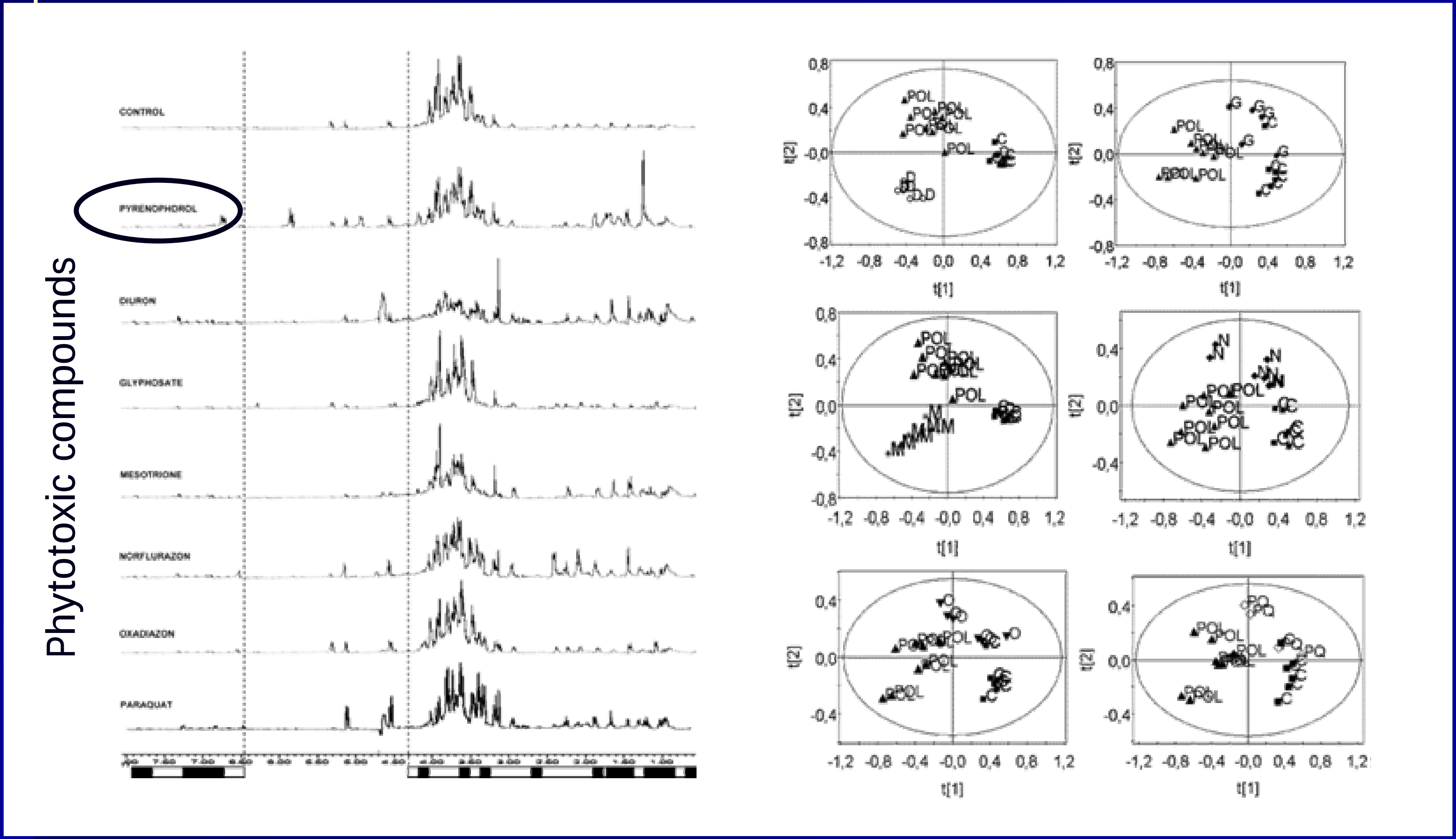
Metabolic profiling

HPLC chromatograms (recorded at 520 nm) of anthocyanins of red ripe strawberries ACHENES: 1 = cyanidin-3-glucoside; 2 = pelargonidin-3-glucoside; 3 = pelargonidin-3-rutinoside; 4 = cyanidin-3-glucoside-malonate; 5 = pelargonidin-3-glucoside-malonate .



Metabolic Fingerprinting

Investigation of the Mode of Action of the Phytotoxin (5S,8R,13S,16R)-(-)-Pyrenophorol Using (1)H Nuclear Magnetic Resonance Fingerprinting (Konstantinos A. Aliferis and Maria Chrysayi-Tokousbalides, JAFC, 2006)



Metabolic FOOTprinting or Exometabolome

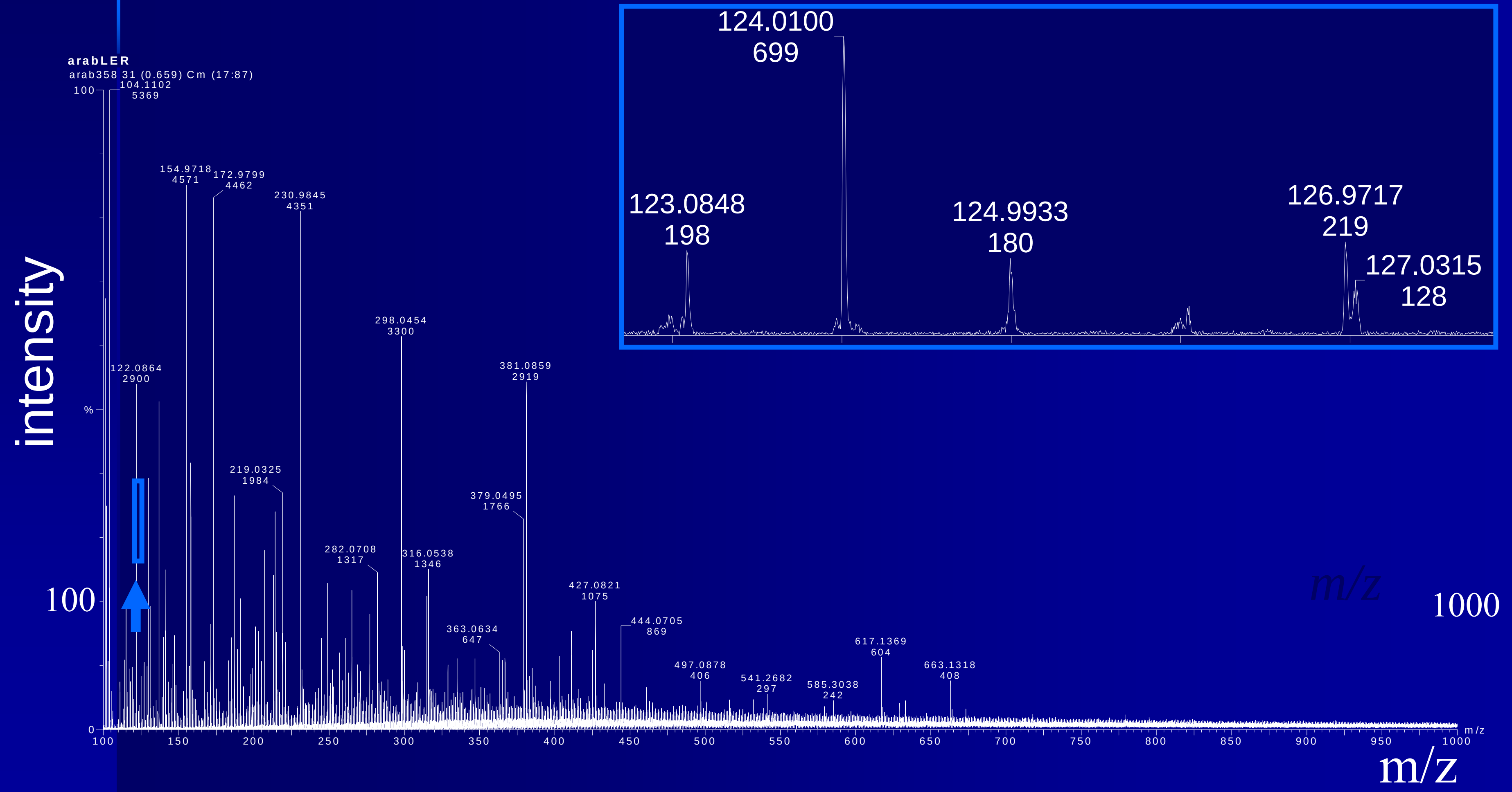
"A Strategy for analyzing the properties of cells or tissues by looking in a high-throughput manner at the metabolites that they excrete or fail to take up from their surroundings (Kell et al, 2005)."

- Similar to fingerprinting, based on pattern recognition
- In Fingerprinting intracellular metabolites analyzed while in Footprinting the culture media (as in the case of yeast).
- Stimulating metabolic changes by adding various carbon compounds or inhibitors

Metabolomics and Other OMICS Approaches

- Transcriptomics: Monitor the expression levels of tens of thousands of genes
- Proteomics: Monitor abundance patterns of thousands of proteins
- Metabolomics: Monitor thousands of low molecular weight metabolites simultaneously
- Bio-informatics: Processing data and extracting biological meaning

Metabolomics: A. Thaliana (Q-Tof-MS)



Metabo-l-omics and Metabo-n-omics

Nicholson et al. 1999

- Metabonomics: “the quantitative measurement of the time – related multi-parametric response of living systems to pathophysioloical stimuli or genetic modification”

Fiehn,

- Metabolomics: “the comprehensive and quantitative analysis of all metabolites”

Approaches are the same, Nomics more metabolic response to drugs and diseases (animal systems and with NMR) while Lomics more bacterial/plants (with MS (GC, LC)

Metabolome Analysis and Nutrition

- Nutritional Metabolomics

- Regulation of metabolic pathways and networks by nutrients and other food components

- Establishment of analytical methods that profile human serum and urinary metabolites to assess nutritional imbalances and disease risk

- Coordination between FOOD SCIENCE and METABOLOMICS

Metabolome Analysis and Nutrition

Extrinsic Factors

Physical activity
Diet
Drugs
Stress
Colonic flora

The Human Metabolome

Intrinsic Factors

Genotype
Age
Tissue turnover
Body composition
Diurnal cycle
Tissue turnover
Health status
Reproductive status
Resting metabolic rate

Factors that could effect the HUMAN Metabolome

Metabolome Analysis and Nutrition

The Human METBOLOME According to HumanCyc Prediction:

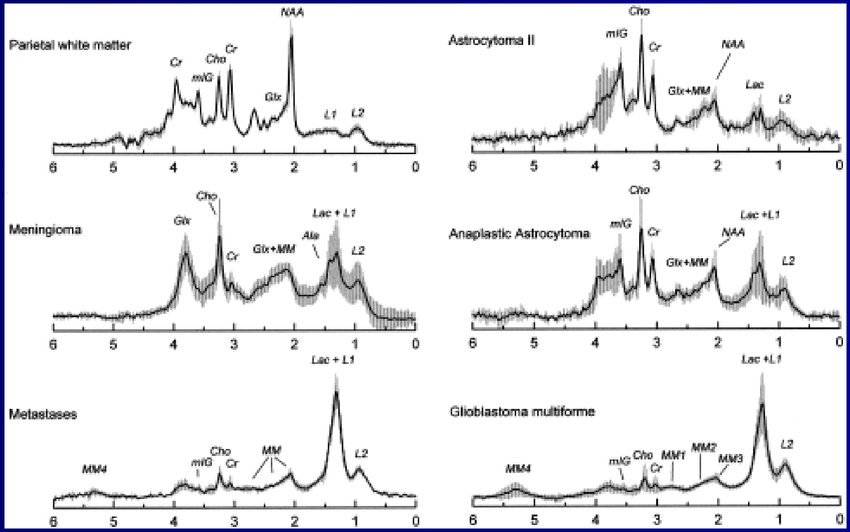
- Enzyme genes: 2,742
- Compounds: 661

- If the numbers are so:

Personal Metabolomics is possible

Metabolome Analysis and Cancer

Tumor Metabolomics in recent years it was based on NMR (non invasive) for clinical diagnosis.



Metabolic profiles of human brain tumors using quantitative in vivo 1H magnetic resonance spectroscopy (MRS; Howe et al., 2003).

Metabolome Analysis and Cancer

- Try to understand the metabolic phenotype of tumor cells

- Interrelationship between respiration and glycolysis investigated for decades

- Hypoxia Inducible Factor-1 (HIF-1), widely expressed in a range of cancers, seems to be of large importance

- HIF-1 controls the response to oxygen and coordinated the up-regulation of genes involved in many metabolic pathways concerned with tumor growth and metabolism

- Metabolomics might explain the differences between tumor and normal cells

Metabolomics-

Next week's class and visit to GC-MS and LC-MS instruments in WIS

Metabolome Diversity and Evolution

The role of Promiscuous Protein Function

Promiscuous activity in a protein is also termed:

- substrate ambiguity
- cross reactivity
- moonlighting activity

Too few genes, too many metabolites?

- In plants more than 200,000 structures have been identified
- Every plant estimated to contain- 4k-20k
- Diversity in modification of the same backbone structure
- Example: 300 different glycosides of the flavonol quercitin
- In Arabidopsis according to AraCyc:

1900 genes encoding enzymes with defined function

Too few genes, too many metabolites?

How to explain METABOLOME size/diversity?

a. DNA level: Alternative reading frames and gene fusion
b. mRNA level: Alternative splicing
c. Protein: Post translational modification and Hetrodimer formation

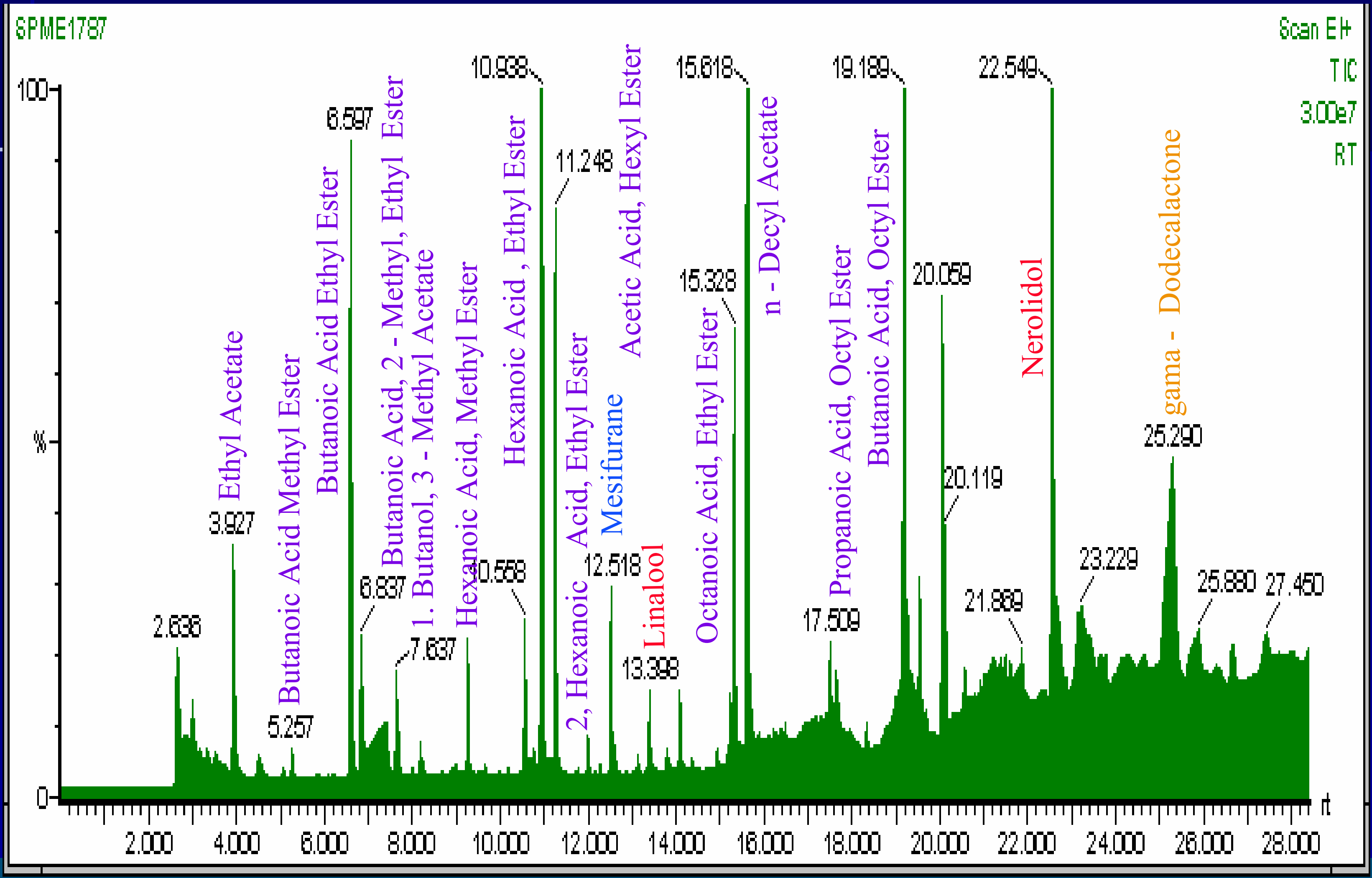
And…… Enzyme Specificity!

Plant Secondary Metabolism: An Excellent Example

Alcohol acyl-CoA transferases

One enzyme…..Multiple substrates…..Multiple products

Volatile Composition of Cultivated Strawberry

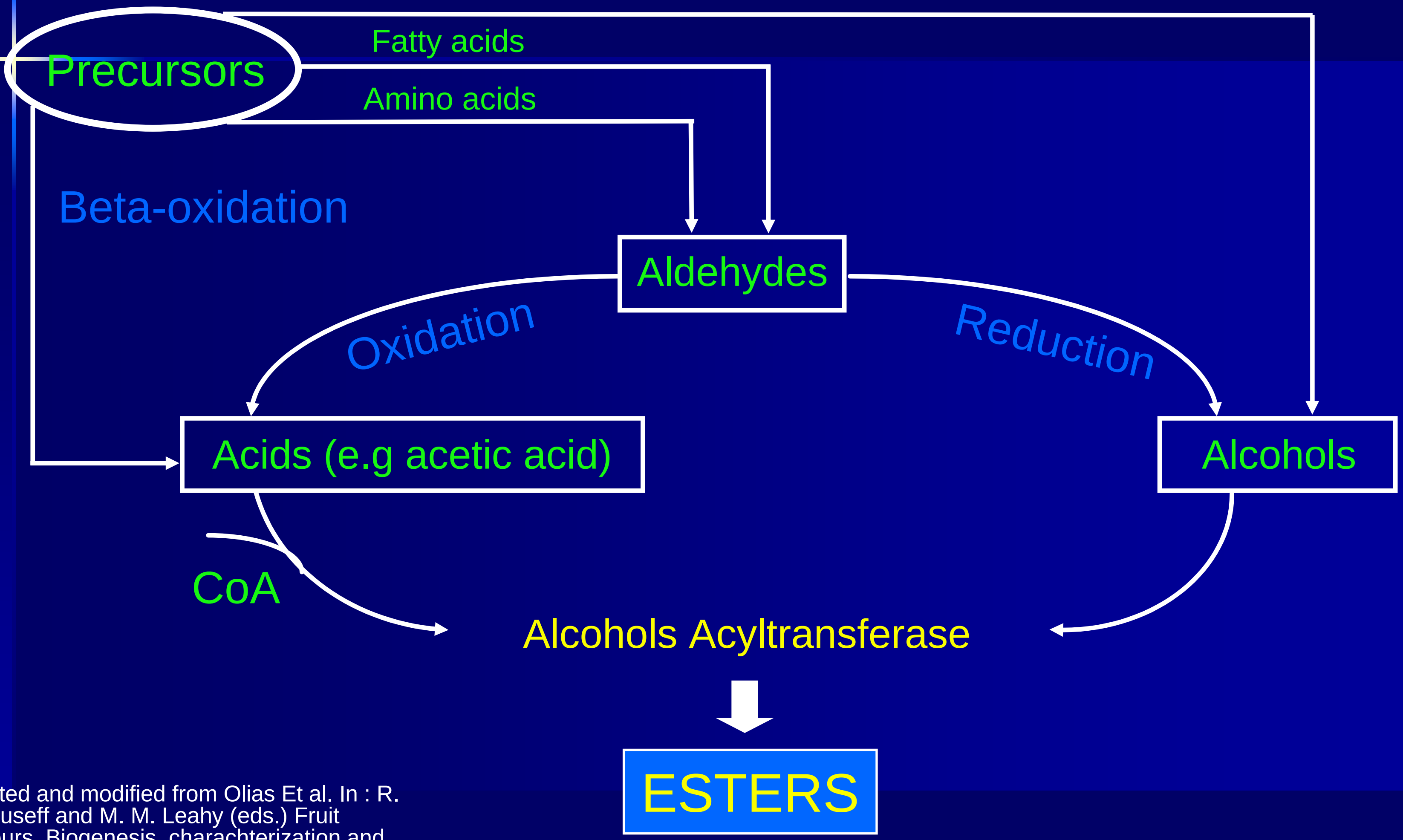


Ester — Furan — Terpene — Lactone

Volatile Esters Contribute to the Aroma of Most Fruit

- Some are responsible for a particular fruit aroma (e.g. banana)

Metabolic Route for the Formation of Esters in Fruit



Adapted and modified from Olias Et al. In : R. L. Rouseff and M. M. Leahy (eds.) Fruit Flavours. Biogenesis, charachterization and authentication (1995).

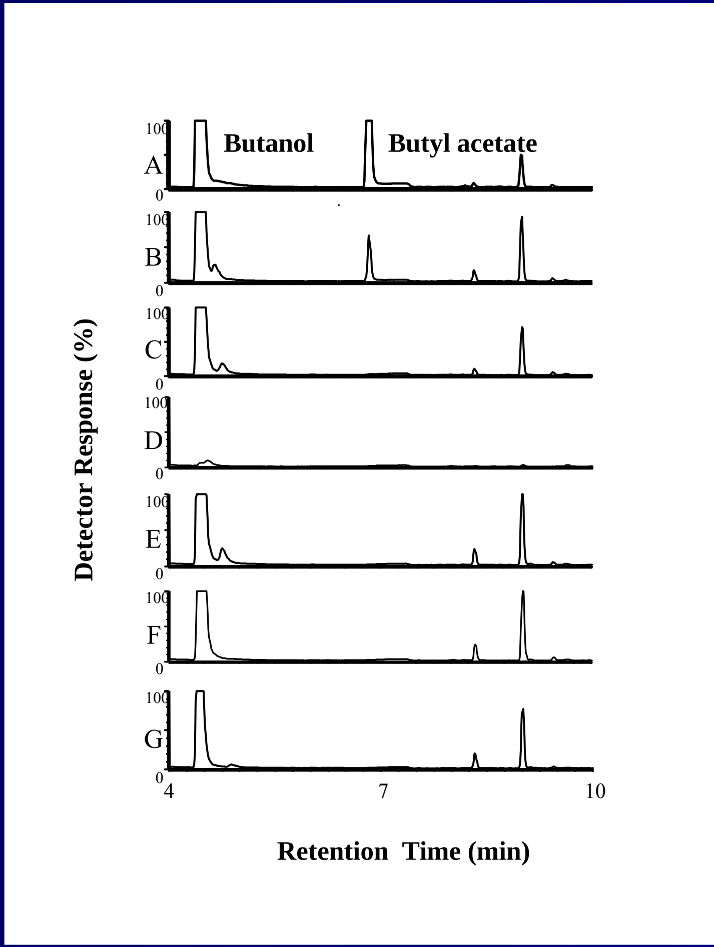
Ester Formation by Alcohol Acyltransferase (AAT)

Ethylalcohol → Ethylacetate

Acetyl-CoA + (AAT)

Isoamylalcohol → Isoamylacetate

Ester Formation by *SAAT* Expressed in *E.coli* Cells



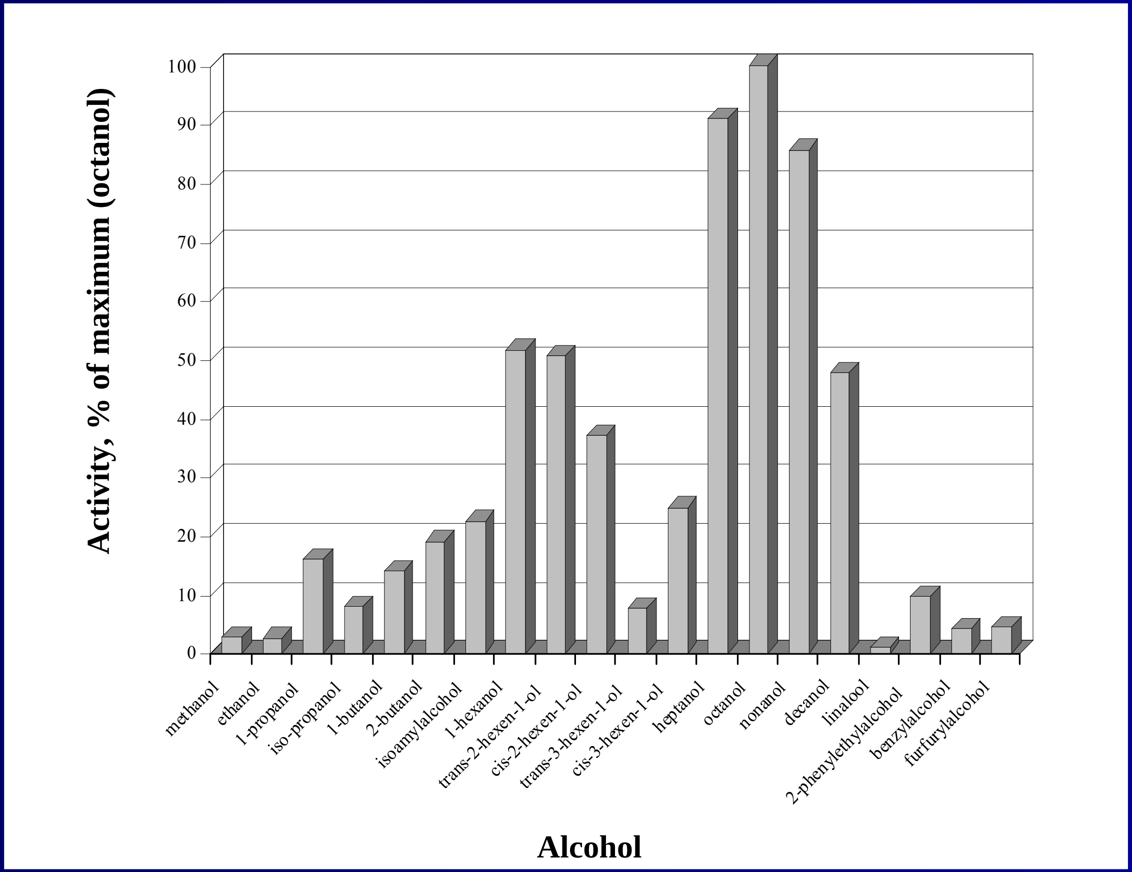
In B:

Butanol + Acetyl CoA

↓

Butylacetate

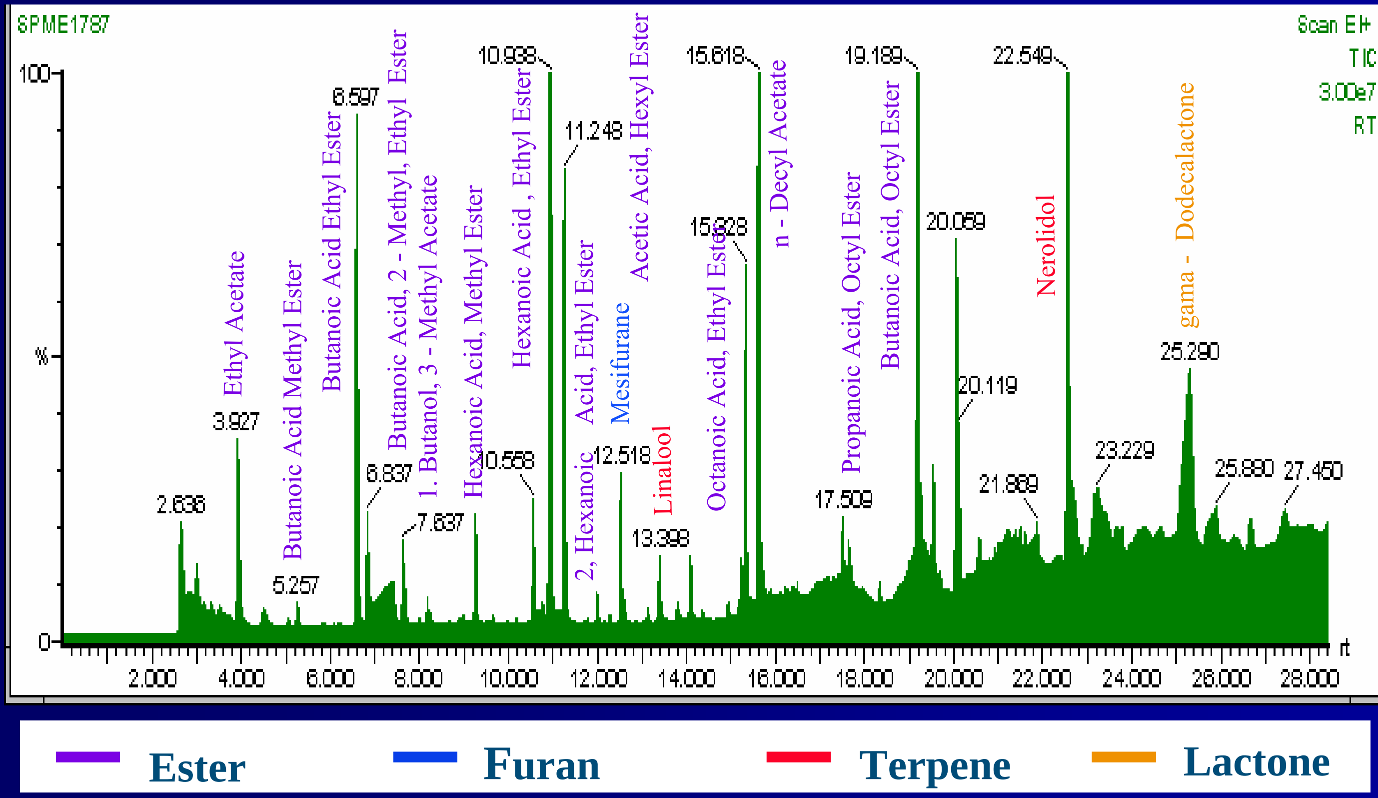
Broad substrate specificity (alcohols)



Broad Substrate Specificity (acyl CoAs)

Acyl CoAs (Carbon no.)	Alcohol	Ester formed	Ester properties
n-propionyl CoA (C3:0)	1-butanol	1-butyl propanoate	ethereal, banana
n-butyryl CoA (C4:0)	1-propanol	1-propyl butyrate	sharp, pungent, rancid, sweaty, sickening
n-butyryl CoA (C4:0)	1-butanol	1-butyl butyrate	fruity, pineapple
isobutyryl CoA (C4:0)	1-butanol	1-butyl isobutyrate	fruity, apple, banana and pineapple
n-crotonoyl CoA (C4:1)	1-butanol	1-butyl crotoate	not described
n-hexanoyl CoA (C6:0)	1-propanol	1-propyl hexanoate	wine-like, cheese
n-decanoyl CoA (C10:0)	1-butanol	1-butyl decanoate	Brandy (Whisky-Cognac)-like odor
benzoyl CoA (C7:0)	1-butanol	1-butyl benzoate	mild floral-balsamic odor

SAAT is a member of a small gene family in strawberry responsible for generating more than 100 different type of esters in the ripe fruit



Plant Secondary Metabolism: An Excellent Example

Terpene Cyclases

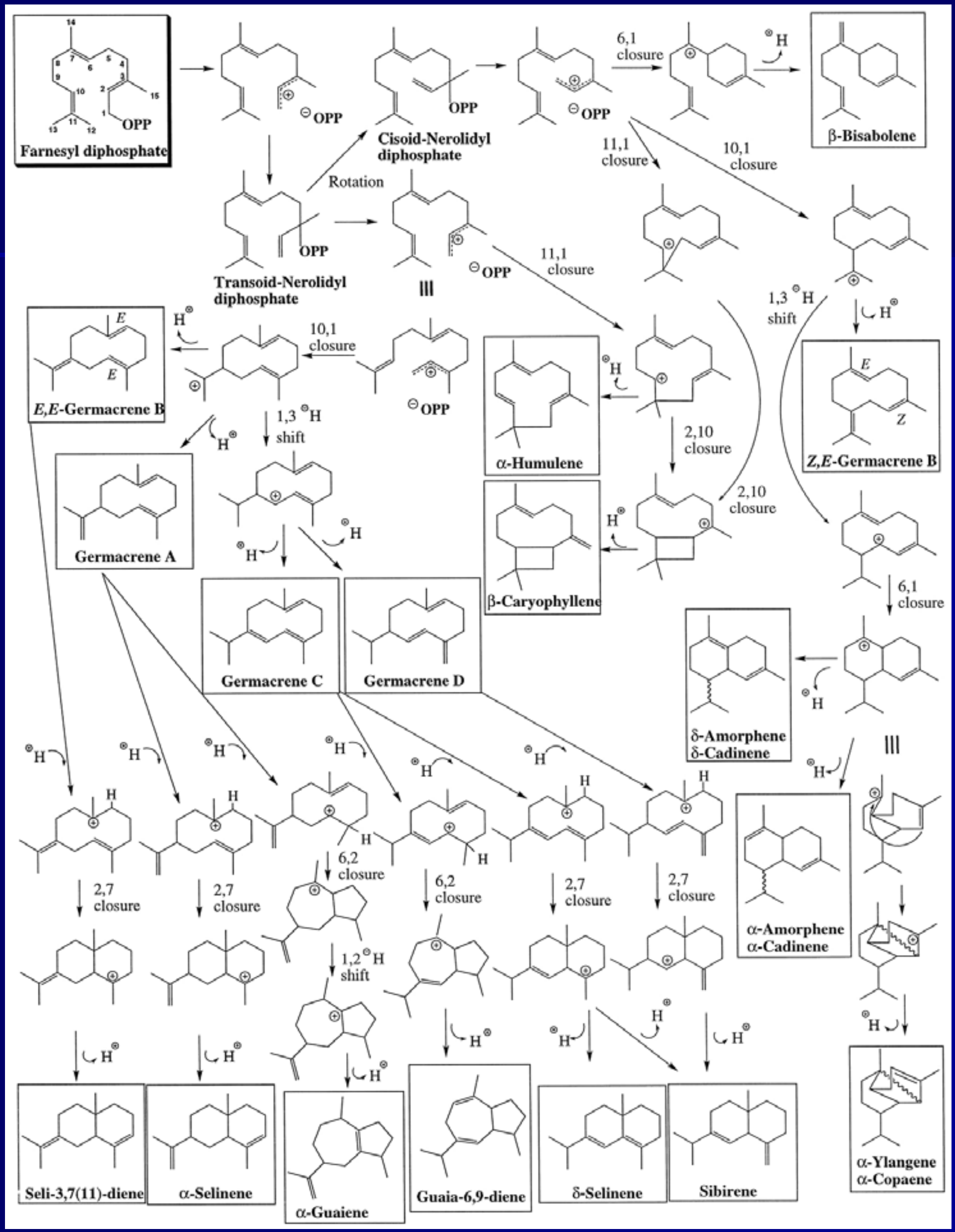
One enzyme…..One substrate…..Multiple products

Plant Secondary Metabolism: An Excellent Example

Trees such as Grand Fir (אשוח):

- Produce oleoresin in response to stem wounding and insect attack
- The turpentine fraction of the oleoresin contains terpenes, mainly mono, sesqui and diterpenes
- In Grand Fir, 38 sesquiterpenes (12.5% of turpentine) and the remaining monoterpenes
- Two terpene synthases expressed in E.coli could synthesize three major products but in total 35 and 53 total sesquiterpenes.

Steele et al., 1998



Farnesyl diphosphate
OPP
Cisoid-Nerolidyl diphosphate
Transoid-Nerolidyl diphosphate
Rotation
6,1 closure
11,1 closure
10,1 closure
β-Bisabolene
1,3 H shift
E,E-Germacrene B
Z,E-Germacrene B
α-Humulene
2,10 closure
β-Caryophyllene
Germacrene A
Germacrene C
Germacrene D
δ-Amorphene
δ-Cadinene
α-Amorphene
α-Cadinene
2,7 closure
6,2 closure
1,2 H shift
Seli-3,7(11)-diene
α-Selinene
α-Guaiene
Guaia-6,9-diene
δ-Selinene
Sibirene
α-Ylangene
α-Copaene

Metabolome Diversity and Evolution

- Promiscuous function of proteins seems to be a major step in the evolution of new protein functions (Aharoni et al., 2005)
- Conflict between the need for mutations for a new function and the deleterious effect of mutations on survival
- Therefore mutations causing promiscuous functions are staring points (no loss of original function)
- Subsequently gene duplication and a new protein occurs

Metabolome Regulation and Evolution

-Past and recent years several discoveries of RNA molecules as regulatory elements in cellular processes:

- antisense
- tRNA – mRNA interactions
- translation control by temp. dependent modulation of RNA structure
- microRNAs, siRNAs

Metabolome Regulation by Riboswitches

- Newly discovered natural elements located at untranslated regions of mRNAs called "Riboswiches"

- Mainly at the 5' end

- Regulate gene expression and translation by binding small metabolites

- Up to date, none of them required protein factors for metabolite binding

Metabolome Regulation by Riboswitches

- Suggested to be descendents of the ancient metabolite sensors in the RNA world

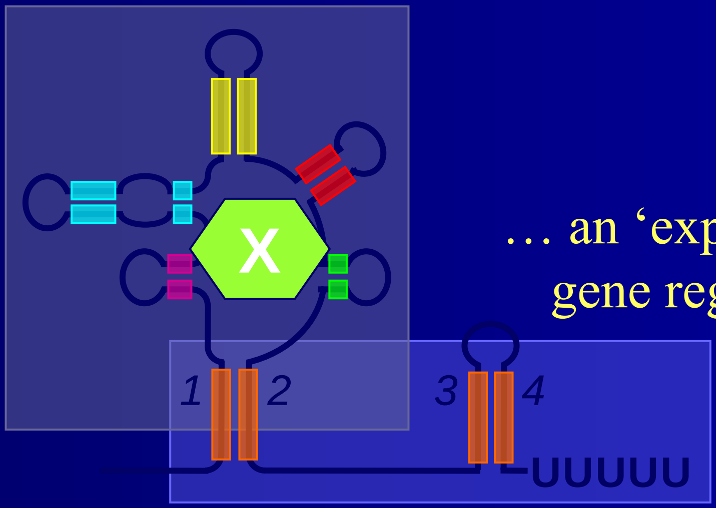
- In the RNA world, RNA handled storage of genetic information (instead of DNA) and also expression (instead of proteins)

- Genes controlled by Riboswitches (RBS) most often encode proteins involved in the biosynthesis or transport of the metabolites being sensed (feedback loop)

The Two Components of Riboswitch Functions

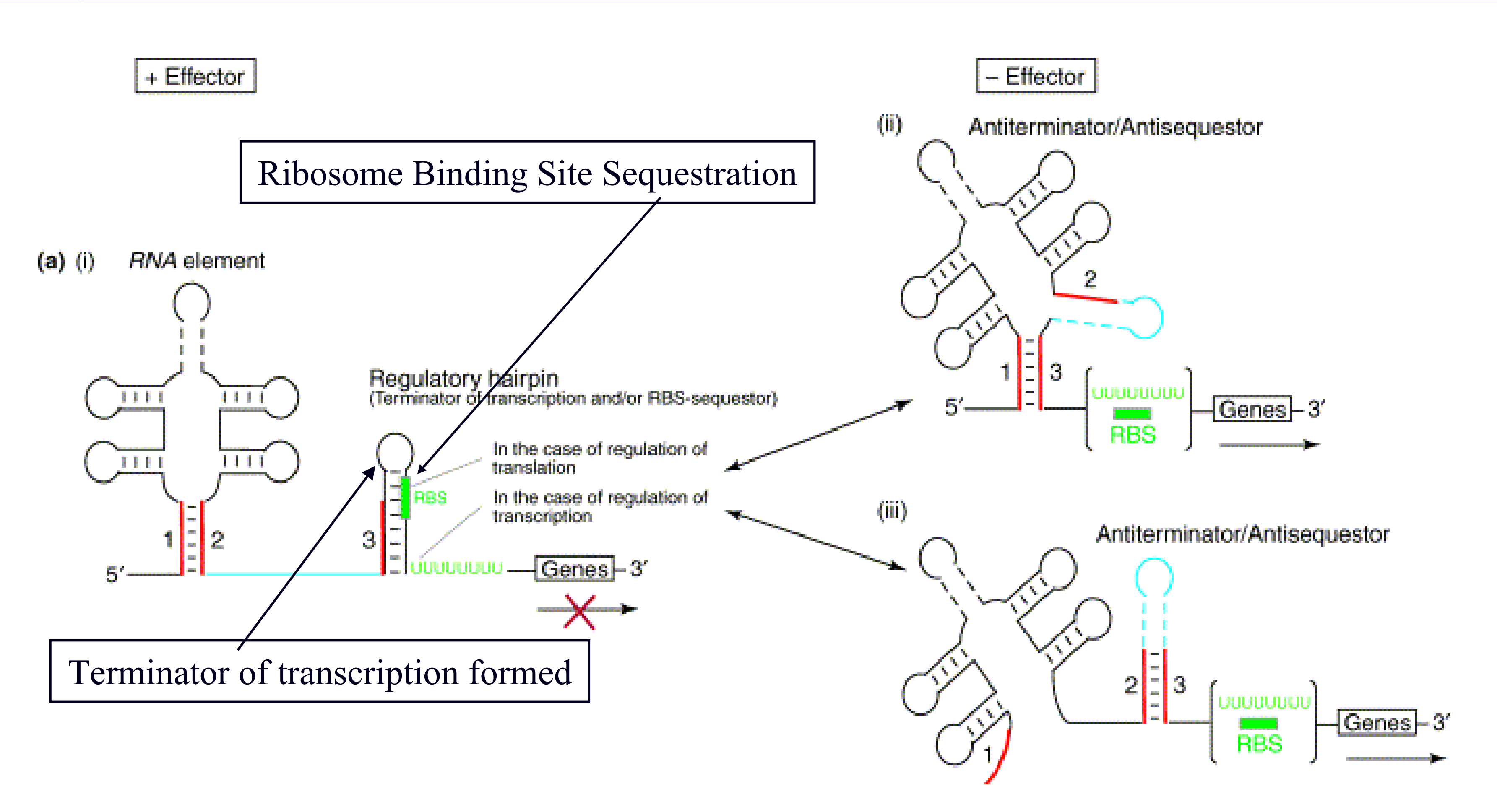
- Direct (protein-free) binding of metabolite to RNA (aptamer)
- Metabolite-dependent regulation of genes (expression platform)

A metabolite-binding ‘aptamer’ domain and …

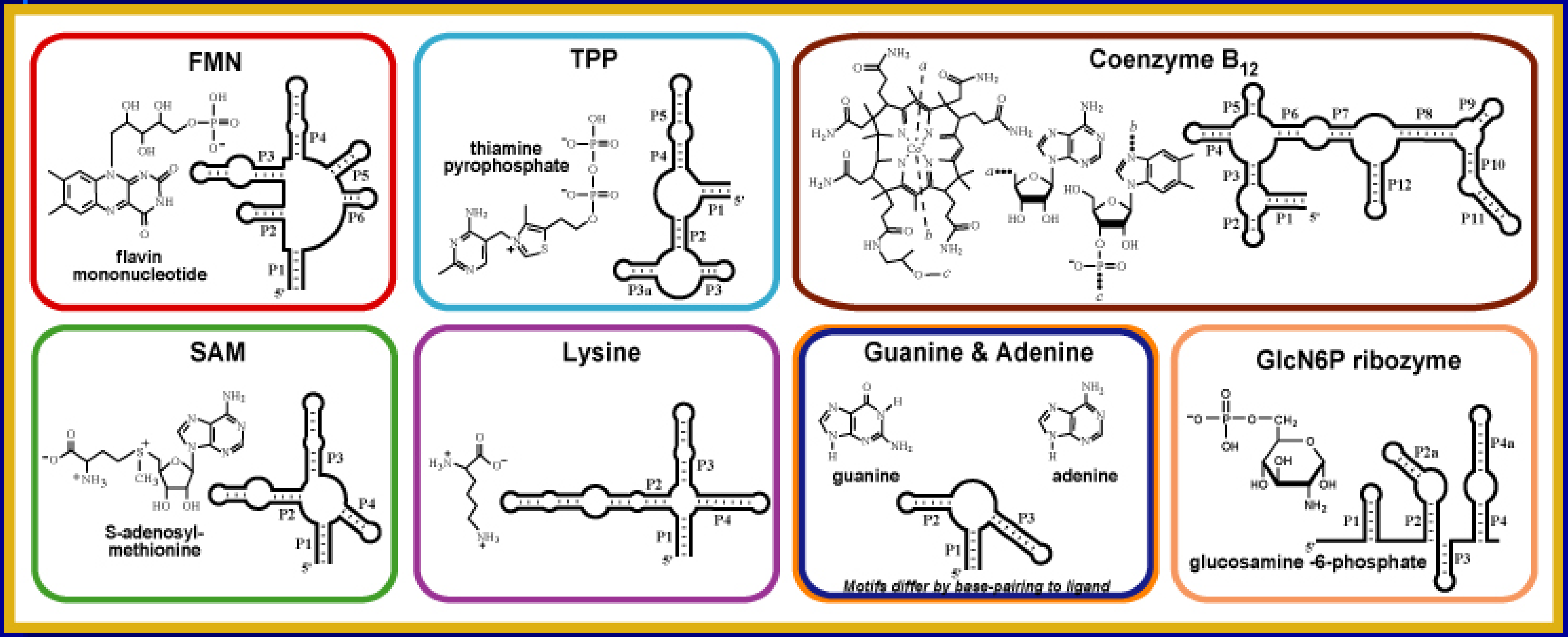


… an ‘expression platform’ for gene regulation.

Metabolome Regulation by Riboswitches

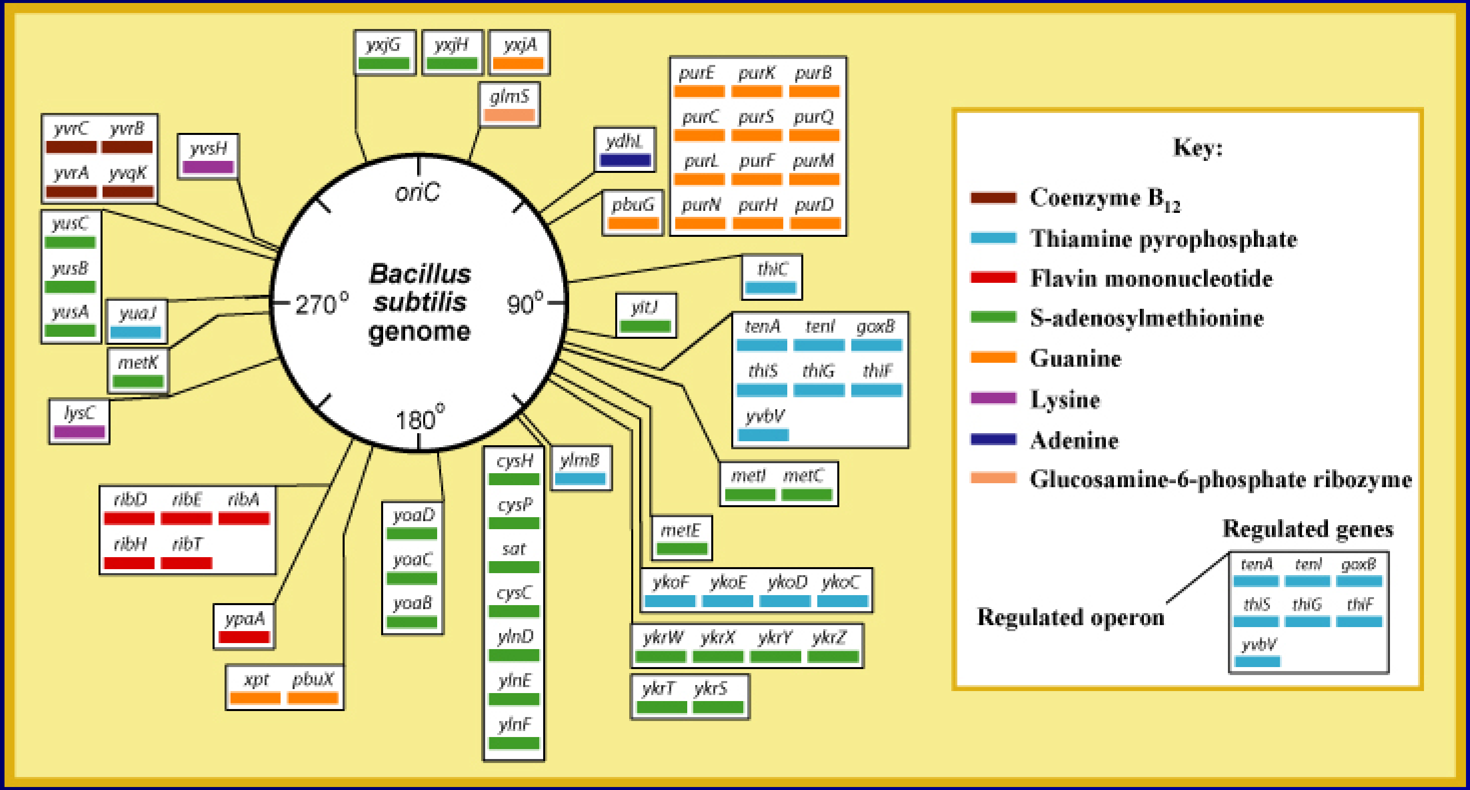


Riboswitch Ligands



Metabolome Regulation by Riboswitches

Nearly 2% of the genes of the model organism *Bacillus subtilis* appear to be controlled by riboswitches



Metabolome in a Specific Cell types & Single Cell

The Metabolome profile could be very different between cell-types

Could even be different between cells of the same type

Can we measure the differences in such a resolution?

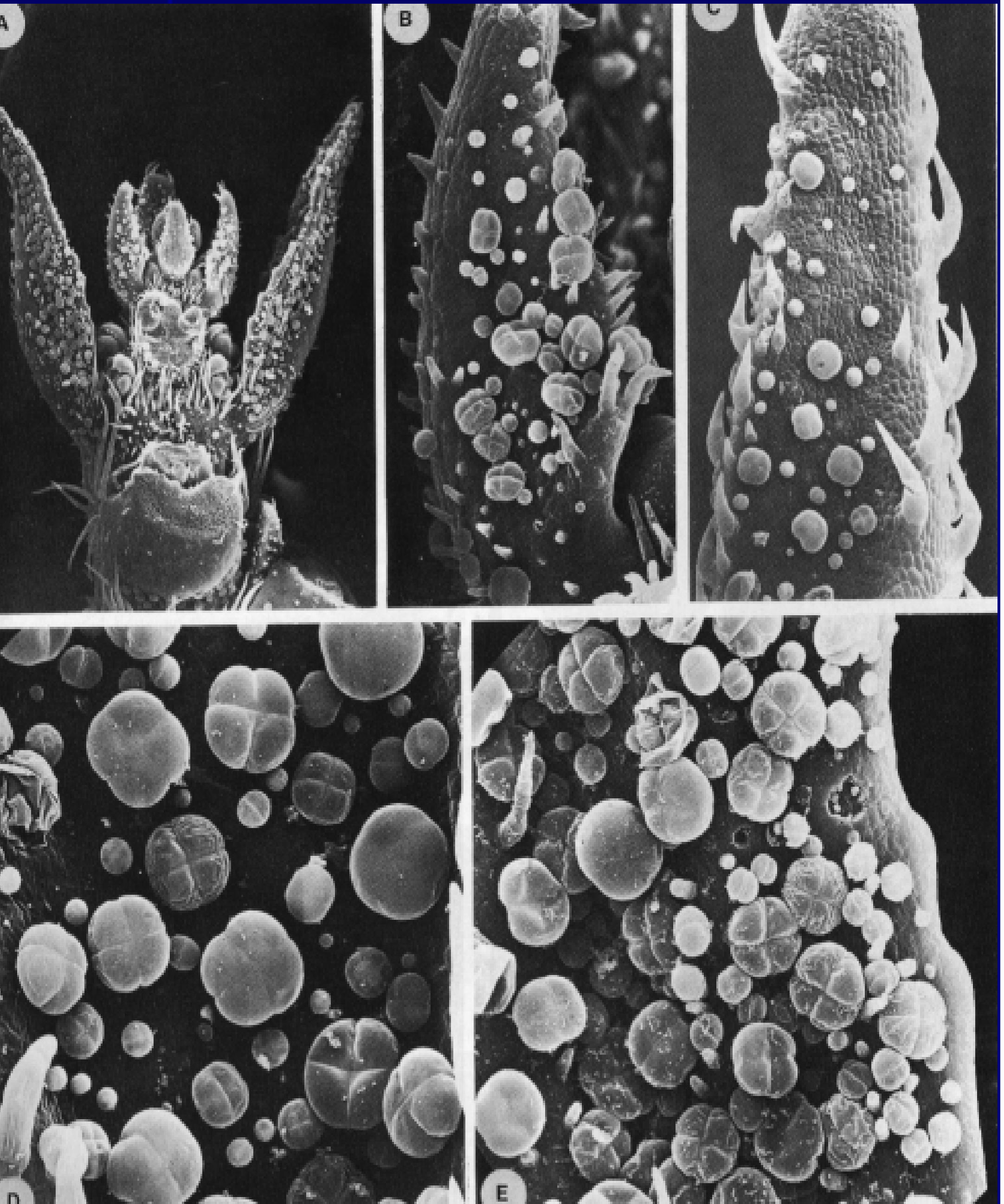
GLANDS in Basil

R-Linalool

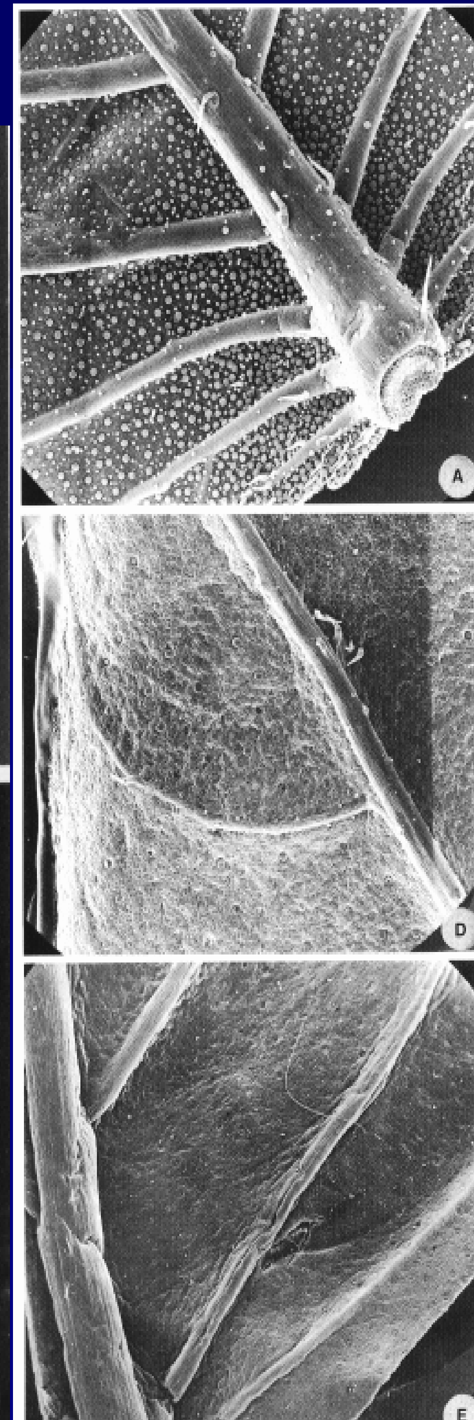
R = OCH_3
Eugenol

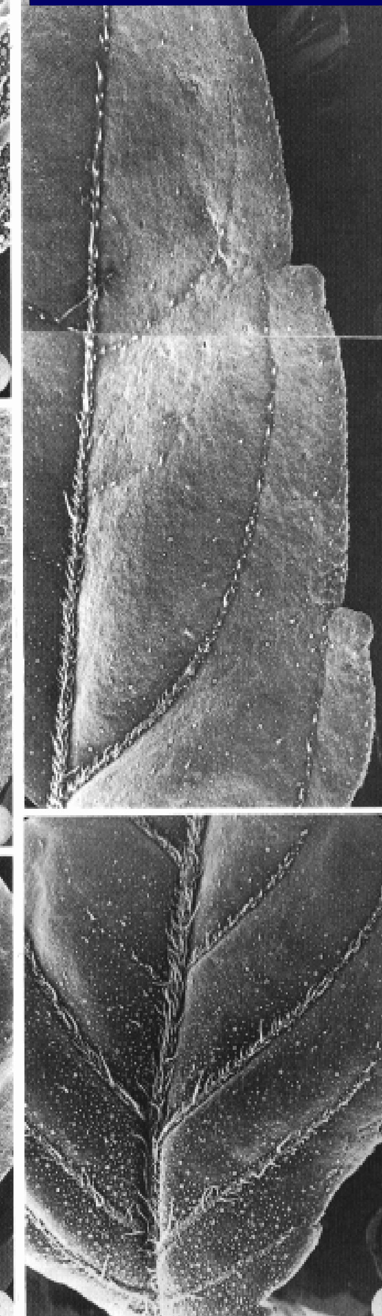
R = H Estragole
R = OCH_3 Methyl Eugenol

Young leaves



Old leaves

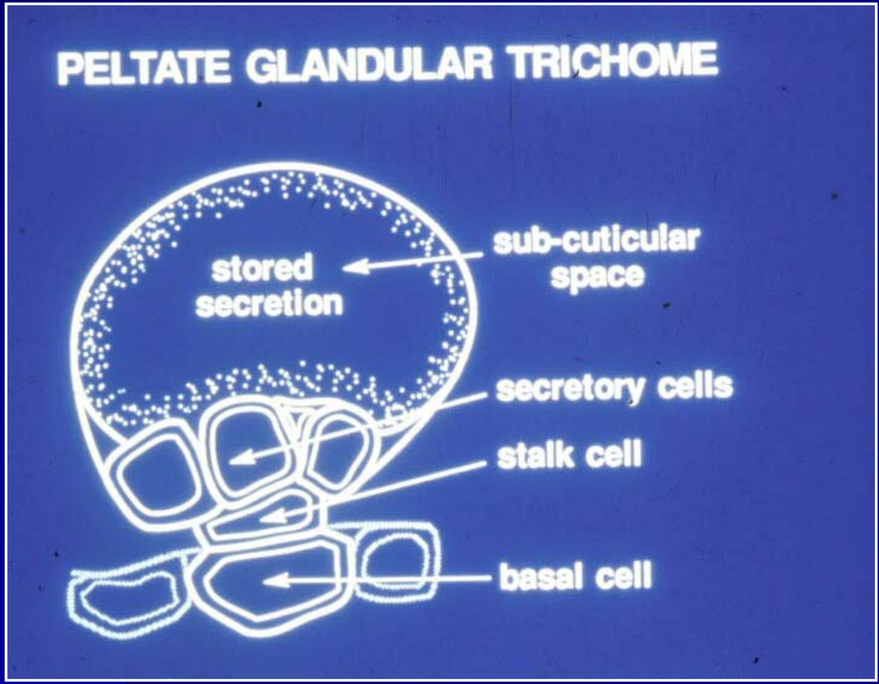


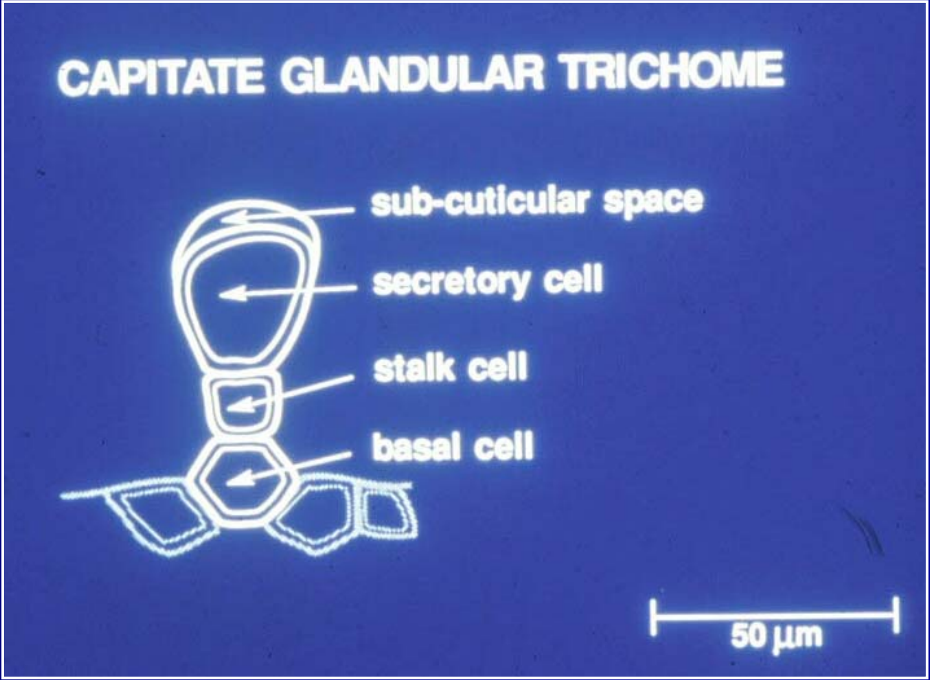


Most secondary metabolites in Basil are produced in the Peltate Glands

Peltate Glands

Capitate Glands

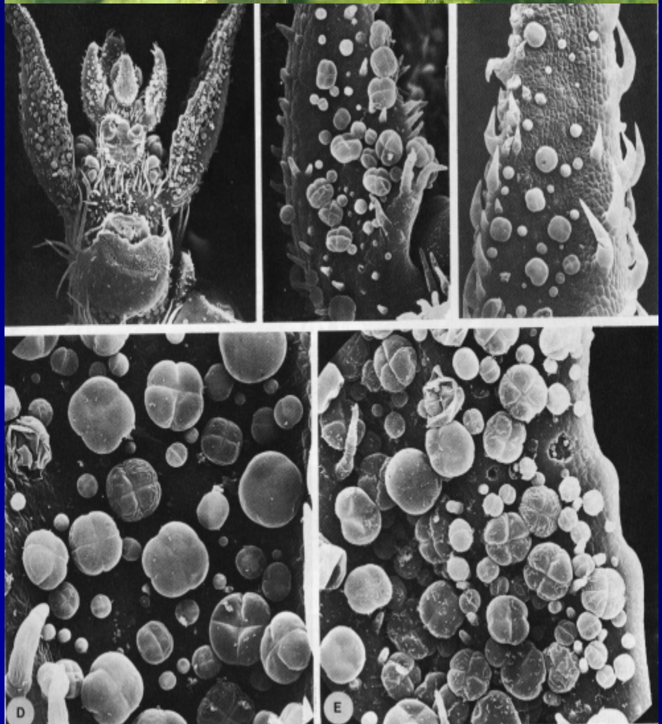


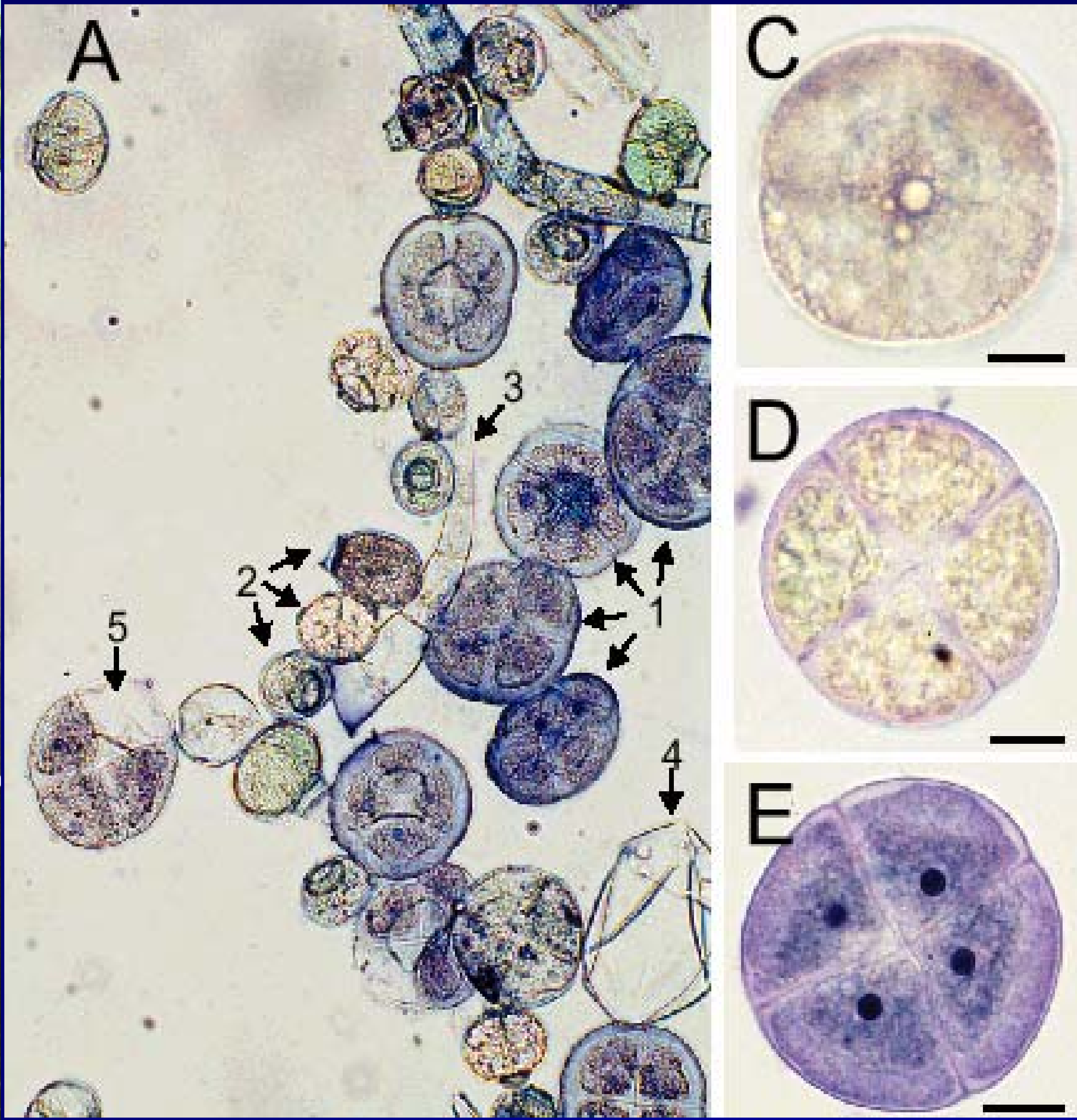



700
600

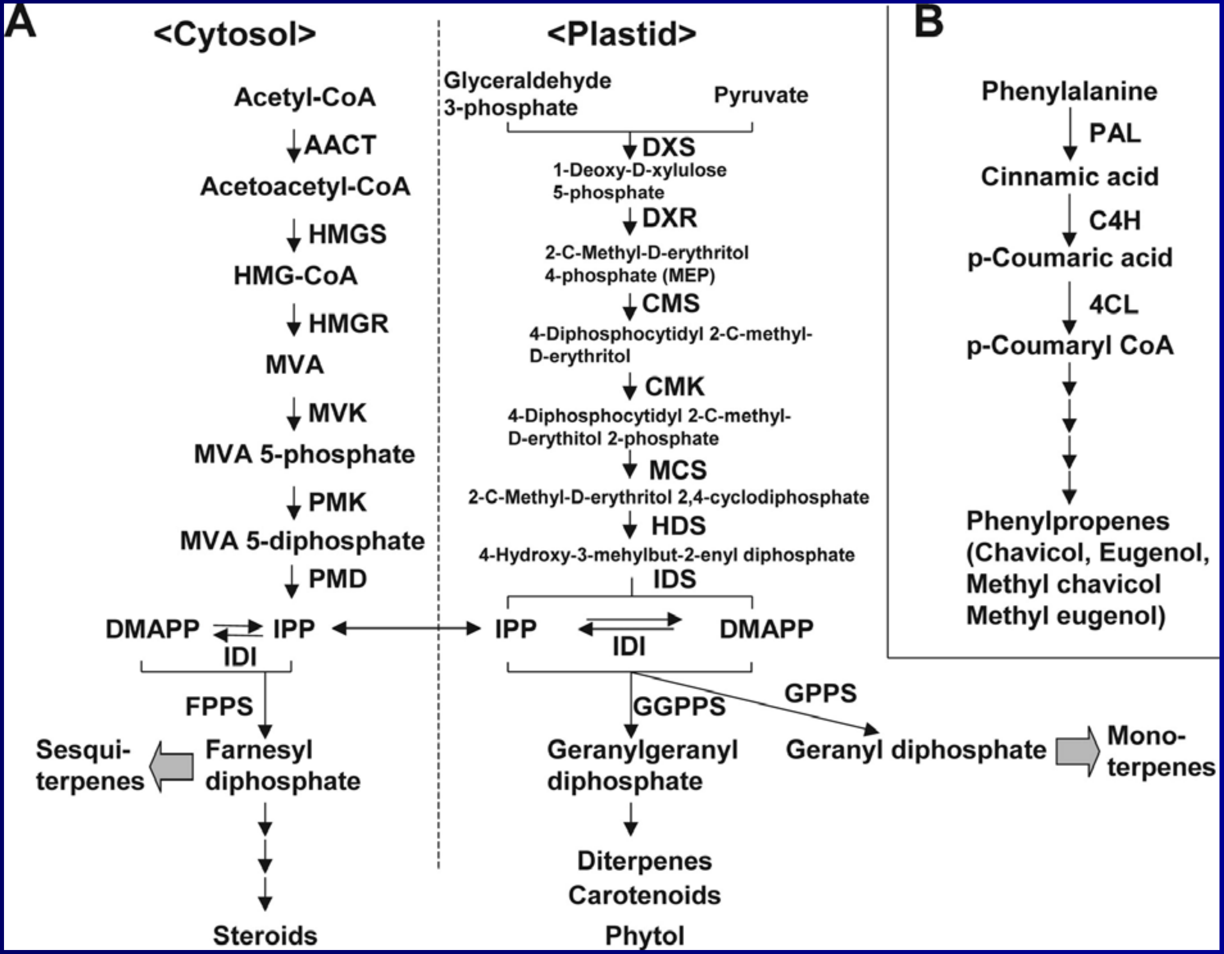

UM FOIL
FOR COOKING & WRAPPING

Peltate Glands Isolated From Sweet Basil



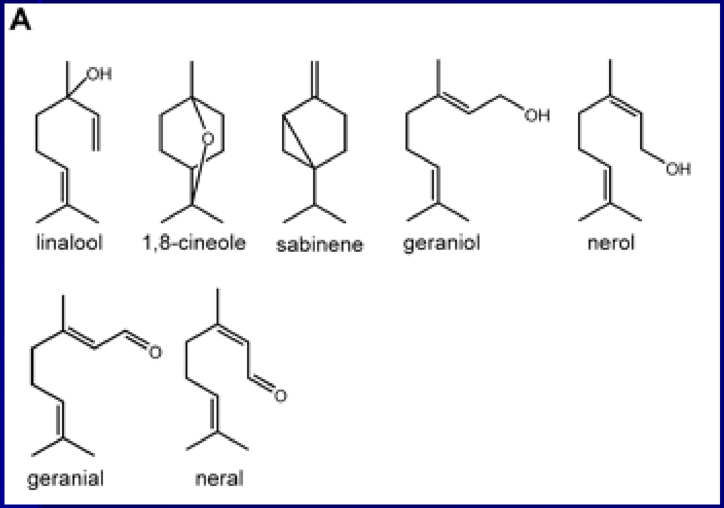


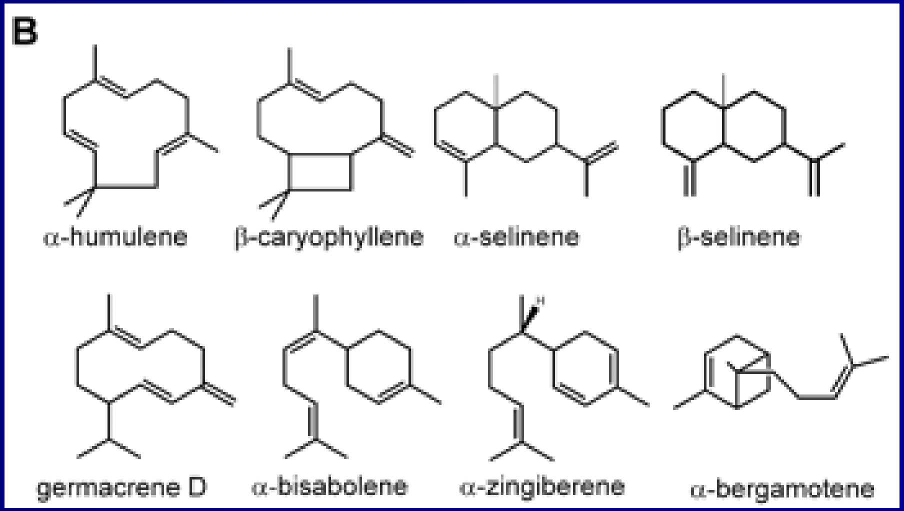
Active Pathways in Peltate Glands (Sweet Basil)



(Iijima et al, 2004)

Terpenoids in Peltate Glands (Sweet Basil)

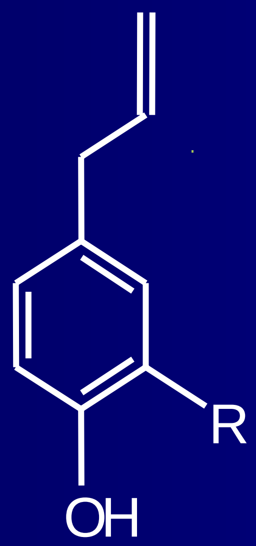


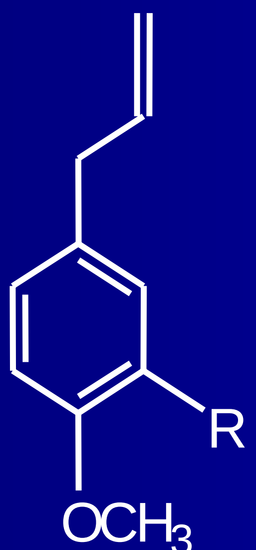


Monoterpenes

Sesquiterpenes

Phenylpropans in Peltate Glands (Sweet Basil)





R = OCH_3
Eugenol

R = H Estragole
R = OCH_3 Methyl Eugenol

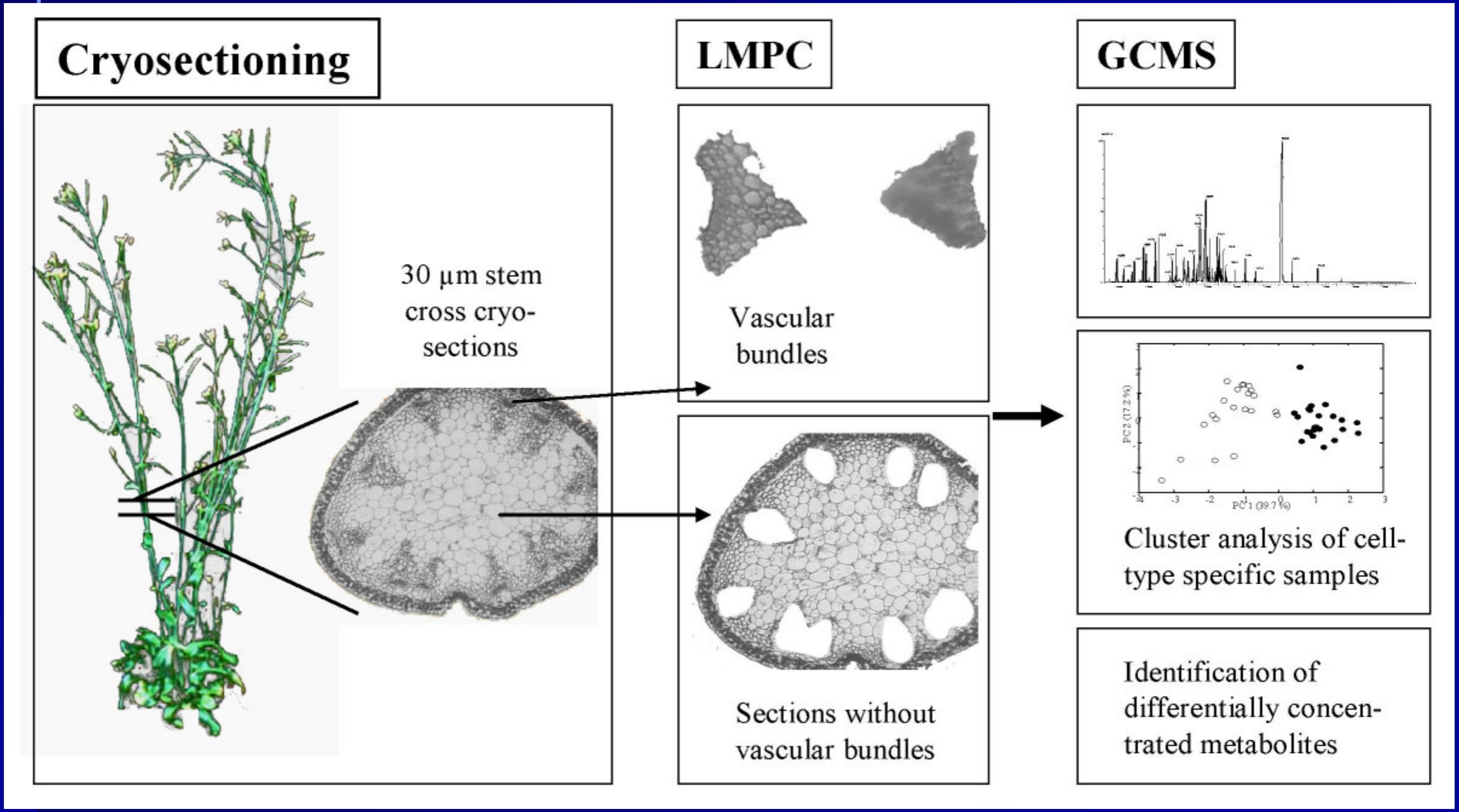
The Metabolome of Vascular Bundles (Schad et al., 2005)

- Using laser microdissection for collecting tissue specific samples

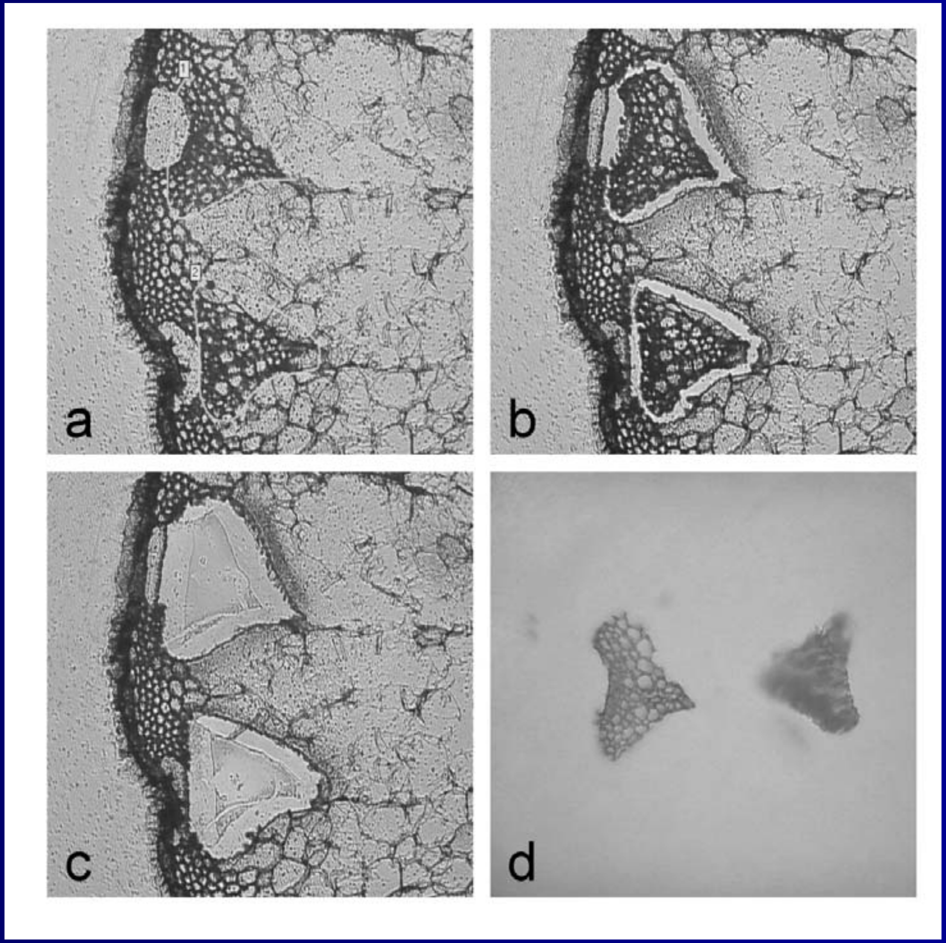
- Standard tissue fixation and embedding protocols do not allow metabolite analysis (fix and dehydrate steps results in loss of small molecules)

- Used cryosectioning- preserving cellular structure without solute exchange steps

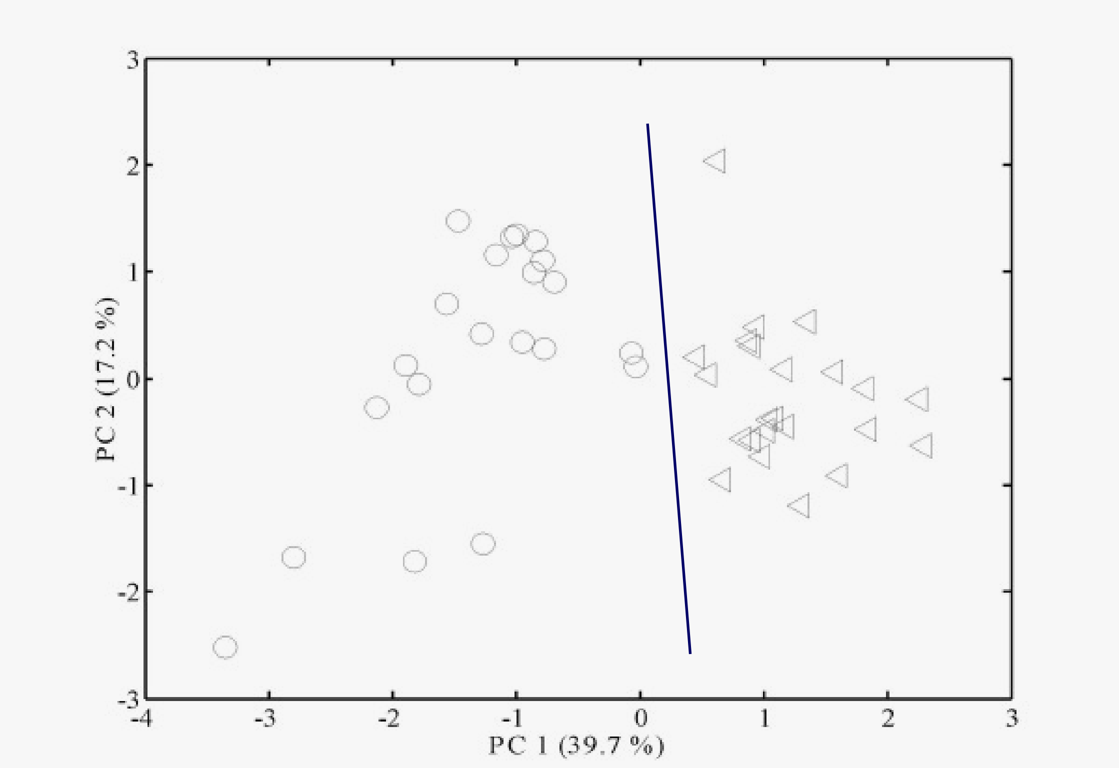
The Metabolome of Vascular Bundles (Arabidopsis)



The Metabolome of Vascular Bundles



The Metabolome of Vascular Bundles



Vascular bundles tissue

Sections without vascular bundles

The Metabolome of Vascular Bundles

- From 100 collected vascular bundles (approx. 5,000 cells), 68 metabolites identified

- Half of the identified, enriched or depleted in vascular bundles compared to the surrounding tissue

Metabolic Engineering

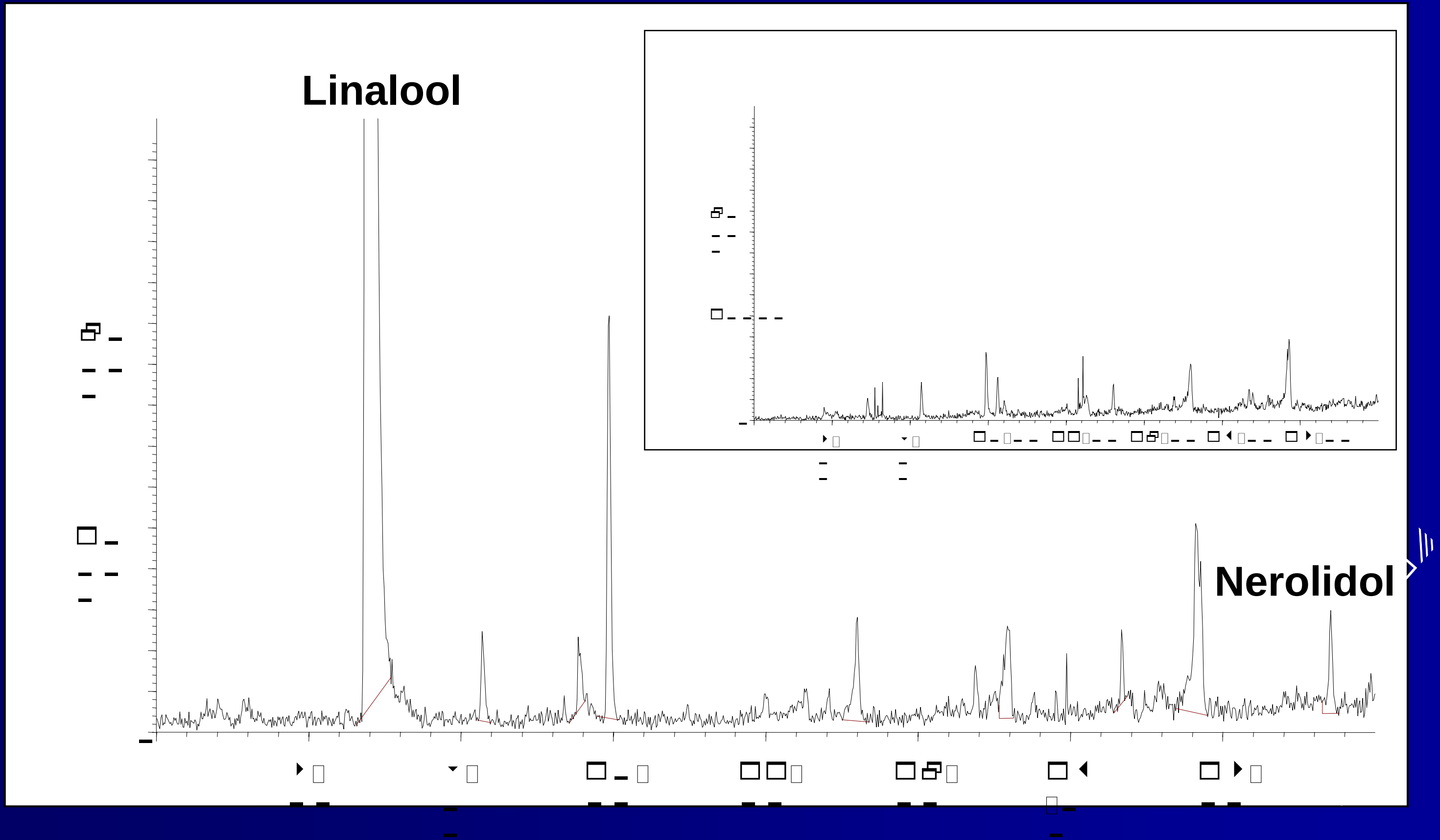
Systematically induce biological changes that will produce desired cellular properties

Metabolic Engineering

Just a few of the possibilities:

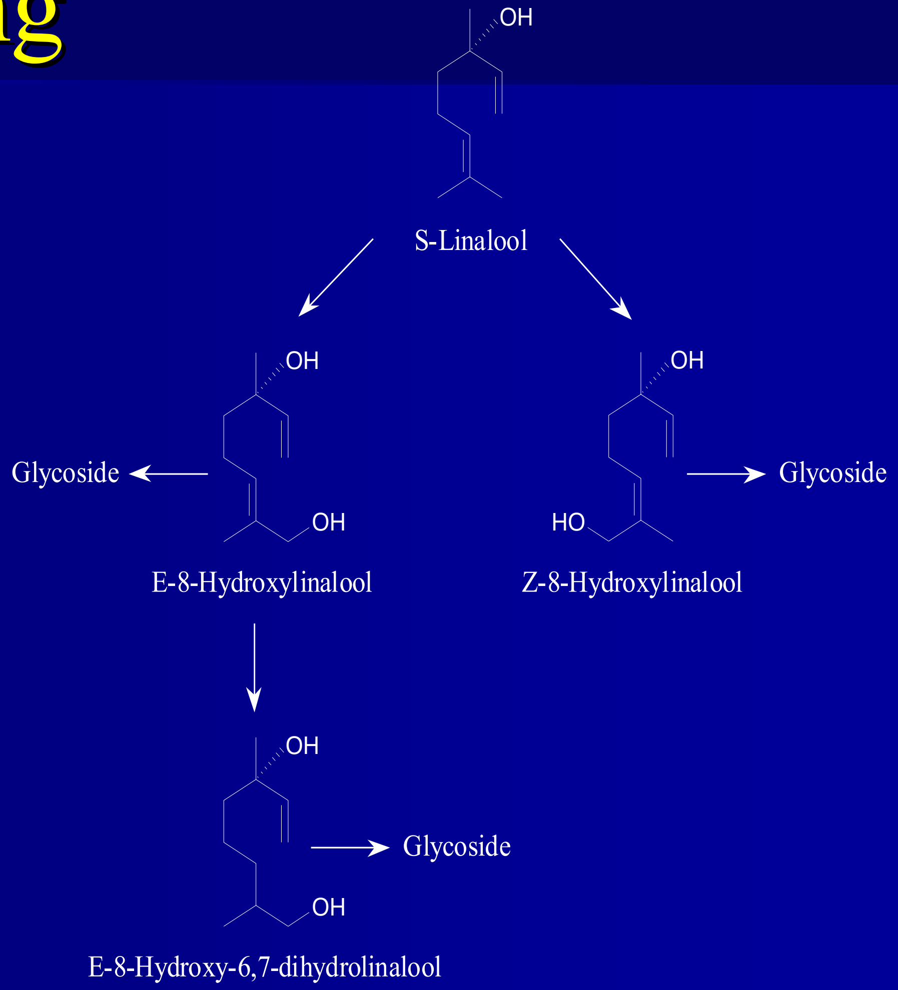
- Block enzyme activity
- Enhance enzyme activity
- Use transcription factors
- Use feedback insensitive genes
- Ectopic gene expression
- Introduce/block multiple genes
- Use directly chemicals/proteins/RNA and not genes

Metabolic Engineering of Flavors from Strawberry fruit to Arabidopsis



Un Expected Effects When Conducting Metabolic Engineering

- Introduced product: linalool
- Modified by endogenous enzymes:
  - P450 hydroxylation (2-3)
  - Double bond reduction
  - Glycosylation (2-3)



Metabolic Engineering Needs Fine Tuning

Metabolic Engineering and Metabolomics

- Metabolic analysis to identify key points of metabolic regulation

- Metabolic analysis to check predictions and generate hypothesis and strategy

Genetically Modified Organisms (GMOs)

- Public concern about GMOs

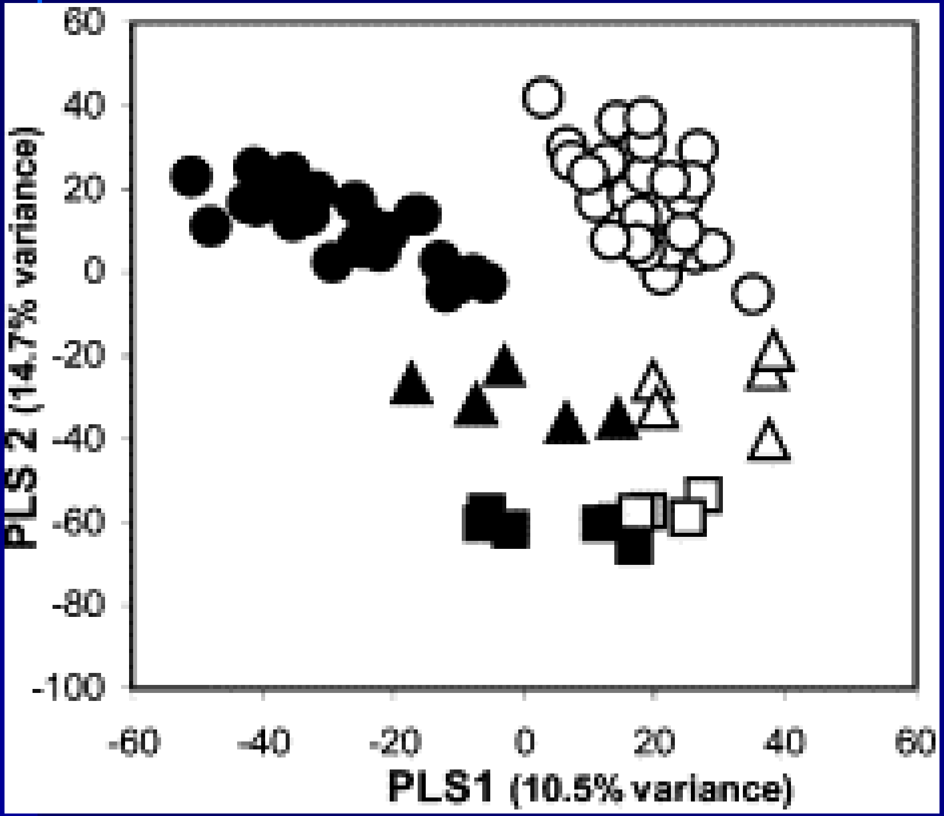
- Can we use Metabolome Analysis to determine similarities or differences between GMO to non-GMOs

Genetically Modified Organisms (GMOs)

'substantial equivalence'

This means that if a GM food can be characterized as substantially equivalent to its 'natural' antecedent (chemically), it can be assumed to pose no new health risks and hence to be acceptable for commercial use

Genetically Modified Organisms (GMOs)

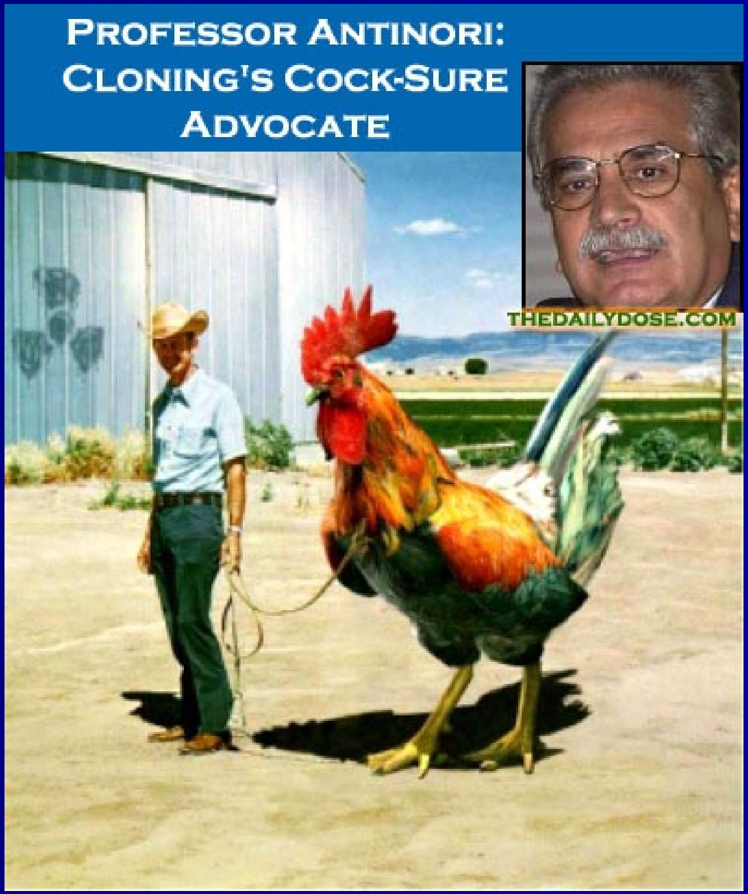


GMO tomato fruit overproducing flavonoids

NMR profiles discriminates between GMO and Non-GMO

Le Gall et al., 2003

Genetically Modified Organisms (GMOs)



See you next week !