Bioenergy beyond biofuels: High-value, low-volume compounds

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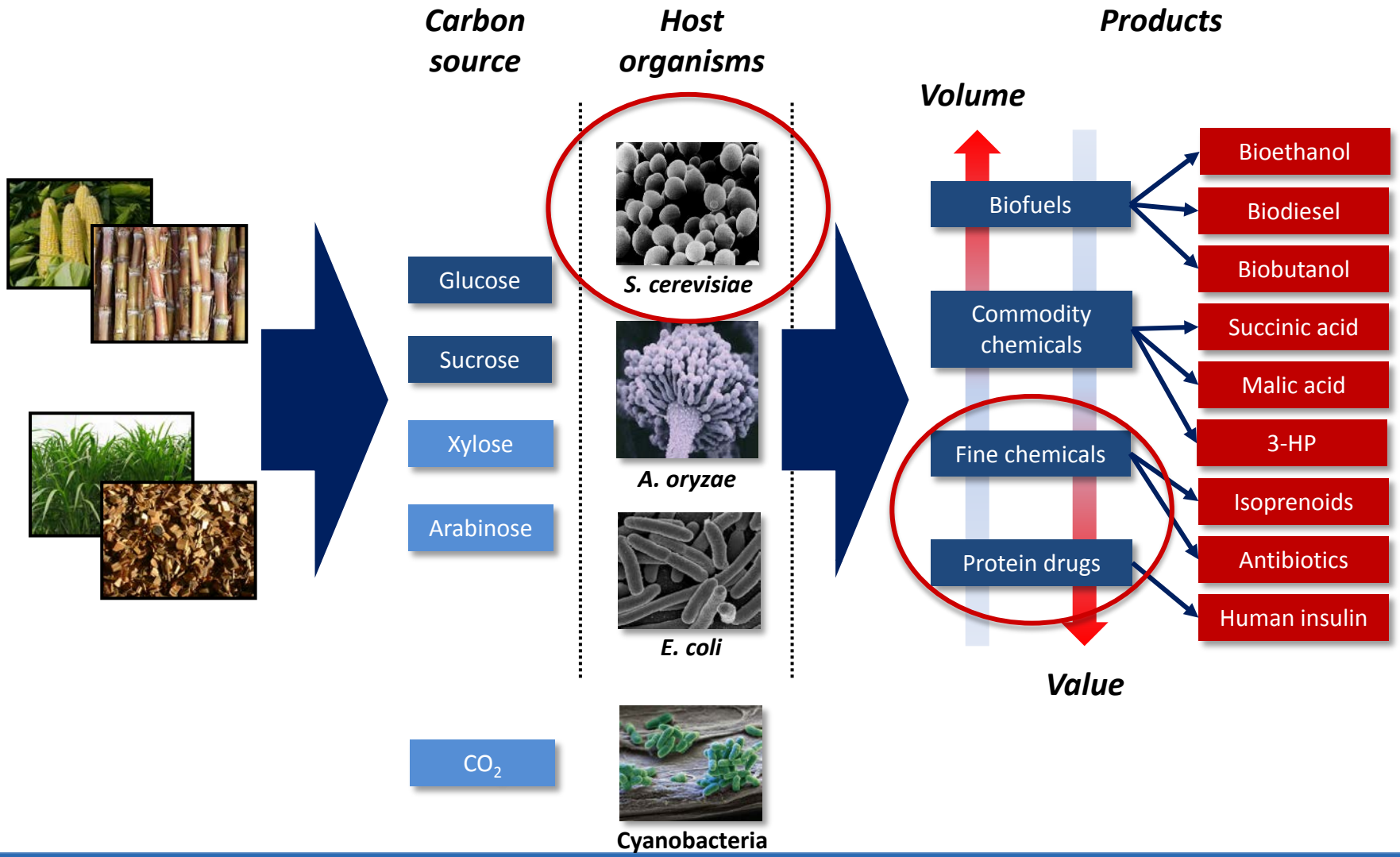
Systems and Synthetic Biology Group

Research topics:

- Modelling of human metabolism
- Metagenomics
- Modelling of microbial metabolism
- Metabolic engineering of yeast
- Yeast cell death
- Signal transduction in bacteria

Cell factories



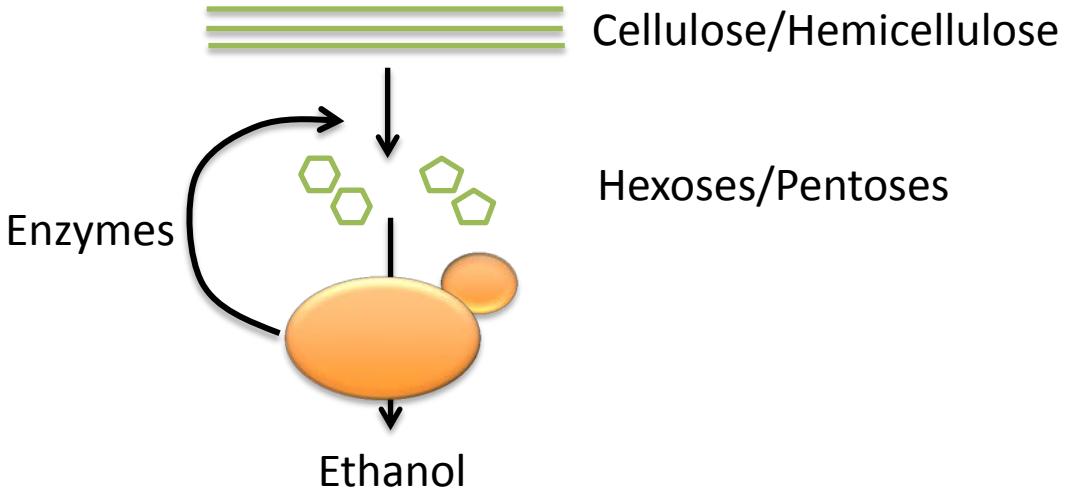
Outline

Production of proteins in yeast
Engineering strategies

Production of isoprenoids as fine chemicals
Artemisinin as example

Protein production in yeast interesting for

- **pharmaceuticals**
- **consolidated bioprocessing**

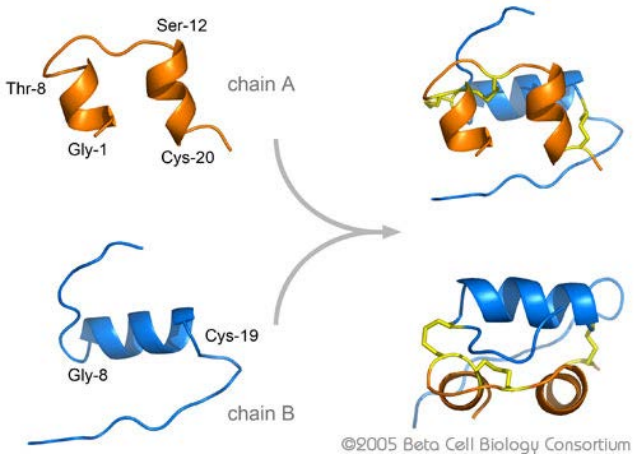


Protein drugs

S. cerevisiae used for production of 15% of biopharmaceutical proteins

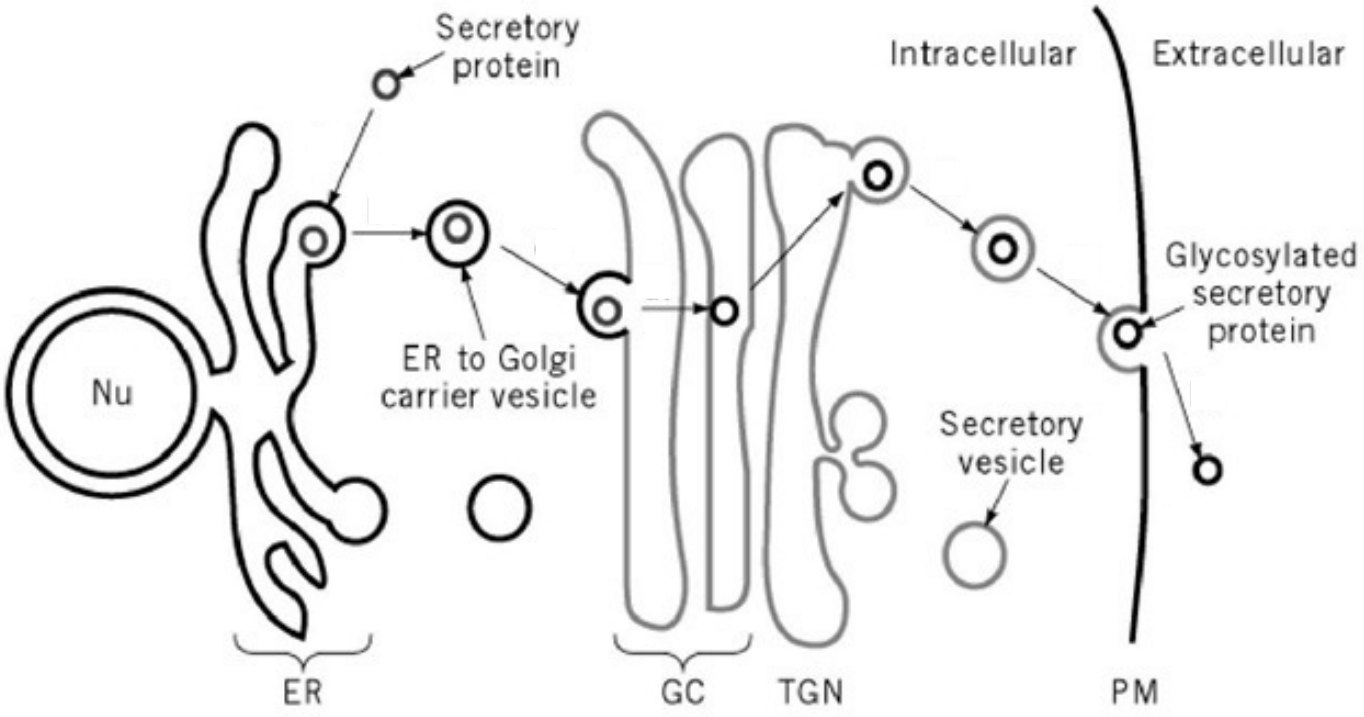
<u>Examples of protein drugs produced in *S. cerevisiae*</u>:

- Insulin
- Glucagon
- Human growth hormone
- Vaccines against hepatitis (A, B), diphteria, tatanus, pertussis, polio, HPV
- Lepirudin (anticoagulant, equivalent to huridin)



intracellular vs. extracellular production

Protein secretion



Protein secretion

Specific challenges

Expression levels
-> Codon optimisation

Codon usage table:

AmAcid	Codon	Number	/1000	Fraction
Gly	GGG	10073.00	5.86	0.11
Gly	GGA	18143.00	10.55	0.21
Gly	GGT	43064.00	25.04	0.49
Gly	GGC	16493.00	9.59	0.19
Glu	GAG	33268.00	19.35	0.29
Glu	GAA	81053.00	47.13	0.71
Asp	GAT	66314.00	38.56	0.65
Asp	GAC	35065.00	20.39	0.35
Val	GTG	18484.00	10.75	0.19
Val	GTA	19556.00	11.37	0.20
Val	GTT	38842.00	22.59	0.40
Val	GTC	20230.00	11.76	0.21
Ala	GCG	10476.00	6.09	0.11
Ala	GCA	27938.00	16.25	0.28
Ala	GCT	37656.00	21.90	0.38
Ala	GCC	22444.00	13.05	0.23
Arg	AGG	15720.00	9.14	0.21
Arg	AGA	37531.00	21.82	0.49
Ser	AGT	23999.00	13.96	0.16
Ser	AGC	16234.00	9.44	0.11
Lys	AAG	54223.00	31.53	0.43
Lys	AAA	72235.00	42.01	0.57
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Protein secretion

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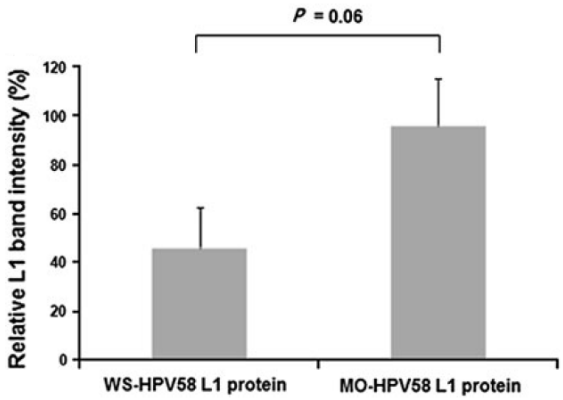

Expression of codon-optimised and non-optimised HPV L1 in yeast (Biotechnol Lett 35:413-421)

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Protein secretion

Specific challenges

Expression levels
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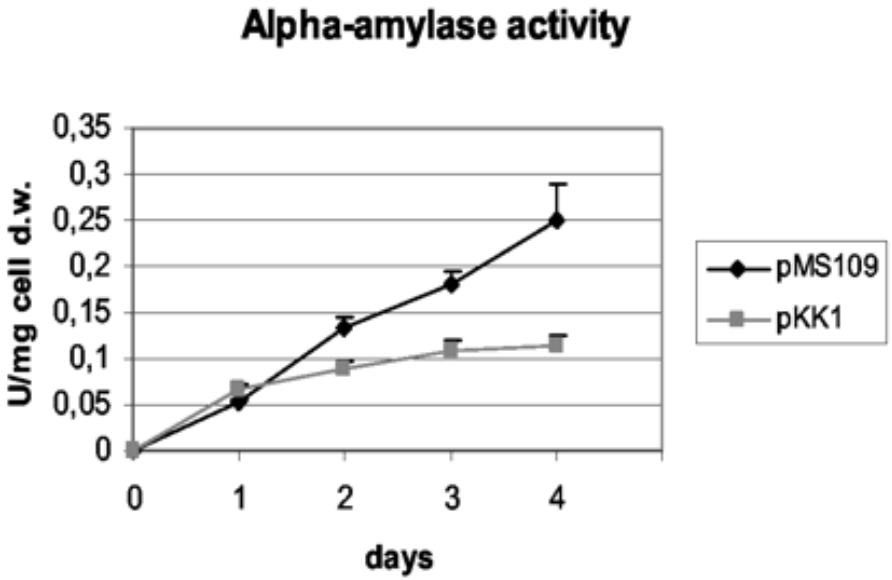
Protein folding
-> Overexpression of chaperones



Overexpression of chaperone-inducing transcription factor (Hac1) in yeast (Appl Environ Microbiol 69:2065-2072)

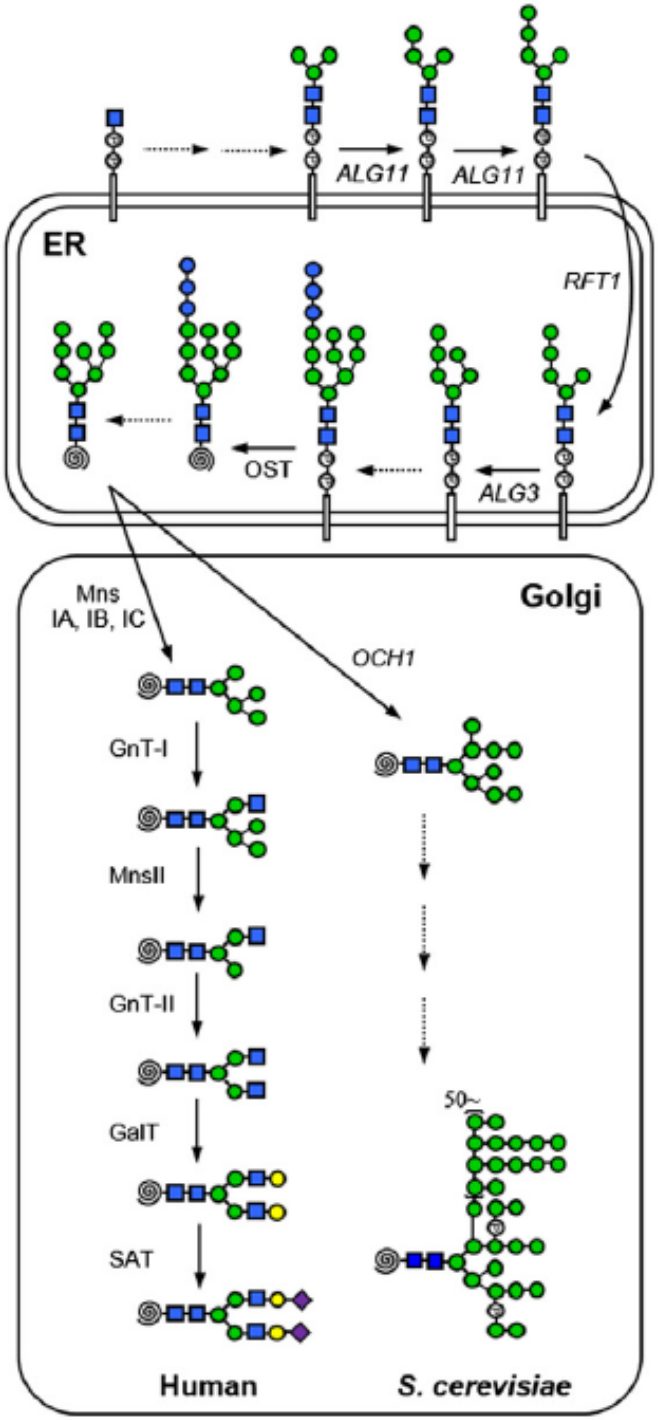
Protein secretion

Specific challenges

Expression levels
-> Codon optimisation

Protein folding
-> Overexpression of chaperones

Post-translational modifications
-> Yeast with human-like glycosylation



Protein secretion

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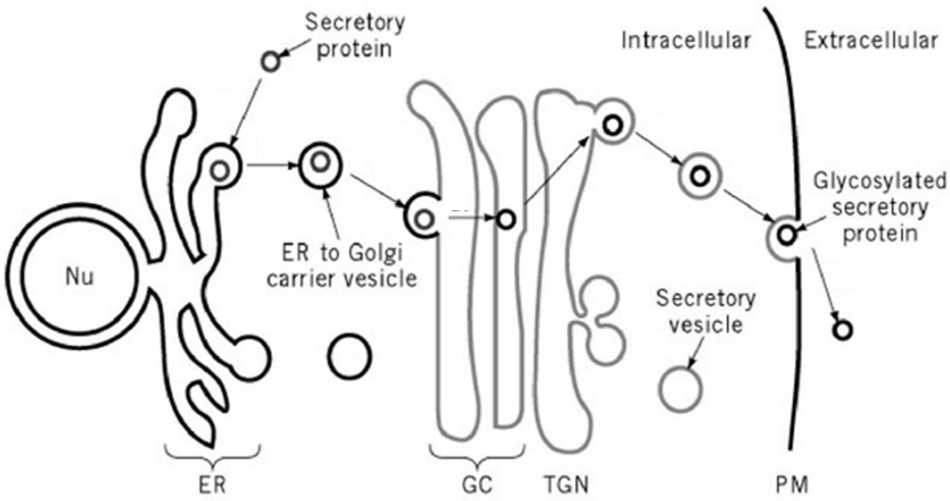


Efficient secretion
-> Up-regulation of protein trafficking
-> Elimination of proteases

However: Strategies often only successful for specific proteins
=> Need for universal approaches

Outline

Production of proteins in yeast
Engineering strategies

Production of isoprenoids as fine chemicals
Artemisinin as example

Isoprenoids

Very large group of secondary metabolites (>50.000 identified)

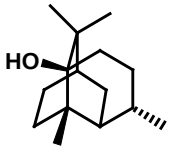
Mainly found in plants

Many with application as fragrances, pigments, vitamins, pharmaceuticals etc.

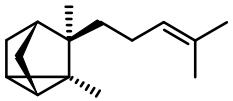
Often produced in small amounts
⇒ Extraction from plants not feasible or expensive

Chemical synthesis may be complicated

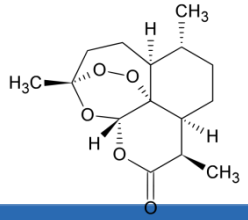
Only few biosynthetic pathways elucidated



patchoulol



santalene

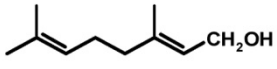


artemisinin

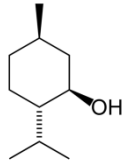
Isoprenoids

isoprene unit
$(C_5H_8)_n$

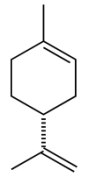
monoterpenoids
n=2



geraniol



menthol

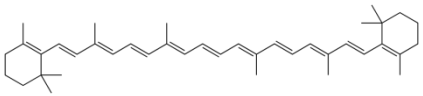

limonene

hemiterpenoids
n=1

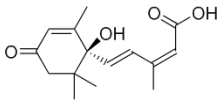
polyterpenoids
n>8


rubber

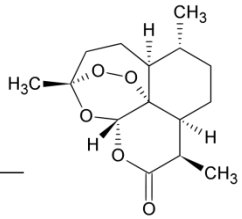
tetraterpenoids
n=8


β-carotene

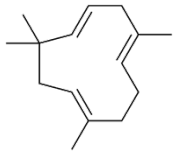
sesquiterpenoids
n=3



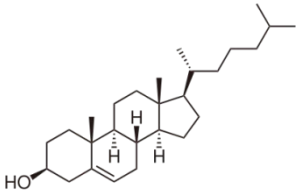
abscisic acid



artemisinin

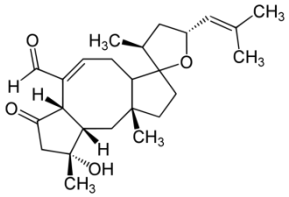

humulene

triterpenoids
n=6



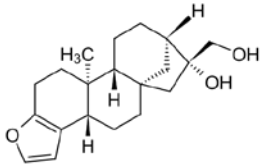
cholesterol

sesterterpenoids
n=5

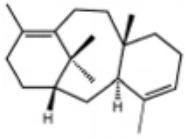


ophiobolin A

diterpenoids
n=4



cafestol



taxadiene