

Establishment of new crops for the production of natural rubber

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Natural rubber is a unique biopolymer of strategic importance that, in many of its most significant applications, cannot be replaced by synthetic alternatives. The rubber tree *Hevea brasiliensis* is the almost exclusive commercial source of natural rubber currently and alternative crops should be developed for several reasons, including: a disease risk to the rubber tree that could potentially decimate current production, a predicted shortage of natural rubber supply, increasing allergic reactions to rubber obtained from the Brazilian rubber tree and a general shift towards renewables. This review summarizes our knowledge of plants that can serve as alternative sources of natural rubber, of rubber biosynthesis and the scientific gaps that must be filled to bring the alternative crops into production.

Introduction

Natural rubber is a biopolymer consisting of isoprene units (C_5H_8)_n linked together in a 1,4 *cis*-configuration (Figure 1). It has unique properties as a polymer owing to its intrinsic structure, its high molecular weight and ill-defined contributions of minor components, such as proteins, minerals, carbohydrates and lipids, which are all present in the latex (a colloidal suspension of rubber particles). Although rubber has been found in over 2500 plant species, the Para rubber tree (*Hevea brasiliensis* Muell. Arg.) [1] is presently the almost only commercial source of natural latex. Box 1 list several scientific, technical and societal issues illustrating gaps in our knowledge and thus the urgency of developing alternative sources.

Natural rubber is of strategic importance because it cannot be replaced by synthetic alternatives in many of its most significant applications. This is owing to its unique properties, which include resilience, elasticity, abrasion and impact resistance, efficient heat dispersion and malleability at cold temperatures [2,3]. Six decades of industrial research have not produced synthetic rubber materials (such as styrene butadiene rubber, butyl rubber, chloroprene or polyisoprene) with price–performance ratios that match those of natural rubber. Consequently, natural rubber is used in over 40 000 products, including more than 400 medical devices, surgical gloves, aircraft tires and countless engineering and consumer products. The market share of natural rubber has increased from close to 30% in the 1970s and 1980s to the present

40%. Over 90% of natural rubber is produced in Asia, particularly in Malaysia, Thailand and Indonesia.

Several factors highlight the urgency of developing novel alternative sources of natural rubber [4]. Synthetic rubbers are derived from petroleum and uncertainties concerning the availability and price of petroleum [5] along with a high demand for rubber from rapidly expanding economies (such as China and India) has led to rapid increases in the prices of both natural and synthetic rubbers. At the same time, rubber-tree plantations are being replaced by more profitable palm-oil plantations to meet the increasing demand for biofuel [6]. Moreover, harvesting rubber is labor intensive and cannot be mechanized, requiring the daily tapping of hundreds of trees by each worker (Figure 2a).

Of strategic concern is South American Leaf Blight (SALB), a fungal disease threatening the rubber tree. SALB has all but ended large-scale *Hevea* rubber production in South America and can be expected to have a similar devastating effect if it spreads to Asia, considering the very narrow genetic basis of rubber trees [7]. Currently, the only barrier to prevent the spread of SALB to Asia is a strict quarantine, which is likely to fail at some point. Extensive efforts in plant breeding have thus far not yielded resistant rubber-tree clones [8]. The strict growing conditions, which limit its cultivation to specific tropical environments, also prevent expansion of the *H. brasiliensis* acreage to non-tropical countries [9].

An additional motivation to develop alternative natural rubber sources is the increase in allergic reactions to the proteins in *H. brasiliensis* latex, which are responsible for moderate to severe allergic reactions. The incidence of such reactions has increased dramatically in the past 15 years and it is now accepted that 1–6% of the general population suffer from latex allergies. Some studies have shown that up to 17% of healthcare workers are at risk of reactions [10].

Alternative plant sources

A need to develop alternative sources of natural rubber has been recognized at various times, leading to research and development programs during which many plants have been investigated [11]. No less than eight botanical families, 300 genera and 2500 species have been identified that produce natural rubber in their latex, although only two species in addition to the Para rubber tree are known to produce large amounts of rubber with high molecular weight [12] (an essential determinant of rubber quality): a shrub named guayule (*Parthenium argentatum* Gray) and

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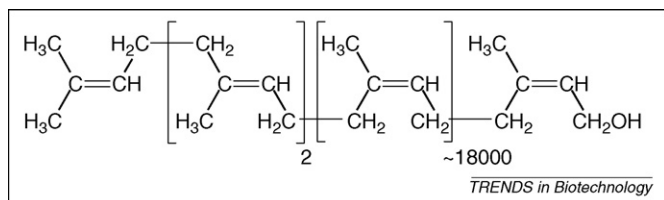


Figure 1. Structure of natural rubber. The molecular weight of an isoprene monomer in natural rubber (C_5H_8) is 68 Da. With farnesyl diphosphate (FPP) as the initiator molecule, the number of *trans*-bonds is two. At an average molecular weight of *H. brasiliensis* rubber of 1200 kDa, *n* is approximately 18 000; in the case of goldenrod (300 kDa), *n* is near 4500.

the Russian dandelion (*Taraxacum koksaghyz*). These plants were considered promising enough as alternative rubber sources that they justified large research programs, especially during WWII. Other alternative rubber-producing plants have not yet been studied in sufficient detail to establish their utility or they produce rubber of inferior quality (Table 1). The ideal rubber-producing crop plant would be an annual, fast-growing plant producing large amounts of biomass with a high concentration of rubber.

Box 1. Outstanding issues in natural rubber production

Main scientific issues

- Identity of rubber polymerase [2]
- Mechanism of polymerization [39]
- Mechanism determining molecular weight [39,55]
- Function of rubber particle-associated proteins [2,35]
- For *H. brasiliensis*: resistance to *Microcyclus ulei* [8]

Technical issues for alternative plant sources of natural rubber

- Full domestication and development of germplasm [56]
- Agronomics, harvesting and transport [16]
- Extraction, processing and utilization of side-products [13]

Societal or strategic issues for *H. brasiliensis*

- Biological threat by *Microcyclus ulei* [7]
- High labor costs and increasing land competition [6]
- Increasing allergic reactions [10]

Annual crops can be planted and plowed-out readily in response to market needs and farmer-production considerations and are also included more readily in on-farm crop rotations and farming systems.



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Figure 2. Sources of natural rubber. (a) Tapping of the Para rubber tree (*H. brasiliensis*); (b) a field of Guayule (*P. argentatum*) at Ehrenberg, Arizona (kindly provided by Yulex Corporation; <http://www.yulex.com/>); (c) Russian dandelion (*T. koksaghyz*) (kindly provided by Dirk Prüfer); (d) Canadian goldenrod (*S. canadensis*).

Table 1. Alternative sources of poly-*cis*-isoprene

Rubber source	Content (%)	Rubber Mw (kD) ^a	Protein Mw (kD) ^b	Production T/Y (year)	Yield (kg ha ⁻¹ y ⁻¹)	R and D related to rubber	Refs
Rubber tree	30–50 in latex, 2% of tree dw	1310	14.6 (REF) 24 (SRPP)	9 000 000 (2005)	500–3000	All facets	[4,36,48,49]
<i>H. brasiliensis</i>							
Guayule	3–12	1280	26 (GHS)	10 000 (1910)	300–2000	Domestication, Processing, Rubber polymerase	[1,4,26,45]
<i>P. argentatum</i> Gray			44 (RPP)				
Russian dandelion	Trace–30	2180	-	3000 (1943)	150–500	WWII emergency projects USSR and USA	[4,21,22]
<i>T. koksaghyz</i>						Domestication	
Rubber rabbitbrush	<7	585	-	-	-	WWII emergency project US	[1,26,50]
<i>Chrysothamnus nauseosus</i>							
Goldenrod	5–12 of root dw	160–240	-	-	110–155	Demonstration project in 1931	[1,26]
<i>Solidago virgaurea minuta</i>							
Sunflower	0.1–1	279	-	Research stage	-	Characterization, genetic engineering	[1,22,26]
<i>Helianthus sp.</i>		69					
Fig tree	4 in latex	190	25, 48?	-	-	Biochemistry	[51]
<i>Ficus carica</i>	0.3 in bark						
<i>Ficus bengalensis</i>	17 in latex	1500	31, 55?	-	-	Biochemistry	[52]
<i>Ficus elastica</i>	18 in latex	1–10	376 (LPR)	-	-	Biochemistry	[2,53,54]
Lettuce	1.6–2.2 in latex	1380		Research stage	-	Characterization, genetic engineering	[12]
<i>Lactuca serriola</i>							

Abbreviations: dw, dry weight; GHS, guayule homologue of SRPP; LPR, large protein from rubber particles; REF, rubber-elongation factor; RPP, rubber-particle protein; SRPP, small rubber particle protein.

^aAverage molecular weight.

^bMain protein(s) associated with rubber particles or implicated in rubber biosynthesis.

Guayule

Guayule, a shrub growing in semi-arid regions in Mexico and the Southern USA (Figure 2b), is the only non-tropical plant that has been used in the early 20th century as a commercial alternative source of natural rubber and is again being developed as a source of hypoallergenic latex by Yulex Corporation. It appears to be a viable alternative to *H. brasiliensis* because it produces relatively high amounts of high-quality rubber with essentially the same molecular weight as the material obtained from the rubber tree (Table 1). In the 1980s, guayule dry rubber was tested successfully in army-truck and -airplane tires [13]. In the context of the emerging requirement for hypoallergenic rubber, guayule rubber is special in that it has a much lower protein content than the *H. brasiliensis*-based product. In addition, only a few proteins are associated with purified rubber particles from guayule compared with the rubber tree. Moreover, rubber particle-associated proteins of guayule do not cross-react with immunoglobulin (Ig)E (Type I latex allergy) and IgG antibodies to *H. brasiliensis* latex proteins [14]. This makes allergic reactions to guayule rubber by consumers sensitized to *H. brasiliensis* rubber unlikely.

Guayule does have several disadvantages, which – in the past – have presented major barriers to its commercialization. It is only domesticated partially and does not tolerate the low winter temperatures in much of Europe and the USA. It is introduced currently as a biannual or multiannual crop, also because most of the rubber is produced during winter months [15]. Stand establishment and optimal harvesting year and method have not been optimized fully. Cultivation systems in which the crop is harvested several times by cutting of the branches and enabling the plant to regrow gives higher yields than simple harvesting of the whole plant after 2–5 years [16]. This system is now used in the commercial cultivation of guayule.

In contrast to *Hevea* rubber production, which is extremely labor intensive, guayule cultivation, harvesting and latex production can be mechanized fully. However, processing of the guayule shrub is technically complicated and involves significant capital and operating costs [13]. This is because the rubber is produced as μ m-size particles in the bark parenchymal cells and not as free-flowing latex as with the rubber tree. To harvest the rubber, the plant material has to be disrupted thoroughly to release the rubber particles from the individual cells. In subsequent steps, the rubber can be separated either by pressing out the latex, followed by centrifugation and creaming steps to purify the latex [17], or by solvent extraction [13]. Major issues during processing guayule for bulk rubber using solvent extraction are mainly technical. For example, the difficult separation of the viscous extractant from the finely dispersed solids, accumulation of terpenes and fine particles in the recycled solvents, separation of a low molecular weight rubber fraction and loss of solvents must be solved.

Processing of guayule for latex used in hypoallergenic products has been commercialized by Yulex Corporation. An issue that needs to be addressed to further develop guayule latex is the presence of resins, which cannot be separated without a solvent-extraction step [13]. The low molecular-weight rubber fraction present in solvent-extracted rubber is not found in latex [18] and the limited oxidative stability of freshly harvested latex can be addressed by stabilization with additives, such as ammonia, KOH (potassium hydroxide) or amine and phenolic antioxidants [13,17].

The price of the guayule rubber or latex is not public, although it is clearly higher than US\$2.60 per kg, the current price of *Hevea* latex. Current cultivation, harvesting and transport practices of guayule alone already lead to estimated costs of US\$2.20 to 2.85 per kg rubber or latex, to

which significant processing costs must be added [19]. However, the higher cost is justified by the added value of hypoallergenic latex used in personal protection (e.g. gloves, condoms) and medical applications (e.g. tubing, catheters). Coproducts from guayule processing range from fuel (the bagasse remaining after rubber extraction), adhesives, coatings, organic pesticides, wood preservatives and specialty chemicals (e.g. resins, low molecular-weight rubber, leaf waxes) and these need to be used to improve the economic viability of guayule rubber.

Russian dandelion

Russian dandelion (*Taraxacum koksaghyz*) was identified in Kazakhstan in the course of a strategic program in 1931–1932 to develop a native source of natural rubber in the USSR [20] (Figure 2c). The root is a source of high quality rubber and was used for rubber production during WWII [1,21]. Tires made from Russian dandelion rubber were as resilient as those produced from *H. brasiliensis* and, furthermore, were better than guayule rubber-derived tires [21], probably owing to the extraordinarily high molecular weight of the dandelion rubber [22].

An attractive feature of the Russian dandelion is that it could be developed as an annual rubber crop for the temperate regions because it can be grown in a similar way to chicory. Although Russian dandelion has laticifers (pipe-like anastomized cell systems, which produce latex) like the rubber tree, the rubber cannot be harvested by tapping; instead, plant roots must be homogenized and the rubber pressed out or extracted.

Turning Russian dandelion into a profitable crop would require an increase of vigor and more favorable agronomic properties, for example, by hybridization with the common dandelion (*T. officinale*). Specific targets include breeding for larger roots that are easier to harvest and increased rubber accumulation. In addition, the low yield per hectare, labor-intensive cultivation, crosses and seed contamination with other dandelions and weed potential should be tackled [e.g. by developing apomictic Russian dandelion (reproducing asexually)] [23]. Modern molecular approaches are required to increase the understanding of rubber biosynthesis, rubber-particle formation, structure and physiology and might enable us to modulate the rubber-synthesis process. For example, DuPont has shown that *T. koksaghyz* genes encoding *cis*-prenyltransferases show similarities to the corresponding *H. brasiliensis* genes [22].

Fast-track breeding methods or the overexpression or downregulation of key genes that are involved in rubber biosynthesis and rubber accumulation are applicable more readily to Russian dandelion with its short life cycle than to species, such as *H. brasiliensis*, which needs several years of growth before clear phenotypes can be determined. Similarly, improvements in rubber production in guayule can only be assessed after 1–2 years. A further advantage of Russian dandelion is that it is amenable both to tissue culture and to transformation and detectable rubber phenotypes can be obtained within 6 months.

One potential disadvantage of Russian dandelion is that rubber particles contain more associated proteins than does *H. brasiliensis*. This raises the possibility that people

might be sensitized to rubber from Russian dandelion, as has happened with *H. brasiliensis* rubber [24]. Therefore, Russian dandelion should also be considered for conventional, non-medical applications, such as tires and other dry rubber applications.

To make rubber from Russian dandelion competitive economically, it is necessary to consider this crop in the context of a biorefinery, in which all components of the plant would be used ideally. Side products from Russian dandelion processing include inulin, the major storage sugar of dandelions (25–40% of dry-root weight), which could be used directly in food and non-food applications. The remaining bagasse could then be fermented to bioethanol and biogas.

Other sources

Several other plants that are able to grow in temperate climates were tested for rubber production, especially in times when price or accessibility of natural rubber was an issue (1920s, WWII, 1970s). When the price of rubber soared in the late 1920s, Thomas Edison, Henry Ford and Harvey Firestone established the Edison Botanic Research Company, which screened over 17 000 plants for quality and quantity of rubber. Extensive research showed that goldenrod (*Solidago altissima*, Figure 2d shows *S. canadensis*), a common weed growing to an average height of 1 m, produced a 5% yield of latex. Through hybridization, Edison produced goldenrod in excess of 3 m, yielding 12% latex. Unfortunately, the resulting rubber was of inferior quality, based on tests with four tires made out of the material by Henry Ford [25]. The reason for the inferior rubber was discovered later when rubber from several goldenrod species was shown to have a molecular weight of only 300 kDa [26] (Table 1).

A recent study showed that lettuce contains small amounts of rubber with a molecular weight similar to that of the rubber tree and guayule. This provides a new opportunity to study rubber biosynthesis in plants on a molecular level, however, its potential as a domestic source of rubber is unclear [12].

An alternative way to improve rubber quality is by generating transgenic plants that yield commercially viable amounts of high molecular-weight rubber. These strategies require a thorough understanding of all biochemical factors affecting rubber yield and quality. Other important issues that need to be addressed are the presence of antinutrients (toxic compounds harming animals feeding on the crop) and the separation from food supplies, as well as acceptance by farmers and the general public because this strategy involves transgenic plants. The feasibility of this approach is being investigated using sunflower [27], which is an agronomically important crop that contains low molecular-weight rubber [28].

Rubber biosynthesis

The identification and characterization of the genes and enzymes involved in rubber biosynthesis have been slow compared with those involved in the synthesis of other biopolymers. This is especially surprising in view of the importance and market value of natural rubber products [2]. To achieve a sustainable production of rubber from

natural sources, our understanding of the molecular mechanism of rubber biosynthesis needs to be improved. This includes factors that are involved directly or indirectly in supplying the building blocks, in polymerization of the isoprene monomers and in providing an appropriate physical environment for rubber-particle and latex formation. Owing to its economic importance, most research and development efforts have focused on *H. brasiliensis*. However, rubber biosynthesis has been investigated to some extent in several other alternative rubber plants discussed previously (Table 1).

Precursors

Essential steps of rubber biosynthesis are indicated in Figure 3. Cytosolic acetyl-CoA is the primary building block of the isoprenoid pathway and for the synthesis of rubber. Acetyl-CoA is converted to isopentenyl diphosphate (IPP) through a pathway involving the intermediate 3-hydroxy-methyl-glutaryl-CoA [29–31]. IPP is transformed into dimethylallyl diphosphate (DMAPP) by isomerization. DMAPP primes the sequential head-to-tail condensations of IPP molecules by *trans*-prenyltransferases to form geranyl diphosphate (GPP, C₁₀, monoterpenoids), farnesyl diphosphate (FPP, C₁₅, sesquiterpenoids) and geranylgeranyl diphosphate (GGPP, C₂₀, diterpenoids).

Initiation of rubber synthesis

Initiation of rubber synthesis has been studied in several plants and a common finding is that the end groups found in rubber are not made up of *cis*-isoprene units, unlike the bulk of the rubber [2]. In goldenrod, two *trans*-isoprene units and a dimethylallyl group were detected [32]. In the case of *H. brasiliensis* leaves (not the rubber particles), two *trans*-isoprene units but no dimethylallyl units were detected. *In vitro* studies have shown that several compounds (DMAPP, GPP, FPP and GGPP) can initiate rubber biosynthesis [33]. However, the low binding constant of FPP compared with other allylic molecules and its synthesis in the cytosol of the laticifer, the same compartment as the rubber transferase, suggest that FPP is the main initiator *in vivo* in *H. brasiliensis* [34], in accordance with the two *trans*-isoprene units detected. FPP also appears to act as the main initiator in rubber biosynthesis in *Ficus elastica* [9]. It must be noted that the end-groups of rubber molecules can only be determined in low molecular-weight rubber (such as rubber from goldenrod and *H. brasiliensis* leaves) and that all *in vitro* studies are carried out with latex fractions containing many proteins and small molecules that might cause artifacts [33].

Rubber polymerization

Rubber particles contain an integral membrane-bound, rubber-synthesizing *cis*-prenyltransferase (CPT) enzyme or enzyme complex (rubber transferase or rubber polymerase, EC 2.5.1.20). This enables the enzyme to access the hydrophilic FPP and IPP molecules in the cytoplasm and the hydrophobic polymer chain extruded from the enzyme can enter the hydrophobic interior of the latex particles. CPT catalyzes the sequential *cis*-1,4-condensation of

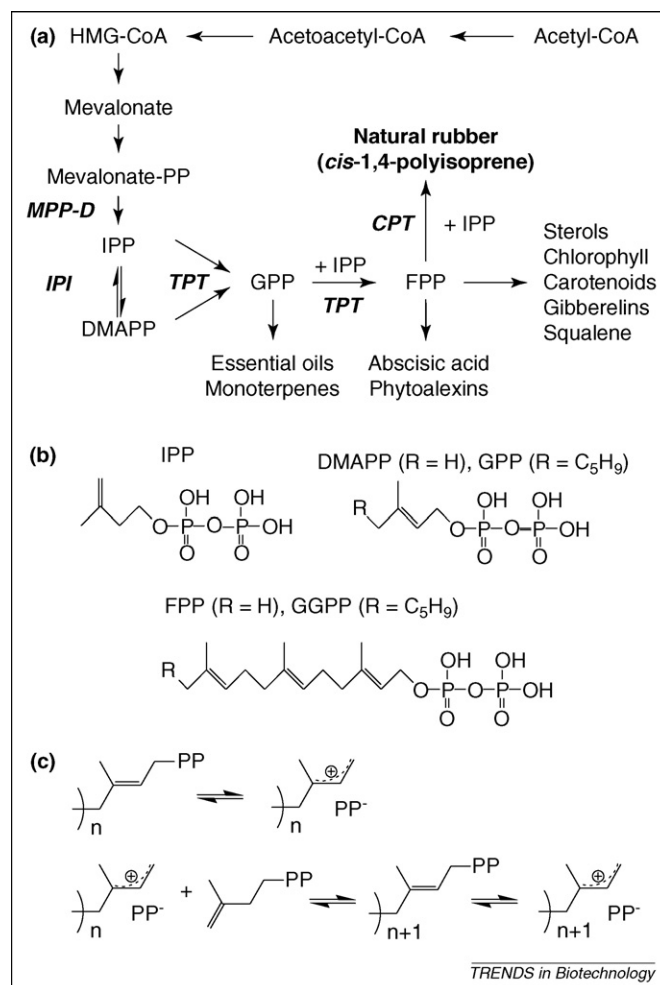


Figure 3. Pathway of rubber biosynthesis from isopentenyl diphosphate. (a) Natural rubber is produced from a side branch of the ubiquitous isoprenoid pathway, with 3-hydroxy-methyl-glutaryl-CoA (HMG-CoA) as the key intermediate derived from acetyl-CoA by the general mevalonic-acid pathway. Mevalonate diphosphate decarboxylase (MPP-D) produces isopentenyl diphosphate (IPP), which is isomerized to dimethylallyl diphosphate (DMAPP) by IPP isomerase (IPI). IPP is then condensed in several steps with DMAPP to produce geranyl diphosphate (GPP), farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP) by the action of a *trans*-prenyltransferase (TPT). The *cis*-1,4-polymerization that yields natural rubber is catalyzed by *cis*-prenyltransferase (CPT), which uses the non-allylic IPP as substrate. (b) Structures of IPP, DMAPP, GPP and FPP. In solution, the diphosphate groups will be partly ionized. (c) Proposed 'living carbocationic polymerization' mechanism (adapted from [39]). In the first step, the diphosphate group of FPP or a growing rubber chain is removed, leaving a carbocationic intermediate. In the second step, this intermediate reacts with IPP to extend the chain. The resulting extended rubber molecule can leave the active site or undergo a next chain-elongation step. The resulting polymer chain has two *trans*- and multiple *cis*-bonds as shown in Figure 1.

cytoplasmic IPP with the allylic diphosphate as an initiator (Figure 3). The identity of the enzyme or enzyme complex required for high-molecular-weight rubber biosynthesis is the most important unsolved and challenging question in rubber biosynthesis. CPT cDNAs have been identified from *H. brasiliensis* latex and their expression in *E. coli* led to the incorporation of IPP into an FPP-initiated polymer consisting of 20 isoprene units [35,36]. In the presence of a latex fraction, the CPT showed much higher activity, producing chains with higher molecular weight (3000–15 000 isoprene units) [37], indicating that additional components present in latex besides CPT are required for high molecular-weight rubber synthesis.

Determinants for rubber molecular weight

An indication for a useable natural rubber is its molecular weight. The rubber tree, guayule, Russian dandelion, lettuce (*Lactuca serriola*) and a few less well characterized tropical plant species, such as *Cryptostegia grandiflora* [1], produce rubber with an average molecular weight over 1000 kDa (Table 1), which correlates with sufficient rubber quality. Other temperate-zone plants that were tested produce rubber with a much lower molecular weight [11,26]. It is not well understood which factors determine the final molecular weight of natural rubber. *In vitro* studies with rubber-tree and guayule *cis*-prenyltransferases indicate that the ratio between initiator FPP and IPP has a major role in determining the length of obtained rubber [14,34,38]: increasing concentrations of FPP lead to a reduction in molecular weight, whereas increasing concentrations of IPP have the opposite effect.

A chemical perspective on the mechanism of rubber polymerization has been presented by Puskas *et al.* [39]. The mechanism of rubber biosynthesis is proposed to be a so-called living carbocationic polymerization, which is consistent with the broad molecular-weight distribution in natural rubber. According to this model, growing rubber molecules leave the active site continuously after the addition of IPP and compete with other rubber molecules or FPP to re-enter the enzyme. Chain growth slows down and stops when the chain reaches a molecular weight hindering further diffusion to the enzyme. In this model, the species-dependent chain length is dependent on the size of the rubber particles, the presence of specific proteins or other factors affecting diffusion, the ratio of initiator FPP to IPP and the affinity of the rubber polymerase for FPP and the growing rubber polymer chains [39]. Clues for the chain-length-determination mechanism of *cis*-prenyltransferases might be also found by comparing related enzymes, such as bacterial, yeast and human CPTs [29,40], or by site-directed mutagenesis of such enzymes to change their processivity [41]. However, these CPTs typically produce short molecules consisting of less than 20 isoprene building blocks.

Rubber particles and associated proteins

Natural rubber is located in subcellular rubber particles in the cytosol of specialized cells called laticifers in the rubber tree and in Russian dandelion [1] or in the cytosol of bark parenchyma cells (as in guayule). The size of the particles in different plant species ranges from 0.2 to 4.0 μm [42]. The particles contain a homogeneous rubber core surrounded by an intact monolayer membrane with a highly species-specific protein and lipid composition. The hydrophilic head groups of the phospholipids and sugar groups attached to the particle-bound proteins render the surface of the particles hydrophilic [2].

The amount of protein associated with rubber particles varies tremendously among plant species, ranging from 4.8% in *Euphorbia lactiflua* to less than 0.1% in guayule [42]. The number of proteins within these complexes also varies significantly, from less than ten for guayule to more than 80 in the case of *H. brasiliensis* [2]. The major proteins associated with rubber particles or suggested to be involved in rubber biosynthesis all have different

sizes and abundances if different species are compared (Table 1).

Two major proteins are associated with rubber particles from *H. brasiliensis*: a 14.6 kD rubber elongation factor (REF) and a 24 kD small rubber particle protein (SRPP). Genes encoding both proteins have been cloned and expressed to elucidate their role in rubber biosynthesis. This also showed that the two proteins show sequence similarities. Although the role of REF in rubber biosynthesis has remained unclear [43], SRPP expressed heterologously stimulates the formation of long chain-length rubber [44]. A guayule homologue of the *H. brasiliensis* SRPP (GHS) stimulated IPP incorporation into high molecular-weight rubbers in the same way as the *H. brasiliensis* SRPP [45]. All three proteins show sequence homology to stress-related proteins in *Arabidopsis* and *Phaseolus vulgaris*, which are plants not known to produce rubber [29]. The major protein associated with guayule rubber particles, comprising approximately 50% of the total protein [46], is a 53 kD cytochrome P450 enzyme of the CYP74 family. It is highly homologous to allene oxide synthase (AOS) and is catalytically active as an AOS [47]. Its role in rubber synthesis is unclear.

In an analysis of the *H. brasiliensis* latex transcriptome, four groups of expressed genes could be distinguished: those encoding (i) rubber particle proteins, such as REF and SRPP; (ii) enzymes involved in rubber precursor synthesis, such as hydroxymethylglutaryl-CoA reductase, and FPP synthase; (iii) defense-related proteins; and (iv) latex allergens (proteins that have unknown functions but have a role in latex allergy). It is interesting to note that the REF and SRPP genes were highly expressed in latex, representing 29% of the total ESTs. The transcriptome analysis did not provide a clear link between *cis*-prenyltransferases and rubber biosynthesis: CPT expression levels in *H. brasiliensis* latex were so low that they were not represented among 1176 ESTs derived from latex. However, this was also true for enzymes, such as mevalonate kinase, phosphomevalonate kinase, diphosphomevalonate decarboxylase and IPP isomerase, which are known to be involved in the synthesis of rubber precursors [35].

Thus, *in vitro* reconstitution of complete long-chain rubber-biosynthesis machinery from its individual components has not been successful, for either guayule or the rubber tree. Until all components are known and identified, the molecular functions of the individual components cannot be established adequately. It is, however, tempting to speculate that the cloned CPT enzymes do represent the elusive rubber polymerases. In this scenario, these enzymes can only produce high molecular-weight rubber when aided by other factors, which might include REF, SRPP, GHS and other proteins.

Conclusions, necessary steps and perspectives

The convergence of numerous factors, including uncertainty about the planet's energy reserves and global warming, has given new impetus to the development of crops for the renewable production of energy and materials. Natural rubber, as one of the most important polymers used in our society, deserves a strong focus. To secure our access to this essential polymer and to decrease

our dependence on petroleum-based synthetic rubber, the mid- to long-term development of alternative sources of natural rubber appears essential. Crops synthesizing rubber could also be used for the sustainable production of other products, by using all resulting by-products, such as converting their lignocellulosic residues into bioethanol or using inulin from Russian dandelion for food applications or the production of bioethanol. Although alternative sources for natural rubber were investigated in the past, it is now necessary to apply advances that have been achieved in molecular plant sciences with the tools of genomics, metabolomics and proteomics, as well as advances at the agronomic level, for example, with marker-assisted breeding, to a much larger extent. This will ensure that progress made towards our understanding of rubber biosynthesis is used for the development of new rubber-producing crops.

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References

- Polhamus, L.G. (1962) *Rubber, Botany, production, and utilization*. Leonard Hill Limited
- Cornish, K. (2001) Similarities and differences in rubber biochemistry among plant species. *Phytochem.* 57, 1123–1134
- Cataldo, F. (2000) Guayule rubber: a new possible world scenario for the production of natural rubber. *Progr. Rubber Plastics Technol.* 16, 31–59
- Mooibroek, H. and Cornish, K. (2000) Alternative sources of natural rubber. *Appl. Microbiol. Biotechnol.* 53, 355–365
- Hirsch, R.L. *et al.* (2006) Peaking of world oil production and its mitigation. *AIChE J.* 52, 2–8
- Belcher, B. *et al.* (2004) Rattan, rubber, or oil palm: Cultural and financial considerations for farmers in Kalimantan. *Econ. Bot.* 58, S77–S87
- Davis, W. (1997) The rubber industry's biological nightmare. *Fortune* 4 August, pp. 86–95
- Le Guen, V. *et al.* (2007) Bypassing of a polygenic *Microcyclus ulei* resistance in rubber tree, analyzed by QTL detection. *New Phytol.* 173, 335–345
- Espy, S.C. *et al.* (2006) Initiator-independent and initiator-dependent rubber biosynthesis in *Ficus elastica*. *Arch. Biochem. Biophys.* 448, 13–22
- Bousquet, J. *et al.* (2006) Natural rubber latex allergy among health care workers: a systematic review of the evidence. *J. Allerg. Clin. Immunol.* 118, 447–454
- Bowers, J.E. (1990) *Natural rubber-producing plants for the United States*, USDA Cooperative State Research Service
- Bushman, B.S. *et al.* (2006) Identification and comparison of natural rubber from two *Lactuca* species. *Phytochem.* 67, 2590–2596
- Schloman, W.W. (2005) Processing guayule for latex and bulk rubber. *Ind. Crops Prod.* 22, 41–47
- da Costa, B.M.T. *et al.* (2006) Magnesium ion regulation of *in vitro* rubber biosynthesis by *Parthenium argentatum* Gray. *Phytochem.* 67, 1621–1628
- Cornish, K. and Backhaus, R.A. (2003) Induction of rubber transferase activity in guayule (*Parthenium argentatum* Gray) by low temperatures. *Ind. Crops Prod.* 17, 83–92
- Foster, M.A. and Coffelt, T.A. (2005) Guayule agronomics: establishment, irrigated production, and weed control. *Ind. Crops Prod.* 22, 27–40
- Cornish, K. *et al.* (2006) Biopolymer extraction from plant materials. US Patent 2006/0149015
- Cornish, K. *et al.* (1993) Fundamental similarities in rubber particle architecture and function in three evolutionarily divergent plant species. *J. Nat. Rubber Res.* 8, 275–285
- van Beilen, J.B. *et al.* (2006) Alternative sources of natural rubber. In *Outputs of the epobio project*, CNAP, University of York
- Ulmann, M. (1951) *Wertvolle Kautschukpflanzen des gemässigten Klimas. Dargestellt auf Grund von Sowjetischer Forschungsarbeiten*. Akademie Verlag
- Whaley, W.G. and Bowen, J.S. (1947) Russian dandelion (*Kok-Saghyz*). An emergency source of natural rubber, United States Department of Agriculture
- Hallahan, D.L. and Keiper-Hrynko, N.M. (2004) *Cis*-prenyltransferases from the rubber-producing plants Russian dandelion (*Taraxacum kok-saghyz*) and sunflower (*Helianthus annuus*). US Patent 2004/044173
- van Dijk, P.J. (2003) Ecological and evolutionary opportunities of apomixis: insights from *Taraxacum* and *Chondrilla*. *Philos. Trans. R. Soc. London. Ser. B* 358, 1113–1120
- Cornish, K. *et al.* (2005) Biotechnological development of domestic rubber producing crops. *Rubber World* 233, 40–44
- Josephson, M. (1959) *Edison: a biography*, McGraw-Hill
- Swanson, C.L. *et al.* (1979) Molecular weights of natural rubbers from selected temperate zone plants. *J. Appl. Polym. Sci.* 23, 743–748
- McMahan, C.M. (2007) ARS Project: Development of domestic natural rubber-producing industrial crops through biotechnology. USDA www.ars.usda.gov/research/projects/projects.htm?ACCN_NO=408518
- Seiler, G.J. *et al.* (1991) Renewable resources from wild sunflowers (*Helianthus* spp. *asteraceae*). *Econ. Bot.* 45, 4–15
- Bouvier, F. *et al.* (2005) Biogenesis, molecular regulation and function of plant isoprenoids. *Prog. Lipid Res.* 44, 357–429
- Fatland, B.L. *et al.* (2005) Reverse genetic characterization of cytosolic acetyl-CoA generation by ATP-citrate lyase in *Arabidopsis*. *Plant Cell* 17, 182–203
- Sirinupong, N. *et al.* (2005) Molecular cloning of a new cDNA and expression of 3-hydroxy-3-methylglutaryl-CoA synthase gene from *Hevea brasiliensis*. *Planta* 221, 502–512
- Ohya, N. and Koyama, T. (2001) Biosynthesis of natural rubber and other natural isoprenoids. In *Isoprenoids* (Koyama, T. and Steinbuechel, A., eds), Wiley VCH
- Stubbe, J. *et al.* (2005) Nontemplate-dependent polymerization processes: polyhydroxyalkanoate synthases as a paradigms. *Annu. Rev. Biochem.* 74, 433–480
- da Costa, B.M.T. *et al.* (2005) Regulation of rubber biosynthetic rate and molecular weight in *Hevea brasiliensis* by metal cofactor. *Biomacromol.* 6, 279–289
- Ko, J.H. *et al.* (2003) Transcriptome analysis reveals novel features of the molecular events occurring in the laticifers of *Hevea brasiliensis* (para rubber tree). *Plant Mol. Biol.* 53, 479–492
- Han, K.H. *et al.* (2000) Genes expressed in the latex of *Hevea brasiliensis*. *Tree Physiol.* 20, 503–510
- Asawatreratanakul, K. *et al.* (2003) Molecular cloning, expression and characterization of cDNA encoding *cis*-prenyltransferases from *Hevea brasiliensis* – a key factor participating in natural rubber biosynthesis. *Eur. J. Biochem.* 270, 4671–4680
- Cornish, K. *et al.* (2000) Rubber molecular weight regulation, *in vitro*, in plant species that produce high and low molecular weights *in vivo*. *Biomacromol.* 1, 632–641
- Puskas, J.E. *et al.* (2006) Natural rubber biosynthesis – a living carbocationic polymerization? *Prog. Polym. Sci.* 31, 533–548
- Takahashi, S. and Koyama, T. (2006) Structure and function of *cis*-prenyl chain elongating enzymes. *Chem. Rec.* 6, 194–205
- Kharel, Y. *et al.* (2006) Manipulation of prenyl chain length determination mechanism of *cis*-prenyltransferases. *FEBS J.* 273, 647–657
- Siler, D.J. *et al.* (1997) Composition of rubber particles of *Hevea brasiliensis*, *Parthenium argentatum*, *Ficus elastica*, and *Euphorbia lactiflua* indicates unconventional surface structure. *Plant Physiol. Biochem.* 35, 881–889
- Dennis, M.S. and Light, D.R. (1989) Rubber elongation factor from *Hevea brasiliensis*: identification, characterization and role in rubber biosynthesis. *J. Biol. Chem.* 264, 18608–18617
- Oh, S.K. *et al.* (1999) Isolation, characterization, and functional analysis of a novel cDNA clone encoding a small rubber particle protein from *Hevea brasiliensis*. *J. Biol. Chem.* 274, 17132–17138
- Kim, I.J. *et al.* (2004) A novel cDNA from *Parthenium argentatum* Gray enhances the rubber biosynthetic activity *in vitro*. *J. Exp. Bot.* 55, 377–385

- 46 Benedict, C.R. *et al.* (1990) The enzymatic synthesis of rubber polymer in *Parthenium argentatum* Gray. *Plant Physiol.* 92, 816–821
- 47 Pan, Z. *et al.* (1995) The major protein of guayule rubber particles is a cytochrome P450. Characterization based on cDNA cloning and spectroscopic analysis of the solubilized enzyme and its reaction products. *J. Biol. Chem.* 270, 8487–8494
- 48 Blanc, G. *et al.* (2006) Efficient *Agrobacterium tumefaciens*-mediated transformation of embryogenic calli and regeneration of *Hevea brasiliensis* Muell Arg. plants. *Plant Cell Rep.* 24, 724–733
- 49 Priya, P. *et al.* (2006) Molecular cloning and characterization of the rubber elongation factor gene and its promoter sequence from rubber tree (*Hevea brasiliensis*): a gene involved in rubber biosynthesis. *Plant Sci.* 171, 470–480
- 50 Weber, D.J. *et al.* (1993) *Chrysothamnus*: a rubber-producing semi-arid shrub. In *New crops* (Janick, J. and Simon, J.E., eds), pp. 355–357, Wiley
- 51 Kang, H. *et al.* (2000) Identification of natural rubber and characterization of rubber biosynthetic activity in fig tree. *Plant Physiol.* 123, 1133–1142
- 52 Kang, H.S. *et al.* (2000) Characterization of natural rubber biosynthesis in *Ficus benghalensis*. *Plant Physiol. Biochem.* 38, 979–987
- 53 Siler, D.J. and Cornish, K. (1993) A protein from *Ficus elastica* rubber particles is related to proteins from *Hevea brasiliensis* and *Parthenium argentatum*. *Phytochem.* 32, 1097–1102
- 54 Cornish, K. and Brichta, J.L. (2002) Some rheological properties of latex from *Parthenium argentatum* Gray compared with latex from *Hevea brasiliensis* and *Ficus elastica*. *J. Polym. Environ.* 10, 13–18
- 55 Cornish, K. and Scott, D.J. (2005) Biochemical regulation of rubber biosynthesis in guayule (*Parthenium argentatum* Gray). *Ind. Crops Prod.* 22, 49–58
- 56 Ray, D.T. *et al.* (2005) Breeding guayule for commercial production. *Ind. Crops Prod.* 22, 15–25

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