

Homeostasis of Isoprenoids in Plants

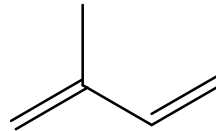


Alain Tissier

Leibniz Institute of Plant Biochemistry
Department of Cell and Metabolic
Biology
Halle, Germany

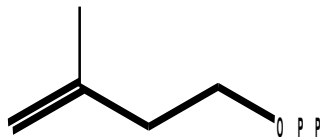
ERA-CAPS First Grantholders
Meeting
Rome, June 12-13

Isoprenoids: compounds that are made entirely or partially of isoprene units

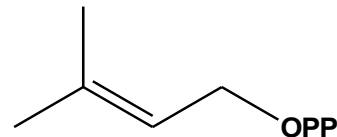


isoprene

All isoprenoids are synthesized from the same building blocks



Isopentenyl diphosphate
(IPP)



dimethylallyl diphosphate
(DMAPP)

GPP, FPP, GGPP

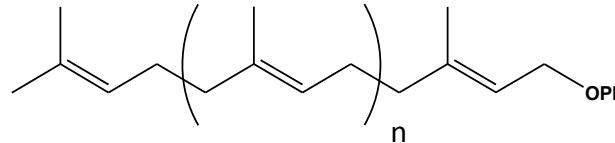
short chain
 $0 \leq n \leq 2$

medium chain
 $3 \leq n \leq 10$

long chain
 $n \geq 11$

„linear“

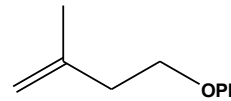
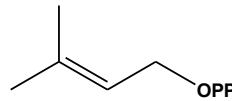
cyclic



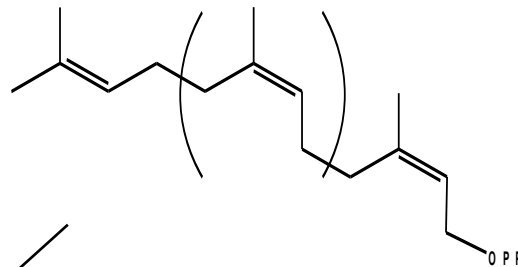
trans-isoprenyls

How isoprenoid
 structural
 diversity is
 generated

Conjugation
 to non-
 isoprenoid
 moieties



cis-isoprenyls



long chain

„linear“

cyclic



short chain



medium chain



The isoprenome

C5-DMAPP → isoprene

C10-GPP → Monoterpenes

menthol eucalyptol pinene linalool borneol limonene

C15-FPP → Sesquiterpenes

Patchoulol Santalols farnesol Nootkatone
Valencene Artemisinine Caryophyllene
Vetiverol

Squalene (C30) → Triterpenes

phytosterols saponins
Brassinosteroids cardiac glycosides

cis-isoprenoids

C50-C100 *cis*-isoprenoids High MW *cis*-isoprenoids

dolichols Rubber
dehydrodolichols

Protein prenylation

C20-GGPP → Diterpenes

Gibberellins Stevioside Forskolin Taxol
Abscissic acid

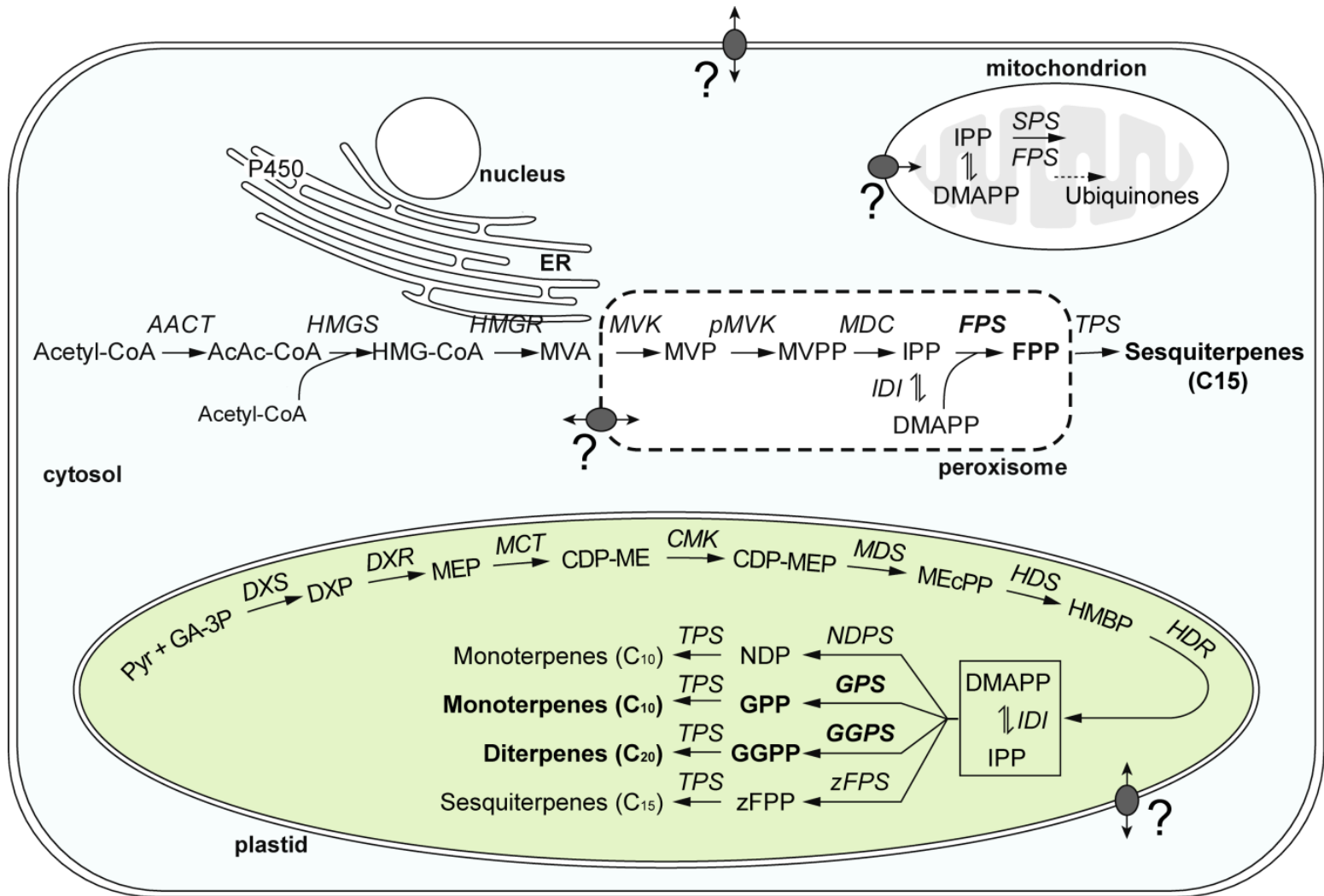
Phytoene (C40) → Carotenoids → Apocarotenoids

Strigolactones Ionones

Phytol-PP → chlorophyll Vitamin E

C30 to C50-PP → Ubiquinones
Plastoquinones

Origin of IPP and DMAPP: the MEV and MEP pathways



Questions

Some isoprenoids are produced in large quantities (structural components, secondary metabolites) while others are produced in extremely low amounts (phytohormones)

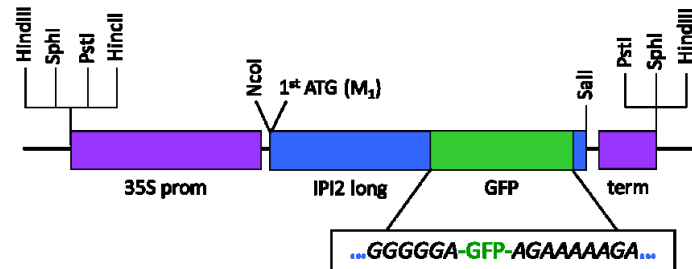
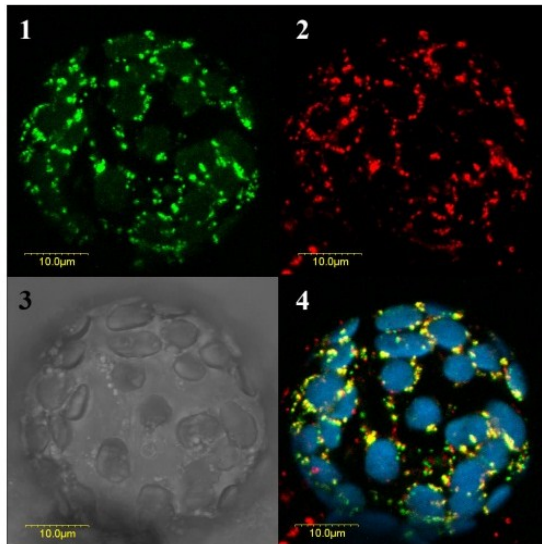
→ How is homeostasis achieved around the precursor pathways?

There is evidence of communication between the MEV and the MEP pathways.

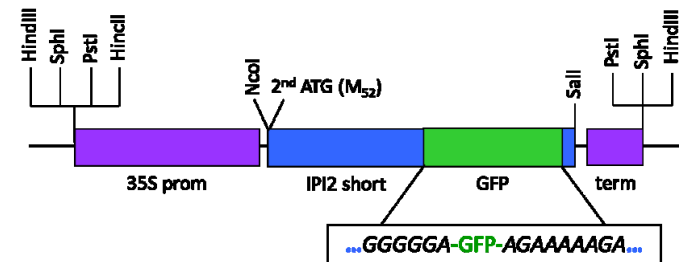
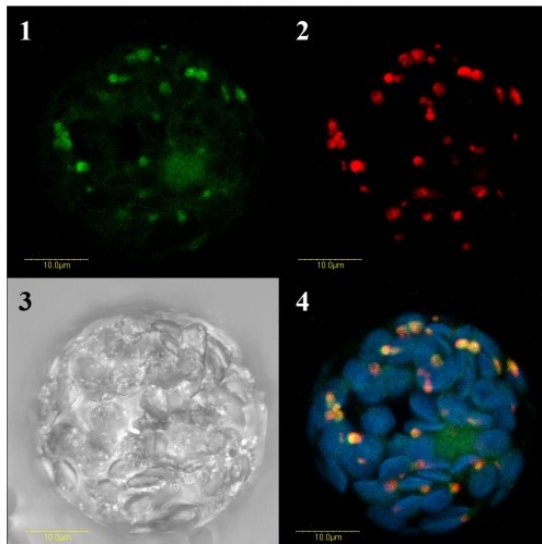
→ What are the transporters?

IPI (isopentenyl diphosphate isomerase) isoforms localize to mitochondria, peroxisomes and plastids

A



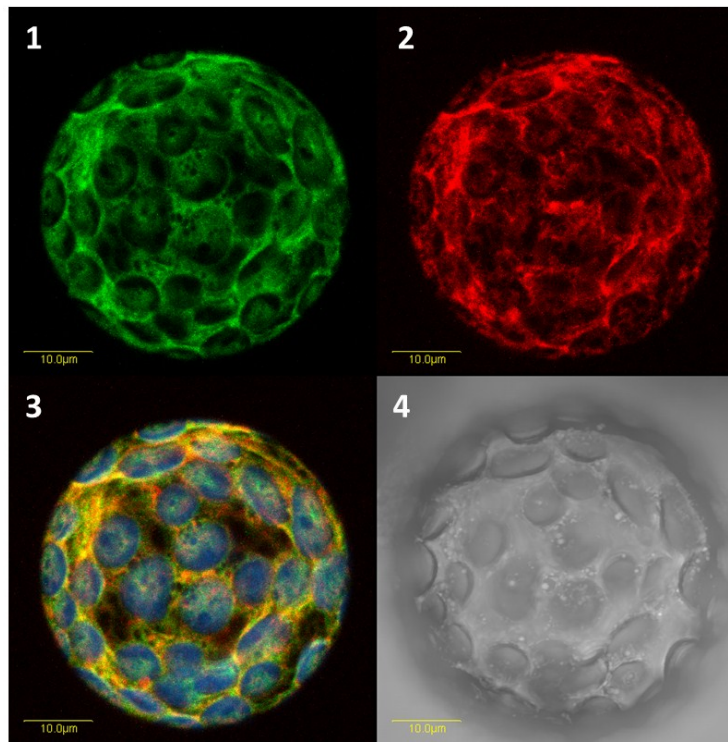
B



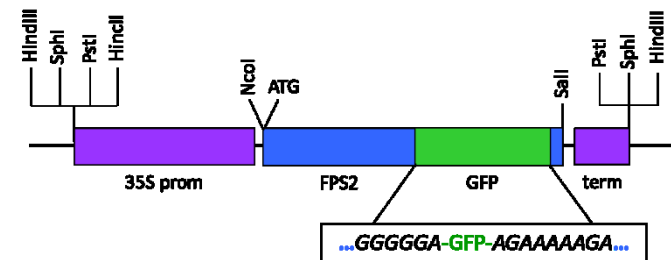
Y. Eyal

FPS-GFP co-localizes with ER-cherry in tobacco

Localization via protein-protein interaction ?

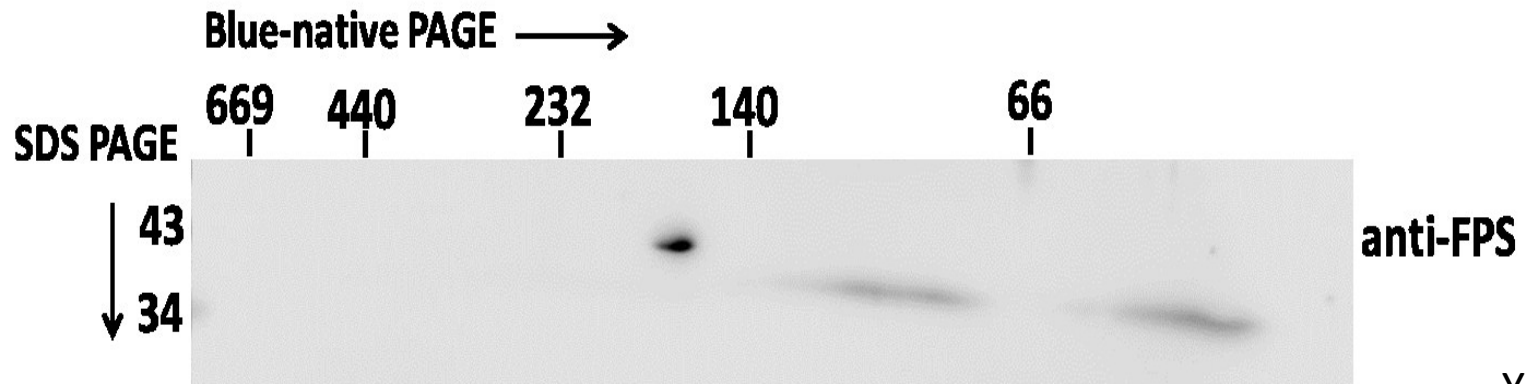


Y. Eyal



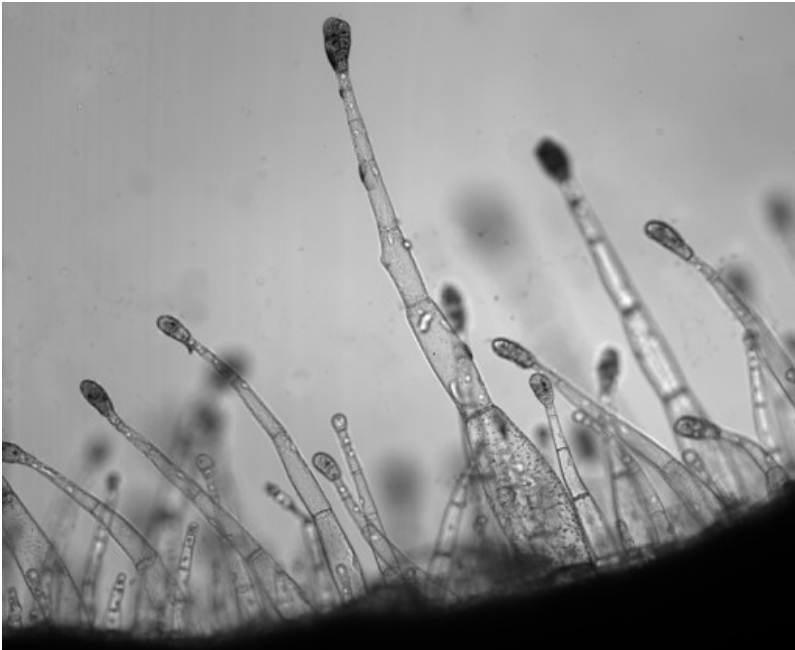
Arabidopsis (cell culture) FPS appears to be a dimer

Evidence for interaction with a additional proteins

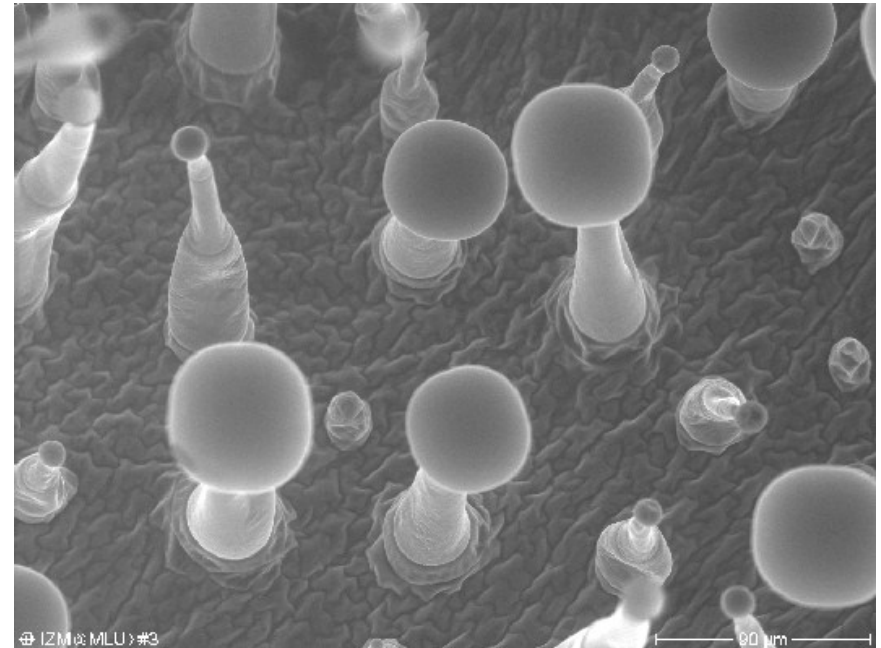


Y. Eyal

Our model systems: glandular trichomes of tobacco and tomato



tobacco
Diterpenoids
(← MEP pathway)



tomato
Sesquiterpenoids and monoterpenoids
(← MEP and MEV pathway)

H.I.P. Consortium



Partner 1

IPB

Leibniz
Institute of
Plant
Biochemistry

Partner 2

VC

Volcani Center
ARO
Yoram Eyal
(Israel)



Partner 3

UCL

Université
catholique de
Louvain
Marc Boutry
(Belgium)

Partner 4

UvA

University of
Amsterdam
Rob Schuurink
(Netherlands)



H.I.P. Project: Research Objectives

Workpackage 1 (WP1): Collection and construction of biological materials

Where are the enzymes located in the cell (a) and in the trichomes (b)?

Do the enzymes interact within the pathways (a) and/or with other proteins (b)?

How is the isoprenoid metabolism distributed in trichomes?

Workpackage 2 (WP2)

Fluorescent protein fusions

Proteomic analysis of trichome subcellular fractions

Targeted and non-targeted Y2H

Native gel assays
Immunoprecipitation

Workpackage 3 (WP3)

Search for transporter(s)

Pathway modification using RNAi and overexpression

Isotope labeling/Inhibitor Treatments - fluxomics

Task 1.1 Isolation and cloning of genes of isoprenoid pathways

MEP
MVA



S. lycopersicum



N. tabacum

MEP
MVA

Genetic resources

- Public databases
- PlantGDB-assembled unique transcripts
- TrichOME database
- Illumina RNA seq. tomato accessions (UvA, IPB)

Task 1.1 Isolation and cloning of genes of isoprenoid pathways

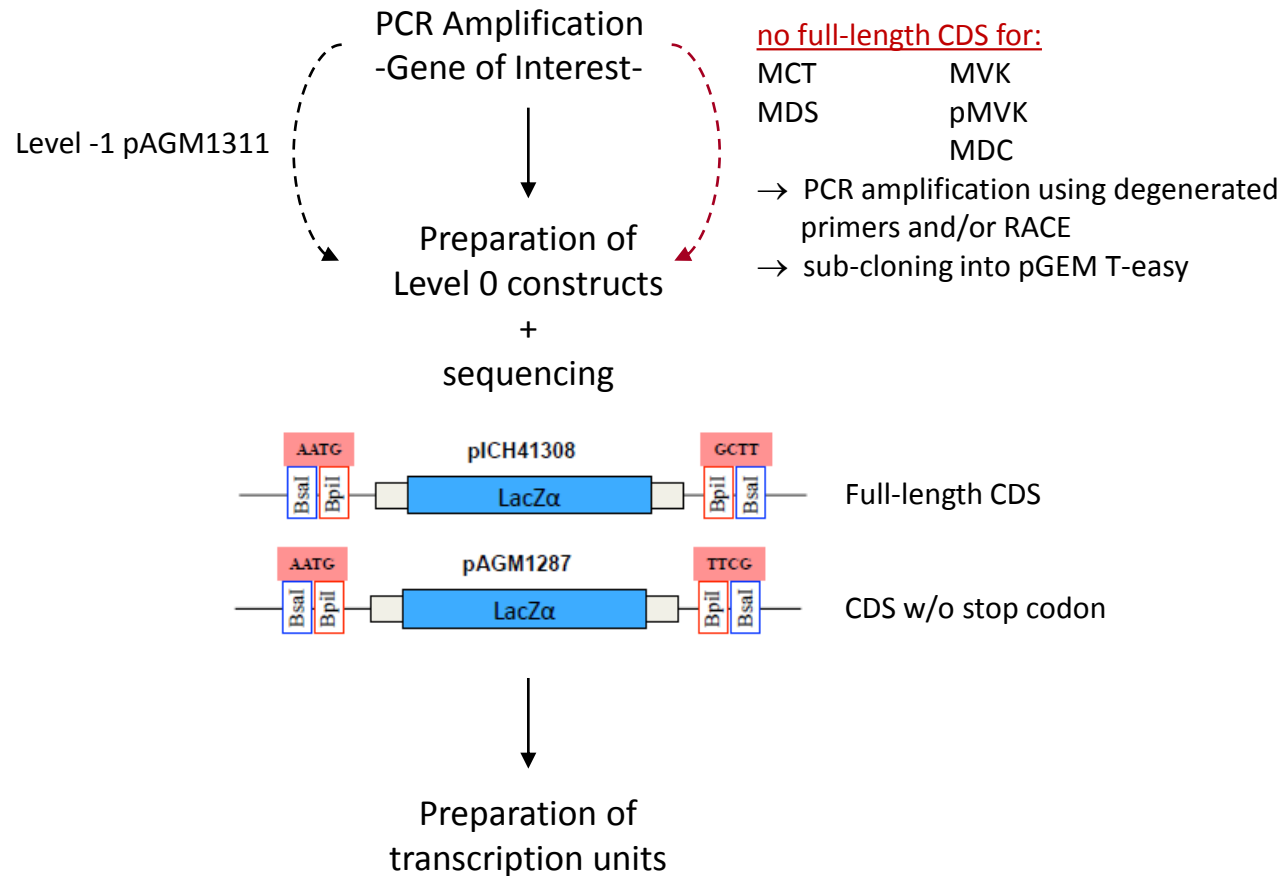
Annotation	Contig name	Accession	Contig length	Contigs coverage	Full-length ORF	Putative localization	Note
DXS	Nicotiana tabacum 1-D-deoxyxylulose 5-phosphate synthase 1 (dxs)	EU650419	2350	95,7%	2154 bp	plastid	Three genes in A. thaliana, but only one functional (At4g15560)
	Nicotiana tabacum 1-deoxy-D-xylulose-5-phosphate synthase (dxs gene) DXS isoform?	FN429979	2386				
	TCNT57154, Trichome db, full-length cds	TRIdb	2691				
	>lcl PUT-173a-Nicotiana_tabacum-28207_27349	PlantGDB	2425				
DXS	Nicotiana tabacum cultivar zhongyan100 1-D-deoxyxylulose 5-phosphate synthase 2	JQ085430	2420	99,3%	2142 bp	plastid	
	>lcl PUT-173a-Nicotiana_tabacum-129126_125010	PlantGDB	1658				
DXR	Nicotiana tabacum cultivar SR1 1-deoxy-D-xylulose-5-phosphate reductoisomerase	DQ839130	1804	98,8%	1422 bp	plastid	Single gene in A. thaliana with two protein variants (different transcription start)
	>lcl PUT-173a-Nicotiana_tabacum-30322_29415	PlantGDB	2278				
MCT	Nicotiana langsdorffii x Nicotiana sanderae MCT mRNA, complete cds	EF636807	939		?	plastid	Single gene in A. thaliana
CMK	Nicotiana tabacum 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (ispE) mRNA	KJ159923	1230	92,4%	1230 bp	plastid	Single gene in A. thaliana
	>lcl PUT-173a-Nicotiana_tabacum-51400_49765	PlantGDB	1125				
	TCNT44160, Trichome db, partial sequence	TRIdb	1186				
	Nicotiana benthamiana 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase mRNA	EF474476	1230				
MDS	Nicotiana tabacum 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase mRNA	KJ001135	1026	93,8%	696 bp	plastid	Single gene in A. thaliana, two splice variants
	Nicotiana langsdorffii x Nicotiana sanderae MDS mRNA, partial cds	EF636808	557				
HDS	Nicotiana benthamiana (E)-4-hydroxy-3-methylbut-2-enyl diphosphate synthase, partial cds	AY497303	804	99,0%	no full-length	plastid	Single gene in A. thaliana, three splice variants
	Nicotiana langsdorffii x Nicotiana sanderae HDS mRNA, partial cds	EF636809	786				
HDR	Nicotiana tabacum chloroplast HDR 1 (ISPH), nuclear gene	KC480443	1386	97,4%	1386	plastid	Single gene in A. thaliana
	Nicotiana tabacum cultivar NC 82 chloroplast HDR 2 (ISPH), nuclear gene	AF159699	1622				
	Nicotiana benthamiana HDR (IspH1) mRNA, complete cds; chloroplast	GQ495614	1386				
	Nicotiana langsdorffii x Nicotiana sanderae HDR mRNA, complete cds	EF636810	1387				
GPS	Nicotiana tabacum geranyl pyrophosphate synthase mRNA, complete cds	KF977582	1248	99,1%	1248	plastid	Single gene in A. thaliana, two splice variants (essential, loss-of-function mutants lethal)
	>lcl PUT-173a-Nicotiana_tabacum-12673_12300	PlantGDB	1726			mito	
	TCNT57777_139507, Trichome db, partial cds	TRIdb	1707				
GGPPS	Nicotiana tabacum geranylgeranyl diphosphate synthase (GGPPS1) mRNA, complete cds	GQ911583	1109	99,5%	1101	plastid	11 genes in A. thaliana ours most similar to GGPPS11
	>lcl PUT-173a-Nicotiana_tabacum-76246_73810	PlantGDB	1328				
GGPPS	Nicotiana tabacum geranylgeranyl diphosphate synthase (GGPPS2) mRNA, complete cds	GQ911584	1192	100,0%	1098	plastid	NtGGPPS2 ✓
	>lcl PUT-173a-Nicotiana_tabacum-61684_59714	PlantGDB	897				

Task 1.1 Isolation and cloning of genes of isoprenoid pathways

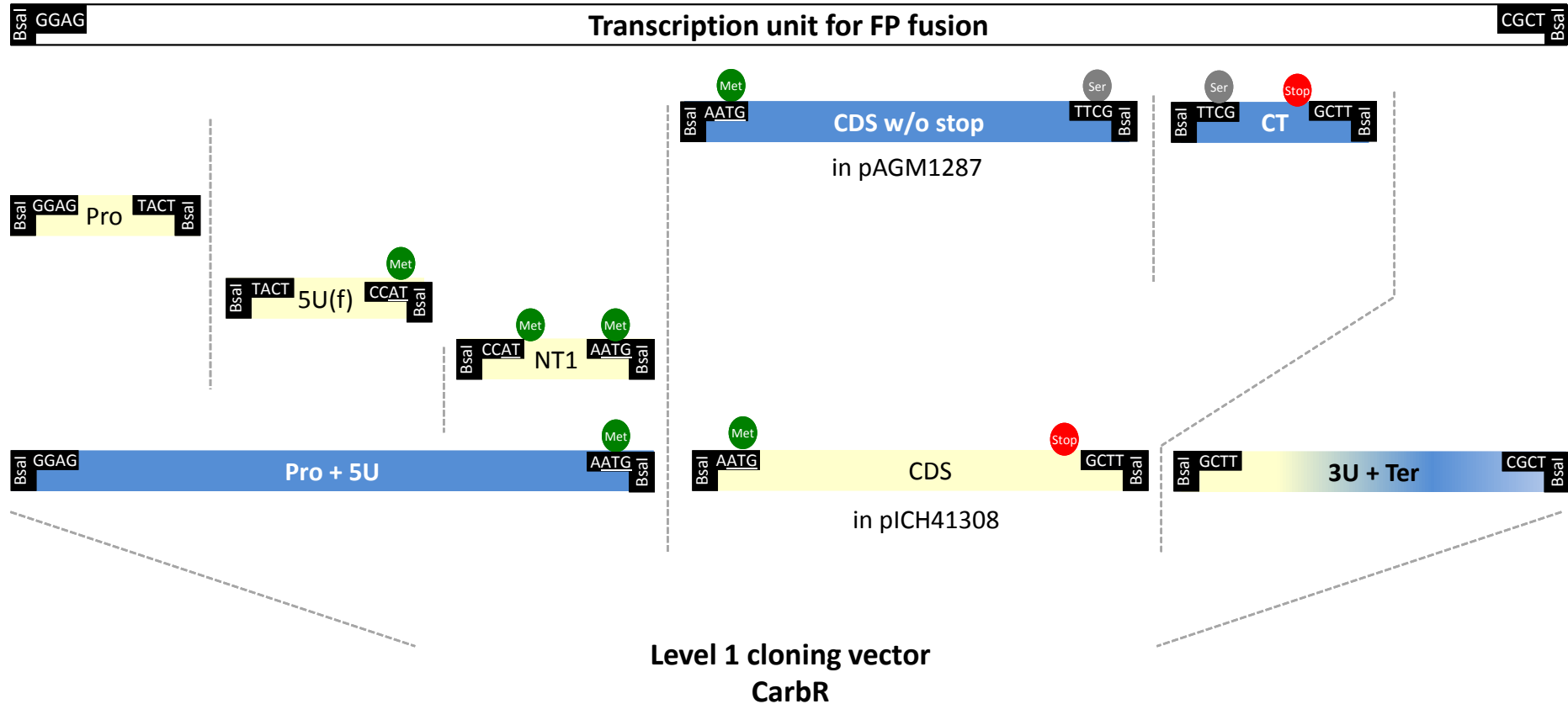
Annotation	Contig name	Accession	Contig length	Contigs coverage	Full-length ORF	Putative localization	Note
AACT	Nicotiana tabacum cytosolic acetoacetyl-coenzyme A thiolase (AACT1) >lcl PUT-173a-Nicotiana_tabacum-31156_30213	AY748245 PlantGDB	1455 1523	100,0%	1215 bp	cyt	Two genes in A. thaliana, each with splice variants; our most similar to At5g48230.2 (AtAACT2) splice variant (essential, loss-of-function mutants are lethal)
AACT	Nicotiana tabacum peroxisomal acetoacetyl-coenzyme A thiolase (AACT2) >lcl PUT-173a-Nicotiana_tabacum-121922_118047	AY748246 PlantGDB	1522 1687	99,9%	1245 bp	perox	most similar to At5g47720.4 splice variant of AtAACT1
HMGS	Nicotiana tabacum 3-hydroxy-3-methylglutaryl coenzyme A synthase >lcl PUT-173a-Nicotiana_tabacum-77184_74723 TCNT50253_136019m, Trichome db, partial cds --> Coverage to KJ001151 79,9%	KJ001151 PlantGDB TRIdb	1773 1272 2070	97,8%	1389 bp	cyt	Single gene, two cytosolic splice variants in A. thaliana, ours most similar to At4g11820.2 splice variant
HMGR	Nicotiana tabacum hydroxy-methylglutaryl-coenzyme A reductase (HMGR1) >lcl PUT-173a-Nicotiana_tabacum-128942_124829 >lcl PUT-173a-Nicotiana_tabacum-27392_26557	U60452 PlantGDB PlantGDB	2473 2484 1884	93,8%	1815 bp	ER	Two genes in Arabidopsis, AtHMGR1 gives 2 proteins (S and L) different at N-terminus
HMGR	Nicotiana tabacum hydroxy-methyl-glutaryl-coenzyme A reductase (HMGR2) >lcl PUT-173a-Nicotiana_tabacum-113075_109473	AF004232 PlantGDB	2377 2401	99,9%	1815 bp	ER	
HMGR	Nicotiana tabacum hydroxy-methyl-glutaryl-coenzyme A reductase (HMGR1)	AF004233	2406		1815 bp	ER	
MVK	Nicotiana sylvestris mevalonate kinase mRNA, complete cds TCNT47203, Trichome db, partial Nicotiana langsdorffii x Nicotiana sanderae mevalonate kinase mRNA, partial cds	KC871597 TRIdb EF636814	1158 516 787	97,6%	no full-length	cyt	Single gene, three cytosolic splice variants in A. thaliana
pMVK	Nicotiana langsdorffii x Nicotiana sanderae pMVK mRNA, partial cds	EF636815	471		no full-length	cyt	Single gene, 2 cytosolic splice variants in A. thaliana
MDC	Nicotiana langsdorffii x Nicotiana sanderae MDC, partial cds >lcl PUT-173a-Nicotiana_tabacum-83195_80530	EF636816 PlantGDB	1463 883	92,0%	no full-length	?	Two genes in A. thaliana, MDC1 located in cytosol
IDI	Nicotiana tabacum ipi1 mRNA for isopentenyl diphosphate isomerase 1, complete cds N. tabacum mRNA for isopentenyl pyrophosphate isomerase >lcl PUT-173a-Nicotiana_tabacum-218133667_131592 TCNT59754_132048, Trichome db, partial cds TCNT41282, Trichome db, partial cds	AB049815 Y09634 PlantGDB TRIdb TRIdb	1107 850 1154 1136 683	91,3%	873	plastid	Two genes with each two protein variants (S and L) in A. thaliana, 1S and 2S (perox.), 1L (mito), 1L and 2L (plast.)
IDI	Nicotiana tabacum ipi2 mRNA for isopentenyl diphosphate isomerase 2, complete cds >lcl PUT-173a-Nicotiana_tabacum-44503_43104 TCNT60911_139500, Trichome db, partial cds TCNT42236, Trichome db, partial cds	AB049816 PlantGDB TRIdb TRIdb	993 1064 948 859	92,9%	708	perox	
FDS	Nicotiana tabacum farnesyl pyrophosphate synthase 1 mRNA, complete cds >lcl PUT-173a-Nicotiana_tabacum-46625_45172	KJ001141 PlantGDB	1406 1358	95,9%	1026	cyt	Two genes, At5g47770 with protein variants (S and L) in A. thaliana, 1L (mito), 2 and 1S (cytosol); our most similar to At4g17190 (AtFPPS2)

Task 1.2 Preparation of constructs for WP2 and WP3

Golden Gate cloning & MoClo

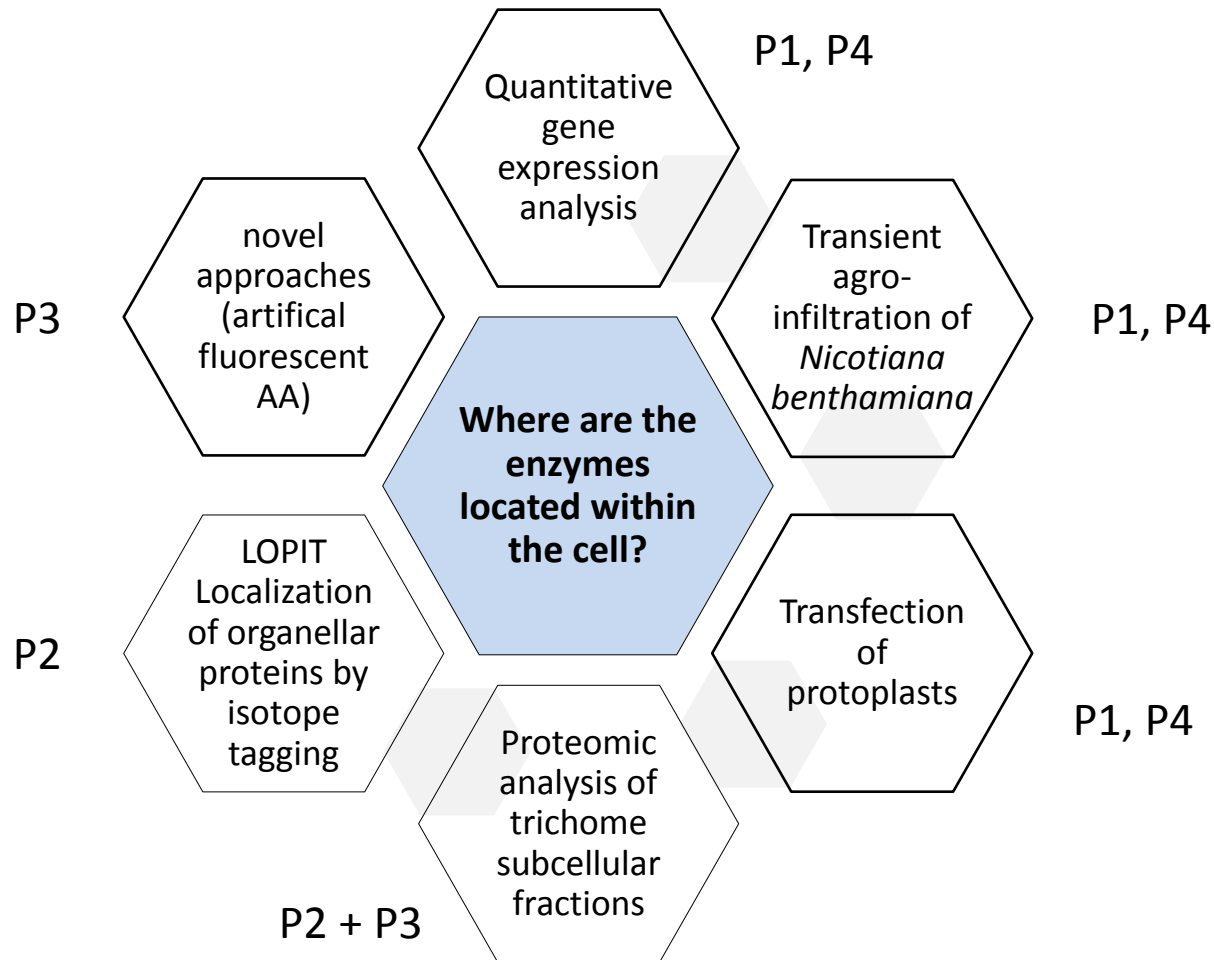


Task 1.2 Preparation of constructs for WP2 and WP3



+ Development of a yeast two-hybrid Golden Gate compatible system

Task 2.1 Subcellular localization

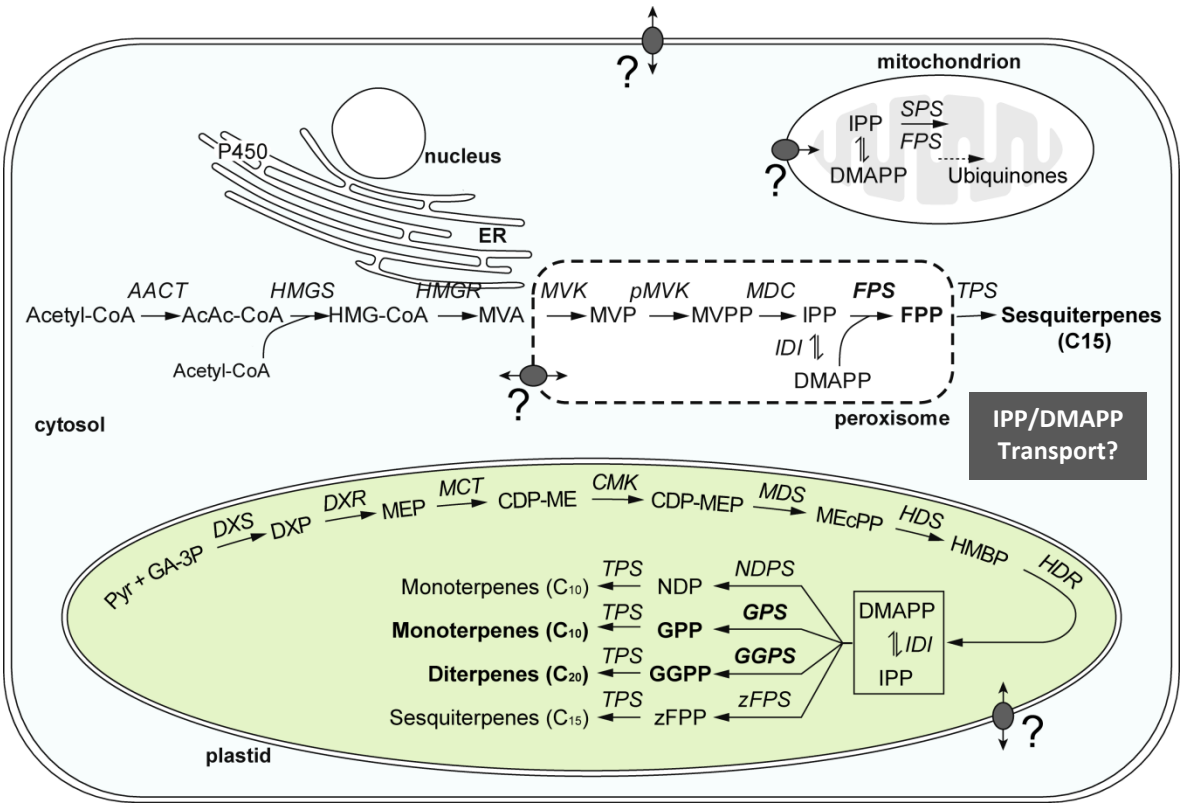


Task 2.2 Protein-protein interactions

- Production of novel antibodies against selected enzymes P1, P3
- Pairwise Y2H within pathway genes P4
- Untargeted Y2H for selected genes (ex: FPS, DXS, GGPPS) P4
- Native gel separation + immunodetection + proteomics P3, P2
- IP + proteomics P3, P2

→ Further characterization of interactors (e.g. impact on enzyme activity or localization)

Task 3.1 Search for genes involved in isoprenoid transport/secretion



Search for gene candidates encoding:

- ABC Transporter
- Lipid Transfer Proteins (LTP)
- MATE proteins
- Major Facilitator
- Proton pumps (antiport)
- ... other transporter families?

Task 3.1 Search for genes involved in isoprenoid transport/secretion

***in silico* prediction**

NGS RNA seq. data from different Tomato accessions (IPB, UvA)

List of gene candidates:

- High expression in trichomes
- Predicted subcellular localization related to compartmentalization of MEP/MVA pathways

Transcriptomic approach

RNA sequencing of *N. tabacum* trichomes

N. tabacum cv. Virginia (wild-type)

N. tabacum Virgin A Mutant (↓ secretion/transport)

→ Differential gene expression analysis + *de novo* assembly

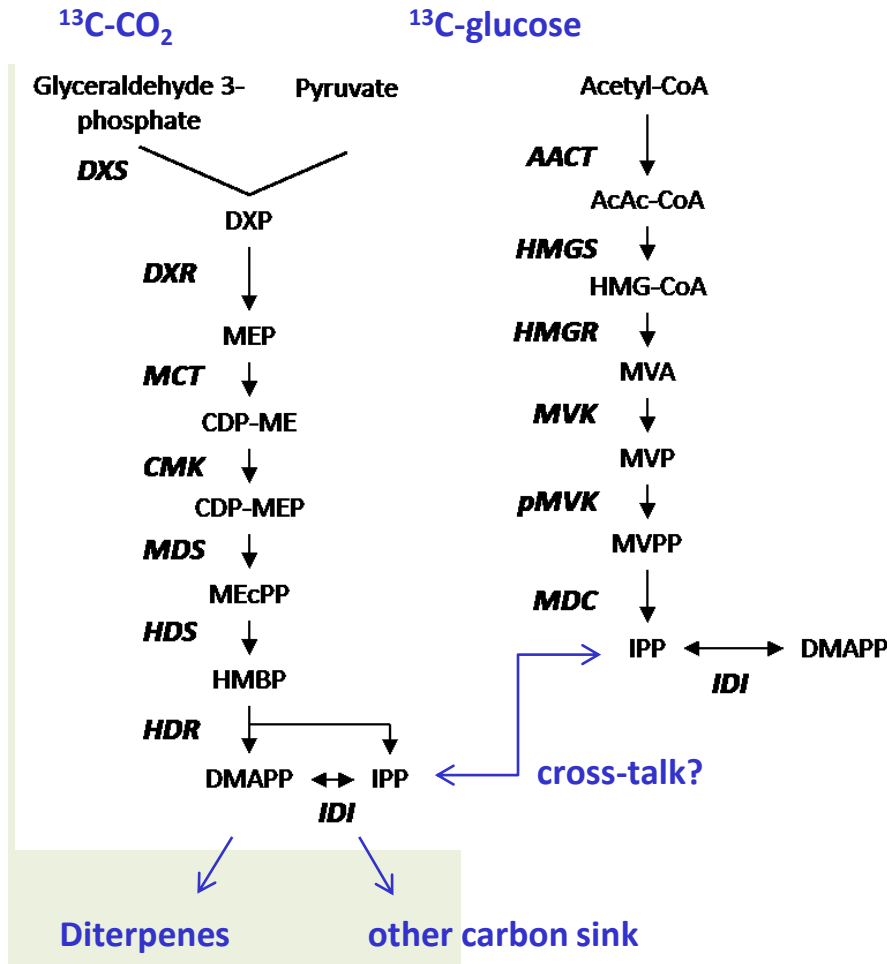
Proteomic approach

Proteomic analysis of membrane fractions

... of different plant lines with altered isoprenoid flux

- due to RNAi or,
- due to defective secretion (Virgin A Mutant) or,
- due to application of pathway inhibitors

Task 3.2 Characterization of pathway flux



How is the isoprenoid metabolism distributed in trichomes?

Analysis of carbon fluxes through MEP and MVA pathways:

- to synthesis of diterpenoids in Tobacco trichomes
- to synthesis of sesquiterpenes in *S. habrochaites* trichomes
- Comparison leaf/trichomes

Use *N. tabacum* mutant lines

- Virgin A. Mutant TI1406
↓ isoprenoid transport/secretion
- no-diTP-3H02 → no diterpenes
- RNAi lines against selected genes of the pathways

Organisation and administrative aspects

- Project officially started on April 1st, 2014
- Each partner has 1 post-doc for the duration of the project
- Consortium Agreement is under review by all partners. Should be signed by the end of the month
- An internet web page is planned (within next 3 months)



Conclusions

- A multidisciplinary approach to the study of isoprenoid precursor pathways
 - New material and avenues for understanding isoprenoid pathway regulation
- Better understanding of metabolic partitioning of isoprenoids
- Application for metabolic engineering of terpenoids

Thanks to



and you for your attention