

# Homeostasis of Isoprenoids in Plants

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ERA-NET for Coordinating  
Action in Plant Sciences



# The H.I.P Consortium



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Biochemistry

**Partner 2**  
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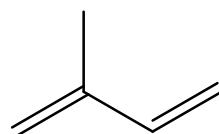
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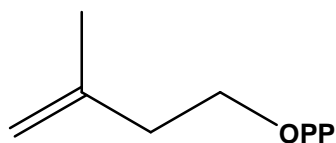
# Isoprenoid building blocks

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

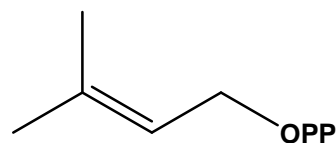


isoprene

All isoprenoids are synthesized from the same building blocks

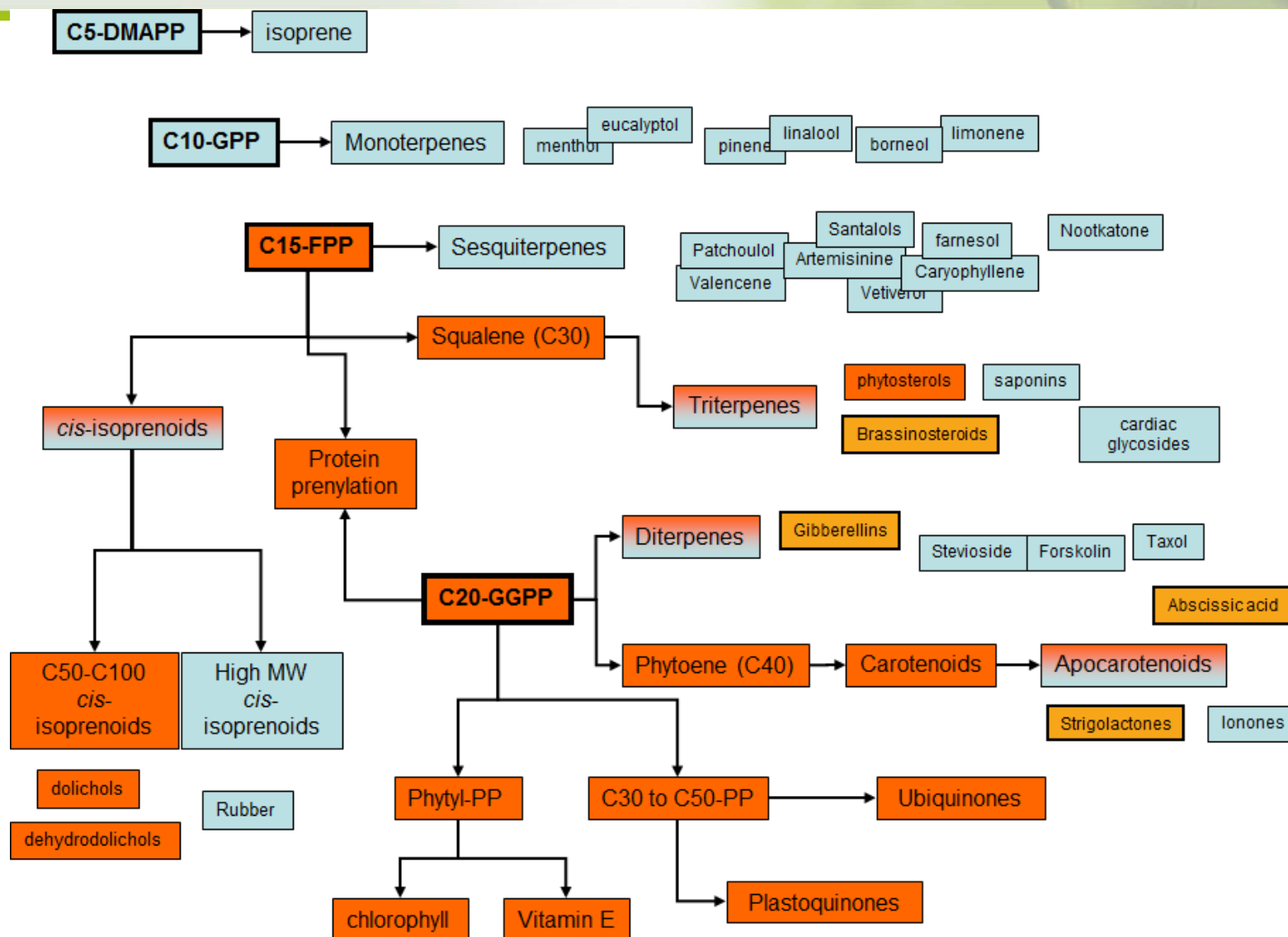


Isopentenyl diphosphate  
(IPP)

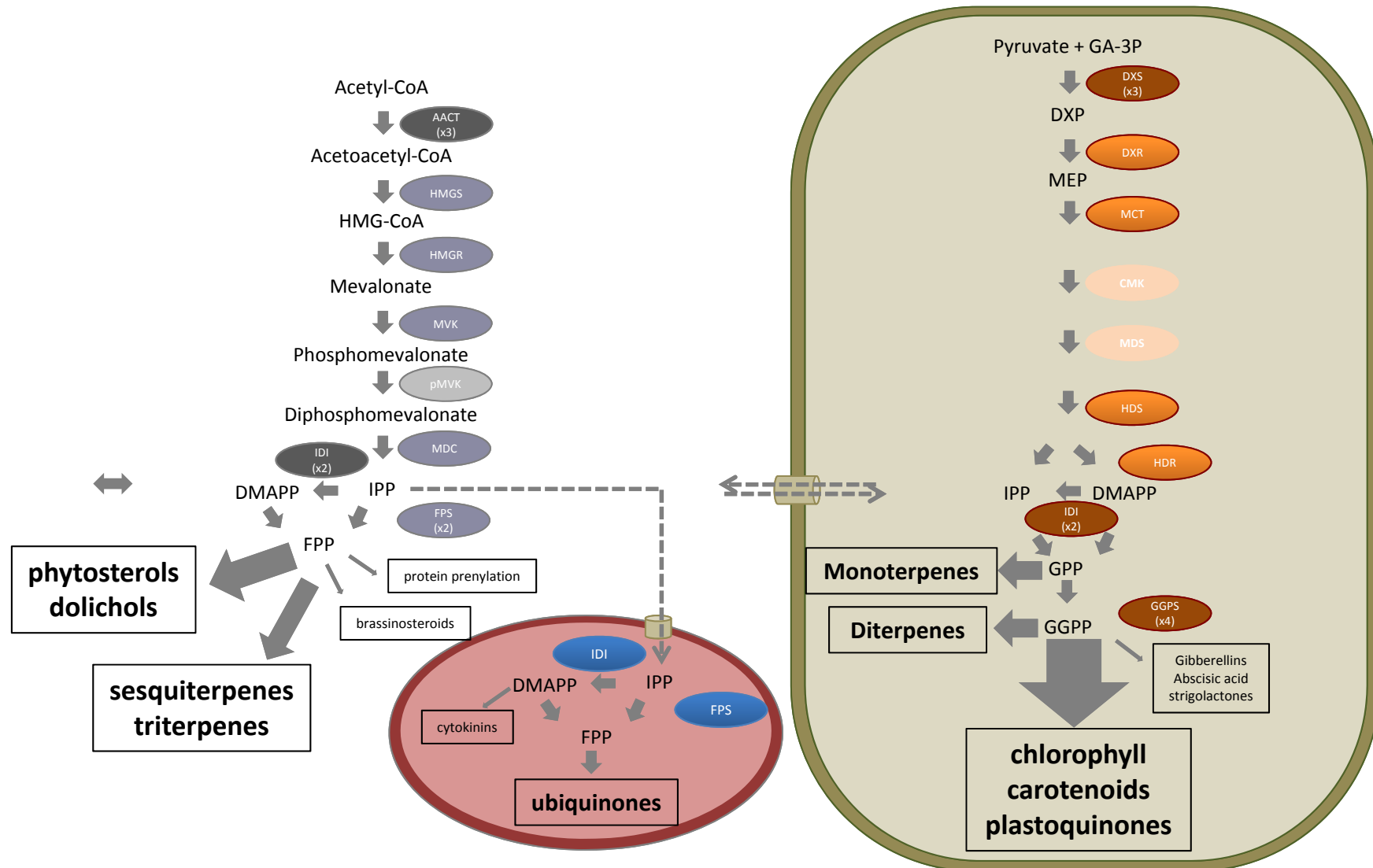


dimethylallyl diphosphate  
(DMAPP)

# The isoprenome



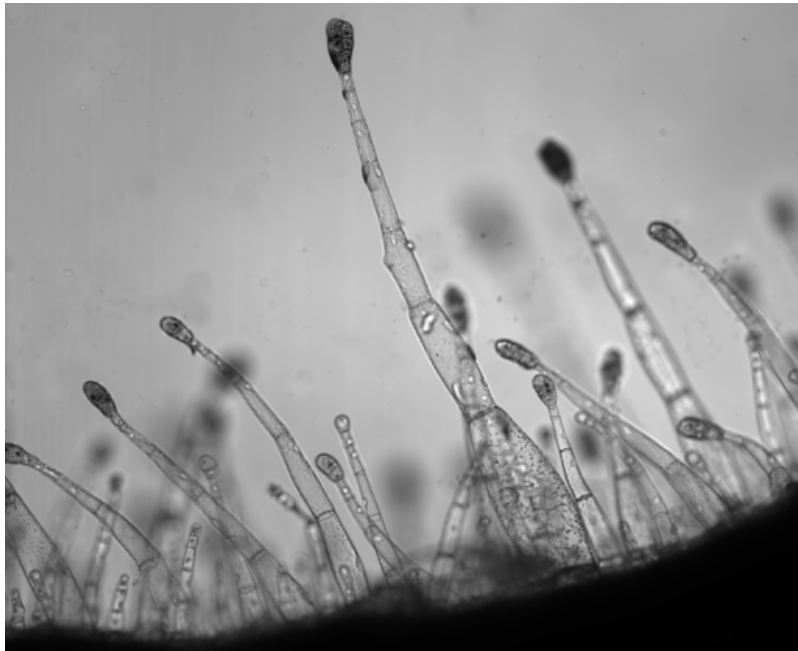
# The precursor pathways



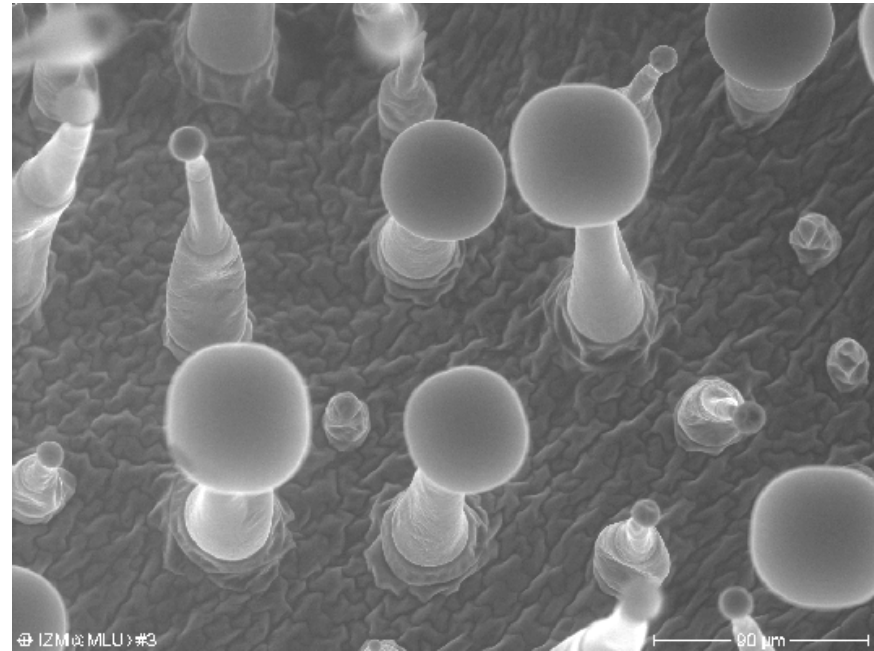
# questions

- How do cells to maintain adequate fluxes for compounds that are in large (e.g. housekeeping) and compounds that are in small concentrations (hormones)?
- How do the precursor pathways (MEP and MEV) communicate with each other?

# our model systems



tobacco  
Diterpenoids  
(← MEP pathway)



tomato  
Sesquiterpenoids and monoterpenoids  
(← MEP and MEV pathway)



# Tasks and WPs

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

Where are the enzymes located in the cell?

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



# Comprehensive gene cloning

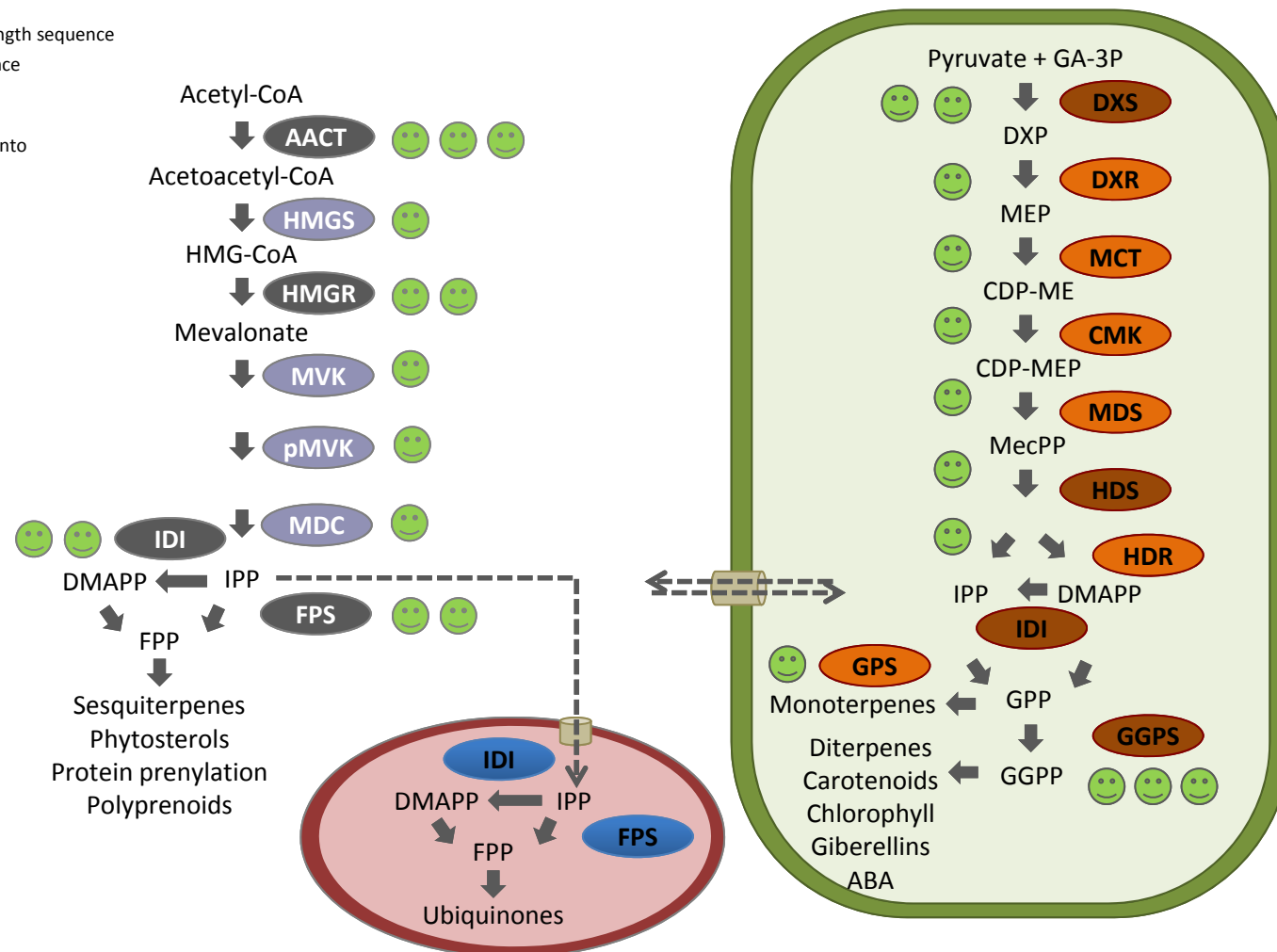
MEV MEP

More than 1 full-length sequence  
1 full-length sequence

From publicly available databases

Tobacco candidates cloned into Golden Gate entry vectors.

Similar results with tomato genes



### ***Ongoing***

- Systematic subcellular localization of MEP/MEV enzymes
- Membrane proteomics in the presence of pathway inhibitors to identify transporters
- Immunoprecipitation to identify protein complexes

### ***Next***

- 2-hybrid (targeted and shotgun)
- Organelle-specific proteomics
- Transcriptomics and proteomics of mutants/knock-downs at specific steps of the MEP/MEV pathway

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Thanks for your attention



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