2. A yeast model for isoprenoid biosynthesis

Wagemaker M.J.M., Springer J., Mooibroek H.

Wageningen UR - Food & Biobased Research, Wageningen, Netherlands

Abstract: Cultivation of rubber producing crops other than the rubber tree (*Hevea brasiliensis*) fit to grow in parts of the world other than Southeast Asia, is seen as a potential alternative resource of natural rubber. Russian dandelion (*Taraxacum koksaghyz*) and guayule (*Parenthenum argentatum*) grow in moderate and dry climates, respectively, and produce high quality rubber. These plant species have been selected as most promising crops for the FP7 subsidized EU-PEARLS research project. Part of the research is aimed at creating more fundamental knowledge concerning the physiology and regulation of (poly) isoprene-biosynthesis necessary for a progressive development in breeding and processing of the rubber producing plants.

Natural rubber, *cis*-1,4-polyisoprene, is formed by condensation of isoprenyl diphosphate molecules (IPP-molecules) to an allylic initiator (farnesyl diphosphate) by the action of a *cis*-prenyltransferase (CPT). The rubber is formed in particles surrounded by a monolayer membrane, onto which the CPT and other, SRPPs, REF and yet undefined, proteins are attached. Recent studies demonstrated that besides CPT, other undefined proteins, co-factors and IPP/allylic initiator ratios play an important role in synthesis of high grade rubber.

Baker's yeast *Saccharomyces cerevisiae* is selected as model-organism and will be used to study and engineer poly-isoprene biosynthesis and the physiological impact of isoprene accumulation. The species serves as a convenient eucaryotic model for functional genomics and proteomics technologies allowing the analysis of heterologous plant genes. Valuable tools include the yeast deletion strain collection, synthetic genetic arrays, microarrays, proteome arrays, and the two-hybrid-system. The yeast endogenous mevalonate (MVA) pathway can be used as a source of (poly)isoprenoid precursors and redirection cellular recourses can be optimized while the downstream branch-point enzymes can be added in a modular fashion to generate cis-1,4-polyisoprene. When expression of a *cpt*-gene is established successfully, complex interactions with other proteins and physical factors can be studied in detail.

Our current efforts to be highlighted include the engineering and heterologous expression of a number of Taraxacum *CPT* genes in yeast and some of the effects observed.

3. Modulation of isoprenoid production in *Nicotiana tabacum* and *Taraxacum koksaghyz.*

Nicole van Deenen[a], J. Post[a], C. Schulze Gronover[b] and Dirk Prüfer

[a]Department for Plant Biochemistry and Biotechnology, Hindenburgplatz 55, Westphalian Wilhelm's University Muenster, 48143 Münster, Germany

[b]Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), branch Münster, Hindenburgplatz 55, 48143 Münster, Germany

nicki.v.d@uni-muenster.de, Tel.: 0049-251-8324716

Abstract: The biosynthetic basis for the formation of natural rubber is the isoprenoid pathway that provides isopentenyl diphosphate (IPP) as the precursor for several different isoprenoids in all higher plant als well as for the formation of the polyisoprenes in rubber producing plants. Two separate, compartmentalized isoprenoids pathways are present in the cell: the cytosolic mevalonic acid (MVA) and the plastid localized methylerythritol phosphate (MEP) pathway, whereas the MVA pathway derived IPP is described to be the main precursor for the formation of the rubber chains.

In the present work, we describe the identification and functional characterisation of MVA pathway genes in the rubber producing plant *T. koksaghyz* and their specific expression