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Natural Rubber Biosynthesis and Physic-Chemical Studies on Plant Derived Latex

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1. Introduction

1.1 Natural rubber is an indispensable biopolymer

Natural rubber is a biopolymer of high economic importance with incomparable performance properties such as high elasticity, resilience and efficient heat dispersion (van Beilen and Poirier, 2007a). This high molecular mass polymer is formed from isopentenyl diphosphate units (IPP) which are linked in *cis*-configuration building poly(*cis*-1,4-isoprene) (Nor and Ebdon, 1998; Wititsuwaannakul et al., 2003; Bushman et al., 2006). 300 to 70,000 isoprene molecules are coupled to form an irregular structure that cannot crystallize under normal conditions mediating the amorphous, rubbery texture (Nor and Ebdon, 1998; Kang et al., 2000a). Upon harvest from plants and processing, along with the biopolymer itself also non-rubber compounds are co-extracted and remain in the natural rubber product (Nor and Ebdon, 1998). Therefore, the final product consists of about 94% poly(*cis*-1,4-isoprene) and 6% non-rubber contents such as proteins and fatty acids (Sakdapipanich, 2007). These contaminations are thought to contribute to the extraordinary characteristics of natural rubber (Nor and Ebdon, 1998).

The plant-derived commodity is required for the production of more than 40,000 consumer products including tires, footwear and medical devices (Davis, 1997; Mooibroek and Cornish, 2000; Hagel et al., 2008). After harvest natural rubber is either kept in solution through addition of solvents and stabilizers or it is coagulated and dried. Solubilised natural rubber is used for products such as gloves or condoms but most of the harvested natural rubber is processed as so called bulk rubber in solid sheets or granules (van Beilen and Poirier, 2007b).

Currently, the sole crop exploited for commercial production of high quality natural rubber in viable quantities is *Hevea brasiliensis* Muell. Arg. (Figure 1A). *H. brasiliensis* – a tree indigenous to the Amazon Basin – is the most recently domesticated plant among major crops. In their natural environment trees grow widely distributed within the forest, but for large-scale rubber production *H. brasiliensis* trees are planted in monoculture (Davis, 1997). The main rubber-producing countries presently are Thailand, Indonesia, Malaysia, India and the People's Republic of China, which together accounted for 89% or 9.33 million tons of the global rubber production in 2005 (Figure 2B) (Hayashi, 2009). With a yield potential of more than 2500 kg ha^{-1} $year^{-1}$ *H. brasiliensis* is a valuable crop in tropical and subtropical countries where its cultivation is possible (Cornish, 2001a; Hayashi, 2009).

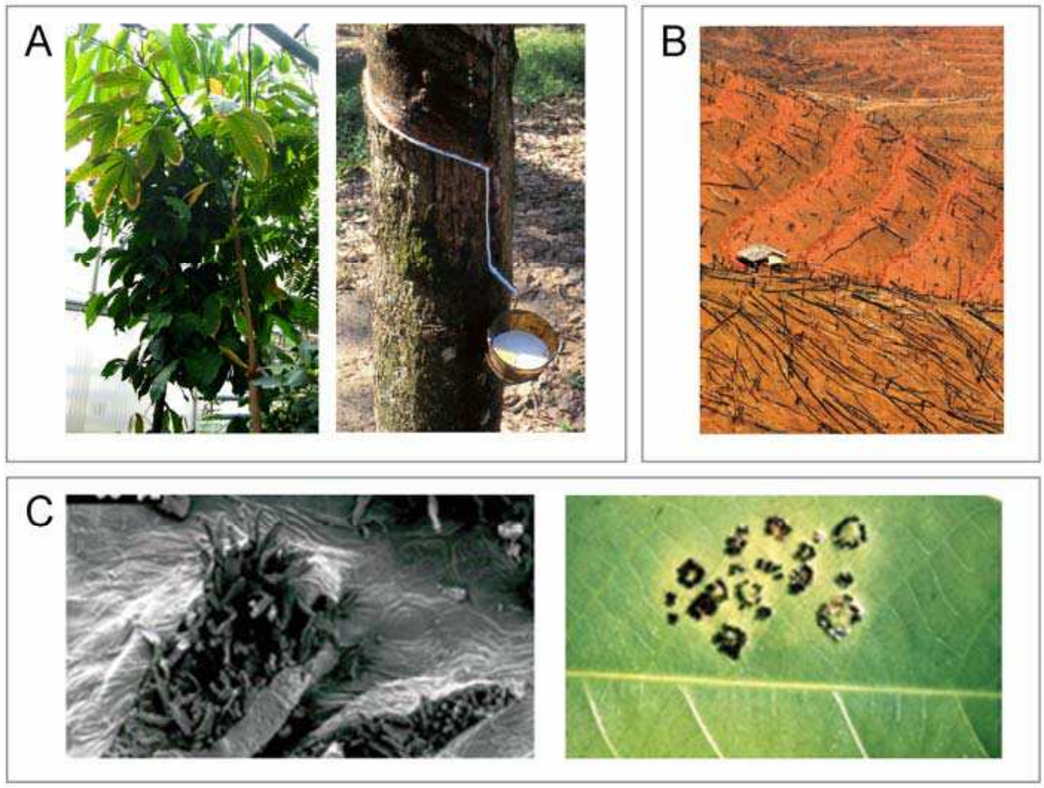


Fig. 1. Cultivation of *H. brasiliensis* A) *H. brasiliensis* tree and common method of harvesting latex from trees by manual tapping the bark and collection of extruding latex in small bins (adapted from uni-stuttgart.de). B) Soil erosion in an area that was cleared for foundation of a rubber plantation (adapted from Qui, 2009). C) Infestation of *H. brasiliensis* with *M. ulei*; left panel: conidiophores of fungi breaking through epidermis of leaf, right panel: lesions on leaf (adapted from Lieberei, 2007).

However, cultivation of *H. brasiliensis* on plantations has severe environmental consequences (Mann, 2009). For example, the People's Republic of China faces problems due to deforestation and conversion of large regions of tropical forest to rubber plantations (Mann, 2009; Qui, 2009; Ziegler, 2009). Primarily, cultivation of *H. brasiliensis* was promising since farmers of the strongly developing country could earn up to five times more than by growing traditional crops such as rice or tea (Qui, 2009). Yet, reshaping of the landscape provoked lowering of the groundwater table coupled with increased soil erosion and subsequent loss of soil quality (Figure 1B) (Mann, 2009; Qui, 2009; Ziegler, 2009).

Next to environmental impacts, biotic and abiotic stresses may lead to massive failure in rubber production from *H. brasiliensis*. The tree is propagated through cuttings whereby all clones cultivated originate from only a small sample of seeds and, therefore, lack genetic diversity (Cornish, 2001a). This uniform genetic background renders *H. brasiliensis* very susceptible to pathogens when planted as monoculture. The most threatening pathogen is the ascomycete *Microcyclus ulei,* which causes defoliation or even death of the tree and which wiped out plantations in the domestic country (Figure 1C) (Davis, 1997; Le Guen et al., 2003). At present, outbreaks of this disease called South American Leaf Blight are rare in the rubber-producing countries of Southeast Asia due to strict pest control standards but an

accidental spread of *M. ulei* might have devastative consequences (Davis, 1997; van Beilen and Poirier, 2007b). Additionally, anthropogenic factors such as the political and economical instability in rubber-producing countries lead to diminishing acreage (Davis, 1997). Growers shift to seemingly more lucrative crops while the global demand on natural rubber increased to more then 10 million tons in 2010. Shortages in rubber supply are already forecasted (Davis, 1997; Cornish, 2001a; van Beilen and Poirier, 2007a).
For all stated reasons, combined with the fact that minor non-rubber components tightly attached to the natural rubber polymer of *H. brasiliensis* cause allergies, countries depending on the import of natural rubber have an interest in establishing alternative domestic rubber crops or engineering alternative substitutes (Cornish, 2001a; Wagner and Breiteneder, 2005; Mooney, 2009).

2. Supplemental commodities and alternative sources of natural rubber

Some petroleum-based materials are potent substitutes for natural rubber. Examples for high-quality synthetic rubbers are polyvinyl chloride (PVC), styrene butadiene and acrylonitrile butadiene, which are commercially produced but cannot match the price-performance ratio of natural rubber (van Beilen and Poirier, 2008). In some special divisions the development of new but application-restricted materials led to the successful replacement of the natural polymer. Progressively, even biodegradable goods could supplement in many applications, which are good alternatives for conventional petroleum-based plastics (Mooney, 2009). These biodegradable goods comprise polymers such as derivative forms of cellulose like cellophane for food packaging, fibrous proteins like silk which are used in Lycra® and Keflar® and hydroxyalkanoate polyesters (PHA) that are gained in fermentation processes (van Beilen and Poirier, 2008; Mooney, 2009). In times of resource constraints these organic compounds that can be totally degraded in municipal landfills might be of high value (Wackernagel et al., 2002).
Nevertheless, neither synthetic rubbers nor biodegradable polymers will be capable to fully replace natural rubber since the biopolymer is unique in its economical competitiveness and no substitute can provide equivalent properties that are required in many industrial applications (van Beilen and Poirier, 2008). All synthetic rubbers produced presently neither have such a high degree of *cis*-bond content (99.5% in natural rubber), nor the high molecular mass, nor contain the non-rubber components that are also responsible for the extraordinary characteristics of natural rubber (Nor and Ebdon, 1998; Hayashi, 2009). Thus, natural rubber still accounts for 40% of the market share and interest in the research of further rubber-producing plants has been renewed (Cornish, 2001a; Mooney, 2009).

2.1 Latex cells

Approximately 2,500 plants synthesize natural rubber (Bonner, 1991). Rubber biosynthesis is restricted to latex cells where the cytoplasm comprises a milky or colorless sap called latex (Schnepf, 1974; Fineran, 1983; Hagel et al., 2008). Mostly, latex cells occur as highly differentiated laticifers – a cell type described in dicotyledonous as well as in monocotyledonous plant orders, ferns and conifers (Metcalfe, 1967; Lewinson, 1991). They seem to have evolved convergently several times as their distribution throughout the whole plant kingdom appears arbitrary, indicating a polyphyletic origin. Based on their morphology laticifers are classified into two major groups (Hagel et al., 2008). Non-articulated laticifers are found as single cells with no direct connection to each other

(Kekwick, 2001). They form giant coenocytic cells that might intrude into different tissues while they grow. Non-articulated laticifers are described, for example, in *Asclepias spp*. and in Moraceae such as *Ficus elastica* and *Nerium oleander* (Mahlberg et al., 1968; Fineran, 1983; Kekwick, 2001). In contrast, articulated laticifers as described in Papaveraceae and Asteraceae, e.g. *Sonchus asper*, and *Taraxacum spp.* or the Euphorbiaceae *H. brasiliensis* are associated to the vascular tissue (Fineran, 1983; Hagel et al., 2008; Sando et al., 2009). They form discrete longitudinal rows of superimposed cells and can form a continuous cytoplasmic network since they fuse with their apical ends and also often generate anastomoses at longitudinal cell walls (Hagel et al., 2008). However, next to the two major morphological types of laticifers, in some plants latex-bearing cells are embedded in the epidermis or bark parenchyma, as it is the case for the desert shrub *Parthenium argentatum* (Backhaus, 1985; Hagel et al., 2008).

The reason why some plants possess latex cells remains elusive and an appropriate physiological function could yet not be identified. Rubber biosynthesis is not a compulsory ability of latex cells since only around 2,500 out of approximately 20,000 laticiferous plant species produce natural rubber as observed, for instance, in *H. brasiliensis*, *Ficus elastica*, *Lactuca sativa*, *P. argentatum* and *T. koksaghyz* (Bonner, 1991; Cornish et al., 1999; Kang et al., 2000b; Bushman et al., 2006; Schmidt et al., 2010). Therefore, latex cells have superimposed functions to rubber biosynthesis. Based on transcriptomic and proteomic studies of various laticiferous plants, a unique metabolism in comparison to other cell types was stated (El Moussaoui et al., 2001; Ko et al., 2003; Chow et al., 2007). Apparently, common to all surveyed laticiferous species, e.g. *Lactuca sativa*, *Ficus spp.*, *H. brasiliensis*, *Calotropis procera*, *Papaver somniferum* and *Morus spp.* – independent of the ability for rubber biosynthesis – is the high abundance of stress- and defense-related proteins in the latex (Decker et al., 2000; El Moussaoui et al., 2001; Stubbe et al., 2005; Kim et al., 2003; Chow et al., 2007; Freitas et al., 2007; Wasano et al., 2009).

2.2 New rubber crops

Among rubber-producing plants only few provide high quality natural rubber. Thereby the quality is defined by the amount and composition of the non-rubber components and essentially by the molecular mass of the poly(*cis*-1,4-isoprene) polymer. For most industrial applications it is crucial that the molecular mass exceeds 4×10^5 Da (Swanson et al., 1979; Bushman, 2006). Therefore, an alternative rubber crop needs to possess commercially viable amounts of high quality rubber, but also has to meet the criteria of rapid growing, large biomass production, domestic cultivation and needs to be an annual crop (Cornish, 2001a). In addition to *H. brasiliensis* only two other rubber-producing species have been described as potential, promising rubber crops – *P. argentatum* and *T. koksaghyz* (van Beilen and Poirier, 2007b; Hayashi, 2009).

P. argentatum is already cultivated in the United States of America for rubber production and is among all potential rubber crops closest to commercial production. The Mexican brush is a perennial plant that can be cultivated in semi-arid regions (Cornish, 2001a). A maximum productivity of 2,000 kg ha^{-1} $year^{-1}$ has been reported (van Beilen and Poirier, 2008). However, the natural rubber gained from bark parenchyma cells needs to be processed soon after harvest since otherwise it becomes unfeasible. Furthermore, a high proportion of low molecular biopolymer lowers the quality and big amounts of resin associated to the natural rubber prevent utilization in bulk rubber applications (Schloman, 2005). Since bulk rubber cannot be gained from *P. argentatum,* which is needed for tire

production that accounts for approximately 70% of total natural rubber consumption, *P. argentatum* is not fully capable of filling the gap in the worldwide increasing demand on natural rubber (Hayashi, 2009). Nevertheless, due to a low abundance and diversity of proteins in the natural rubber product from *P. argentatum,* the Yulex Corporation is successfully marketing *P. argentatum* rubber as hypoallergenic and thus useful in medical applications (Cornish, 2001a; van Beilen and Poirier, 2008; Hamilton and Cornish, 2010).
Taraxacum species infest habitats in all temperate regions of the northern hemisphere (Holm et al., 1997; van Djik, 2003). Among 500 species *T. officinale* is the most common (van Djik, 2003). Its typical jagged leaves build a rosette lying close to the ground with single heads of flowers consisting of numerous strapshaded bright yellow florets. A major tap root with an average diameter of 2.5 cm produces large amounts of latex but an all-encompassing vessel network of laticifers spans the whole plant body (Steward-Wade et al., 2002).

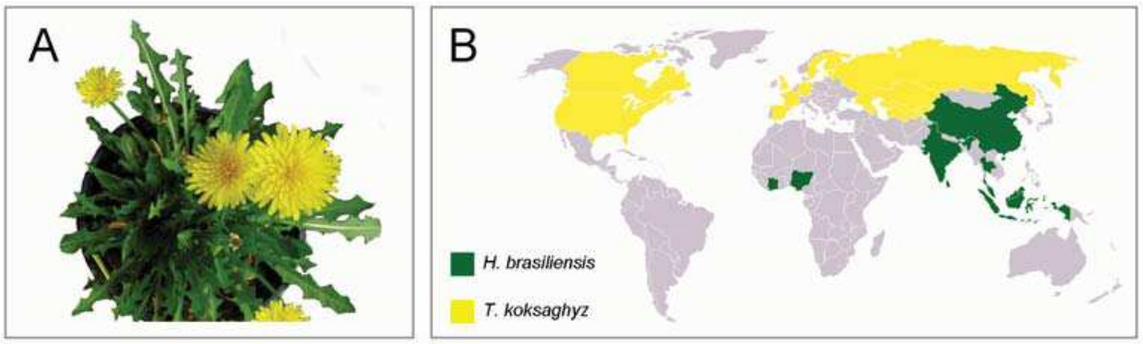


Fig. 2. *Taraxacum brevicorniculatum* as an alternative source of natural rubber. A) Adult *T. brevicorniculatum* plant. B) Countries cultivating *H. brasiliensis* are marked in green on a map of the world; countries that successfully used *T. koksaghyz* as a rubber crop during and after World War II are shaded in yellow (adapted from Suomela, 1950).

However, in *T. officinale* natural rubber is only observed in tiny amounts whereas in its close relative *T. koksaghyz* (Russian dandelion) high quantities have been described (Figure 2A). *T. koksaghyz* was discovered as a potential rubber crop during World War II in East-Kazakhstan and was brought to cultivation in several countries including the United States of America, Spain, the United Kingdom, Germany, Sweden and the former Uzbek Soviet Socialist Republic (Uzbek SSR) (Figure 2B) (Suomela, 1950). Natural rubber production from *T. koksaghyz* compensated for the missing import of *H. brasiliensis* natural rubber, which was due to an import embargo enforced by the Japanese (Javorsky, 1944; Suomela, 1950; van Beilen and Poirier, 2007b).
Wild-growing plants of *T. koksaghyz* are described as diploid with sexual reproduction that exhibit an immense phenotypic variance in terms of morphology but also in the amount and quality of natural rubber. For cultivation a tetraploid accession was chosen which contained up to 37% of natural rubber in its latex (Javorsky, 1944). In general, cultivation of *T. koksaghyz* was facilitated since damage due to pathogens was almost not observed and within five months plants were grown to optimal size and rubber content for harvest (Suomela, 1950). Nevertheless, cultivation of *T. koksaghyz* could not be justified after import of natural rubber from the tropics was resumed. Since cultivation of *T. koksaghyz* was labour-intensive and a yield of no more than 100 kg ha^{-1} $year^{-1}$ in Germany was obtained *T. koksaghyz* could not compete in price with *H. brasiliensis* (Suomela, 1950).

At present, cultivation of a closely related *Taraxacum* species – *T. brevicorniculatum* – as a rubber crop might be of economic profit. *T. brevicorniculatum* is a triploid plant that was shown to contain approximately 32% of high quality rubber with a unimodal mass distribution of 2 × 10^6 Da in its latex (Table 1) (Schmidt et al., 2010). Even after extensive storage of harvested plant material up to 90% of the natural rubber can be recovered and exhibits the same good quality parameters after processing as natural rubber from *H. brasiliensis*. This allows application as solubilised as well as bulk rubber (Suomela, 1950; van Beilen and Poirier, 2007b; Schmidt et al., 2010).
Nevertheless, a severe problem with rubber production from *T. koksaghyz* and *T. brevicorniculatum* is the separation of the biopolymer from the plants´ biomass. Due to fast coagulation of the latex the natural rubber agglutinates with the remaining biomass and only by dint of chemicals, purification is possible (Suomela, 1950). However, it was shown that latex coagulation in *T. brevicorniculatum* is due to the activity of the most abundant protein in latex, a polyphenol oxidase (Wahler et al., 2009). Through breeding of *Taraxacum* plant lines with reduced PPO activity in the latex the problem of fast latex coagulation can be circumvented, i.e. such plants might be employed as a new rubber crop. Rubber yields of 1000 kg ha^{-1} $year^{-1}$ are expected.

species	cultivation area	rubber content in latex (w/v) [%]	Mw of poly(*cis*-1,4-isoprene) [Da]	by-products
H. brasiliensis[*1]	Southeast Asia	30	1x10^5 and 2x10^6	rubber wood
P. argentatum[*2,*3]	South USA, Central America	6	<1×10^6 and >1×10^6	bagasse
T. koksaghyz[*4]	all temperate regions	32	2x10^6	inulin

[*1] Tangpakdee et al., 1996; [*2] van Beilen and Poirier, 2007; [*3] McIntyre et al., 2001; [*4] Schmidt et al., 2010

Table 1. Rubber-producing plant species with the potential for cultivation as rubber crops. Indicated are the climatic requirements, the latex content, the molecular mass of the natural rubber and valuable by-products.

3. Rubber biosynthesis

Despite an emerging strong interest in rubber-producing plants the molecular mechanism of rubber biosynthesis has not been studied in detail. Natural rubber is a huge linear biopolymer with IPP as the monomeric subunit. As IPP is the monomer of all isoprenoids, rubber biosynthesis is just one of numerous biosynthetic pathways using IPP (Kharel and Koyama, 2003). Plant isoprenoids comprise around 23,000 compounds and are the most diverse class of natural compounds including substances such as gibberellins, carotenoids, chlorophyll side chains, plastochinone side chains, sesquiterpenes, sterols, brassinosteroids, dolichol and mitochondrial ubiquinone side chains (Lichtenthaler et al., 1997; Lichtenthaler, 1999; Newman and Chappell, 1999; Kasahara et al., 2002; Nagata et al., 2002; Kasahara et al., 2004).
IPP is produced via two biosynthetic pathways in higher plants. In the cytosol the well described mevalonate (MVA) pathway synthesizes IPP from acetyl-CoA (Spurgeon and Porter, 1981). Another metabolic pathway for IPP synthesis is called 1-deoxy-D-xylulose-5-phosphate/2-*C*-methyl-D-erythritol-4-phosphate (DOXP/MEP) pathway, which is

located in the plastids (Rohmer et al., 1996; Lichtenthaler et al., 1997). Both pathways form IPP and its isomer dimethylallyl diphosphate (DMAPP). Radiolabeling of intermediates of the cytosolic MVA pathway provided evidence that synthesized IPP is incorporated into natural rubber (Skilleter and Kekwick, 1971). Although expression of an enzyme of the DOXP/MEP pathway could be proven in laticifers of *H. brasiliensis*, in feeding experiments using [1-^{13}C]1-deoxy-D-xylulose triacetate, an intermediate of the MEP pathway, no rubber molecules could be detected that carry an isotope label (Ko et al., 2003; Sando et al., 2008). However, incorporation of IPP derived from the DOXP/MEP pathway into natural rubber cannot be excluded, because crossover of both pathways under certain conditions (e.g. depletion of MVA pathway enzymes) could be demonstrated in *Arabidopsis thaliana* and tobacco Bright Yellow-2 cells (BY-2) (Kasahara et al., 2002; Hemmerlin et al., 2003).

For initiation of rubber biosynthesis a priming allylic diphosphate is needed (Cornish, 2001a). IPP is isomerized to DMAPP by IPP-isomerase and is used as a substrate by *trans*-prenyltransferase (TPT) to synthesize an allylic initiator molecule (Priya et al., 2006; Koyama et al., 1996). It could be shown that initiation of rubber biosynthesis is most efficient with farnesyl diphosphate (FPP) in *H. brasiliensis*, *Ficus elastica* and *P. argentatum* in *in vitro* experiments (Xie et al., 2008). ^{13}C nuclear magnetic resonance (NMR) analysis revealed a *trans-trans-cis* sequence, typical for FPP, at the initiating terminal of rubber molecules in *H. brasiliensis* (Tanaka et al., 1996; Tangpakdee, 1996). These results combined with the finding that FPP is synthesized in the cytosol of *H. brasiliensis* indicate that FPP is the most likely initiator molecule (da Costa et al., 2006). The sequential condensation of the non-allylic IPP in *cis*-configuration proceeds during rubber biosynthesis at the priming allylic substrate (Cornish, 2001a; Kharel and Koyama, 2003).

The polymerization of IPP to natural rubber is thought to be located at specific organelles. The natural rubber polymer is found as suspended particles encased by a contiguous monolayer biomembrane (Cornish and Backhaus, 1990; Cornish et al., 1999). In articulated laticifers and latex cells of *P. argentatum* these particles are found within the cytosol, while localization to the vacuome is described in non-articulated laticifers (Wilson and Mahlberg, 1980; Cornish, 2001a). Within the monolayer biomembrane of these rubber particles, the hydrophilic phospholipid headgroups point towards the cytosol to compartmentalize the hydrophobic rubber molecules (Archer et al., 1963; Cornish et al., 1999). The colloidal stability of rubber particles is maintained since they have an overall negative surface charge that leads to a charge-to-charge repulsion preventing coalescence (Southorn and Yip, 1969; Cornish et al., 1999). With regard to the architecture rubber particles resemble oil bodies (Cornish et al., 1999). Hence, it is likely that rubber particles originate, likewise to other vesicle-like structures carrying insoluble material, from the endoplasmic reticulum (ER) (Hermann, 2008). This view is supported by the finding that phosphatidylcholine, phosphatidylethanolamin and glycoproteins are abundant in the monolayer biomembrane as it is described for oil bodies and typical for the cytoplasmic part of the ER bilayer biomembrane (Cornish et al., 1999; Hermann, 2008).

Rubber biosynthesis is catalyzed at the surface of rubber particles by integrated proteins or protein complexes. After incubation of rubber particles from *H. brasiliensis* or *P. argentatum* with [1-^{14}C]IPP, radiolabelled rubber could be observed (Archer et al., 1963; Benedict et al., 1990; Cornish and Backhaus, 1990; Siler and Cornish, 1993).

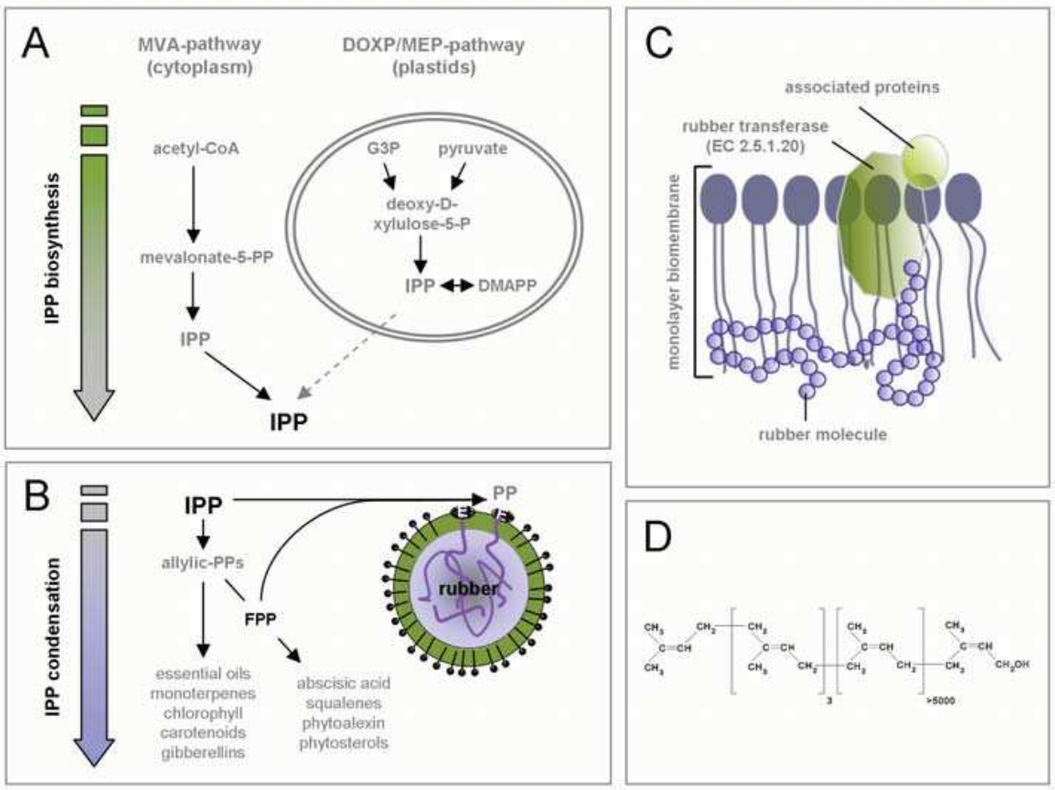


Fig. 3. Biosynthesis of poly(*cis*-1,4-isoprene) at rubber particles A) The monomeric subunit of natural rubber IPP is synthesized by the MVA pathway and the DOXP/MEP pathway in higher plants from acetyl-CoA or glyceraldehydes-3-phosphate and pyruvate, respectively. B) IPP is used for the synthesis of isoprenoids such as allylic diphosphates, as side chains of chlorophylls, and natural rubber. C) Natural rubber is synthesized and regulated by the activity of rubber particle-associated proteins such as a rubber transferase (EC 2.5.1.2.0) and other proteinaceous factors at the monolayer biomembrane surface of rubber particles. D) chemical structure of the natural rubber polymer – poly(*cis*-1,4-isoprene). DOXP/MEP - 2-1-deoxy-D-xylulose-5-phosphate/2-C-methyl-D-erythritol-4-phosphate, G3P – glyceraldehyde 3-phosphate, IPP – isopentenyl diphosphate, MVA – mevalonate, PP- diphosphate

The enzyme catalyzing the rubber molecule elongation is designated as rubber transferase (EC 2.5.1.20) (Cornish, 2001b). Even after extensive research the enzymatic nature of the rubber transferase remains elusive and no sequence is deposited at the comprehensive enzyme information system BRENDA (Chang et al., 2009). Since natural rubber is a poly(*cis*-1,4-isoprene) it is speculated that the rubber transferase is similar to *cis*-prenyltransferases (CPT). This view is supported by data about the biosynthesis of isoprenoids, which are synthesized through the action of prenyltransferases catalyzing the transfer of IPP to prenyl groups (Oh et al., 2000; Kharel and Koyama, 2003). Prenyltransferases are divided into two classes due to their amino acid sequence, the resulting protein fold and due to the reactions they mediate: *trans*-prenyltransferases catalyze the formation of short chain prenyls in *trans*-configuration such as the priming allylic molecule used for the initiation of rubber biosynthesis (Tarshis et al., 1994; Fujihashi et al., 2001). In contrast, CPTs catalyze the transfer of IPP units to substrates in *cis*-configuration and some can mediate the formation

of long-chain polyprenylphosphates. For example, dehydrodolichyl phosphates, which act as a sugar carrier lipid in the biosynthesis of GPI-anchored proteins, are synthesized in *Saccharomyces cerevisiae* through the catalytic action of the long-chain CPT RER2 (Sato et al., 1999). In *H. brasiliensis*, two *CPTs* (*HRT1* and *HRT2*) were cloned, which were mainly expressed in laticifers. HRT2 could be demonstrated to exhibit IPP-condensation activity when heterologously expressed in *Escherichia coli* and co-incubated with *H. brasiliensis* latex extract. Thereby, the observed product mainly was polyisoprene of a molecular mass of 2×10^5 to 10^6 Da (Asawatreratanakul et al., 2003). These data suggest that the rubber transferase might indeed be a CPT. However, immunogold labeling of rubber particles from *H. brasiliensis* using an antibody raised against a synthetic peptide with a consensus sequence for a highly conserved region of CPTs failed to detect CPTs on rubber particles and unlike CPTs, the rubber transferase does not synthesize products of predetermined size but can synthesize products of a wide range of molecular mass (Castillion and Cornish, 1999; Singh et al., 2003; da Costa et al., 2005). However, the varying size of the natural rubber product was shown not to be solely dependant on the rubber transferase. Through *in vitro* studies measuring the incorporation of labeled allylic diphosphate and IPP into isolated rubber particles from *H. brasiliensis*, *P. argentatum* and *Ficus spp.*, it was demonstrated that the concentration of these two substrates determine the initiation of new natural rubber molecules and for this reason also the termination of chain elongation (Castillon and Cornish, 1999). In general, if IPP supply is limited, with an increasing concentration of FPP, more FPP is incorporated into rubber particles, inducing the initiation of new natural rubber molecules and simultaneously leading to a decrease in molecular mass of natural rubber molecules. *Vice versa*, under limiting concentration of FPP an increasing concentration of IPP raises the molecular mass since condensation of IPP to the natural rubber chain is not terminated (Castillion and Cornish, 1999; da Costa et al., 2005). Furthermore, elongation of natural rubber molecules seems also to be dependent on the presence of divalent cations. Incorporation of IPP only occurs if divalent cations such as Mg^{2+} are supplied as co-fatcors (Cornish et al., 2000; Kang et al., 2000b; Scott et al., 2003; da Costa et al., 2005; da Costa et al., 2006). Analysis of the internal concentrations of FPP, IPP and Mg^{2+} could reflect the correlation between rubber molecular mass and rubber transferase activity since the FPP to IPP ratio and the Mg^{2+} concentration were in the optimal range for the specific rubber transferase in surveyed species (da Costa et al., 2005). Therefore, it was stated that these are the main factors for high molecular mass of natural rubber molecules in *H. brasiliensis* and *P. argentatum* (Castillon and Cornish, 1999; Kang et al., 2000b; Scott et al., 2003; da Costa et al., 2006).

Next to sufficient supply of substrate and metal co-factor for rubber transferase activity, additional proteins appear to be involved in rubber biosynthesis. A positive effect was observed when small rubber particle protein (SRPP) and rubber elongation factor (REF), both proteins belonging to the REF superfamily, were added to washed rubber particles in *H. brasiliensis* (Oh et al., 1999; Wititsuwannnakul et al., 2003). As shown by immunogold labeling SRPPs are the most abundant proteins on rubber particles in *H. brasiliensis* (Oh et al., 1999; Singh et al., 2003; Sando et al., 2009). Other factors have not been identified as crucial for rubber biosynthesis so far. Hence, the actual biomolecular process of rubber biosynthesis and its regulation have not been elucidated in detail.

Rubber biosynthesis in *T. brevicorniculatum* and *T. koksaghyz* is very likely to follow the described processes. Rubber particles of *T. koksaghyz* could be shown to share the architecture as described for rubber particles of all surveyed rubber-producing plants and

exhibit rubber transferase activity when included in [1-^{14}C]IPP incorporation assays with Mg^{2+} and FPP (Schmidt et al., 2010). This is consistent with previously reported results gained from studies of biosynthetic activity of rubber particles in *P. argentatum*, *Ficus spp*. and *H. brasiliensis* (Kang et al., 2000b; Cornish, 2001a). Furthermore, several proteins such as CPTs, SRPPs and REF, sharing high sequence similarity with the *H. brasiliensis* proteins, have been identified in the latex of *Taraxacum spp.* (Schmidt et al., 2009). Thus *T. koksaghyz* and *T. brevicorniculatum* could due to their rapid life cycle and the possibility for genetic modifications serve as model plants for researching rubber biosynthesis.

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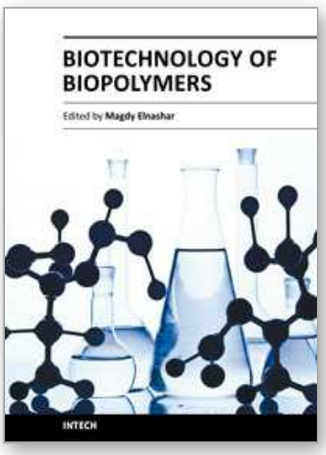
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The book "Biotechnology of Biopolymers" comprises 17 chapters covering occurrence, synthesis, isolation and production, properties and applications, biodegradation and modification, the relevant analysis methods to reveal the structures and properties of biopolymers and a special section on the theoretical, experimental and mathematical models of biopolymers. This book will hopefully be supportive to many scientists, physicians, pharmaceutics, engineers and other experts in a wide variety of different disciplines, in academia and in industry. It may not only support research and development but may be also suitable for teaching. Publishing of this book was achieved by choosing authors of the individual chapters for their recognized expertise and for their excellent contributions to the various fields of research.

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