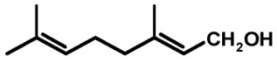
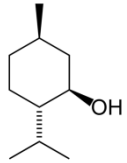
Isoprenoids

isoprene unit
$(C_5H_8)_n$

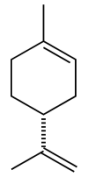
monoterpenoids
n=2



geraniol



menthol

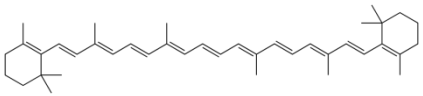

limonene

hemiterpenoids
n=1

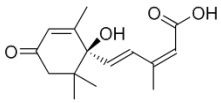
polyterpenoids
n>8


rubber

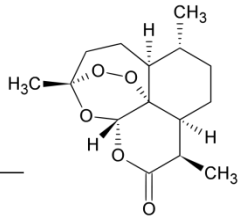
tetraterpenoids
n=8


β-carotene

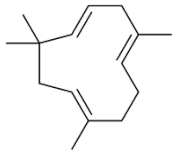
sesquiterpenoids
n=3



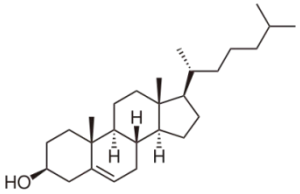
abscisic acid



artemisinin

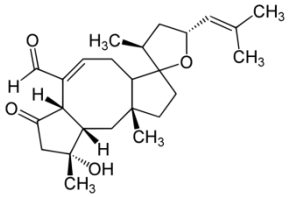

humulene

triterpenoids
n=6



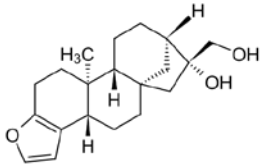
cholesterol

sesterterpenoids
n=5

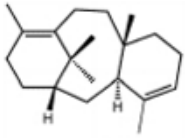


ophiobolin A

diterpenoids
n=4



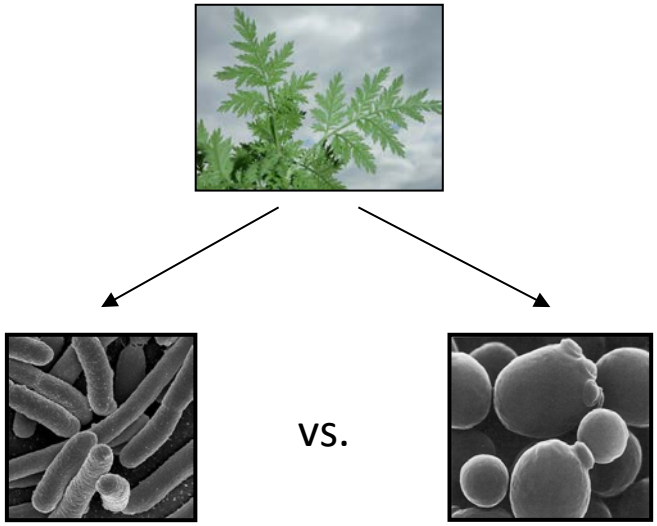
cafestol



taxadiene

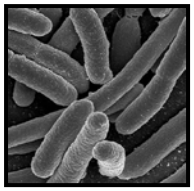
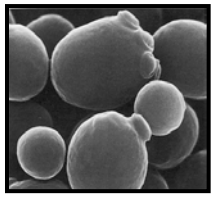
Isoprenoids

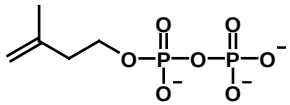
Pathway transfer from plant to microorganism



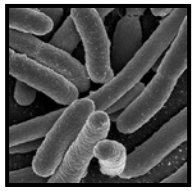
Isoprenoids

2 metabolic pathways

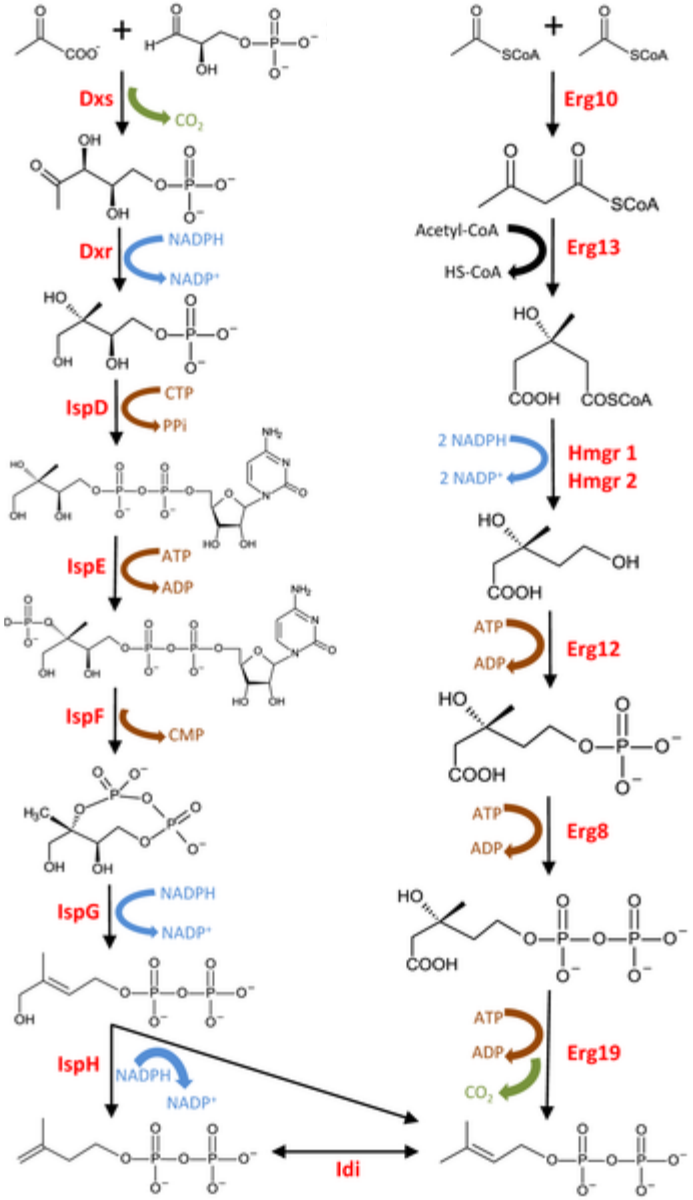
non-mevalonate pathway (DXP pathway, MEP pathway)	mevalonate pathway (MVA pathway)
	
eubacteria plants (plastides) apicomplexa	eukaryotes plants (cytosol) archaea, few eubacteria
	
precursors: pyruvate + glyceraldehyde-3-phosphate	precursor: acetyl-CoA
1 glucose per IPP	1.5 glucose per IPP



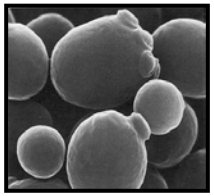
IPP



MEP pathway

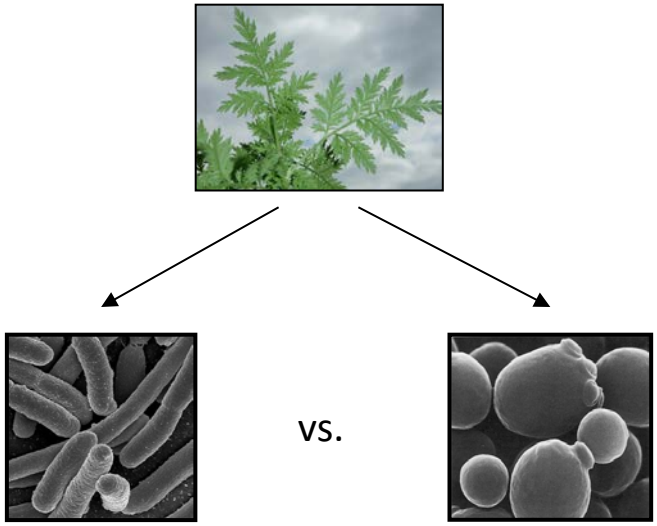


MVA pathway



Partow *et al*. (2012) PLoS ONE 7:e52498

Isoprenoids

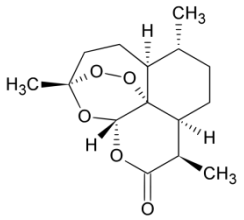


Advantage *E. coli*: more efficient pathway -> higher theoretical yields

Advantage *S. cerevisiae*: better expression host for auxiliary enzymes (P450 monooxygenases)

=> choice may depend on product

Artemisinin



Antimalarial drug precursor

Derivatives used in antimalarial combination therapies

Derived from sweet wormwood plant (*Artemisia annua*)

Fluctuating price and availability

⇒ Semi-synthetic artemisinin project:

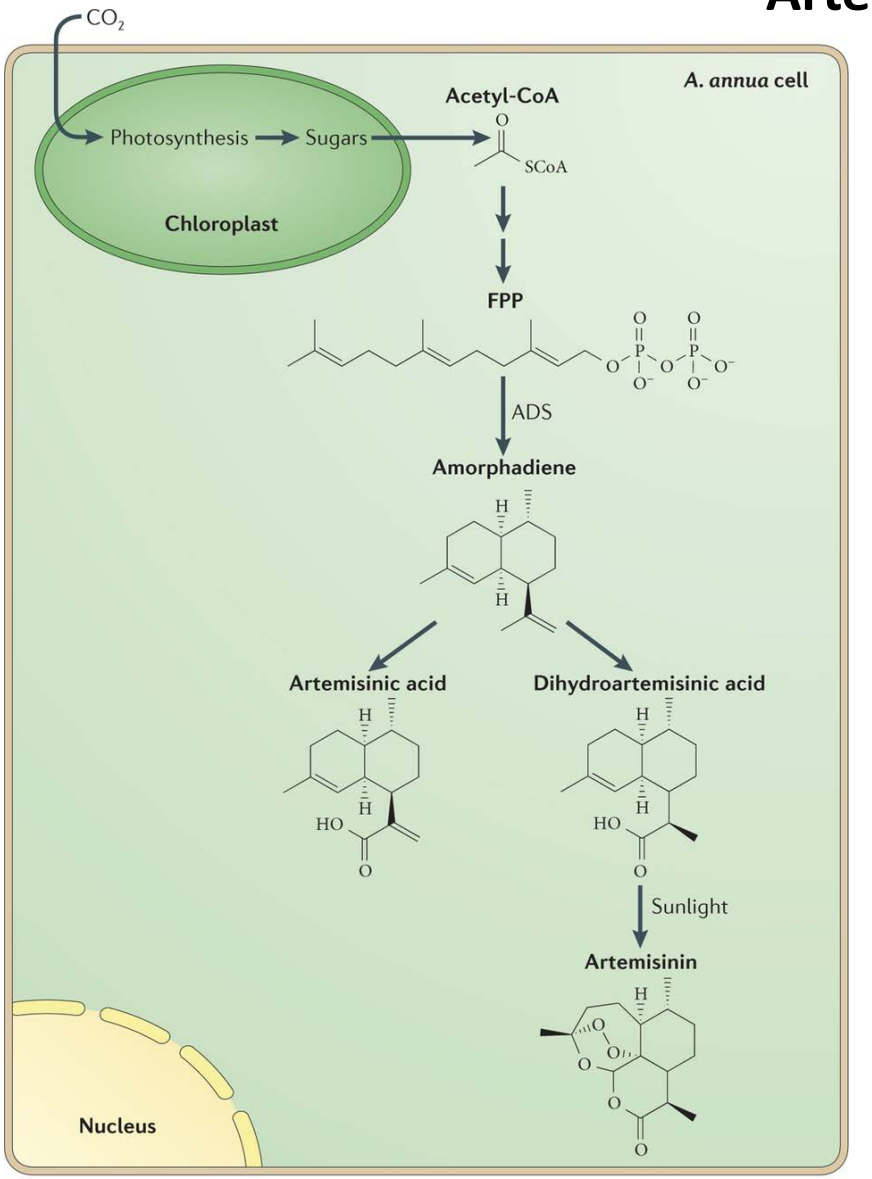
Started in 2004

Funded by Bill and Melinda Gates Foundation

Collaboration between UC Berkeley, Amyris, Institute for OneWorld Health

Paddon & Keasling (2014) Nature Rev 12:355-367

Artemisinin



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Plant pathway:

Conversion of FPP tp amorphadiene via amorphadiene synthase (ADS)

2 oxidation products: artemisinic acid, dihydroartemisinic acid

spontaneous conversion of dehydroartemisinic acid to artemisinin

Project aim:

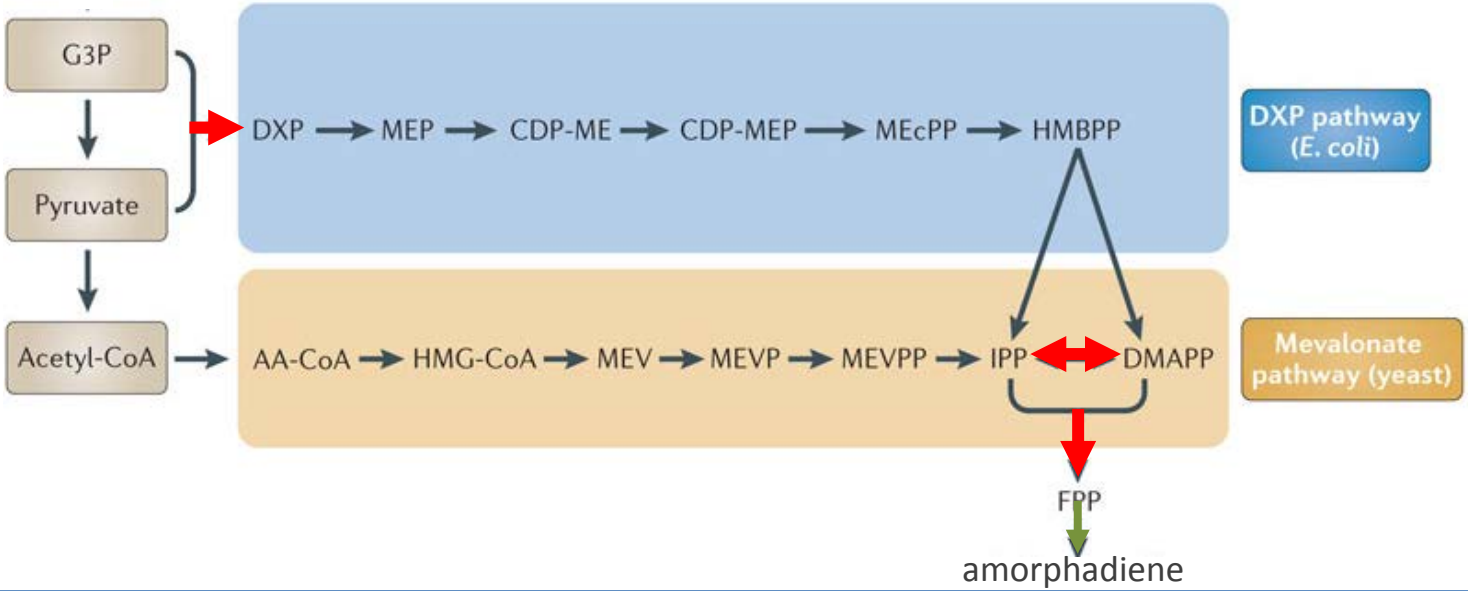
Production of artemisinic acid in *E. coli*

Chemical conversion to artemisinin

Artemisinin

1. Amorphadiene production in *E. coli*

- Use of plant terpene synthases with limited success
- Overexpression of rate-limiting DXP pathway enzymes with limited success (probably due to internal regulatory mechanisms)



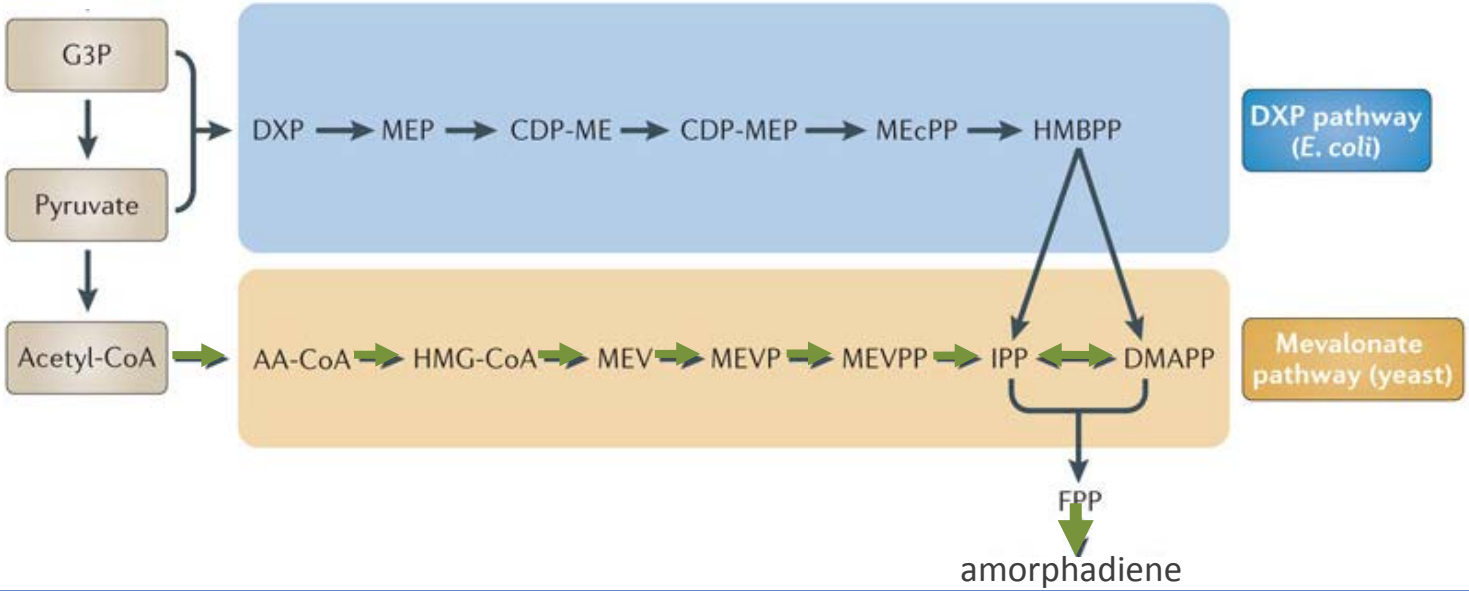
Paddon & Keasling (2014) Nature Rev 12:355-367

Artemisinin

1. Amorphadiene production in *E. coli*

- Use of plant terpene synthases with limited success
- Overexpression of rate-limiting DXP pathway enzymes with limited success

⇒ Expression of codon-optimised ADS
⇒ Expression of yeast MVA pathway -> 0.5 g/l amorphadiene



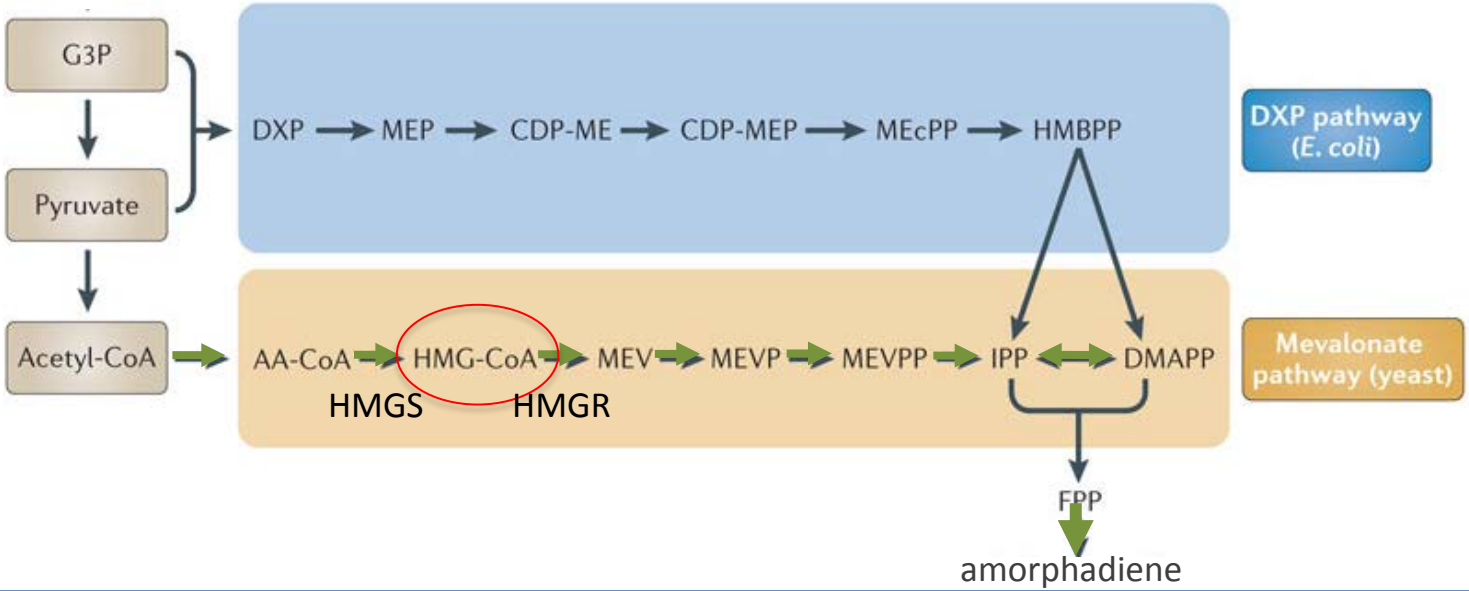
Paddon & Keasling (2014) Nature Rev 12:355-367

Artemisinin

1. Amorphadiene production in *E. coli*

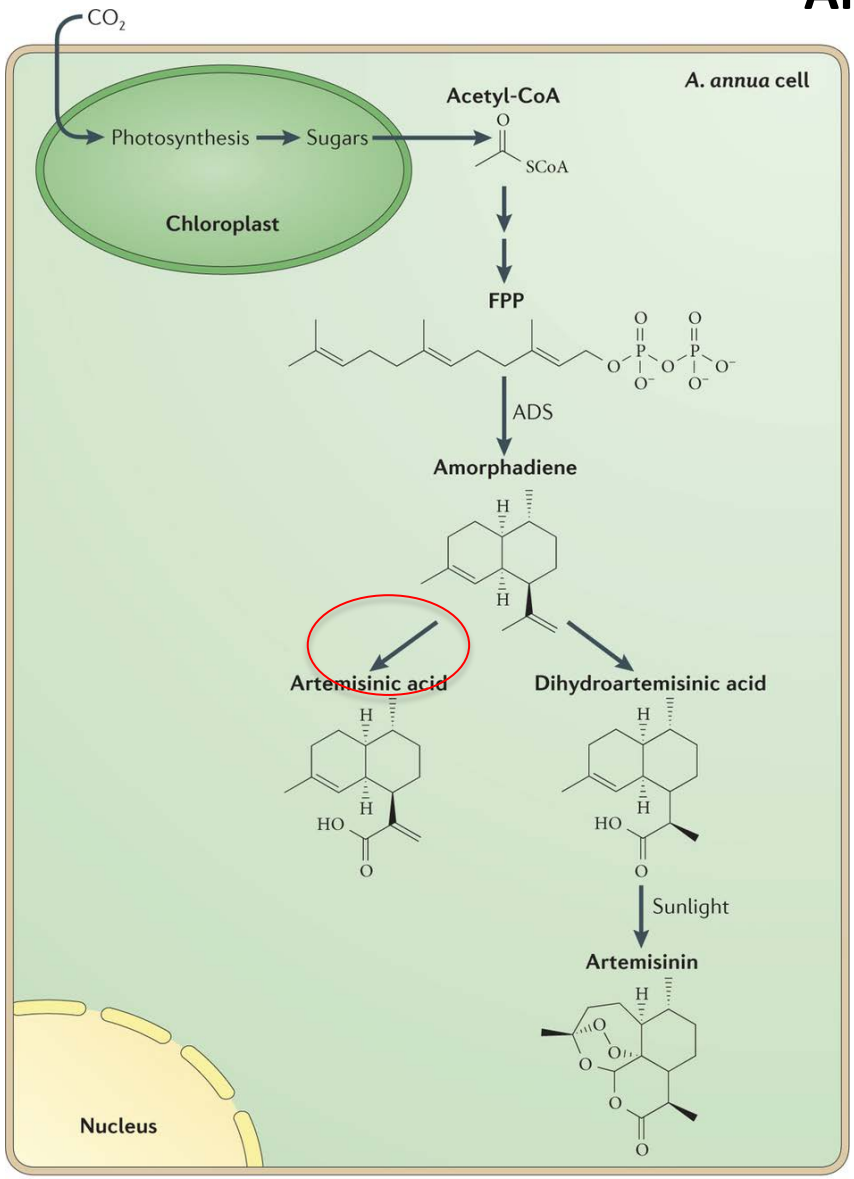
Problem: Imbalanced enzyme expression leading to HMG-CoA accumulation and growth inhibition

Solution: Balanced expression of HMGS and HMGR, replacement of yeast enzymes with bacterial enzymes
Fermentation optimisation -> 25 g/l amorphadiene



Paddon & Keasling (2014) Nature Rev 12:355-367

Artemisinin



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2. Switch to yeast

a) Identification of enzyme converting amorphadiene to artemisinic acid:

CYP71AV (P450 monooxygenase)

-> 100 mg/l artemisinic acid in primarily engineered strain

Paddon & Keasling (2014) Nature Rev 12:355-367

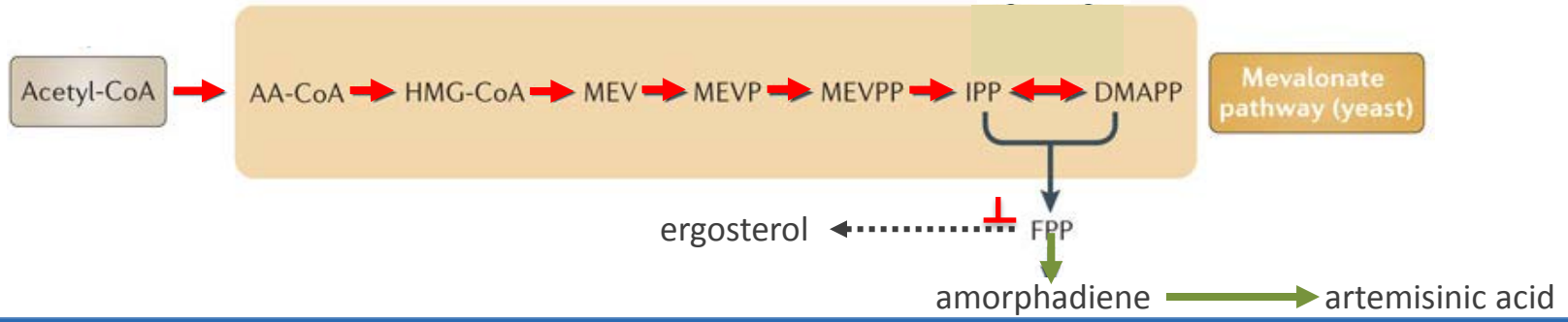
Artemisinin

2. Switch to yeast

b) Pathway optimisation

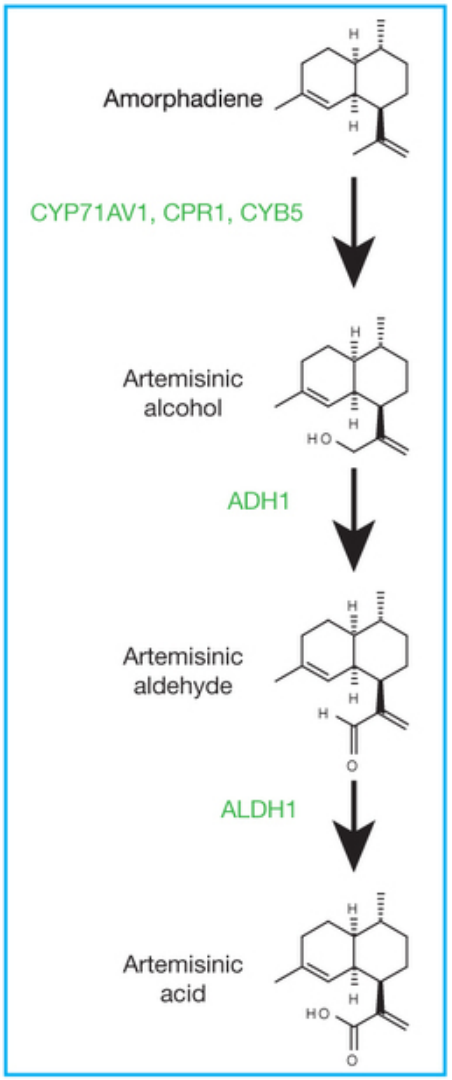
- Overexpression of all MVA pathway genes
- Downregulation of first step of ergosterol formation (gene under regulation of methionine- or copper-regulatable promoter)
- Fermentation optimisation -> 40 g/l amorphadiene

but: amorphadiene production 10x higher than artemisinic acid production



Paddon & Keasling (2014) Nature Rev 12:355-367

Artemisinin



2. Switch to yeast

c) Optimisation of oxidation step

Engineered strains with low viability due to oxidative stress
(poor coupling between reductase CPR1 and CYP71AV1)

=> Reduction of CPR1 expression, additional expression of cytochrome b_5

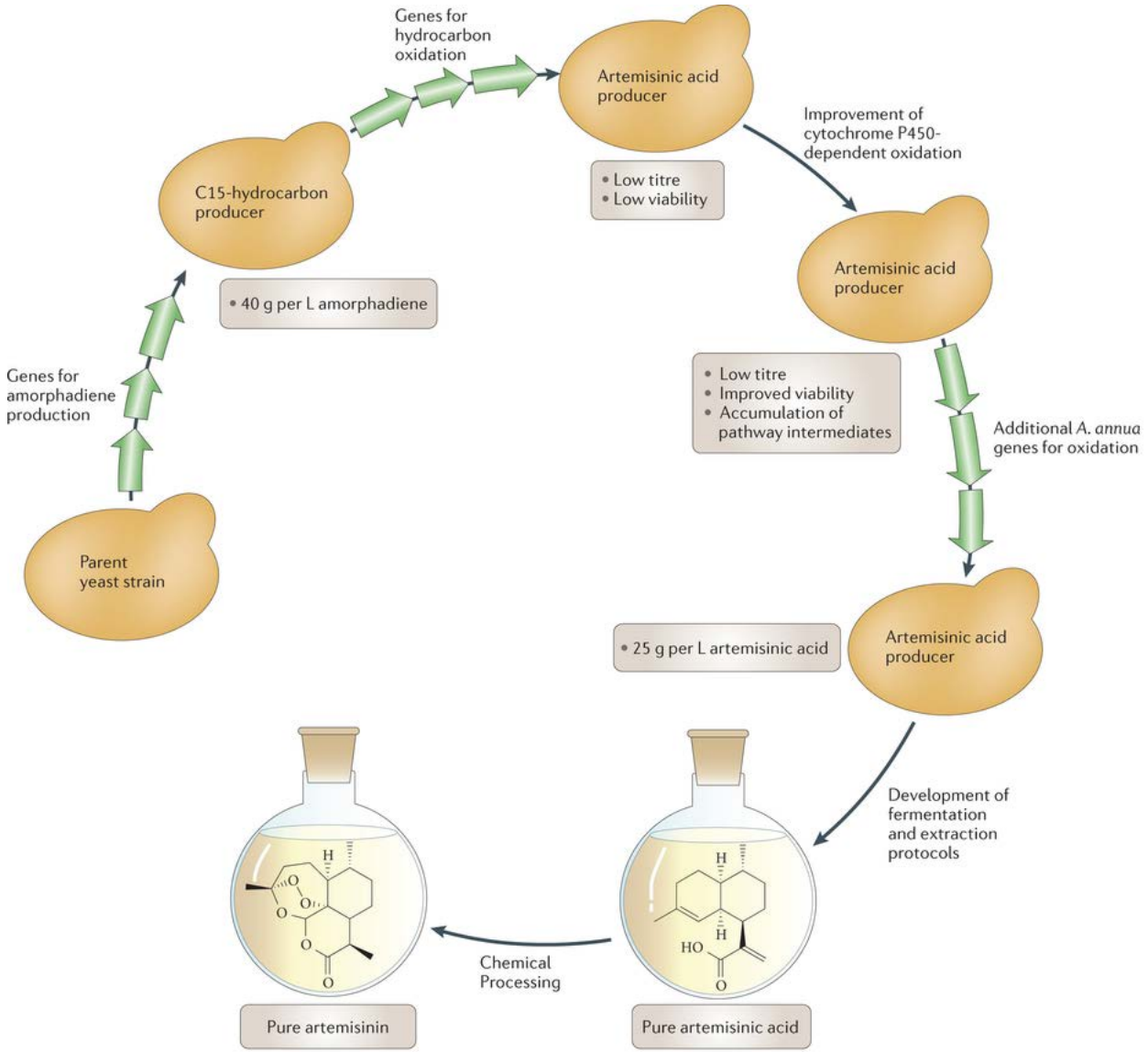
Accumulation of oxidation intermediates

⇒ Additional expression of *A. annua* aldehyde dehydrogenase ALDH1 and alcohol dehydrogenase ADH1

-> 25 g/l artemisinic acid

Paddon *et al*. (2013) Nature 496:528-532

Artemisinin



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Paddon & Keasling (2014) Nature Rev 12:355-367

Conclusion (Part I)

Yeast as a suitable host for production of high-value compounds

Engineering strategies often product specific and time consuming

$\Rightarrow$ Need for better predictability, universal strategies, platform strains

Systems biology for the improvement of microbial fermentations

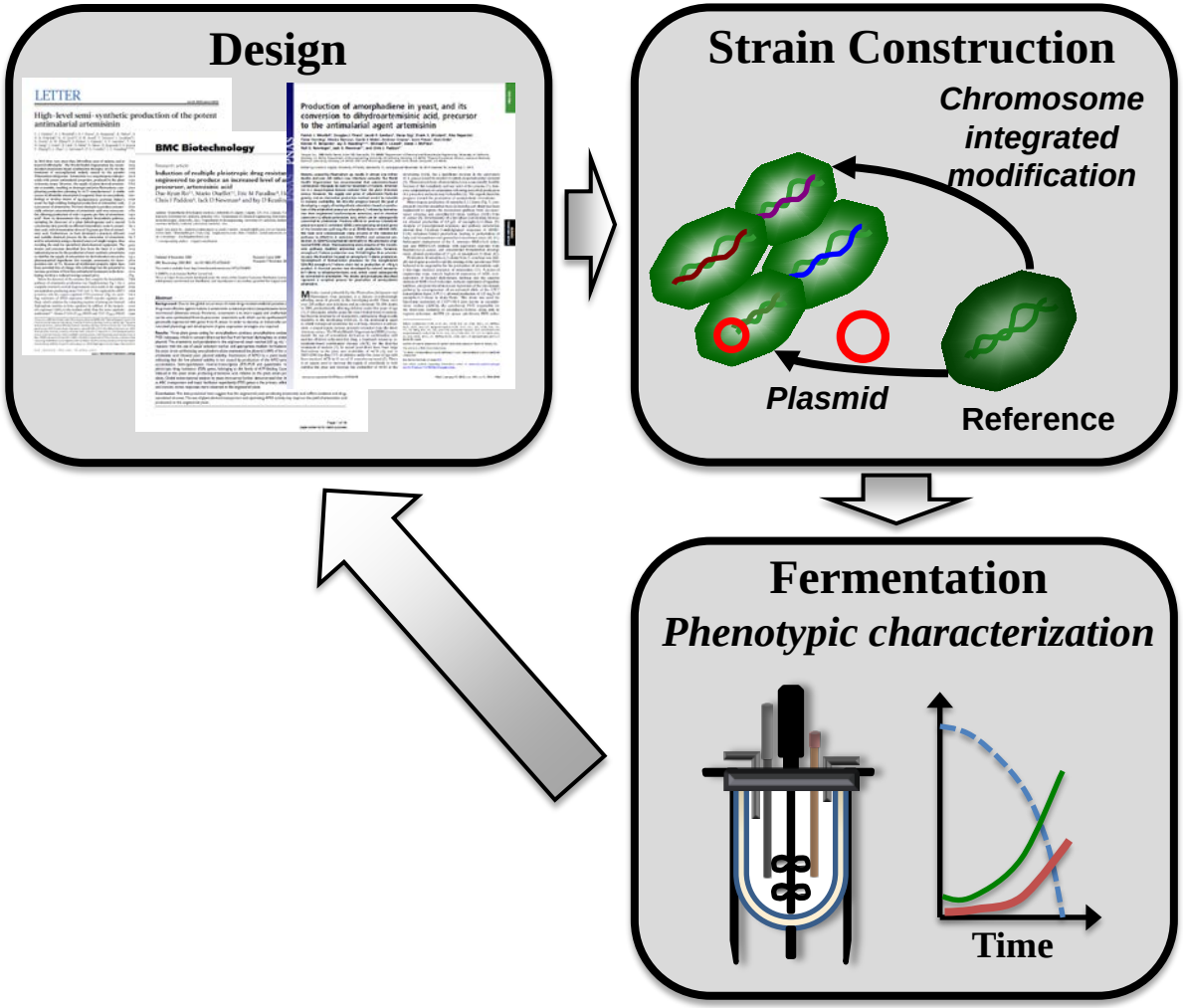
Verena Siewers

Systems and Synthetic Biology

Chalmers University of Technology

Campinas, 12 October 2014

The metabolic engineering cycle



Metabolic Eng. (2004) 6:204-211