Plant Biotechnology Journal
Open Access

Plant Biotechnology Journal (2019) **17**, pp. 2041–2061 doi: 10.1111/pbi.13181

Review

Natural rubber biosynthesis in plants, the rubber transferase complex, and metabolic engineering progress and prospects

Sam Cherian[1,2], Stephen Beungtae Ryu[1,3,*] and Katrina Cornish[4,5,*]

[1]*Plant Systems Engineering Research Centre, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Yuseong-gu,Daejeon, Korea*
[2]*Research & Development Center, DRB Holding Co. LTD, Busan, Korea*
[3]*Department of Biosystems and Bioengineering, KRIBB School of Biotechnology, Korea University of Science and Technology (UST), Daejeon, Korea*
[4]*Department of Horticulture and Crop Science, The Ohio State University, Wooster, OH, USA*
[5]*Department of Food, Agricultural and Biological Engineering, The Ohio State University, Wooster, OH, USA*

Received 3 December 2018;
revised 24 May 2019;
accepted 29 May 2019.
**Correspondence* (Tel +1 330 263 3982; fax +1 330 263 3777 (K.C.); emails cornish.19@osu.edu (K.C.) and Tel +82 42 8604295; fax +82 42 8604489 (S.B.R.); emails cornish.19@osu.edu (K.C.) and sbryu@kribb.re.kr (S.B.R.))

Keywords: allylic pyrophosphate, *cis*-prenyl transferase, guayule, *Hevea brasiliensis*, isopentenyl pyrophosphate, natural rubber, *Parthenium argentatum*, rubber dandelion, rubber transferase, *Taraxacum kok-saghyz*.

Summary

Natural rubber (NR) is a nonfungible and valuable biopolymer, used to manufacture ~50 000 rubber products, including tires and medical gloves. Current production of NR is derived entirely from the para rubber tree (*Hevea brasiliensis*). The increasing demand for NR, coupled with limitations and vulnerability of *H. brasiliensis* production systems, has induced increasing interest among scientists and companies in potential alternative NR crops. Genetic/metabolic pathway engineering approaches, to generate NR-enriched genotypes of alternative NR plants, are of great importance. However, although our knowledge of rubber biochemistry has significantly advanced, our current understanding of NR biosynthesis, the biosynthetic machinery and the molecular mechanisms involved remains incomplete. Two spatially separated metabolic pathways provide precursors for NR biosynthesis in plants and their genes and enzymes/complexes are quite well understood. In contrast, understanding of the proteins and genes involved in the final step(s)—the synthesis of the high molecular weight rubber polymer itself—is only now beginning to emerge. In this review, we provide a critical evaluation of recent research developments in NR biosynthesis, *in vitro* reconstitution, and the genetic and metabolic pathway engineering advances intended to improve NR content in plants, including *H. brasiliensis*, two other prospective alternative rubber crops, namely the rubber dandelion and guayule, and model species, such as lettuce. We describe a new model of the rubber transferase complex, which integrates these developments. In addition, we highlight the current challenges in NR biosynthesis research and future perspectives on metabolic pathway engineering of NR to speed alternative rubber crop commercial development.

Introduction

Over 2500 plant species produce natural rubber (NR; Metcalfe, 1967; Bowers, 1990). However, only a small subset produce significant quantities of economically viable high-quality rubber (van Beilen and Poirier, 2007a,b; Mooibroek and Cornish, 2000; Table 1). NR (*cis*-1,4-polyisoprene) is an irreplaceable high molecular weight biopolymer, which is a critical, nonfungible, raw material vital to industries such as transportation, medicine and defence. Its unique physical properties, which include high elasticity, resilience, impact and abrasion resistance, efficient heat dispersion and malleability in cold temperatures, make NR an important raw material in the manufacture of many different rubber and latex products. However, the para rubber tree (*Hevea brasiliensis* Müll, Arg.), grown in tropical plantations as clonal, bud-grafted, scions on seedling rootstocks, is the only source of commercially produced NR (Cornish, 2017). The ever-increasing industrial demand for NR coupled with the vulnerability of the *H. brasiliensis* rubber production system, the current deforestation moratorium, and severe allergic reactions to proteins present in *H. brasiliensis* latex and rubber products have empowered researchers to domesticate and develop alternative rubber crops amenable to mechanized agriculture in temperate regions (van Beilen and Poirier, 2007a,b; Cornish, 2001a, 2017; Cornish *et al.*, 2015; Schmidt *et al.*, 2010a,b). At this time, a number of established and start-up companies are, or recently have been, involved in alternative rubber products and crops, including the following: (i) *P. argentatum*: Apollo, Bridgestone, Cooper, EnergyEne, Ford, Goodyear, Nokian, PanAridus, Guayule Australia; *T. kok-saghyz*: American Sustainable Rubber, Bridgestone, Continental, Ford, Goodyear, KeyGene, Kultivat, Ling-long, NovaBioRubber, Sumitomo, and there are undoubtedly others.

Despite the emerging strong interest and investment in alternative rubber crops, and a good understanding of NR enzymology (Archer and Audley, 1987; Cornish, 2001a,b; Cornish *et al*. 2000; Cornish and Scott, 2005; Cornish and Xie, 2012; da Costa *et al*., 2004, 2006; Espy *et al*., 2006; Kang *et al*., 2000a,b), our current understanding of the protein chemistry and the molecular mechanisms involved in rubber biosynthesis is far less complete and has not been recently reviewed. Therefore, here we review the recent biotechnological/metabolic pathway engineering advances made towards improving natural rubber

Table 1 Properties of potential alternative NR yielding plants compared to *Hevea brasiliensis*

Plant Species	Source and rubber content (%)	Rubber Mw (kg/mol)	Production (kiloton/y) (year)	Yield (kg/ha/y)	Rubber properties					References
					Gel	Allergenic proteins	Tensile strength	Modulus	Elongation	
Rubber tree *Hevea brasiliensis*	Bark laticifer 30–50 (w/w dwt) of latex 2 (w/w dwt) of tree	1310	12 760 (2017)	500–3000	Yes	Yes	High	High	Medium	Tangpakdee *et al*. (1996), van Beilen and Poirier (2007b), Cornish (2017)
Guayule *Parthenium argentatum*	Bark parenchymal cells 3–12 (w/w dwt) of bark	1280	10 (1910)	300–2000	No	No	High	Low	High	McIntyre *et al*. (2001), van Beilen and Poirier (2007b), Abdel-Haleem *et al*. (2017), Cornish (2017)
Rubber dandelion *Taraxacum kok-saghyz*	Root laticifer 15–30 (w/w dwt) of breeding line root 3–9 (w/w dwt) of wild-type root	2180	3 (1943)	150–900	Yes	Yes	High	High	Medium	Whaley and Bowen (1947), van Beilen and Poirier (2007b), Josefsson (1953), Kreuzberger *et al*. (2016), Cornish (2017), Bates *et al*. (2019)
Lettuce *Lactuca serriola*	Stem laticifer 2–8 (w/w dwt) of latex	1380	–	–	–	–	–	–	–	van Beilen and Poirier (2007b), Bell *et al*. (2015), McKeon and Brichta (2018)
Ficus tree *Ficus carica* *F. bengalensis* *F. elastica*	Bark laticifer 4 17 18 (w/w dwt) of latex	 190 1500 1–10	 – – –	– –	–	–	–	–	–	Kang *et al*. (2000a,b), van Beilen and Poirier (2007b)
Sunflower *Helianthus sp*.	Bark parenchymal cells 0.1–1 (w/w dwt) of bark	69 or 279	–	–	–	–	–	–	–	Swanson *et al*. (1979), van Beilen and Poirier (2007b)
Goldenrod *Solidago levenworthii, S. virgaurea minuta*	Leaf laticifer 5–12 (w/w dwt) of leaf	160–240	–	110–155	–					Swanson *et al*. (1979), van Beilen and Poirier (2007b)
Rubber rabbitbrush *Ericameria nauseosa*	Shoot cells 1.5–6.5 (w/w dwt) of shoot	585	–		–					Weber *et al*. (1993), van Beilen and Poirier (2007b), Hathwaik (2012)

yield and quality (specifically high molecular weight NR) in rubber plants. Additionally, we highlight the current challenges and future perspectives in metabolic pathway engineering of natural rubber biosynthesis in these potential rubber crops.

Alternative natural rubber crops

Among several rubber-producing species, *Parthenium argentatum* (Gray; guayule) and *Taraxacum kok-saghyz* (Rodin; TK, rubber dandelion, Russian/Siberian/Kazakh dandelion) have gained increasing attention in recent years, as potentially commercially viable rubber crops (Arias *et al*., 2016; van Beilen and Poirier, 2007a,b; Benedict *et al*., 2008; Buranov and Elmuradov, 2010; Mooibroek and Cornish, 2000). We briefly describe the commercial viability and quality parameters of alternative rubber crops, in comparison with *H. brasiliensis*, in the following paragraphs.

Guayule, *Parthenium argentatum*

Parthenium argentatum is a perennial shrub, native to the Chihuahuan desert of Mexico and Texas (Benedict *et al*., 2008; Coffelt and Ray, 2010). *P. argentatum* NR is high molecular weight and can be used similarly to *H. brasiliensis* rubber, but it combines comparable strength with greater softness and higher stretchiness, and does not contain the proteins present in *H. brasiliensis* latex that can cause severe allergic reactions in human beings (Cornish, 1996; Hamilton and Cornish, 2010; Siler *et al*. 1996). Its combination of allergy safety and outstanding film properties can be targeted to medical and consumer applications, such as medical gloves, catheters and balloons, as well as other high-margin products like weather balloons and lineman's gloves (Cornish, 2017). Unlike *H. brasiliensis* and *T. kok-saghyz*, which produce rubber particles in latex of laticifers, *P. argentatum* rubber particles are contained within bark parenchyma cells requiring mechanical extraction to produce latex or solvent extraction to produce rubber (Cornish and Schloman, 2004). NR yield and the overall production system need improvement before *P. argentatum* can be commercially competitive in mainstream markets (Eranki *et al*., 2017; Soratana *et al*., 2017). Also, although this is a desert species, *P. argentatum* crops require 1000–2200 mm of total water applied (irrigation plus rainfall) for high NR and biomass yields (Bucks *et al*., 1985a,b; Foster and Coffelt, 2005; Hunsaker and Elshikha, 2017). The agronomic, technical and economic feasibility of the *P. argentatum* commodity chain has been assessed in Europe, under the EU-PEARLS project (van Loo *et al*., 2012; Palu *et al*., 2013; Sfeir *et al*., 2012, 2014; Snoeck *et al*., 2011, 2015), and in the United States (Eranki *et al*., 2017; Soratana *et al*., 2017). Improved germplasm availability, through molecular and conventional breeding efforts, remains essential (Veatch *et al*., 2005). Also, established farming practices, processing companies willing to buy *P. argentatum* crops from growers, and the extraction and supply of purified rubber of high quality to rubber product-manufacturing companies, are some of the key bottlenecks still facing *P. argentatum* development as a new industrial rubber crop (Cornish, 2017).

Rubber dandelion, *Taraxacum kok-saghyz*

Taraxacum kok-saghyz is an herbaceous plant, native to Kazakhstan and Uzbekistan, and its rubber is very similar in quality to *H. brasiliensis* rubber (Cornish *et al*., 2015). It can be established from transplants or direct seeding, can be grown as an annual crop and is broadly adapted to temperate regions (Luo *et al*., 2017; McAssey *et al*., 2016; Ramirez-Cadavid *et al*., 2017; Whaley and Bowen, 1947). However, *T. kok-saghyz* is largely undomesticated, and has several inherent problems, such as its need for steady moisture content during germination, slow growth rate, poor competitiveness with weeds, meaningful rubber yield only measurable at maturity, a high degree of heterozygosity and self-incompatibility (Hodgson-Kratky and Wolyn, 2015; Warmke, 1943). Therefore, further improvement in rubber yield and agronomic fitness, through molecular and conventional breeding efforts, is essential before *T. kok-saghyz* can become a commercially viable and competitive rubber crop (Cornish, 2017; Kreuzberger *et al*., 2016; Luo *et al*., 2017; Ramirez-Cadavid *et al*., 2017). Burgeoning interest in *T. kok-saghyz* rubber research has led to field-scale trials to evaluate its performance (Arias *et al*., 2016; Buranov and Elmuradov, 2010; Cornish, 2017; Kreuzberger *et al*., 2016; Ramirez-Cadavid *et al*., 2017; Tata *et al*., 2012).

Model plants

The triploid, apomictic *T. brevicorniculatum* Koroleva (TB), a vigorous dandelion often co-located with *T. kok-saghyz*, has proved useful as an analog for *T. kok-saghyz* because it is much less variable and produces some rubber. Both species have been used for rapid genetic analysis and gene function studies in recent years (Collins-Silva *et al*., 2012; van Deenen *et al*., 2011; Hillebrand *et al*., 2012; Lin *et al*., 2017; Nowicki *et al*., 2019; Post *et al*., 2012; Schmidt *et al*., 2010a; Tata *et al*., 2012).

Lactuca sativa L. (lettuce) also synthesizes high molecular weight NR, especially during bolting, comparable to that of *H. brasiliensis* (Table 1) and, like the *Taraxacum* species, has the advantage of a short life cycle (3-5 months), making it amenable to *in vivo* gene function studies through reverse genetic approaches (Chakrabarty *et al*., 2015; Table 1). Also, prickly lettuce (*Lactuca serriola* L.), the likely progenitor of cultivated lettuce, may be promising as a potential new crop plant for rubber production (Bell *et al*., 2015).

Isoprenoid biosynthesis

From a metabolic perspective, the isoprenoid biosynthetic pathway involves three phases: (i) formation of isopentenyl pyrophosphate (IPP), (ii) condensation of IPP to synthesize linear isoprenoids, and (iii) condensation, coupled to chain elongation and/or cyclization and modification (Takahashi and Koyama, 2006). In the first phase, the basic isoprenoid unit IPP is formed, which is followed by the isomerization of IPP into dimethylallyl pyrophosphate (DMAPP). In the second phase, linear, usually *trans*, isoprenoids are formed by the sequential condensation of IPP (nonallylic) to DMAPP (allylic) to form longer allylic pyrophosphates (APP) than DMAPP through catalysis by prenyl transferases (Figure 1). In a wider sense, prenyl transferases include all enzymes that catalyse the transfer of allylic prenyl groups to acceptor molecules, such as IPP, aromatic intermediates of quinones or specific proteins (Takahashi and Koyama, 2006). The prenyl transferases responsible for each linear isoprenoid (with a specific number of isoprene units) strictly recognize the prenyl chain lengths of the allylic substrates and regulate the size and stereochemistry of the ultimate products (Takahashi and Koyama, 2006). The carbon chain length specific to naturally occurring linear isoprenoids ranges from C_{10} in geranyl (*trans*; Burke *et al*., 1999) and neryl (*cis*) pyrophosphate to C_{45} in

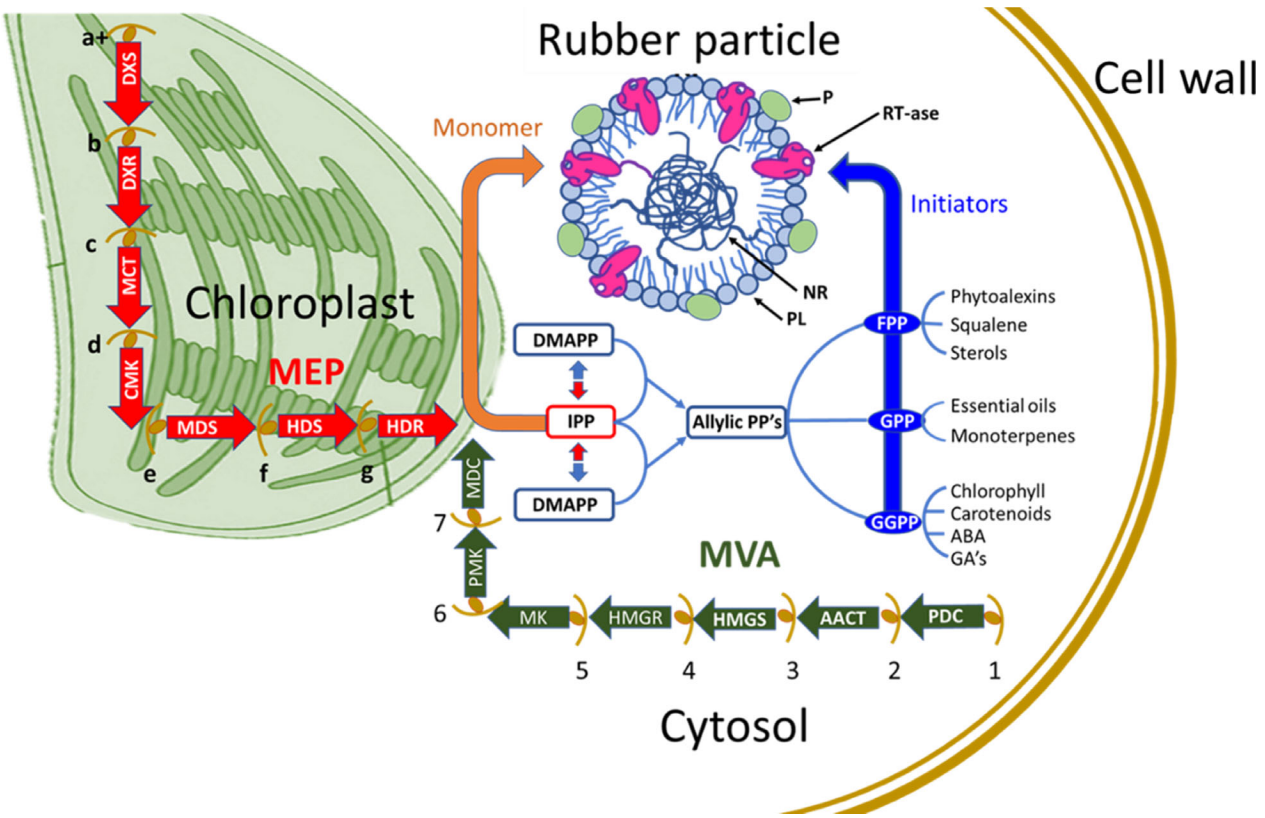

Figure 1 The metabolic route map for natural rubber (*cis*-1,4-polyisoprene) biosynthesis in plants, including the pathways for substrate synthesis, and their locations. Isopentenyl pyrophosphate (IPP), the monomeric subunit for rubber biosynthesis (orange arrow) is synthesized by two pathways, the mevalonic acid pathway (MVA, cytosolic, green arrows and numerals) and the methylerythritol pathway (MEP, plastidic, red arrows and lower case letters) from acetyl-CoA or glyceraldehyde-3-phosphate and pyruvate, respectively. IPP and its stereoisomer dimethylallyl pyrophosphate (DMAPP) condense to form several allylic pyrophosphates (APPs), namely geranyl pyrophosphate (GPP, C_{10}), farnesyl pyrophosphate (FPP, C_{15}) and geranyl geranyl pyrophosphate (GGPP, C_{20}). These APPs can be used as rubber chain initiators (blue arrow), FPP being the most common initiator, and are also the building blocks for terpenes such as chlorophyll, sterols, plant growth regulators, essential oils and so forth. Natural rubber biosynthesis is catalysed by rubber transferase complexes (magenta) bound to the proteolipid uni-lamella membrane (light blue) of cytosolic rubber particles, and rubber is compartmentalized to the rubber particle interior. Key: MVA enzymes: PDC, pyruvate dehydrogenase complex; AACT, acetyl coenzyme A acetyltransferase; HMGS, hydroxymethylglutaryl coenzyme A synthase; HMGR, hydroxymethylglutaryl coenzyme A reductase; MK, mevalonate kinase; PMK, phosphomevalonate kinase; MDC, diphosphomevalonate decarboxylase. MVA substrates: 1. pyruvate; 2. acetyl coenzyme A; 3. acetoacetyl coenzyme A; 4. hydroxymethylglutaryl coenzyme A; 5. mevalonate; 6. phosphomevalonate; 7. diphosphomevalonate. MEP enzymes: DXS, 1-deoxy-$_D$-xylulose 5-phosphate synthase; DXR, 1-deoxy-$_D$-xylulose 5-phosphate reductoisomerase; MCT, 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase; CMK,4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol kinase; MDS, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase; HDS, 4-hydroxy-3-methylbut-2-enyl diphosphate synthase; HDR, 4-hydroxy-3-methylbut-2-enyl diphosphate reductase. MEP substrates: a+, pyruvate and D-glyceraldehyde 3-phosphate; b. 1-deoxy-D-xylulose 5-phosphate; c. 2-C-methyl-D-erythritol 4-phosphate; d. 4-(cytidine 5′-diphospho)-2-C-methyl-D_erythritol; e. 2-phospho-4-(cytidine 5′-diphospho)-2-C-methyl-D- erythritol; f. 2-C-methyl-D_erythritol 2,4-cyclodiphosphate; g. (E)-4-hydroxy-3-methylbut-2-enyl diphosphate. RP, rubber particle; RT-ase, rubber transferase complex; P, non-RT-ase rubber particle-associated proteins; complexes; PL, proteolipid unilamella membrane; NR, natural rubber polymers.

solanesyl pyrophosphate (Ducluzeau *et al*., 2012; Hirooka *et al*., 2003; Ohnuma et al., 1991, 1992). The longer dolichol phosphate is more variable ($C_{55–100}$) in size but is still finite (Pan et al., 2000). Rubber, on the other hand, has variable length and can be greater than $C_{100\ 000}$ for rubber polymers of 2000 kg/mol or more (Cornish, 2016).

The mevalonate and 2-C-methyl-D-erythritol 4-phosphate IPP biosynthetic pathways

Plants use two pathways, namely the mevalonate (MVA) pathway and the methylerythritol (MEP) pathway in different compartments (cytosol and plastid, respectively) to synthesize IPP, the rubber monomer. The first enzymes in both pathways use intermediates derived from sugar metabolism via central metabolism as substrates (pyruvate and glyceraldehyde 3-phosphate, or acetyl-CoA, for the MEP and MVA pathways, respectively; Whited *et al*., 2010; Figure 1).

In the cytoplasmic MVA pathway, cytosolic acetyl-CoA (derived either from sucrose or glucose and fructose) is the primary substrate. The MVA pathway operates six major steps catalysed by the following enzymes: acetyl-CoA acetyltransferase (AACT), 3-hydroxy-3-methyl-glutaryl-coenzyme A synthase (HMGS), HMG-CoA reductase (HMGR), mevalonate kinase (MVK), phosphomevalonate kinase (PMK) and mevalonate diphosphate decarboxylase (MVD; Figure 1; Bouvier *et al*., 2005; Sirinupong *et al*., 2005). HMGR was identified as a key rate-limiting enzyme of the MVA pathway (Chappell *et al*., 1995; Stermer *et al*., 1994), but compelling experimental evidence, such as metabolic

flux analysis, is still needed to verify this (Lange *et al*., 2015; Rodríguez-Concepción, 2006, 2010; Rodríguez-Concepción and Boronat, 2015). The enzyme HMGS catalysing the irreversible conversion of acetoacetyl-CoA to HMG-CoA also seems to be a committed step in the MVA pathway in plants (Meng *et al*., 2017; Suvachittanont and Wititsuwannakul, 1995; Tang *et al*., 2016) and also may be rate-limiting.

In contrast, the plastidic MEP pathway operates eight consecutive enzymes to form IPP and DMAPP from the precursors, pyruvate and D-glyceraldehyde-3-phosphate (Cordoba *et al*., 2009; Figure 1). All the genes coding the enzymes of this pathway, in plants, are of prokaryotic origin (Cordoba *et al*., 2009). The first step of the MEP pathway is rate limiting and is catalysed by 1-deoxy-D-xylulose 5-phosphate synthase (DXS, EC 2.2.1.7) converting the precursors pyruvate and glyceraldehyde 3-phosphate into 1-deoxy-D-xylulose 5-phosphate (DXP; Sprenger *et al*., 1997; Cordoba *et al*., 2009). A recent study indicates that DXS is the enzyme with highest flux control coefficient (the main rate-determining step) in the *Arabidopsis thaliana* MEP pathway (Wright *et al*., 2014). Further condensation of pyruvate and D-glyceraldehyde-3-phosphate leads to IPP production.

In the cytosol, IPP is isomerized to DMAPP by IPP-isomerase. DMAPP is then condensed with IPP in several steps by *trans*-prenyl-transferases (TPTs) to form geranyl pyrophosphate (GPP, C_{10}, monoterpenoids), farnesyl pyrophosphate (FPP, C_{15}, sesquiterpenoids and triterpenoids) and geranylgeranyl pyrophosphate (GGPP, C_{20}, diterpenoids; van Beilen and Poirier, 2007b). These *trans*-short-chain prenyl pyrophosphates serve as initiators (allylic primer substrates) for additional IPP condensation and the final product formation (rubber molecules as well as other isoprenoids; Figure 1). The enzyme rubber transferase (RT-ase) or rubber polymerase (EC 2.5.1.20) catalyses such a reaction to polymerize *cis*-1.4-polyisoprene and can accept *trans* and *cis*-allylic pyrophosphates as initiators (e.g. Cornish, 1993; Figure 1; Archer and Audley, 1987; Bushman *et al*., 2006; van Beilen and Poirier, 2007b; Cornish, 1993, 2001a,b; Cornish and Backhaus, 1990; Cornish and Xie, 2012; Yamashita *et al*., 2016).

Cross-talk between the MVA and MEP pathways

Currently, it is clear that multiple feedback mechanisms regulate both the MVA and MEP pathways (Cordoba *et al*., 2009; Espenshade and Hughes, 2007; Rodríguez-Concepción, 2006; Rodríguez-Concepción and Boronat, 2015). If IPP can flow from the plastid to the cytosol, plastidic IPP overproduction may be a means of negating IPP substrate limitations for cytosolic RT-ases (see later discussion on IPP pool sizes).

Several studies have demonstrated that metabolic cross-regulation or cross-talk between cytosol and plastids occurs (Arigoni *et al*., 1997; Chow *et al*., 2012; De-Eknamkul and Potduang, 2003; Kumar *et al*., 2012; Nagata *et al*., 2002; Yang and Orihara, 2002). For example, in *H. brasiliensis*, quantitative real-time polymerase chain reaction (qRT-PCR) analysis of MEP and MVA pathway gene transcripts indicated that the MEP pathway was an alternate source of IPP for NR synthesis in the laticifer cytosol of a clone with low carotenoid production, but not in a clone with high carotenoid levels (Chow *et al*., 2012; Lau *et al*., 2016).

Similar results were obtained when the plastidic MEP pathway was inhibited by fosmidomycin, a specific inhibitor of DXP reductoisomerase (Kumar *et al*., 2012; Loreto *et al*., 2004; Mandel *et al*., 1996; Sharkey *et al*., 2001), where some metabolites from the cytosolic MVA pathway flowed into the plastids (Kumar *et al*., 2012; Nagata *et al*., 2002). However, the IPP transported from the cytosol into plastids did not fully compensate for lack of a functioning MEP pathway (Bouvier *et al*., 2005; Kumar *et al*., 2012). A metabolic engineering study used a multigene approach in order to express all six MVA pathway genes in chloroplasts of *Nicotiana tabacum* (tobacco) plants (Kumar *et al*., 2012). The transplastomic plants, expressing all six MVA genes, had unimpeded growth on fosmidomycin (Kumar *et al*., 2012). Increased levels of mevalonate and cytoplasmically synthesized carotenoids also were found in the transplastomic plants, indicating the likely shuttling of excess IPP from the chloroplast to the cytosol, augmenting the cytosolic IPP pool. The importance of chloroplasts in IPP production and accumulation was exploited in a study on artemisinin synthesis and accumulation in *N. tabacum* (Malhotra *et al*., 2016) where the compartmental separation of IPP and artemisinin biosynthesis led to significant secondary product increases.

However, cross-talk of isoprenoid intermediates between cytosolic and plastidic compartments was negligible in *A. thaliana* (Lange *et al*., 2015). When global gene expression patterns were analysed and pools of isoprenoid metabolites quantified in *A. thaliana* seedlings subjected to specific inhibitors (lovastatin for the MVA pathway and fosmidomycin for the MVA pathway; Laule *et al*., 2003), there was no correlation between the patterns of gene expression and metabolite changes.

Apart from IPP, cross-talk also is evident for other common isoprenoid precursors such as GPP, FPP and GGPP (Gutensohn *et al*., 2013; Hemmerlin *et al*., 2012; Laule *et al*., 2003; Mendoza-Poudereux *et al*., 2015), which can serve as rubber molecule initiators. However, although studies of whole plants and isolated plastids suggest a putative transporter, this has not been identified (Bouvier *et al*., 2005; Flugge and Gao, 2005; Soler *et al*., 1993) and the mechanisms regulating the cross-talk are not yet known. This lack of detailed understanding is likely due to confounding effects of multiple factors, including translation, post-translational modification, and other posttranscriptional processes that operate to modulate flux (Kumar *et al*., 2012; Lange *et al*., 2015; Laule *et al*., 2003).

It is also possible that pH gradients may be involved in IPP translocation, as appears to be the case in movement of protonated abscisic acid between compartments (Baier and Hartung, 1988; Cornish and Zeevaart, 1985). A similar protonation of IPP under acidic conditions may allow it similarly to move across the plastidic membrane without a specific transporter.

Natural rubber biosynthesis

The basic mechanism of rubber formation is conserved across plant species, but species-specific differences exist in tissue localization, regulation of rubber synthesis, and the molecular weight and composition of the rubber produced (Cornish *et al*., 1993, Cornish *et al*. 2000, Cornish, 2001a,b). Synthesis of the nonallylic IPP and the allylic pyrophosphates (APPs) also is ubiquitous (Chen *et al*., 2011). Biochemical studies have demonstrated that rubber biosynthesis requires a divalent cation cofactor (magnesium *in vivo*, although manganese also works *in vitro*) and integral or tethered membrane localization (Cornish, 2001a,b; Cornish and Xie, 2012; Scott *et al*. 2003). In general, two mechanisms have been proposed for NR biosynthesis: (i) the *de novo* formation of NR via sequential *cis*-1,4-condensation of IPP, via a carbocationic reaction (Puskas *et al*., 2006), onto short-chain allylic pyrophosphates as priming substrates and (ii) addition of IPP at the α-terminus of partially polymerized rubber molecules

or polyprenyl pyrophosphates (Cornish, 2001a,b; Espy *et al*., 2006; Rojruthai *et al*., 2010; Tong *et al*., 2017; Wallrapp *et al*., 2013; Yamashita *et al*., 2016; Figure 1). It should be noted that the active end of an elongating rubber molecule is an APP moiety.

Proteins and genes involved in NR substrate biosynthesis

MVA, MEP and downstream pathways

Conventionally, the genes involved in the synthesis of IPP and rubber polymer formation are coined "rubber biosynthesis genes" (Chow *et al*., 2007; Tang *et al*., 2016) and many have been characterized and manipulated in relation to NR biosynthesis (Table 2).

The recent *H. brasiliensis* genome assembly revealed 94 rubber biosynthesis-related genes belonging to 20 gene families (Tang *et al*., 2016). Among them, 18 belong to the MVA pathway, 22 to the MEP pathway, 15 to initiator synthesis in the cytosol and 39 to putative rubber particle-associated "rubber elongation" genes [which include 18 REF (rubber elongation factor)/SRPP (small rubber particle protein) genes]. However, of the 22 MEP genes, only two *DXS genes* (*DXS7* and *DXS10*) showed substantial expression in latex. In contrast, at least one gene for each MVA pathway enzyme was abundantly expressed in latex, suggesting that the MVA pathway is the primary contributor of IPP for *H. brasiliensis* rubber biosynthesis (Sando *et al*., 2008a,b; Tang *et al*., 2016). The genome assembly results also found that three of the identified gene families (*REF/SRPP, CPT* (*cis*-prenyl transferase) and *DXS*) each contained more than ten genes (Tang *et al*., 2016).

All six genes of the *H. brasiliensis* MVA pathway genes have been cloned and their expression levels analysed. However, the multiple genes encoding HMGS and HMGR have different expression patterns. *HbAACT1, HMGS1, HbMVK, HbPMK* and *HbMVD* were highly expressed in latex and complemented MVA pathway deletion mutations in yeast (Sando *et al*., 2008a,b). HMGS activity was positively correlated with the rubber content of latex (Sirinupong *et al*., 2005), and *HMGR1* was reportedly involved in rubber biosynthesis (Chye *et al*., 1992; Venkatachalam *et al*., 2009).

The genome assembly of *T. kok-saghyz* revealed a total of 102 candidate rubber biosynthesis-related genes belonging to all six steps of the MVA pathway (40 genes), all eight of the MEP pathway (23 genes), as well as 19 genes for initiator synthesis and 20 genes for rubber particle-associated "rubber elongation" proteins (Lin *et al*., 2017). Many transcripts (472) shared homology with 49 of the 50 known rubber biosynthesis-related genes and 21 036 potential rubber yield-related SNPs were noted (Luo *et al*., 2017).

When these genes were compared to their homologs in rubber-producing (*H. brasiliensis*) and nonrubber-producing [*Cynara cardunculus* var. *scolymus* (Globe artichoke)] plant species, the gene number was similar for enzymes in the MEP pathway and rubber initiator synthesis, but was greater for the MVA pathway and rubber elongation proteins in the rubber-producing plant (Lin *et al*., 2017).

In *T. brevicorniculatum*, three HMGRs (*TbHMGR1-3*) were identified and their functional analysis indicated that *TbHMGR1* is involved in the regulation of precursors for rubber biosynthesis (van Deenen *et al*., 2011). *TkHMGR1* and *TkHMGR2* from the *T. kok-saghyz* draft genome were predominantly expressed in roots, with highest expression in root latex (Lin *et al*., 2017). Expression of two genes upstream of HMGR, namely the adenosine triphosphate (ATP) citrate lyase (*ACL*) and the acetoacetyl-CoA thiolase (*AACT*), was correlated with synthesis of precursors of isoprenoid synthesis in *T. brevicorniculatum* latex. Additionally, overexpression of *A. thaliana ACL, AACT* and *HMGR* in *T. brevicorniculatum* latex caused increased activity and accumulation of all three enzymes, as well as increases in sterol, pentacyclic triterpene, *cis*-1,4-polyisoprene and squalene to potentially industrially relevant (up to 32 mg/g root dry weight) levels (Pütter *et al*., 2017). Similarly, the overexpression of full-length or truncated *HbHMGR1* increased sterol accumulation in *N. tabacum* plants (Harker *et al*., 2003; Schaller *et al*., 1995).

Recent transcriptome analysis has led to the identification of 1709 new EST (expressed sequence tags) SSRs (simple sequence repeats), and a total of 78 SNP (single nucleotide polymorphisms) markers were validated in the MVA and MEP rubber biosynthesis pathways of *H. brasiliensis* bark tissue (Mantello *et al*., 2014). In contrast to *H. brasiliensis*, transcriptome analysis (Luo *et al*., 2017) and marker trait association analysis of rubber yield-related traits in *T. kok-saghyz* (Luo *et al*., 2018) found more SNP markers related to high rubber concentration in the MEP pathway genes, indicating a stronger involvement of the MEP pathway than the MVA pathway in rubber yield in this species. Also, more SNPs were found in genes involved in inulin production than in rubber biosynthesis (Luo *et al*., 2017), suggesting a high degree of conservation of rubber biosynthesis genes (Luo *et al*., 2017).

Parthenium argentatum HMGR activity and RT-ase activity increased in cold temperature and were correlated with a rapid increase in rubber formation (Cornish and Backhaus, 2003; Ji *et al*., 1993). However, overexpression of HMGR only caused increased rubber yield in young *P. argentatum* plants under controlled environment; these increases were not maintained in field-grown transgenics, although an interesting correlation of expression level and heat tolerance was observed (Dong *et al*., 2013). Transcriptome analysis of cold-acclimated *P. argentatum* ESTs (a total of 11 748) found that most of the ESTs were from genes encoding stress-related proteins, while only just 1% of the ESTs were identified as rubber biosynthesis-related (Ponciano *et al*., 2012). Also, as in *H. brasiliensis*, a multigene family encodes the *P. argentatum* HMGS and HMGR (Ponciano *et al*., 2012).

Several TPT genes were overexpressed in two lines of *P. argentatum* and the interspecific hybrid line AZ101 (*P. argentatum* × *P. tomentosum*; Veatch *et al*., 2005). These included FPP synthase (*FPS*), GPPS synthase (*GGPS*) and a mutated form of GGPP, which produced a mixture of hexa (C_{30}) and hepta (C_{35}) APPs (*HHPS*; Veatch *et al*., 2005). Prenyl transferase activity increased in the transgenic plants, but different effects on plant growth and isoprenoid production were observed. In the transgenic *P. argentatum* field plants, more rubber molecules were synthesized than in wild-type plants, but they were of lower molecular weight. Thus, although more molecules were initiated by the increased APP, there appeared to be insufficient IPP to grow them to full length. Rubber content and molecular weight were little affected in the hybrid line by APP overexpression, but total terpene resins nearly doubled, indicating different metabolic regulation in the hybrid and nonhybrid accessions. Heterologous expression of chloroplast-localized *GGPS* isolated from *Helianthus annuus* (sunflower) in *T. brevicorniculatum*, *N. tabacum* and *A. thaliana* conferred fast plant growth, early flowering and increased seed yield with elevated gibberellin content and

Table 2 Natural rubber biosynthesis pathway genes/proteins identified and characterized

Gene/protein name	Gene origin	Approach or method	Performance	Pathway or protein	References
ATP citrate lyase (ACL), Acetoacetyl-COA thiolase (AACT) and 3-hydroxy-methyl-glutaryl-CoA reductase (HMGR)	*A. thaliana*	Overexpression in *T. brevicorniculatum*	Overexpression of the three genes resulted in an increase in pentacyclic triterpene and *cis*-1,4-isoprene levels	MVA pathway	Pütter *et al*. (2017)
HMGR1	*H. brasiliensis*	Overexpression in transgenic Arabidopsis plants	Transgenic plants were morphologically distinct from wild-type plants	MVA	Venkatachalam *et al*. (2009)
REF1, SRPP1, REF3, REF7	*H. brasiliensis*	Genome assembly	These four genes were highly expressed in latex; four different SRPPs were expressed in the latex.	RP-associated Protein	Tang *et al*. (2016)
HbEIN3-1 to *HbEIN3-4*	*H. brasiliensis*	Genome annotation	*HbEIN3-1* had high levels of transcript abundance in the latex, and four *HbEIN3*s responded to ethylene and jasmonic acid treatments suggesting their involvement in ET and JA signalling.	Protein	Yang *et al*. (2015)
CPT, REF, SRPP, RBSP, RBIP and others	*H. brasiliensis*	Latex transcriptome/qRT-PCR	Ethylene increased *cis*-polyisoprene synthesis. Hybrid contigs showed 11 classes of metabolic destinations for IPP, including *cis*-polyisoprene.	CPT and RP-associated protein	Chow *et al*. (2012)
REF, SRPP, HRT1, HRT2	*H. brasiliensis*	EST and RT-PCR analysis	These genes were highly expressed as latex-specific genes and *cis*-acting regulatory elements were identified in promoter regions of these genes.	CPT and RP-associated proteins	Aoki *et al*. (2014)
REF	*H. brasiliensis*	BAC/SDS-PAGE and MS analysis	Two different forms of REF are mainly and tightly located on the surface of large RPs.	RP-associated protein	Dai *et al*. (2017)
REF, SRPP, HRBP (CBP), CPT	*H. brasiliensis*	Transient expression in tobacco	SRPP can recruit CPT to the ER and interaction of CPT with HRBP leads to both proteins relocating to the plasma membrane	interactions among RP proteins	Brown *et al*. (2017)
HRT1, HRT2	*H. brasiliensis*	Heterologous expression in yeast and Arabidopsis	The recombinant proteins showed distinct CPT activity and produced polyisoprenoids with chain lengths of C_{80}-C_{100}, but no rubber	CPT enzymes	Takahashi *et al*. (2012)
HRT1, HRT2	*H. brasiliensis*	Overproduction of recombinant protein in *E.coli*, heterologous expression in yeast, RT-PCR, activity assay	Recombinant HRT2 protein may have synthesized medium-chain polyprenyl diphosphate intermediates as well as long-chain rubber in the presence of RPs.	RT-ase	Asawatreratanakul *et al*. (2003)
HbAACT1,2, HbHMGS1,2 HbHMGR1,4,5 HbMVK, HbPMK, HbMVD	*H. brasiliensis*	RT-PCR and loss-of-function complementation in yeast	Many of these genes were highly expressed in latex, and individual functions of the genes were tested in yeast	MVA	Sando *et al*. (2008a)
CPTL2, CPT3	*L. sativa*	RNA interference/transient expression in tobacco/expression in yeast microsome	*CPTL2* RNAi resulted in reduced rubber content. Yeast microsomes with CPTL2/CPT3 had enhanced synthesis of short *cis*-polyisoprenes, but not rubber.	CPT-like enzyme, CPT	Qu *et al*. (2015)
LsCPT3, LsCPTL2, *NgBR* homologue HRT1-REF bridging protein (*HRBP (CBP)*)	*H. brasiliensis, L. sativa*	Cell-free translation-coupled protein introduction system onto WRPs, PR proteomics and interaction network analysis	The results support that the key factor for the reconstitution of RT-ase activity is not an unknown interacting protein for CPT but proper introduction of CPT (HRT1) as a form of complex with REF and HRBP.	CPT-like enzyme/ protein	Yamashita *et al*. (2016)

Table 2. Continued

Gene/protein name	Gene origin	Approach or method	Performance	Pathway or protein	References
LsSRPP1-8	*L. sativa*	EST, qRT-PCR and RNA interference	Two *LsSRPPs* (*LsSRPP4* and *LsSRPP8*) were highly expressed in latex; *LsSRPP4* and *LsSRPP8* RNAi had no effect NR amount, molecular weight or polydispersity	RP-associated protein	Chakrabarty *et al*. (2015)
PaFPS	*P. argentatum*	Immunoblots and FPS activity assay	Two FPS isoforms were identified, and at least one is on the RPs.	RP-associated protein	Pan *et al*. (1996)
PaCPT1-3 and *PaCBP*	*P. argentatum*	Co-expression, yeast mutant complementation, activity assays	Co-expression of *PaCBP* and *PaCPT* led to dolichol synthesis but not rubber in yeast. PaCPT3 is a likely catalytic subunit in RT-ase	Protein complexes involving CPT	Lakusta *et al*. (2019)
PaAOS (PaCYP74)	*P. argentatum*	RNAi, overexpression	Involved in RP expansion and phytohormone regulation.	RP-associated protein	Placido *et al*. (2019)
GHS	*P. argentatum*	cDNA isolation and heterologous expression in *E.coli*	The recombinant protein enhanced rubber biosynthetic activity *in vitro*	RP-associated protein	Kim *et al*. (2004)
TbREF	*T. brevicorniculatum*	Mass spectrometry/RNA interference	The TbREF was homologous to TbSRPPs, and the promoter was active in laticifers. Rubber content was significantly reduced by TbREF RNAi, correlating with lower TbCPT protein and activity in latex.	RP-associated protein	Laibach *et al*. (2015)
TbRTA (CPTL)	*T. brevicorniculatum*	Knockdown by RNA interference	*TbRTA* RNAi inhibited rubber particle formation and prevented rubber biosynthesis, without affecting dolichol accumulation or protein glycosylation in the latex	Rubber transferase	Epping *et al*. (2015)
TbbZIP.1/TbSRPP	*T. brevicorniculatum*	Yeast one-hybrid system/transactivation experiments in tobacco mesophyll protoplasts	*TbbZIP.1* regulated *TbSRPP* gene expression in response to ABA, reflecting overlapping roles in rubber biosynthesis and stress responses.	Transcription factor protein	Fricke *et al*. (2013)
TbCPT1-3	*T. brevicorniculatum*	RNA interference	*TbCPT1-3* RNAi reduced rubber biosynthesis and increased triterpenes and inulin levels.	Rubber transferase	Post *et al*. (2012)
TbSRPPs	*T. brevicorniculatum*	RNA interference, Down-regulation of protein expression	RNA inference of *Tb SRPPs* affected integrity of rubber particles and rubber content	RP-associated protein	Hillebrand *et al*. (2012)
Tb and Tk 1-SST, 1-FFT and 1-FEH	*T. brevicorniculatum, T. kok-saghyz*	Full-length cDNA isolation and analysis of root mRNA levels (qPCR), overexpression of 1-FEH	*Tk 1-SST, 1-FFT* gene expression was correlated to amount and degree of polymerization (DP) of inulin during summer. *Tk1-FEH* overexpression led to inulin degradation and root rubber increase.	Inulin pathway enzymes	Stolze *et al*., 2017;
TkCPT1-3, TkSRPP1-5	*T. kok-saghyz*	Heterologous expression in *N. tabacum* and yeast, semi-quantitative RT-PCR analysis	CPTs associated with RPs were able to produce polyprenols in yeast; TkCPT1 was predominantly expressed in latex.	CPT enzyme	Schmidt *et al*. (2010a,b)
TkSRPP3	*T. kok-saghyz*	Overexpression and RNA interference	*TkSRPP3*-overexpressing lines have slightly higher levels of root rubber; *TkSRPP3* RNAi significantly reduced rubber content and rubber molecular weight.	RP-associated protein	Collins-Silva *et al*. (2012)
TbHMGR1-3, Tb HMGS, TbMVK, TbMVD	*T. brevicorniculatum*	Full-length cDNA isolation and complementation assay using an IPP auxotroph mutant of *E.coli*, transient expression assay in *Nicotiana benthamiana* leaves	High expression of *TbHMGR1, Tb HMGS, and TbMVK* in the latex and functionality of *TbHMGR1-3* confirmed in *E.coli*. Transient assays demonstrated that *TbHMGR1-2* can regulate the MVA pathway by stimulating sterol formation	MVA	van Deenen *et al*. (2011)

reduced carotenoid content, but no significant change in rubber content was observed (Tata *et al*., 2016).

Transcription factor regulatory genes

Transcription factors (TFs) are important genes regulating biosynthetic pathways involving secondary metabolites (Endt *et al*., 2002; Wang *et al*., 2013). Studies on transcription factor genes and their roles in regulating rubber biosynthesis in plants appear limited, but negative results suggest that some negative studies have not been reported. A MADS-box TF gene (*HbMADS4,* inducible by methyl jasmonate (MeJA) and ethylene, and preferentially expressed in latex) was recently isolated from *H. brasiliensis*. A yeast one-hybrid experiment, which used the *HbSRPP* promoter as bait, demonstrated that overexpression of HbMADS4 suppressed *HbSRPP* promoter activity in transgenic *N. tabacum* plants (Li *et al*., 2016). Similarly, GUS activity was suppressed when *pHbSRPP::GUS* was co-expressed with *CaMV35S::HbWRKY1*, in *N. tabacum*, making it likely that the transcription factor, WRKY, behaves as a negative regulator of *HbSRPP* in NR biosynthesis (Wang *et al*., 2013, 2015).

Transcription factor genes identified from ESTs of *H. brasiliensis* (e.g., the putative MYB transcription factor from EST clone Hebr-BJ14) had highly specific latex expression (~1000 times higher in latex than stems or leaves; Aoki *et al*., 2014). The *T. brevicorniculatum TbbZIP.1* transcription factor gene also was predominantly expressed in laticifers. The activity of this TF, which was dependent upon ABA concentration, promoted the expression of *TbSRPP* genes, suggesting a dual role for this gene in rubber biosynthesis and stress adaptation (Fricke *et al*., 2013; Table 2). The observed effects may have been mediated by ABA-inducible protein kinases. TF gene expression in laticifers suggests that they may play an overarching role in rubber biosynthesis.

Transcriptome analysis of *P. argentatum* (Ponciano *et al*., 2012; Stonebloom and Scheller, 2019) has revealed a range of genes more highly expressed in cold-induced tissue in which rubber biosynthesis is synthesized at a greater rate than in plants grown at warmer temperatures (Cornish and Backhaus, 1990, 2003). A cluster of 30 differentially expressed contigs had homologies to DNA-binding proteins or transcription factors and might be involved in cold-induced rubber biosynthesis (Stonebloom and Scheller, 2019).

Rubber particles

Rubber particle architecture

Biosynthesis of NR takes place at the surface of rubber particles produced in the cytosol of cells, whether these are specialized laticifers, as in *H. brasiliensis* bark and *T. kok-saghyz* roots, or in adapted bark parenchyma cells, as in *P. argentatum*. Rubber is compartmentalized in the rubber particle interior as an end product (Cornish, 2001a,b; Schmidt *et al*., 2010b; Figure 1), which cannot be catabolized during the life of the plant. Rubber particle size (0.08–2 μm in *H. brasiliensis*, 0.2–8 μm in *T. kok-saghyz*, 0.2–6.5 μm in *Ficus sps* and 0.7–2 μm in *P. argentatum*) and composition vary among different plant species, although ultrastructural analysis indicates a common globular structure (Abdul Ghaffar *et al*., 2016; Cornish, 2001b; Cornish *et al*., 1993; Gomez and Hamzah, 1989; Nawamawat *et al*., 2011; Schmidt *et al*., 2010b; Siler *et al*., 1997; Singh *et al*., 2003; Wood and Cornish, 2000). *H. brasiliensis* produces large rubber particles (LRP) and small rubber particles (SRP) rather than a continuous size distribution (Singh *et al*., 2003; Wood and Cornish, 2000). Similarly, two distinct rubber particle morphologies were reported in *T. kok-saghyz*, namely irregularly shaped cytoplasmic rubber particles and globular and smooth vacuolar rubber particles (Abdul Ghaffar *et al*., 2016).

Three models have been proposed to describe the structure of the rubber particle lipid–protein surface membrane, namely: (i) a general model in which all rubber particles consist of a homogeneous rubber core enclosed by a contiguous, monolayer biomembrane (Cornish, 2001a,b; Cornish *et al*., 1999), made up of proteins and lipids. Here, hydrophilic amino acids, glycosylated moieties and polar lipid head groups interact with the aqueous cytosol, while hydrophobic protein regions and lipid chains form the interior of the monolayer membrane and are compatible with the hydrophobic rubber polymers. The shell interfacial model is basically the same as this general model (Rochette *et al*., 2013); (ii) a mixed monolayer (patchy) model, which is similar to the general model but in which the membrane includes patches of rubber polymer (Berthelot *et al*., 2014; Nawamawat *et al*., 2011; Siler *et al*., 1997); and (iii) the *H. brasiliensis*-specific protein film model, in which the lipid chains of a continuous monolayer of fatty acids interact with the rubber core, their polar head groups interact with amphiphilic proteins, while the hydrophilic regions of those proteins interact with the cytosol (Wadeesirisak *et al*., 2017). This model may be true for particles with high protein content, but cannot be applied to *P. argentatum* due to lack of sufficient protein to form a contiguous layer (Siler *et al*., 1997).

The nonrubber components present in rubber particles are highly species-specific and can impact the properties of rubber produced by different plants (Junkong *et al*., 2017; Monadjemi *et al*., 2016). For instance, an unusual furanoid fatty acid is a major constituent of *H. brasiliensis* rubber particle membranes, whereas the membranes of *F. elastica* particles contain very-long-chain FAs (Siler *et al*., 1997). These long-chain FAs, combined with integral membrane proteins, form stiff membranes instead of the fluid rubber particle membranes in most other species, including *H. brasiliensis* and *P. argentatum* (Cornish *et al*., 1999; Wood and Cornish, 2000).

Rubber particle proteins

As mentioned above, rubber particles are the location of the enzymes and proteins specifically involved in rubber polymerization (Cornish and Backhaus, 1990). The RT-ase complex likely includes proteins involved in substrate binding, catalysis, molecular weight regulation and correct channelling of the rubber polymer into the rubber particle interior. However, many additional proteins also are associated with rubber particles. These include membrane-bound proteins and other proteins merely rubber particle-associated which can be readily displaced: 186 rubber particle-bound proteins were identified in *H. brasiliensis* (Dai *et al*., 2013). In the absence of reproducible solubilized RT-ase activity, it is challenging to unequivocally identify which proteins are directly involved in the regulation of rubber biosynthesis and genetic approaches have played a key role in identification attempts. However, some rubber particle proteins appear to be involved in the structure and integrity of the rubber particle. SRPP and REF do appear to play a significant role in latex coagulation and rubber particle stabilization in *H. brasiliensis* (Dennis and Light, 1989; Wititsuwannakul *et al*., 2008). Especially, SRPP has been proposed to stabilize rubber particles by displaying a kind of 'covering effect' over the lipid head groups without disturbing membrane integrity (Berthelot *et al*., 2014;

Wadeesirisak *et al*., 2017). This idea was further supported by studies of lipid droplets that compartmentalize triacylglycerol rather than rubber polymers, in which homologs of HbSRPP were identified as lipid droplet-associated proteins and were required for the maintenance of lipid droplets in stress conditions (Gidda *et al*., 2013, 2016; Horn *et al*., 2013; Pyc *et al*., 2017). SRPP (24 kD) in *H. brasiliensis* rubber particles is one of two major acidic proteins, the other being REF (14.6 kD; Dennis and Light, 1989; Yeang *et al*., 1996; Oh *et al*., 1999; Priya *et al*., 2006; Aoki *et al*., 2014). The two proteins share significant homology [approximately 72% amino acid homology (Oh *et al*., 1999)]. *P. argentatum* and *T. kok-saghyz* rubber particles both contain SRPP (HbSRPP) orthologs, PaGHS and TkSRPPs, respectively (Collins-Silva *et al*., 2012; Kim *et al*., 2004).

The most abundant *P. argentatum* rubber particle-bound protein is allene oxide synthase (AOS; also known as rubber particle protein, RPP; Backhaus *et al*., 1991; Pan *et al*., 1995; Ponciano *et al*., 2012) Overexpression of AOS in tobacco created small globules (Backhaus, pers. com.) and RNAi down-regulation of AOS (CYP74) led to smaller rubber particles and increased rubber biosynthesis in *P. argentatum* (Placido *et al*., 2019), seemingly in contrast to a report (not repeated) that solubilized AOS could synthesize rubber *in vitro* (Benedict *et al*., 1989). The expansion of particles may be limited by the amount of particle-bound AOS, leading to increased RT-ase activity. This is analogous to the proposed role of the *H. brasiliensis* REF and SRPP, which associate and may stabilize the particles when they expand as newly synthesized rubber molecules are compartmentalized to the particle interior (Dai *et al*., 2017). The main SRPP homolog in cold-acclimated *P. argentatum* ESTs has 99.9% and 60% amino acid similarity to a previously isolated *P. argentatum* protein and to *H. brasiliensis* SRPP, respectively (Backhaus *et al*., 1991; Pan *et al*., 1995; Ponciano *et al*., 2012). Five SRPPs (TbSRPP1-TbSRPP5) were identified in *T. brevicorniculatum* (initially designated as *T. kok-saghyz*, due to erroneous identification; Schmidt *et al*., 2010a). The corresponding genes, except *TbSRPP2*, were expressed in root laticifers, and the proteins (composed mainly of SRPP3 and SRPP5) were rubber particle-bound (Hillebrand *et al*., 2012; Wahler *et al*., 2012). A farnesyl pyrophosphate synthase also appears to be bound to the *P. argentatum* rubber particle membrane (Pan *et al*., 1996), but this may not be functional in particles with RT-ase activity because kinetic studies could only detect a single IPP using enzyme (Cornish, 2001a,b; Cornish and Scott, 2005).

Both REF and SRPP are allergenic proteins (named HbREF/Hevb3 and HbSRPP/Hevb1) in the context of human type I latex allergy (Yeang *et al*., 1996). REF is abundantly located on the surface of both small and large *H. brasiliensis* rubber particles (Dennis and Light, 1989; Oh *et al*., 1999; Schmidt *et al*., 2010a; Yeang *et al*., 1996) as well as in the soluble fraction of latex. These proteins also are immunochemically similar to proteins on *T. kok-saghyz* rubber particles (Cornish *et al*., 2015). *In H. brasiliensis*, SRPP is predominantly localized on small rubber particles, in phloem, and to a lesser degree on large particles but, unlike REF, is not found in the soluble latex protein fraction (Sando *et al*., 2009). Other factors, like glycosylation, are likely associated with the soluble REF form because the sequence of REF indicates that it, like SRRP, is highly hydrophobic. These proteins have different affinity for the monolayer of rubber particles: a subfraction of REF seems to be an integral membrane protein while another subfraction of REF is readily solubilized, and a third is soluble as mentioned above. In contrast, SRPP binds at the membrane surface and may form a kind of proteinaceous coating (Berthelot *et al*., 2012, 2014). It also has been proposed that REF may displace membrane lipids forming a type of mixed micelle (Berthelot *et al*., 2014).

Recent genome analysis of *H. brasiliensis* revealed that SRPP and REF have eight and nine different genes, respectively, the largest numbers reported in any plant genome for single proteins (Lau *et al*., 2016). Another genome analysis identified 18 REF/SRPP genes in *H. brasiliensis* (SRPP1 to 10 and REF1 to 8, respectively; Tong *et al*., 2017). REF1 (138 amino acids) and SRPP1 (204 amino acids), the most abundant isoforms (Tong *et al*., 2017), encode the well-characterized *H. brasiliensis* REF and SRPP particle proteins (Dennis and Light, 1989; Oh *et al*., 1999).

Rubber particle proteins implicated in rubber transferase (RT-ase) activity

RT-ases form a distinct subgroup of the *cis*-prenyltransferase (CPT) family (Akhtar et al., 2013; Asawatreratanakul *et al*., 2003; Cornish *et al*., 2001; Epping *et al*., 2015; Espy *et al*., 2006; Hemmi et al., 2001; Kharel and Koyama, 2003; Oh *et al*., 2000; Schmidt *et al*., 2010b; Yamashita *et al*., 2016). RT-ases have low substrate affinity for IPP, which prevents rubber biosynthesis from causing critical IPP deficits—rubber can only be made when IPP is in excess of cellular metabolic requirements (Cornish, 2001a,b; da Costa *et al*., 2004, 2006). Different types of CPTs are localized differently, possess different substrate-binding requirements and constants, and produce different product sizes. RT-ase is the only CPT capable of producing high molecular weight (>1000 kg/mol) *cis*-polyisoprene (da Costa *et al*., 2004, 2006; Espy *et al*., 2006; Kharel *et al*., 2001; Kharel and Koyama, 2003; Kharel *et al*., 2006; Ko *et al*., 2001; Liang *et al*., 2002; Post *et al*., 2012; Shimizu *et al*., 1998; Shridas *et al*., 2003; Surmacz *et al*., 2015, Teng and Liang, 2012), and nonrubber-producing CPTs have been confused with, or misidentified as, RT-ase (Cornish, 1993). The *H. brasiliensis* rubber particle-bound 35-kD CPT (Asawatreratanakul *et al*., 2003) can be removed from the particles without a concomitant loss of RT-ase activity (Cornish, unpublished data). CPTs are involved in sterol synthesis and so are required to form cell membranes (possibly including those of rubber particles). Also, the direct product of most CPTs are relatively short-chain *cis*-allylic pyrophosphates, which, if soluble, can function as initiators of rubber molecular biosynthesis. Thus, CPTs can affect rubber biosynthesis *without* being RT-ase itself (Cornish, 1993; Light and Dennis, 1989; Light et al., 1989).

A CPT-binding protein (CBP) was collectively discovered, in investigations not all related to rubber biosynthesis, via the identification of a new CPT, conserved across the eukaryotic lineages in human, *L. sativa*, *T. kok-saghyz* and *Lycopersicon esculentum* (tomato), by four independent research groups (Brasher *et al*., 2015; Epping *et al*., 2015; Park *et al*., 2014; Qu *et al*., 2015, respectively). A unique heteromeric CPT protein complex was found to exist in eukaryotes quite unlike the simple prokaryotic CPTs. The heteromeric eukaryotic CPT protein complex is composed of a catalytic CPT and an unusual nonenzymatic protein, designated as NgBR (Nogo-B receptor) in humans, CPTL (CPT-like protein) in *L. sativa*, CPTBP (CPT-binding protein) in *L. esculentum* and RTA (RT-ase activator) in *T. brevicorniculatum* (Brasher *et al*., 2015; Epping *et al*., 2015; Kwon *et al*., 2016; Park *et al*., 2014; Qu *et al*., 2015). The fourth nonenzymatic protein (RTA) also is named CBP (CPT-binding protein), and, for clarity, this is the abbreviation we use below as we combine these

reports. Thus, CBP directly interacts with enzymatically active CPTs and CPT/CBP complexes appear to be involved in both dolichol and NR biosynthesis (Brasher *et al*., 2015; Epping *et al*., 2015; Qu *et al*., 2015; Lakusta *et al*., 2019; Table 2).

In *T. brevicorniculatum,* CBP interacts with CPTs on the surface of rubber particles (Epping *et al*., 2015). CBP may perform two important functions: linking CPT to the rubber particle, and also stabilizing the entire complex to protect CPT from degradation, thus ensuring efficient rubber synthesis and accumulation (Epping *et al*., 2015). The CBP-RNAi transgenic lines generated were unable to synthesize long-chain *cis*-1,4-polyisoprene), suggesting that assembly of the RT-ase complex requires both the CPT and CBP (Epping *et al*., 2015).

Likewise, an unusual *cis*-prenyltransferase-like 2 (CPTL2) protein, with some homology to the human CBP, was needed for rubber biosynthesis in lettuce, but the yeast expressed CPTL2/CPT3 was not active *in vitro*, and no rubber was made (Qu *et al*., 2015). The recent *T. kok-saghyz* genome analysis identified eight *CPTs* and two *CPTL* genes encoding rubber particle proteins and *CPTL1* was highly expressed in latex as expected for rubber particle-bound proteins (Lin *et al*., 2017). However, the *L. sativa* CPTL2 did not reactivate RT-ase activity in detergent-washed and inactivated *H. brasiliensis rubber particle*s (Yamashita *et al*., 2016) possibly because it was already replaced by CPR (HRBP, Hevea REF Bridging Protein), leaving no room for additional insertion.

Two *H. brasiliensis* CPT-like proteins, designated as putative RT-ases (HRT1 and HRT2), are predominantly expressed in latex (Aoki *et al*., 2014). HRT2 protein increased new rubber molecule synthesis when added to the washed bottom fraction from centrifuged *H. brasiliensis* latex (Asawatreratanakul *et al*., 2003). However, rubber particles were still present in this assay and CPT could have stimulated rubber biosynthesis through the initiator system (by producing *cis*-APP initiators). Also, both HRT1 and HRT2 need latex-specific co-factor(s) for activity (Takahashi *et al*., 2012). However, partially deproteinized purified rubber particles, which could synthesize very little rubber from exogenous IPP and FPP, regained significant RT-ase activity when HRT1, or *L. sativa* CPT3, was added back to the rubber particles (Yamashita *et al*., 2016) and high molecular weight rubber was produced. These proteins may have reattached to the rubber particles by interacting with still present SRPP tethered by residual HRBP (a protein binding protein which has a transmembrane domain; Qu *et al*., 2015).

Other proteinaceous or architectural components of rubber particles are essential to the HRT1-mediated reconstitution of RT-ase activity, because RT-ase activity could not be reconstituted in liposomes (Yamashita *et al*., 2016). This indicates that *H. brasiliensis* rubber particle-specific components are required for activity, and may include integral proteins, and/or other membrane constituents, such as the unusual furanoid fatty acid, predominant in *H. brasiliensis* rubber particle membranes (Siler *et al*., 1997). The HRT1, CBP and possibly REF proteins only affected RT-ase activity, not polymer molecular weight, and all the rubber produced, including by the control, was of typically high molecular weight. It is also important to note that several CPTs tested in this system did not lead to RT-ase activity, including the HRT2 (Yamashita *et al*., 2016). Also, in *H. brasiliensis*, SRPP, REF and CBP are expressed in the endoplasmic reticulum (ER) and appear to be involved in rubber particle ontogeny. Also, ER-located SRRP interacts with REF and can recruit a cytosolic CPT to the ER membrane (Brown *et al*., 2017). Although this CPT (CPT6, HRT2) is not as clear a RT-ase catalytic protein candidate as HRT1 (Yamashita *et al*., 2016), this report does provide strong direct evidence for an ER origin of rubber particles.

CPT genes were reported to be required for rubber biosynthesis in *T. kok-saghyz, L. sativa* and *H. brasiliensis* (Epping *et al*., 2015; Qu *et al*., 2015; Yamashita *et al*., 2016; Table 2), but their ability to produce allylic pyrophosphate initiators (Cornish, 1993, 201a, b) can make interpretation of such experiments difficult. Four *CPT*-like sequences were identified in a *P. argentatum* cold-acclimated EST collection (Ponciano *et al*., 2012), and recently, three *CPT* genes (*PaCPT1-3*) and one *CBP* (*PaCBP*) also were identified in this species (Lakusta *et al*., 2019). When PaCBP was co-expressed with each PaCPT in turn they formed a complex which could synthesize dehydrodolichyl-PP in microsomes (Lakusta *et al*., 2019). Although no rubber was made, *PaCPT3* was both highly expressed in rubber-producing stem tissue and was further induced by cold stress, as was *PaCBP*. Thus, these proteins may be involved in RT-ase activity in this nonlaticferous species. However, proteomic analysis of *P. argentatum* rubber particles did not find CPT homologues (Kajiura *et al*., 2018), indicating that the RT-ase catalytic protein is in low abundance.

Also, three CPTs (*TkCPT1-3*) highly homologous to each other (~98%) were expressed in *T. brevicorniculatum* laticifers and root tissue and produced short-chain *cis*-1,4-polyisoprene (Schmidt *et al*., 2010a,b). However, these had low homology with other CPTs (~53% with HRT1, ~52% with HRT2, ~31% with REF2 (Sato *et al*., 1999), and ~21% with ACPT; Lin *et al*., 2017; Table 2). *T. brevicorniculatum* rubber particle CPTs complemented yeast mutants deficient in CPT activity and maintained activity in *N. tabacum* protoplasts (Schmidt *et al*., 2010b). Inhibition of CPT by RNAi reduced *cis*-1,4-polyisoprene content and led to an almost complete absence of long-chain molecules (Post *et al*., 2012), suggesting a role for this CPT in molecular weight regulation. HMGR (the key rate-limiting enzyme of the MVA pathway) also was inhibited in latex of the *TbCPT*-RNAi plants via an uncharacterized feedback mechanism. Of course, when HMGR activity is reduced, the supply of IPP decreases and the rate of rubber biosynthesis declines, and this is probably the cause of the observed low rubber phenotype (Post *et al*., 2012). Also, HMGR inhibition caused upstream precursors, such as acetyl-CoA, to accumulate. Feedback regulation effects led to a corresponding increase in the storage carbohydrate, inulin (up to 20%), in transgenic *TbCPT*-RNAi plants (Post *et al*., 2012). Similarly, *T. brevicorniculatum* rubber and inulin (biosynthesis and degradation) levels were negatively correlated and depended on corresponding enzymatic activities at different periods of the growing season (Hillebrand *et al*., 2012; Tata *et al*., 2012). An inverse relationship between inulin and rubber also was observed in *T. kok-saghyz* (Arias *et al*., 2016; Cornish and Xie, 2012; Kreuzberger *et al*., 2016).

The role of REF in rubber biosynthesis remains obscure due to conflicting reports. This may partly be caused by the presence of both membrane-associated and soluble forms of REF. Two REF isoforms bind more tightly to large rubber particles of *H. brasiliensis* than the most abundant form of REF (Dai *et al*., 2017). In the HRT-1 study, RT-ase activity was little affected by additional CBP except when this was added to HRT 1 and rubber particles *with* REF, when activity increased significantly (Yamashita *et al*., 2016). Cornish also has noted a strong correlation between CHAPS (0%–1%) solubilization of REF and loss of RT-ase activity (r^2 = 0.948, df = 5) from purified *H. brasiliensis* rubber particles. Nonetheless, REF does not seem to play a role in rubber

polymer synthesis or elongation as originally believed (Dennis and Light, 1989). The gel-purified rubber particles used in that study contained many proteins (revealed by silver staining), and the anti-REF IgG was not purified (Cornish, 1993; Dennis and Light, 1989) when used to inhibit rubber biosynthesis: unpurified sera contains components which strongly inhibit RT-ase active (Cornish, 2001b). Although REF and SRPP have multiple isoforms, identified by 2D gels of latex from *H. brasiliensis* clones of different productivity, few of them were correlated with yield or response to ethylene stimulation (Tong *et al*., 2017). RNA interference (RNAi) of REF expression in *T. brevicorniculatum* confirmed that REF was not required for rubber biosynthesis in this species (Laibach *et al*., 2015). Rubber particle numbers were reduced, however, and REF may provide a needed structural component in rubber particle biogenesis and development. Similarly, RNAi down-regulation of *TbSRPP3* severely impacted rubber content, which was affected to a lesser extent by down-regulation of the other *TbSRPP*s (Collins-Silva *et al*., 2012; Hillebrand *et al*., 2012; Table 2). Molecular weight was significantly reduced in all the RNAi lines in one study (Collins-Silva *et al*., 2012) but not the other (Hillebrand *et al*., 2012). A role for SRPP in generation of high molecular weight rubber, but not rubber biosynthesis itself, is backed up by an apparent lack of SRPP homologs in *Ficus carica and F. benghalensis*, which both produce only low molecular weight rubber (Singh *et al*., 2003). SRPP homologues are present in the high molecular mass rubber producers *H. brasiliensis*, *P. argentatum, T. brevicorniculatum* and *T. kok-saghyz*.

The RT-ase protein complex

The RT-ase enzyme/enzyme complex catalysing rubber biosynthesis (Cornish, 2001b) remains poorly understood. In this section, we have integrated the published reports of proteins apparently involved in RT-ase activity, as discussed above, with structural information either directly obtained or inferred from enzyme kinetics to inform a new schema of the rubber particle-bound RT-ase complex (Figure 2). This model is drawn to assemble the components in such a way that they could interact and perform their putative functions, as discussed below.

Hevea brasiliensis, *P. argentatum* and *F. elastica* rubber particles contain very large membrane-associated proteins (Cornish *et al*., 1993), which have monomeric sizes of 241 000, 287 000 and 360 000 Da, respectively (Cornish *et al*., 2018). Binding studies, with labelled rubber substrates, demonstrate that these very large proteins are part of the RT-ase complex (Cornish *et al*., 2018) and the native *F. elastica* protein was shown to be a dimer (Cornish and Siler, 1996; Cornish *et al*., 1994). Some interspecific surface commonalities have been demonstrated immunochemically (Cornish *et al*., 1994; Siler and Cornish, 1993), also supporting a similar function of the large proteins among these species. Thus, it appears that the RT-ase complex contains a large dimeric protein scaffold, half of which is shown in the schema [brown half ring, with grey cross-hatch (Figure 2)]. The scaffold "doughnut-like" dimeric protein tightly, but noncovalently, holds two small specific substrate-binding proteins (Cornish *et al*., 2018) in position at the entrance of this channel, shown as the blue/red proteins (Figures 2 and 3). The size of the smaller of the two proteins ranges from 1600 to 1800 Da depending on the species, while the larger ranges from 3650 to 3990 Da. It seems likely that the tritiated benzophenone allylic pyrophosphates labelled both the allylic and nonallylic pyrophosphate binding sites, because it has been proved that competitive inhibitors of allylic pyrophosphate initiators can bind at both sites (Mau *et al*., 2003). However, since we only have the minimized structure of the smaller of the two proteins, and it is not yet possible to tell which small protein binds IPP and which binds FPP, the same structure is shown for both in the schemas (Figures 2 and 3).

Enzyme kinetic analysis, and protein purification and quantification studies, demonstrated that there are three of each binding protein per scaffold monomer (Cornish *et al*., 2018), even though only two of each are shown on the schema for the sake of clarity (Figure 2). However, the tritiated benzonphene substrates used to identify the two specific binding proteins did not bind to CPT, SRPP or REF-type proteins in these experiments. This suggests that the catalytic site of the RT-ase complex either is not a CPT (which seems unlikely) or that it is adjacent to the substrate-binding sites, sufficiently close to perform the condensation reaction but not aligned to the benzophenone photoaffinity amino acid labelling moiety at the far terminus of the substrate (see right-hand active site in Figure 2). Thus, the CPT-type catalytic site [shown on the schema as blue amino acids, in the white globular protein representation of the CPT-like protein (Figure 2, and expanded in Figure 3)] is positioned adjacent to these binding sites so that the condensation reaction between IPP and the elongating rubber molecule to take place.

In the schema (Figure 2), an integral membrane CBP (in pink; Qu *et al*., 2015; Epping *et al*., 2015; Brasher *et al*., 2015) is shown integrally attached to the monomer of the dimeric scaffold (in brown) and positioned adjacent to the CPT-like proteins (in white), where it could act as a binding protein.

The rubber particle-bound protein SRPP plays a role in molecular weight regulation (Collins-Silva *et al*., 2012) and so must also form a subunit of the RT-ase complex (Figure 2, depicted as a blue globular protein). SRPP must be positioned sufficiently close to the RT-ase active site to affect polymer size. Thus, SRPP [blue globular protein (Figure 2)] is placed in the immediate vicinity of both the binding protein and the RT-ase catalytic site where it could affect the chain transfer reaction, that is, speed or impede the displacement of the elongating rubber chain by a new initiator.

However, although the exact position of SRPP and REF, their conformation, and proximity to the growing rubber polymer, is not yet known (Berthelot *et al*., 2014; Collins-Silva *et al*., 2012; Guo *et al*., 2014; Li *et al*., 2016; Wadeesirisak *et al*., 2017), due to lack of a definitive role in molecular weight regulation or biosynthesis, and the conflicting results discussed above, the integral REF is placed on the schema [green globular protein (Figure 2)] in a position more distant from the active site than SRPP. Membrane-associated and soluble REF forms also are shown because these different isoforms clearly exist (Dai *et al*., 2017).

The *H. brasiliensis* HRT1 appears to be part of a surface-bound complex held at the rubber particle surface by a transmembrane tethered CPB protein (HMBR protein, Yamashita *et al*., 2016) and is indicated on the schema by the CPT-like white globular protein associated with the integral (pink) CPB protein (Figure 2). The *P. argentatum* rubber particle protein (RPP or AOS) also has a transmembrane tethering domain (Pan *et al*., 1995) and can form very large multimers (Siler and Cornish, 1994). Electron paramagnetic resonance spin labelling confirmed that neither *H. brasiliensis* nor *P. argentatum* have much integral rubber particle membrane protein, but this was in strong contrast to *F. elastica* and *Euphorbia lactiflua* rubber particles, both of which

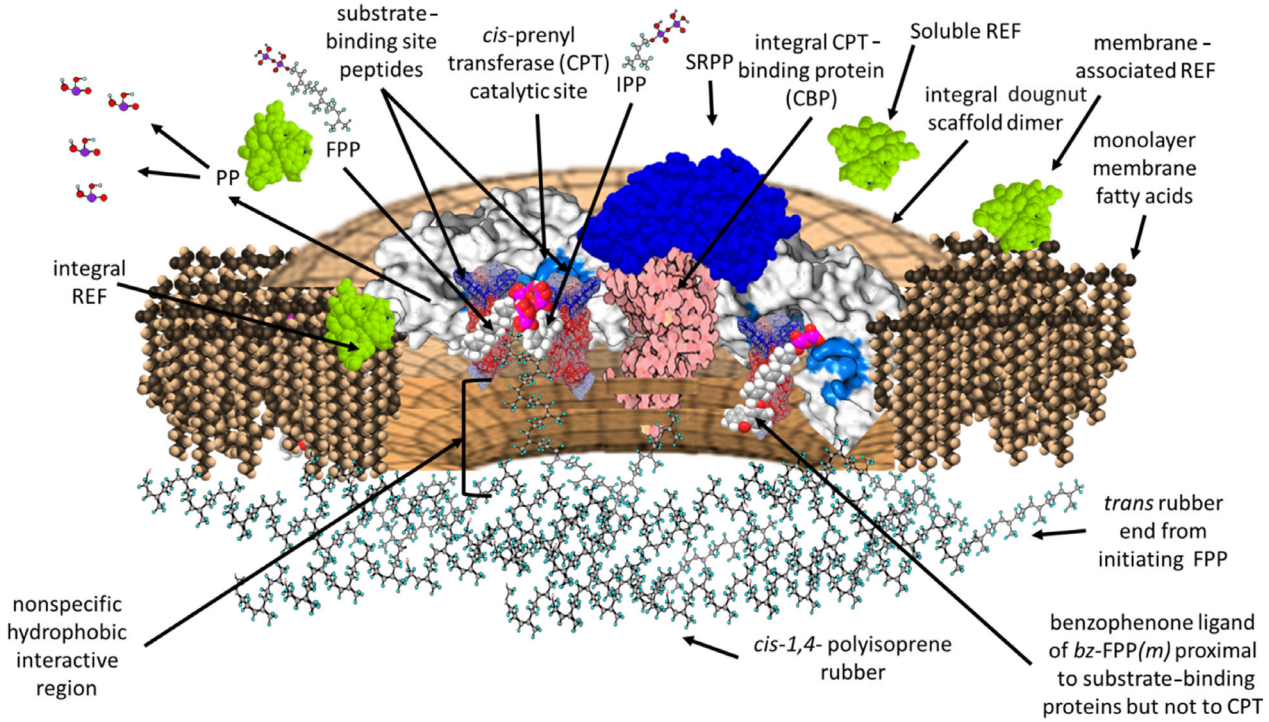

Figure 2 A new, schematic model of the rubber transferase complex (RT-ase) embedded in the monolayer biomembrane of a rubber particle. The schema is a hypothetical cross-section unilamella particle membrane with fatty acids in light and dark brown, and an embedded, integrated model of the hypothetical RT-ase complex based on the published literature, which is discussed in detail with the pertinent references in the text associated with this figure. The RT-ase complex schema contains a scaffold protein indicated by the large half ring (brown, with grey grid lines), and proteins believed to be involved in rubber biosynthesis mostly shown as space-filled globular proteins (REF in green, SRRP in blue, a CPT-type protein in white, the integral CPT-binding protein (CBP) in pink), except for the two small substrate-binding proteins. These are represented by the actual confirmation predicted by the amino acid sequence of the smaller of the two, with a hydrophobic region in blue and a hydrophobic region in red. This is because the amino acid sequence of the larger of the two small proteins is not yet known, but both bind the same allylic pyrophosphate substrates suggesting homology between their binding sites. The IPP and FPP substrates in the binding site are indicated by space-filled molecular models while the remaining substrates, polymers and released pyrophosphates are depicted as ball-and-stick models. Only two RT-ase active sites are drawn for the sake of clarity although kinetic data and quantitative protein analysis indicate that there are three per scaffold monomer. The rightermost active site contains a space-filled benzophenone labelled substrate demonstrating how UV-induced covalent attachment of the ligand could bind to the small binding proteins but not to the CPT catalytic site. The schema also indicates the nonspecific hydrophobic binding region which interacts with the hydrocarbon chains of initiators larger than DMAPP and of the elongating rubber molecule. Key: Moving from left to right of the figure: REF, rubber elongation factor, green globular protein (integral, membrane-associated and soluble forms are shown); PP, pyrophosphate, released with each condensation reaction; FPP, farnesyl pyrophosphate (allylic pyrophosphate rubber molecule initiator); CPT, *cis*-prenyl transferase; IPP, isopentenyl pyrophosphate (nonallylic pyrophosphate monomer); SRPP, small rubber particle protein; CBP, *cis*-prenyl transferase binding protein; *bz*-FPP(*m*), meta benzophenone derivatized FPP.

have significant amounts of integral membrane protein (Cornish *et al*., 1999).

Whether or not the RT-ase complex in a particular species is tethered to the rubber particle member surface or is more integrally embedded in the membrane, once the RT-ase has catalysed the condensation reaction, the part of the complex proximal to the active site region plays a significant role in both rubber compartmentalization and rate of reaction. The specific RT-ase binding sites recognize and bind DMAPP and IPP (Figure 3), and the C_5 allylic pyrophosphate moiety of initiators larger than DMAPP or of the elongating rubber polymer (which also is an allylic pyrophosphate). The kinetics of RT-ase activity, probed with APP initiators of different size and stereochemistry, using rubber particles purified from *Hevea brasiliensis*, *Parthenium argentatum* and *Ficus elastica*, indicate that the RT-ase complex contains a central channel through which the growing rubber polymers pass as they traverse the uni-lamella membrane of the rubber particles into the rubber particle interior [half ring structure, coloured brown with grey cross-hatches (Figure 2) for a detailed explanation, see Cornish, 2001a,b; Cornish and Scott, 2005;]. The length of the hydrophobic region is slightly shorter in *P. argentatum* than in *H. brasiliensis* and *F. elastica* RT-ase, which was demonstrated kinetically (Cornish, 2001a,b; Cornish and Scott, 2005) and structurally by paramagnetic resonance spectroscopy (Cornish *et al*., 1999). Also, it has been demonstrated that the rate of rubber biosynthesis increases with the number of RT-ase catalytic sites occupied by elongating rubber molecules (up to six binding sites per dimeric scaffold, as discussed above), probably due to increased hydrophobicity of the channel microenvironment being more favourable to the passage of the hydrophobic rubber chains (Cornish *et al*., 2018).

The schema (Figure 2) is not an actual RT-ase, but it does provide a predictive model which unifies the majority of the reports on RT-ase. The large scaffold proteins may be a type of

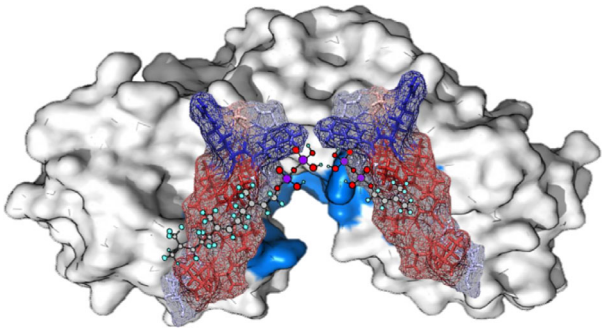

Figure 3 A close-up schema of the putative relationship of the two specific substrate-binding proteins, and the CPT catalytic site, to a farnesyl pyrophosphate initiator and an isopentenyl pyrophosphate monomer. The white globular protein is CPT with the catalytic site indicated in blue. The same minimized structure is shown for both small proteins, shown in red, blue and grey, because both bind the same allylic pyrophosphates, shown as ball-and-stick structures (although catalysis will only occur if IPP is in the correct binding site), the minimized structure of the larger small proteins is not known, and it is not yet possible to tell which small protein binds IPP and which FPP.

CBP-like proteins and could obviate the need for the smaller CBPs in some rubber-producing species. As described in this review, it is clear that rubber particle composition, RT-ase kinetics and the size of the rubber polymers produced vary considerably among species. The underlying differences in the species-specific RT-ase complexes have not yet been elucidated, and the degree and impact of protein glycosylation, while undoubtedly relevant, has not been touched upon. The precise relationship of these structural and binding proteins to the scaffold protein, small binding proteins, CPT/CBP, SRPP and REF, and other as-yet-unknown subunits, will require both solubilization of active RT-ase complexes and subsequent X-ray crystallographic revelation of their structures.

NR quality and its relationship to molecular weight

The quality of NR is an important determinant for industrial use, and it varies in different plant species. At a molecular level, NR quality is affected by polymer molecular weight and macromolecular structure, gel content (cross-linked rubber insoluble in organic solvents), and to the composition and amount of nonrubber components like lipids and proteins (Cornish, 2001b; Cornish and Blakeslee, 2011; Schmidt *et al*., 2010b; Tangpakdee and Tanaka, 1997). Molecular weight is arguably the most important parameter underlying rubber quality, with quality positively correlated to molecular weight. Although a large group of rubber-producing plants are known, only a subset, including *H. brasiliensis*, *T. kok-saghyz*, *P. argentatum*, *Cryptostegia grandiflora* (Madagascar rubber vine), *L. sativa* (lettuce) and some *Ficus* and *Euphorbia* species produce rubber with an average molecular weight over 1000 kg/mol (van Beilen and Poirier, 2007b; Table 1). RT-ases are a specific family of CPTs with distinct substrate-binding constants (K_m's) and are able to synthesize NR with varying molecular weights depending on the available substrate and cofactor concentrations (da Costa *et al*., 2004, 2006). All RT-ases investigated to date have high K_m^{IPP}'s, preventing rubber (a terminal C sink) from polymerizing IPP in competition with essential IPP-requiring enzymes. Thus, rubber is made when IPP is in excess of their requirements. *In vitro* studies of *H. brasiliensis, F. elastica* and *P. argentatum* rubber particle-bound RT-ases have shown that rubber molecular weight is greatly affected by the ratio of the initiator FPP and IPP as well as their specific concentrations; increasing FPP concentration, under limiting IPP concentrations, causes a reduction in molecular weight, while increasing IPP concentrations under limiting FPP concentration has the opposite effect (Cornish *et al*. 2000; Cornish, 2001a,b; Cornish and Scott, 2005; da Costa *et al*., 2004, 2006). The concentration of the metal ion cofactor/activator also alters the molecular weight of the rubber produced by *H. brasiliensis*, *F. elastica, P. argentatum, L. sativa* and *T. kok-saghyz* (Scott *et al*. 2003; Bushman *et al*., 2006; Cornish and Blakeslee, 2011; Collins-Silva *et al*., 2012). Thus, the laticifer environment may play a significant molecular weight regulatory role. Species-specific *in vitro* molecular weight regulation studies indicate that additional proteins, such as SRPP and REF, may be involved in the regulation of polymer chain elongation and mature rubber molecular mass (Asawatreratanakul *et al*., 2003; van Beilen and Poirier, 2007a; Collins-Silva *et al*., 2012; Cornish, 2001b; Dennis and Light, 1989; Kim *et al*., 2004; Laibach *et al*., 2015; Puskas *et al*., 2006; Wadeesirisak *et al*., 2017). Only the *P. argentatum* RT-ase, so far, has been shown to exert some direct control over rubber molecular weight (Cornish and Scott, 2005). It is possible that this ability is related to the lack of laticifers in this species, and other nonlaticiferous rubber plants [e.g. *Ericameria nauseosa* (grey rabbit brush)] may prove to have similar types of RT-ases.

Involvement of rubber particle proteins and CPTs in functions other than rubber synthesis

In addition to a role in rubber molecular weight regulation, SRPPs also may be induced by stress and play a role in stress tolerance in plants (Balbuena *et al*., 2011; Fricke *et al*., 2013). SRPP orthologs isolated from a nonrubber-producing plant, *Capsicum annuum* (pepper), have significant amino acid homologies to putative stress-related proteins, and when CaSRP1 was overexpressed in *A. thaliana*, the transgenic plants exhibited enhanced growth rates and drought tolerance (Kim *et al*., 2010). Similarly, the SRPPs identified in *T. brevicorniculatum* had high sequence homologies among themselves and with HbSRPP, and also with the *A. thaliana* stress-related protein, AtSRP (Schmidt *et al*., 2010a). A SRPP ortholog was differentially expressed in cold-acclimated *H. annuus*, which is a rubber-producing species (Balbuena *et al*., 2011). In *P. argentatum*, RT-ase activity and rubber production are induced by cold temperatures (Cornish and Backhaus, 2003; Madhavan *et al*., 1989; Ponciano *et al*., 2012; Salvucci *et al*., 2010). It is possible that SRPP levels in *P. argentatum* are post-transcriptionally regulated and SRPP may play a role in cold tolerance (Ponciano *et al*., 2012).

The recent co-localization of CPTs in rubber particles with SRPPs in *T. brevicorniculatum* provides further evidence for a link between rubber biosynthesis and stress responses (Hillebrand *et al*., 2012; Post *et al*., 2012; Schmidt *et al*., 2010b). The *HbSRPP* promoter contains light-dependent and stress-responsive regulatory elements and is upregulated by tapping (Guo *et al*., 2014; Sookmark *et al*., 2002). A promoter of *HbSRPP* with a different sequence to the *HbSRPP* promoter was functional in the laticifers of transgenic *T. brevicorniculatum* and also was induced by stresses such as light, cold and tapping (Tata *et al*., 2012). Thus, the TbSRPPs may be involved in both rubber biosynthesis and plant stress responses. Similarly, as mentioned earlier, a bZIP

transcription factor (TbbZIP.1) in *T. brevicorniculatum* regulates genes encoding SRPPs in an ABA-dependent manner and contributes to stress tolerance (Fricke *et al.*, 2013; Table 2).

In addition, expression of the AOS (CYP74) protein in *P. argentatum* was inversely correlated with biomass and photosynthetic rate in cold temperatures. The phytohormone salicylic acid played a role in the regulation of AOS expression (Placido *et al.*, 2019).

Challenges and future perspectives for metabolic improvement of NR yield

Although great strides have been made in our understanding of isoprenoid biosynthetic pathways, as well as of the genes and proteins involved in rubber biosynthesis, a comprehensive understanding of the molecular mechanisms of natural rubber biosynthesis remains elusive. At present, the simplest explanation given for NR biosynthesis is that specialized CPTs, perhaps evolved from dolichol synthase and/or undecaprenol-PP synthase (Cunillera et al., 2000), lost their specific oligomeric chain termination motifs, allowing them to catalyse the condensation of more than 10 000 IPPs (Kwon *et al.*, 2016). Complementation and heterologous expression studies in bacteria, yeast and plants have not led to production of long-chain rubber molecules and have had limited enhancement of isoprenoid end product levels (Cornish and Xie, 2012; Epping *et al.*, 2015; Kwon *et al.*, 2016; Pütter *et al.*, 2017; Qu *et al.*, 2015; Schmidt *et al.*, 2010b; Stolze *et al.*, 2017; Zhang *et al.*, 2008). Employing *cis*-genic (host gene sequences only) or marker-free vectors in genetic transformation experiments may reduce adverse effects and improve the final products (Tester and Langridge, 2010). Similar approaches also may be useful for improving rubber yield and molecular weight.

It is clear that the RT-ase has multiple components, some of which are integral membrane components, and some of which are associated with the rubber particles by their strong noncovalent interaction with membrane proteins (Cornish *et al.*, 2018; Yamashita *et al.*, 2016). The complexity of rubber biosynthesis machinery, and its substrates and activator(s) have, thus far, prevented full reconstitution of the RT-ase complex and its RT-ase activity (van Beilen and Poirier, 2007a,b; Cornish *et al.*, 2018; Kwon *et al.*, 2016).

An in-depth analysis of metabolic flux between the cytosolic MVA pathway and plastidic MEP pathway, including compartmental cross-talk and feedback loops, would be very helpful in guiding choice of the most appropriate IPP-generating pathway to genetically manipulate to create transgenic plants with improved natural rubber content. The failure of overexpressed isoprenoid biosynthesis genes/proteins to increase end product yield may be due to negative feedback mechanisms or inadequate knowledge about additional, or downstream, rate-limiting enzymes (Liao *et al.*, 2016). The advent of new genome editing technology, and other advanced metabolic, proteomic and genomic tools, should allow alternative approaches to investigate the roles of single or multiple rubber-related genes and potentially increase product yields (Gao *et al.*, 2015; Iaffaldano *et al.*, 2016; Piatek *et al.*, 2015).

The current emergence of synthetic biology and its synergy with metabolic engineering offers tremendous opportunities to create tailor-made cell factories for efficient production of desired chemicals (Nielsen and Keasling, 2011; Paddon and Keasling, 2014). The available genomic, transcriptomic and proteomic data from rubber-producing plants (Lin *et al.*, 2017; Luo *et al.*, 2017; Tang *et al.*, 2016; Wang *et al.*, 2015) may inform effective strategies to increase rubber substrate pools and the amount of the catalyst (RT-ase) itself. However, if such studies include determination of the reaction kinetics of RT-ase, this would greatly assist interpretation of the results. Molecular advances, in conjunction with advances at the breeding and agronomic levels, appropriate harvesting techniques and extraction processes (Buranov and Elmuradov, 2010; Cornish, 1996; Ramirez-Cadavid *et al.*, 2017), may increase NR yield to commercially viable levels in alternative rubber crops in the coming years.

Acknowledgements

The authors thank the Cooperative Research Program for Agriculture Science & Technology Development (PJ01368201 & PJ013486), Rural Development Administration, Republic of Korea and in part of KRIBB Project Program. S.C. was supported by the Brain Pool Program of Korean government and by the joint project (PI, S.B.R.) of KRIBB and DRB Holding Co. LTD. The authors also thank the United States Department of Agriculture, National Institute of Food and Agriculture (Hatch project 230837). We thank Dr. Xiaohui Liu for the space-filled molecular structures used in Figure 2. We thank Dr. Colleen M. McMahan for her critical review of this manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

References

Abdel-Haleem, H., Foster, M., Ray, D. and Coffelt, T. (2017) Phenotypic variations, heritability and correlations in dry biomass, rubber and resin production among guayule improved germplasm lines. *Ind. Crops Prod.* **112**, 691–697.

Abdul Ghaffar, M.A., Meulia, T. and Cornish, K. (2016) Laticifer and rubber particle ontogeny in *Taraxacum kok-saghyz* (Rubber Dandelion) roots. *Microsc. Microanal.* **22**, 1034–1035.

Akhtar, T.A., Matsuba, Y., Schauvinhold, I., Yu, G., Lees, H.A., Klein, S.E. and Pichersky, E. (2013) The tomato *cis* prenyltransferase gene family. *Plant J.* **73**, 640–652.

Aoki, Y., Takahashi, S., Takayama, D., Ogatac, Y., Sakurai, N., Suzuki, H., Asawatreratanakul, K. *et al.* (2014) Identification of laticifer-specific genes and their promoter regions from a natural rubber producing plant *Hevea brasiliensis*. *Plant Sci.* **225**, 1–8.

Archer, B.L. and Audley, B.G. (1987) New aspects of rubber biosynthesis. *Bot. J. Linn. Soc.* **94**, 181–196.

Arias, M., Herrero, J., Ricobaraza, M., Hernández, M. and Ritter, E. (2016) Evaluation of root biomass, rubber and inulin contents in nine *Taraxacum koksaghyz* Rodin populations. *Ind. Crops Prod.* **83**, 316–321.

Arigoni, D., Sagner, S., Latzel, C., Eisenreich, W., Bacher, A. and Zenk, M.H. (1997) Terpenoid biosynthesis from 1-deoxy-D-xylulose in higher plants by intramolecular skeletal rearrangement. *Proc. Natl. Acad. Sci. USA*, **94**, 10600–10605.

Asawatreratanakul, K., Zhang, Y.W., Wititsuwannakul, D., Wititsuwannakul, R., Takahashi, S., Rattanapittayaporn, A. and Koyama, T. (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.

Backhaus, R.A., Cornish, K., Chen, S.-F., Huang, D.-S. and Bess, V.H. (1991) Purification and characterization of an abundant rubber particle protein from guayule. *Phytochemistry*, **30**, 2493–2497.

Baier, M. and Hartung, W. (1988) Movement of abscisic acid across the plasmalemma and the tonoplast of guard cells of *Valerianella locusta*. *Plant Biol*. **101**, 332–337.

Balbuena, T.S., Salas, J.J., Martı'nez-Force, E., Garce's, R. and Thelen, J.J. (2011) Proteome analysis of cold acclimation in sunflower. *J. Proteome Res.* **10**, 2330–2346.

Bates, G.M., McNulty, S.K., Amstutz, N.D., Pool, V.K. and Cornish, K. (2019) Planting density and harvest season effects on actual and potential latex and rubber yields in *Taraxacum kok-saghyz*. *Hort. Sci.* in press.

van Beilen, J. and Poirier, Y. (2007a) Guayule and Russian dandelion as alternative sources of natural rubber. *Crit. Rev. Biotechnol.* **27**, 217–231.

van Beilen, J. and Poirier, Y. (2007b) Establishment of new crops for the production of natural rubber. *Trends Biotechnol.* **25**, 522–529.

Bell, J.L., Burke, I.C. and Neff, M.M. (2015) Genetic and biochemical evaluation of natural rubber from Eastern Washington Prickly Lettuce (*Lactuca serriola* L.). *J. Agri. Food Chem.* **63**, 593–602.

Benedict, C.R., Madhavan, S., Greenblatt., G.A., Venkatachalam, K.V. and Foster, M.A. (1989) The enzymatic synthesis of rubber polymer in *Parthenium argentatum* Gray. *Plant Physiol.* **92**, 816–821.

Benedict, C.R., Greer, P.J. and Foster, M.A. (2008) The physiological and biochemical responses of guayule to the low temperature of the Chihuahuan Desert in the biosynthesis of rubber. *Ind. Crops Prod.* **27**, 225–235.

Berthelot, K., Lecomte, S., Estevez, Y., Coulary-Salin, B., Bentaleb, A., Cullin, C., Deffieux, A. *et al*. (2012) Rubber elongation factor (REF), a major allergen component in *Hevea brasiliensis* latex has amyloid properties. *PLoS ONE*, **7**, e48065.

Berthelot, K., Lecomte, S., Estevez, Y., Zhendre, V., Henry, S., Thevenot, J., Dufourc, E.J. *et al*. (2014) Rubber particle proteins, HbREF and HbSRPP, show different interactions with model membranes. *Biochim. Biophys. Acta*, **1838**, 287–299.

Bouvier, F., Rahier, A. and Camara, B. (2005) Biogenesis, molecular regulation and function of plant isoprenoids. *Prog. Lip. Res.* **44**, 357–429.

Bowers, J.E. (1990) *Natural Rubber-Producing Plants for the United States. USDA, Cooperative State Research Service and National Agricultural Library*. Beltsville, Maryland: NAL.

Brasher, M.I., Surmacz, L., Leong, B., Pitcher, J., Swiezewska, E., Pichersky, E. and Akhtar, T.A. (2015) A two-component enzyme complex is required for dolichol biosynthesis in tomato. *Plant J.* **82**, 903–914.

Brown, D., Feeney, M., Ahmadi, M., Lonoce, C., Sajari, R., Si Cola, A. and Frigerio, L. (2017) Subcellular localization and interactions among rubber particle proteins from *Hevea brasiliensis*. *J. Exp. Bot.* **68**, 5045–5055.

Bucks, D.A., Nakayama, F.S., French, O.F., Legard, W.W. and Alexander, W.L. (1985a) Irrigated guayule – evapotranspiration and plant water stress. *Agric. Water Manag.* **10**, 61–79.

Bucks, D.A., Roth, R.L., Nakayama, F.S. and Gardner, B.R. (1985b) Irrigation water, nitrogen, and bioregulation for guayule production. *Trans. ASAE*, **28**, 1196–1205.

Buranov, A.U. and Elmuradov, B.J. (2010) Extraction and characterization of latex and natural rubber from rubber-bearing plants. *J. Agric. Food Chem.* **58**, 734–743.

Burke, C.C., Wildung, M.R. and Croteau, R. (1999) Geranyl diphosphate synthase: cloning, expression, and characterization of this prenyltransferase as a heterodimer. *Proc. Natl. Acad. Sci. USA*, **96**, 13062–13067.

Bushman, B.S., Scholte, A.A., Cornish, K., Scott, D.J., Brichta, J.L., Vederas, J.C., Ochoa, O. *et al*. (2006) Identification and comparison of natural rubber from two *Lactuca* species. *Phytochemistry*, **67**, 2590–2596.

Chakrabarty, R., Qu, Y. and Ro, D.K. (2015) Silencing the lettuce homologs of small rubber particle protein does not influence natural rubber biosynthesis in lettuce (*Lactuca sativa*). *Phytochemistry*, **113**, 121–129.

Chappell, J., Wolf, F., Proulx, J., Cuellar, R. and Saunders, C. (1995) Is the reaction catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A reductase a rate-limiting step for isoprenoid biosynthesis in plants? *Plant Physiol.* **109**, 1337–1343.

Chen, F., Tholl, D., Bohlmann, J. and Pichersky, E. (2011) The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. *Plant J.* **66**, 212–229.

Chow, K.S., Wan, K.L., Isa, M.N., Bahari, A., Tan, S.H., Harikrishna, K. and Yeang, H.Y. (2007) Insights into rubber biosynthesis from transcriptome analysis of *Hevea brasiliensis* latex. *J. Exp. Bot.* **58**, 2429–2440.

Chow, K.S., Mohd-Noor, M., Bahari, A., Ghazali, A.K., Alias, H., Zainuddin, Z.M., Hoh, C.C. *et al*. (2012) Metabolic routes affecting rubber biosynthesis in *Hevea brasiliensis* latex. *J. Exp. Bot.*, **63**, 1863–1871.

Chye, M.-L., Tan, C.-T. and Chua, N.-H. (1992) Three genes encode 3-hydroxy-3-methylglutaryl-coenzyme A reductase in *Hevea brasiliensis*: *hmgs1* and *hmgs3* are differentially expressed. *Plant Mol. Biol.* **19**, 473–484.

Coffelt, T.A. and Ray, D.T. (2010) Cutting height effects on guayule latex, rubber, and resin yields. *Ind. Crops Prod.* **32**, 264–268.

Collins-Silva, J., Nural, A.S., Skaggs, A., Scott, D.J., Hathwai, U., Woolsey, R., Schegg, K. *et al*. (2012) Altered levels of the *Taraxacum kok-saghyz* (Russian dandelion) small rubber particle protein, TkSRPP3, result in qualitative and quantitative changes in rubber metabolism. *Phytochemistry*, **79**, 46–56.

Cordoba, E., Salmi, M. and Leo, P. (2009) Unravelling the regulatory mechanisms that modulate the MEP pathway in higher plants. *J. Exp. Bot.* **60**, 2933–2943.

Cornish, K. (1993) The separate roles of plant *cis* and *trans* prenyl transferases in *cis*-1,4-polyisoprene biosynthesis. *Eur. J. Biochem.* **218**, 267–271.

Cornish, K. (1996) *Hypoallergenic natural rubber products from Parthenium argentatum (Gray) and other non-Hevea brasiliensis species*. US Patent 5,580,942.

Cornish, K. (2001a) Similarities and differences in rubber biochemistry among plant species. *Phytochemistry*, **57**, 1123–1134.

Cornish, K. (2001b) Biochemistry of natural rubber, a vital raw material, emphasizing biosynthetic rate, molecular weight and compartmentalization, in evolutionarily divergent plant species. *Nat. Prod. Rep.* **18**, 182–189.

Cornish, K. (2016) *Rubber Production, Encyclopedia of Applied Plant Sciences*, Second edition, Vol. **3**, 410–419.

Cornish, K. (2017) Alternative natural rubber crops: why should we care? *Technol. Innov.* **18**, 245–256.

Cornish, K. and Backhaus, R.A. (1990) Rubber transferase activity in rubber particles of guayule. *Phytochemistry*, **29**, 3809–3813.

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.

Cornish, K. and Blakeslee, J.J. (2011) *Rubber Biosynthesis in Plants*. The AOCS Lipid Library. http://lipidlibrary.aocs.org/plantbio/plantlip.html

Cornish, K. and Schloman, Jr, W.W., (2004) *Rubber, Guayule*. Encyclopedia of Polymer Science and Technology. https://doi.org/10.1002/0471440264.pst486

Cornish, K. and Scott, D.J. (2005) Biochemical regulation of rubber biosynthesis in guayule (*Parthenium argentatum Gray*). *Ind. Crops Prod.* **22**, 49–58.

Cornish, K. and Siler, D.J. (1996) Characterisation of cis-prenyl transferase activity localised in a buoyant fraction of rubber particles from *Ficus elastica* latex. *Plant Physiol. Biochem.* **34**, 377–384.

Cornish, K. and Xie, W. (2012) Natural rubber biosynthesis in plants: rubber transferase. *Methods Enzymol.* **515**, 63–82.

Cornish, K. and Zeevaart, J.A.D. (1985) Movement of abscisic acid into the apoplast in response to water stress in *Xanthium strumarium* L. *Plant Physiol.* **78**, 623–626.

Cornish, K., Siler, D.J., Grosjean, O.K. and Goodman, N. (1993) Fundamental similarities in rubber particle architecture and function in three evolutionarily divergent plant species. *J. Nat. Rubber Res.* **8**, 275–285.

Cornish, K., Siler, D.J. and Grosjean, O.K. (1994) Immunoinhibition of rubber particle-bound *cis*-prenyl transferases in *Ficus elastica* and *Parthenium argentatum* (Gray). *Phytochemistry* **35**, 1425–1428.

Cornish, K., Wood, D.F. and Windle, J.J. (1999) Rubber particles from four different species, examined by transmission electron microscopy and electron-paramagnetic-resonance spin labeling, are found to consist of a homogeneous rubber core enclosed by a contiguous, monolayer biomembrane. *Planta*, **210**, 85–96.

Cornish, K., Castillón, J. and Scott, D.J..(2000) Rubber molecular weight regulation, in vitro, in plant species that produce high and low molecular weights in vivo. *Biomacromolecules* **1**, 632–641.

Cornish, K., Brichta, J.L., Yu, P., Wood, D.F., McGlothlin, M.W. and Martin, J.A. (2001) Guayule latex provides a solution for the critical demands of the non-allergenic medical products market. *Agro-Food-Industry Hi-Tech*, **12**, 27–31.

Cornish, K., Xie, W., Kostyal, D., Shintani, D. and Hamilton, R.G. (2015) Immunological analysis of the alternate natural rubber crop *Taraxacum kok-saghyz* indicates multiple proteins cross-reactive with *Hevea brasiliensis* latex allergens. *J. Biotechn. Biomat.* **5**, 201–207.

Cornish, K., Scott, D.J., Xie, W., Mau, C.D.J., Zheng, Y.F., Liu, X.-H. and Prestwich, G.D. (2018) Unusual subunits are directly involved in binding substrates for natural rubber biosynthesis in multiple plant species. *Phytochemistry*, **156**, 55–72.

da Costa, B.M.T., Keasling, J.D. and Cornish, K. (2004) Regulation of rubber biosynthetic rate and molecular weight in *Hevea brasiliensis* by metal cofactor. *Biomacromolecules*, **6**, 279–289.

da Costa, B.M.T., Keasling, J.D., McMahan, C.M. and Cornish, K. (2006) Magnesium ion regulation of in vitro rubber biosynthesis by *Parthenium argentatum* Gray. *Phytochemistry*, **67**, 1621–1628.

Cunillera, N., Arro, M., Fores, O., Manzano, D. and Ferrer, A. (2000) Characterization of dehydrodolichyl diphosphate synthase of *Arabidopsis thaliana*, a key enzyme in dolichol biosynthesis. *FEBS Let.* **477**, 170–174.

Dai, L., Kang, G., Li, Y., Nie, Z., Duan, C. and Zeng, R. (2013) In-depth proteome analysis of the rubber particle of *Hevea brasiliensis* (para rubber tree). *Plant Mol. Biol.* **82**, 155–168.

Dai, L., Nie, Z., Kang, G., Li, L. and Zheng, R. (2017) Identification and subcellular localization analysis of two rubber elongation factor isoforms on *Hevea brasiliensis* rubber particles. *Plant Physiol. Biochem.* **111**, 96–106.

De-Eknamkul, W. and Potduang, B. (2003) Biosynthesis of b-sitosterol and stigmasterol in *Croton sublyratus* proceeds via a mixed origin of isoprene units. *Phytochemistry*, **62**, 389–398.

van Deenen, N., Bachmann, A.-L., Schmidt, T., Schaller, H., Sand, J., Prűfer, D. and Gronover, C.S. (2011) Molecular cloning of mevalonate pathway genes from *Taraxacum brevicorniculatum* and functional characterisation of the key enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *Mol. Biol. Rep.*. **39**, 4337–4349.

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.

Dong, N., Ponciano, G., McMahan, C.M., Coffelt, T.A., Johnson, L., Creelman, R.A., Whalen, M.A. *et al*. (2013) Overexpression of 3-hydroxy-3-methylglutaryl coenzyme A reductase in *Parthenium argentatum* (guayule). *Ind. Crops Prod.* **46**, 15–24.

Ducluzeau, A.L., Wamboldt, Y., Elowsky, C.G., Mackenzie, S.A., Schuurink, R.C. and Basset, G.J. (2012) Gene network reconstruction identifies the authentic *trans*-prenyl diphosphate synthase that makes the solanesyl moiety of ubiquinone-9 in Arabidopsis. *Plant J.* **69**, 366–375.

Endt, D.V., Kijne, J.W. and Memelink, J. (2002) Transcription factors controlling plant secondary metabolism: what regulates the regulators? *Phytochemistry*, **61**, 107–114.

Epping, J., van Deenen, N., Niephaus, E., Stolze, A., Fricke, J., Huber, C., Eisenreich, W. *et al*. (2015) A rubber transferase activator is necessary for natural rubber biosynthesis in dandelion. *Nature Plant*, **1**, 15048.

Eranki, P.L., El-Shikha, D., Hunsaker, D.J., Bronsonb, K.F. and Landis, A.E. (2017) A comparative life cycle assessment of flood and drip irrigation for guayule rubber production using experimental field data. *Ind. Crops Prod.* **99**, 97–108.

Espenshade, P.J. and Hughes, A.L. (2007) Regulation of sterol synthesis in eukaryotes. *Annu. Rev. Genet.* **41**, 401–427.

Espy, S.C., Keasling, J.D., Castillón, J. and Cornish, K. (2006) Initiator-independent and initiator-dependent rubber biosynthesis in *Ficus elastica*. *Arch. Biochem. Biophys.* **448**, 13–22.

Flugge, U.-I. and Gao, W. (2005) Transport of isoprenoid intermediates across chloroplast envelope membranes. *Plant Biol.* **7**, 91–97.

Foster, M.A. and Coffelt, T.A. (2005) Guayule agronomics: establishment, irrigated production, and weed control. *Ind. Crops Prod.* **22**, 27–40.

Fricke, J., Hillebrand, A., Twyman, R.M., Prufer, D. and Gronover, C.S. (2013) Abscisic acid-dependent regulation of small rubber particle protein gene expression in *Taraxacum brevicorniculatum* is mediated by TbbZIP1. *Plant Cell Physiol.* **54**, 448–464.

Gao, J., Wang, G., Ma, S., Xie, X., Wu, X., Zhang, X., Wu, Y. *et al*. (2015) CRISPR/Cas9-mediated targeted mutagenesis in *Nicotiana tabacum*. *Plant Mol. Biol.* **87**, 99–110.

Gidda, S.K., Watt, S.C., Collins-Silva, J., Kilaru, A., Arondel, V., Yurchenko, O., Horn, P.J. *et al*. (2013) Lipid droplet-associated proteins (LDAPs) are involved in the compartmentalization of lipophilic compounds in plant cells. *Plant Signal. Behav.* **8**, e27141.

Gidda, S.K., Park, S., Pyc, M., Yurchenko, O., Cai, Y., Wu, P., Andrews, D.W. *et al*. (2016) Lipid droplet-associated proteins (LDAPs) are required for the dynamic regulation of neutral lipid compartmentation in plant cells. *Plant Physiol.* **170**, 2052–2071.

Gomez, J.B. and Hamzah, S. (1989) Particle size distribution in *Hevea* latex - some observations on the electron microscopic method. *J. Nat. Rubber Res.* **4**, 204–211.

Guo, D., Li, H.L., Tang, X. and Peng, S.Q. (2014) Molecular and functional characterization of the HbSRPP promoter in response to hormones and abiotic stresses. *Transgenic Res.* **23**, 331–340.

Gutensohn, M., Orlova, I., Nguyen, T.T., Davidovich-Rikanati, R., Ferruzzi, M.G., Sitrit, Y., Lewinsohn, E. *et al*. (2013) Cytosolic monoterpene biosynthesis is supported by plastid-generated geranyl diphosphate substrate in transgenic tomato fruits. *Plant J.* **75**, 351–363.

Hamilton, R.G. and Cornish, K. (2010) Immunogenicity studies of guayule and guayule latex in occupationally-exposed Yulex plant workers. *Ind. Crops Prod.* **31**, 197–201.

Harker, M., Holmberg, M., Clayton, J.C., Gibbard, C.L., Wallace, A.D., Rawlins, S., Hellyer, S.A. *et al*. (2003) Enhancement of seed phytosterol levels by expression of an N-terminal truncated Hevea brasiliensis (rubber tree) 3-hydroxy-3-methylglutaryl-CoA reductase. *Plant Biotechnol. J.* **1**, 113–121.

Hathwaik, U.I. (2012) *Molecular biochemical characterization of rubber biosynthetic machinery in Hevea and dandelion, and evaluation of rabbitbrush as a potential domestic rubber crop*.Ph.D. thesis. U of Nevada, Reno.

Hemmerlin, A., Harwood, J.L. and Bach, T.J. (2012) A raison d'être for two distinct pathways in the early steps of plant isoprenoid biosynthesis? *Prog. Lipid Res.* **51**, 95–148.

Hemmi, H., Yamashita, S., Shimoyama, T., Nakayama, T. and Nishino, T. (2001) Cloning, expression, and characterization of *cis*-polyprenyl diphosphate synthase from the thermoacidophilic archaeon *Sulfolobus acidocaldarius*. *J. Bacteriol.* **183**, 401–404.

Hillebrand, A., Post, J.J., Wurbs, D., Wahler, D., Lenders, M., Krzyzanek, V., Prűfer, D. *et al*. (2012) Down-regulation of small rubber particle protein expression affects integrity of rubber particles and rubber content in *Taraxacum brevicorniculatum*. *PLoS ONE*, **7**, e41874.

Hirooka, Y., Bamba, T., Fukusaki, E. and Kobayashi, A. (2003) Cloning and kinetic characterization of *Arabidopsis thaliana* solanesyl diphosphate synthase. *Biochem. J.* **370**, 679–686.

Hodgson-Kratky, K.J.M. and Wolyn, D.J. (2015) Cytoplasmic male sterility in Russian dandelion. *J. Am. Soc. Hort. Sci.* **140**, 580–586.

Horn, P.J., James, C.N., Gidda, S.K., Kilaru, A., Dyer, J.M., Mullen, R.T., Ohlrogge, J.B. *et al*. (2013) Identification of a new class of lipid droplet-associated proteins in plants. *Plant Physiol.* **162**, 1926–1936.

Hunsaker, D.J. and Elshikha, D.M. (2017) Surface irrigation management for guayule rubber production in the US desert Southwest. *Agri. Water Manag.* **185**, 43–57.

Iaffaldano, B., Zhang, Y. and Cornish, K. (2016) CRISPR/Cas9 genome editing of rubber producing dandelion *Taraxacum kok-saghyz* using *Agrobacterium rhizogenes* without selection. *Ind. Crops Prod.* **98**, 356–362.

Ji, W., Benedict, C.R. and Foster, M.A. (1993) Seasonal variation in rubber biosynthesis, 3- hydroxy-3-methylglutaryl-coenzyme A reductase, and rubber transferase activities in *Parthenium argentatum* in the Chihuahuan Desert. *Plant Physiol.* **103**, 535–542.

Josefsson, A. (1953) Förädlingsarbeten med gummimaskros. *Sveriges Utsädesförenings Tidsskrift*, 293–385.

Junkong, P., Cornish, K. and Ikeda, Y. (2017) Characteristics of mechanical properties of sulphur cross-linked guayule and dandelion natural rubbers. *RSC Adv.* **7**, 50739–50752.

Kajiura, H., Suzuki, N., Mouri, H., Watanabe, N. and Nakazawa, Y. (2018) Elucidation of rubber biosynthesis and accumulation in the rubber producing shrub, guayule (*Parthenium argentatum* Gray). *Plant*, **247**, 513–526.

Kang, H., Kang, M.Y. and Han, K.-H. (2000a) Identification of natural rubber and characterization of rubber biosynthetic activity in fig tree. *Plant Physiol.* **123**, 1133–1142.

Kang, H., Kim, Y.S. and Chung, G.C. (2000b) Characterization of natural rubber biosynthesis in *Ficus benghalensis*. *Plant Physiol. Biochem.* **38**, 979–987.

Kharel, Y. and Koyama, T. (2003) Molecular analysis of *cis*-prenyl chain elongating enzymes. *Nat. Prod. Rep.* **20**, 111–118.

Kharel, Y., Zhang, Y.-W., Fujihashi, M., Miki, K. and Koyama, T. (2001) Identification of significant residues for homoallylic substrate binding of *Micrococcus luteus* B-P 26 undecaprenyl diphosphate synthase. *J. Biol. Chem.* **276**, 28459–28464.

Kharel, Y., Takahashi, S., Yamashita, S. and Koyama, T. (2006) Manipulation of prenyl chain length determination mechanism of cis-prenyltransferases. *FEBS J.* **273**, 647–657.

Kim, I.J., Ryu, S.B., Kwak, Y.S. and Kang, H. (2004) A novel cDNA from *Parthenium argentatum* Gray enhances the rubber biosynthetic activity *in vitro*. *J. Exp. Bot.* **55**, 377–385.

Kim, Y.K., Young, S.S., Lee, H. and Kim, W.T. (2010) Constitutive expression of CaSRP1, a hot pepper small rubber particle protein homolog, resulted in fast growth and improved drought tolerance in transgenic Arabidopsis plants. *Planta*, **232**, 71–83.

Ko, T.-P., Chen, Y.-K., Robinson, H., Tsai, P.-C., Gao, Y.-G., Chen, A.P., Wang, A.H. *et al*. (2001) Mechanism of product chain length determination and the role of a flexible loop in *Escherichia coli* undecaprenyl-pyrophosphate synthase catalysis. *J. Biol. Chem.* **276**, 47474–47482.

Kreuzberger, M., Hahn, T., Zibek, S., Schiemann, J. and Thiele, K. (2016) Seasonal pattern of biomass and rubber and inulin of wild Russian dandelion (*Taraxacum koksaghyz* L. Rodin) under experimental field conditions. *Eur. J. Agro.* **80**, 66–77.

Kumar, S., Hahn, F.M., Baidoo, E., Kahlon, T.S., Wood, D.F., McMahan, C.M., Cornish, K. *et al*. (2012) Remodeling the isoprenoid pathway in tobacco by expressing the cytoplasmic mevalonate pathway in chloroplasts. *Metab. Eng.* **14**, 19–28.

Kwon, M., Kwon, E.-J.G. and Ro, D.K. (2016) *cis*-Prenyltransferase and polymer analysis from a natural rubber perspective. *Methods Enzymol.* **576**, 121–145.

Laibach, N., Hillebrand, A., Twyman, R.M., Prüfer, D. and Gronover, C.S. (2015) Identification of a *Taraxacum brevicorniculatum* rubber elongation factor protein that is localized on rubber particles and promotes rubber biosynthesis. *Plant J.* **82**, 609–620.

Lakusta, A.M., Kwon, M., Kwon, E.G., Sonebloom, S., Scheller, H.V. and Ro, D. (2019) Molecular studies of the protein complexes involving *cis*-prenyl transferase in guayule *Parthenium argentatum*), an alternative rubber-producing plant. *Front. Plant Sci.* **10**, 165.

Lange, I., Poirier, B.C., Herron, B.K. and Lange, B.M. (2015) Comprehensive assessment of transcriptional regulation facilitates metabolic engineering of isoprenoid accumulation in Arabidopsis. *Plant Physiol.* **169**, 1595–1606.

Lau, N.S., Makita, Y., Kawashima, M., Taylor, T.D., Kondo, S., Othman, A.S., Shu-Chien, A.C. *et al*. (2016) The rubber tree genome shows expansion of gene family associated with rubber biosynthesis. *Sci. Rep.* **6**, 28594.

Laule, O., Fürholz, A., Chang, H.S., Zhu, T., Wang, X., Heifetz, P.B., Gruissem, W. *et al*. (2003) Crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci.* **100**, 6866–6871.

Li, H.L., Wei, L.R., Guo, D., Wang, Y., Zhu, J.H., Chen, X.T. and Peng, S.Q. (2016) HbMADS4, a MADS-box transcription factor from *Hevea brasiliensis*, negatively regulates HbSRPP. *Front. Plant Sci.* **7**, 1709.

Liang, P.H., Ko, T.P. and Wang, A.H. (2002) Structure, mechanism, and function of prenyltransferases. *Eur. J. Biochem.* **269**, 3339–3354.

Liao, P., Hemmerlin, A., Bach, T.J. and Chye, M.L. (2016) The potential of the mevalonate pathway for enhanced isoprenoid production. *Biotechn. Adv.* **34**, 697–713.

Light, D.R. and Dennis, M.S. (1989) Purification of a prenyltransferase that elongates *cis*-polyisoprene rubber from the latex of *Hevea brasiliensis*. *J. Biol. Chem.* **264**, 18589–18597.

Light, D.R., Lazarus, R.A. and Dennis, M.S. (1989) Rubber elongation by farnesyl pyrophosphate synthases involves a novel switch in enzyme stereospecificity. *J. Biol. Chem.* **264**, 18598–18607.

Lin, T., Xu, X., Ruan, J., Liu, S., Wu, S., Shao, X., Wang, X. *et al*. (2017) Genome analysis of *Taraxacum kok-saghyz* Rodin provides new insights into rubber biosynthesis. *Natl. Sci. Rev.* **5**, 78–87.

van, Loo, R., Snoeck, D., Pioch, D., Chapuset, T., Visser, P. and Palu, S. (2012) *Agronomic performance of guayule as alternative source of rubber and latex in Europe: genetic variation and effects of irrigations and fertilization, EU-PEARLS Congress BioRubber for Europe in Global Perspective, September 24-25, Wageningen, The Netherlands, 2012*.

Loreto, F., Pinelli, P., Brancaleoni, E. and Ciccioli, P. (2004) ^{13}C Labeling reveals chloroplastic and extrachloroplastic pools of dimethylallyl pyrophosphate and their contribution to isoprene formation. *Plant Physiol.* **135**, 1903–1907.

Luo, Z., Iafaldano, B.J., Zhuang, X., Fresnedo-Ramírez, J. and Cornish, K. (2017) Analysis of the first *Taraxacum kok-saghyz* transcriptome reveals potential rubber yield related SNPs. *Sci. Rep.* **7**, 9939.

Luo, Z., Iaffaldano, B.J., Zhuang, X., Fresnedo-Ramirez, J. and Cornish, K. (2018) Variance, inter-trait correlation, heritability and marker-trait association of rubber yield related characteristics in *Taraxacum kok-saghyz*. *Plant Mol. Biol. Rep.*. **36**, 576–587.

Madhavan, S., Greenblatt, G.A., Foster, M.A. and Benedict, C.R. (1989) Stimulation of isopentenyl pyrophosphate incorporation into polyisoprene in extracts from guayule plants (*Parthenium argentatum* Gray) by low temperature and 2-(3,4-dichloro-phenoxy)-triethylamine. *Plant Physiol.* **89**, 506–511.

Malhotra, K., Subramaniyan, M., Rawat, K., Kalamuddin, M., Qureshi, M.I., Malhotra, P., Mohmmed, A. *et al*. (2016) Compartmentalized metabolic engineering for artemisinin biosynthesis and effective malaria treatment by oral delivery of plant cells. *Mol. Plant*, **9**, 1464–1477.

Mandel, M.A., Feldmann, K.A., Herrera-Estrella, L., Rocha-Sosa, M. and Leon, P. (1996) *CLA1*, a novel gene required for chloroplast development, is highly conserved in evolution. *Plant J.* **9**, 649–658.

Mantello, C.C., Cardosa-Silva, C.B., da Silva, C.C., de Souza, L.M., Scaloppi Junior, E.J., Goncalves, P.S., Vicentini, R. *et al*. (2014) *De novo* assembly and transcriptome analysis of the rubber tree (*Hevea brasiliensis*) and SNP markers development for rubber biosynthesis pathways. *PLoS ONE*, **9**, e-102665.

Mau, C.J.D., Garneau, S., Scholte, A.A., Vederas, J.C. and Cornish, K. (2003) Protein farnesyltransferase inhibitors interfere with farnesyl diphosphate binding by rubber transferase. *Eur. J. Biochem.* **270**, 1–7.

McAssey, E.V., Gudger, E.G., Zuellig, M.P. and Burke, J.M. (2016) Population genetics of the rubber-producing Russian dandelion (*Taraxacum kok-saghyz*). *PLoS ONE*, **11**, e0146417.

McIntyre, D., Stephens, H.L., Schloman, W.W. Jr and Bhowmick, A.K. (2001) Guayule rubber. In *Plastics Engineering*, vol. **61**, p. 1–27. New York: Marcel Dekker.

McKeon, T.A. and Brichta, J. (2018) An improved method for fractionation of small quantities of lettuce latex. *Am. J. Plant Sci.* **9**, 2004–2007.

Mendoza-Poudereux, I., Kutzner, E., Huber, C., Segura, J., Eisenreich, W. and Arrillaga, I. (2015) Metabolic cross-talk between pathways of terpenoid backbone biosynthesis in spike lavender. *Plant Physiol. Biochem.* **95**, 113–120.

Meng, X., Song, Q., Ye, J., Wang, L. and Xu, F. (2017) Characterization, function, and transcriptional profiling analysis of 3-hydroxy-3-methylglutaryl-CoA synthase gene (GbHMGS1) towards stresses and exogenous hormone treatments in *Ginkgo biloba*. *Molecules*, **22**, 1706.

Metcalfe, C.R. (1967) Distribution of latex in the plant kingdom. *Econ. Bot.* **21**, 115–127.

Monadjemi, S.M.A., McMahan, C.M. and Cornish, K. (2016) Effect of Non-rubber constituents on guayule and Hevea rubber intrinsic properties. *J. Res. Updates Polym. Sci.* **5**, 87–96.

Mooibroek, H. and Cornish, K. (2000) Alternative sources of natural rubber. *Appl. Microbiol. Biotechnol.* **53**, 355–365.

Nagata, N., Suzuki, M., Yoshida, S. and Muranaka, T. (2002) Mevalonic acid partially restores chloroplast and etioplast development in Arabidopsis lacking the non-mevalonate pathway. *Planta*, **216**, 345–350.

Nawamawat, K., Sakdapipanich, J.T., Ho, C.C., Ma, Y., Song, J. and Vancso, J.G. (2011) Surface nanostructure of *Hevea brasiliensis* natural rubber latex particles. *Colloids Surf. A Physicochem. Eng. Asp.* **390**, 157–166.

Nielsen, J. and Keasling, J.D. (2011) Synergies between synthetic biology and metabolic engineering. *Nat. Biotechn.* **29**, 693–695.

Nowicki, M., Zhao, Y., Boggess, S.L., Fluess, H., Paya-Milans, M., Staton, M.E., Houston, L.C. *et al*. (2019) *Taraxacum kok-saghyz* (rubber dandelion)

genomic microsatellite loci reveal modest genetic diversity and cross-amplify broadly to related species. *Sci. Rep.* **9**, 1915.

Oh, S.K., Kang, H., Shin, D.H., Yang, J., Chow, K.S., Yeang, H.Y., Wagner, B. *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.

Oh, S.K., Han, K.H., Ryu, S.B. and Kang, H. (2000) Molecular cloning, expression, and functional analysis of a *cis*-prenyltransferase from *Arabidopsis thaliana*. *J. Biol. Chem.* **275**, 18482–18488.

Ohnuma, S., Koyama, T. and Ogura, K. (1991) Purification of solanesyl-diphosphate synthase from *Micrococcus luteus*. A new class of prenyltransferase. *J. Biol. Chem.* **266**, 23706–23713.

Ohnuma, S., Koyama, T. and Ogura, K. (1992) Chain length distribution of the products formed in solanesyl diphosphate synthase reaction. *J. Biochem.* **112**, 743–749.

Paddon, C.J. and Keasling, J.D. (2014) Semi-synthetic artemisinin: a model for the use of synthetic biology in pharmaceutical development. *Nat. Rev. Microbiol.* **12**, 355–367.

Palu, S., Pioch, D., Amor, A., Tardan, E., Chapuset, T., Snoeck, D. and Bonfils, F. (2013) *The Challenge of Guayule: an Alternative Source of Natural Rubber a Model of Bio-refinery*. International Rubber Research and Development Board Conference London, UK, June 2013.

Pan, Z., Durst, F., Werck-Reichhart, D., Gardner, H.W., Camara, B., Cornish, K. and Backhaus, R.A. (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.

Pan, Z., Herickhoff, L. and Backhaus, R.A. (1996) Cloning, characterization, and heterologous expression of cDNAs for farnesyl diphosphate synthase from the guayule rubber plant reveals that this prenyltransferase occurs in rubber particles. *Arch. Biochem. Biophys.* **332**, 196–204.e.

Pan, J.-J., Chiou, S.-T. and Liang, P.-H. (2000) Product distribution and pre-steady-state kinetic analysis of *Escherichia coli* undecaprenyl pyrophosphate synthase reaction. *Biochemistry*, **39**, 10936–10942.

Park, E.J., Grabińska, K.A., Guan, Z., Stránecký, V., Hartmannová, H., Hodaňová, K., Barešová, V. *et al*. (2014) Mutation of Nogo-B receptor, a subunit of *cis*-prenyltransferase, causes a congenital disorder of glycosylation. *Cell Metab.* **20**, 448–457.

Piatek, A., Ali, Z., Baazim, H., Li, L., Abulfaraj, A., Al-Shareef, S., AouiDa, M. *et al*. (2015) RNA-guided transcriptional regulation in planta via synthetic dCas9-based transcription factors. *Plant Biotechnol. J.* **13**, 578–589.

Placido, D., Dong, N., Dong, C., Cruz, V.M.V., Dierig, D., Cahoon, R.E., Kang, B. *et al*. (2019). Downregulation of a CYP74 rubber particle protein increases natural rubber production in *Parthenium argentatum*. *Front. Plant Sci.* in press.

Ponciano, G., McMahan, C.M., Xie, W., Lazo, G.R., Coffelt, T.A., Collins-Silva, J., Nural-Taban, A. *et al*. (2012) Transcriptome and gene expression analysis in cold-acclimated guayule (*Parthenium argentatum*) rubber-producing tissue. *Phytochemistry*, **79**, 57–66.

Post, J., van Deenen, N., Fricke, J., Kowalski, N., Wurbs, D., Schaller, H., Eisenreich, W. *et al*. (2012) Laticifer-specific *cis*-prenyltransferase silencing affects the rubber, triterpene, and inulin content of *Taraxacum brevicorniculatum*. *Plant Physiol.* **158**, 1406–1417.

Priya, P., Venkatachalam, P. and Thulaseedharan, A. (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.

Puskas, J.E., Gautriaud, E., Deffieux, A. and Kennedy, J.P. (2006) Natural rubber biosynthesis – a living carbocationic polymerization? *Prog. Polym. Sci.* **31**, 533–548.

Pütter, K.M., van Deenen, N., Unland, K., Prüfer, D. and Gronover, S.S. (2017) Isoprenoid biosynthesis in dandelion latex is enhanced by the overexpression of three key enzymes involved in the mevalonate pathway. *BMC Plant Biol.* **17**, 88.

Pyc, M., Cai, Y., Gidda, S.K., Yuchenko, O., Park, S., Kretzschmar, F.K., Ischebeck, T. *et al*. (2017) Arabidopsis lipid droplet-associated protein (LDAP) – interacting protein (LDIP) influences lipid droplet size and neutral lipid homeostasis in both leaves and seeds. *Plant J.* **92**, 1182–1201.

Qu, Y., Chakrabarty, R., Tran, H., Kwon, E.-J.G., Kwon, M., Nguyen, T.-D. and Ro, D.-K. (2015) A lettuce *(Lactuca sativa*) homolog of human Nogo-B receptor interacts with *cis*-prenyltransferase and is necessary for natural rubber biosynthesis. *J. Biol. Chem.* **290**, 1898–1914.

Ramirez-Cadavid, D.A., Cornish, K. and Michel, F.C. Jr. (2017) *Taraxacum kok-saghyz* (TK): compositional analysis of a feedstock for natural rubber and other bioproducts. *Ind. Crops Prod.* **107**, 624–640.

Rochette, C.N., Crassous, J.J., Drechsler, M., Gaboriaud, F., Eloy, M., de Gaudemaris, B. and Duval, J.F. (2013) Shell structure of natural rubber particles: evidence of chemical stratification by electrokinetics and cryo-TEM. *Langmuir*, **29**, 14655–14665.

Rodríguez-Concepción, M. (2006) Early steps in isoprenoid biosynthesis: multilevel regulation of the supply of common precursors in plant cells. *Phytochem. Rev.* **5**, 1–15.

Rodríguez-Concepción, M. (2010) Supply of precursors for carotenoid biosynthesis in plants. *Arch. Biochem. Biophys.* **504**, 118–122.

Rodríguez-Concepción, M. and Boronat, A. (2015) Breaking new ground in the regulation of the early steps of plant isoprenoid biosynthesis. *Curr. Opin. Plant Bio.* **25**, 17–22.

Rojruthai, P., Sakdapipanich, J.T., Takahashi, S., Hyegin, L., Noike, M., Koyama, T. and Tanaka, Y. (2010) *In vitro* synthesis of high molecular weight rubber by Hevea small rubber particles. *J. Biosci. Bioeng.* **109**, 107–114.

Salvucci, M.E., Barta, C., Byers, J.A. and Canarini, A. (2010) Photosynthesis and assimilate partitioning between carbohydrates and isoprenoid products in vegetatively active and dormant guayule: physiological and environmental constraints on rubber accumulation in a semiarid shrub. *Physiol. Plant.* **140**, 368–379.

Sando, T., Takaoka, C., Mukai, Y., Yamashita, A., Hattori, M., Ogasawara, N., Fukusaki, E. *et al*. (2008a) Cloning and characterization of mevalonate pathway genes in a natural rubber producing plant, *Hevea brasiliensis*. *Biosci. Biotech. Bioch.* **72**, 2049–2060.

Sando, T., Takeno, S., Watanabe, N., Okumoto, H., Kuzuyama, T., Yamashita, A., Hattori, M. *et al*. (2008b) Cloning and characterization of the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway genes of a natural-rubber producing plant, *Hevea brasiliensis*. *Biosci. Biotechnol. Biochem.* **72**, 2903–2917.

Sando, T., Hayashi, T., Takeda, T., Akiyama, Y., Nakazawa, Y., Fukusaki, E. and Kobayashi, A. (2009) Histochemical study of detailed laticifer structure and rubber biosynthesis-related protein localization in *Hevea brasiliensis* using spectral confocal laser scanning microscopy. *Planta*, **230**, 215–225.

Sato, M., Sato, K., Nishikawa, S., Hirata, A., Kato, J. and Nakano, A. (1999) The yeast *RER2* gene, identified by endoplasmic reticulum protein localization mutations, encodes *cis*-prenyltransferase, a key enzyme in dolichol synthesis. *Mol. Cell. Biol.* **19**, 471–483.

Schaller, H., Grausem, B., Benveniste, P., Chye, M.-L., Tan, C.-T., Song, Y.-H. and Chua, N.-H. (1995) Expression of the *Hevea brasiliensis* (H.B.K.) Müll. Arg. 3-Hydroxy-3-Methylglutaryl-coenzyme a Reductase 1 in tobacco results in sterol overproduction. *Plant Physiol.* **109**, 761–770.

Schmidt, T., Hillebrand, A., Wurbs, D., Wahler, D., Lenders, M. and Gronover, C.S. (2010a) Molecular cloning and characterization of rubber biosynthetic genes from *Taraxacum koksaghyz*. *Plant Mol. Biol. Rep.* **28**, 277–284.

Schmidt, T., Lenders, M., Hillebrand, A., van Deenen, N., Munt, O., Reichelt, R., Eisenreich, W. *et al*. (2010b) Characterization of rubber particles and rubber chain elongation in *Taraxacum koksaghyz*. *BMC Biochem.* **11**, 11.

Scott, D.J., da Costa, B.M.T., Espy, S.C., Keasling, J.D. and Cornish, K..(2003) Activation and inhibition of rubber transferases by metal cofactor and pyrophosphate substrate. *Phytochemistry* **64**, 121–132.

Sfeir, N., Snoeck, D., Van Loo, R., Chapuset, T., García García, J. and Lancon, F. (2012) *Feasibility of guayule commodity chain in the Mediterranean region*. EU-PEARLS Congress BioRubber for Europe in Global Perspective 24–25 September, Wageningen, The Netherlands, 2012.

Sfeir, N., Chapuset, T., Palu, S., Lançon, F., Amor, A., García, J. and Snoeck, D. (2014) Technical and economic feasibility of a guayule commodity chain in Mediterranean Europe. *Ind. Crops Prod.* **59**, 55–62.

Sharkey, T.D., Wiberley, A.E. and Donohue, A.R. (2001) Isoprene emission from plants: why and how. *Annals Bot.* **101**, 5–18.

Shimizu, N., Koyama, T. and Ogura, K. (1998) Molecular cloning, expression, and purification of undecaprenyl diphosphate synthase. No sequence

similarity between *e*- and *z*-prenyl diphosphate synthases. *J. Biol. Chem.* **273**, 19476–19481.

Shridas, P., Rush, J.S. and Waechter, C.J. (2003) Identification and characterization of a cDNA encoding a long-chain *cis*-isoprenyltranferase involved in dolichylmonophosphate biosynthesis in the ER of brain cells. *Biochem. Biophys. Res. Commun.* **312**, 1349–1356.

Siler, D.J. and Cornish, K. (1993) A protein from *Ficus elastica* rubber particles is related to proteins from *Hevea brasiliensis* and *Parthenium argentatum*. *Phytochemistry*, **32**, 1097–1102.

Siler, D.J. and Cornish, K. (1994) Identification of *Parthenium argentatum* rubber particle proteins immunoprecipitated by an antibody that specifically inhibits rubber transferase activity. *Phytochemistry*, **36**, 623–627.

Siler, D.J., Cornish, K. and Hamilton, R.G..(1996) Absence of cross-reactivity of IgE antibodies from Hevea brasiliensis latex allergic subjects with a new source of natural rubber latex from guayule (Parthenium argentatum). *J. Allergy Clin. Immunol.* **98**, 895–902.

Siler, D.J., Goodrich-Tanrikulu, M., Cornish, K., Stafford, A.E. and Mckeon, T.A. (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.

Singh, A.P., Wi, S.G., Chung, G.C., Kim, Y.S. and Kang, H. (2003) The micromorphology and protein characterization of rubber particles in *Ficus carica*, *Ficus benghalensis* and *Hevea brasiliensis*. *J. Exp. Bot.* **54**, 985–992.

Sirinupong, N., Suwanmanee, P., Doolittle, R.F. and Suvachitanont, W. (2005) Molecular cloning of a new cDNA and expression of 3-hydroxy-3-methylglutaryl-CoA synthase gene from *Hevea brasiliensis*. *Planta*, **221**, 502–512.

Snoeck, D., Van Loo, E.N., Chapuset, T. and Palu, S. (2011) *Agronomic evaluation of guayule cultivation in two Mediterranean areas – Spain and France*. AAIC 23rd Annual Meeting: Challenges and Opportunities for Industrial Crops, September 11–14, Fargo, USA, 2011.

Snoeck, D., Chapuset, T., García, J.G., Sfeir, N. and Palu, S. (2015) Feasibility of a guayule commodity chain in the Mediterranean region. *Ind. Crops Prod.* **75**, 159–164.

Soler, E., Clastre, M., Bantignies, B., Marigo, G. and Ambid, C. (1993) Uptake of isopentenyl diphosphate by plastids isolated from *Vitis vinifera* L. cell suspensions. *Planta*, **205**, 324–329.

Sookmark, U., Pujade-Renaud, V., Chrestin, H., Lacote, R., Naiyanetr, C., Seguin, M., Romruensukharom, P. *et al*. (2002) Characterization of polypeptides accumulated in the latex cytosol of rubber trees affected by the tapping panel dryness syndrome. *Plant Cell Physiol.* **43**, 1323–1333.

Soratana, K., Rasutis, D., Azarabadi, H., Eranki, P.L. and Landis, A.E. (2017) Guayule as an alternative source of natural rubber: A comparative life cycle assessment with Hevea and synthetic rubber. *J. Cleaner Prod.* **159**, 271–280.

Sprenger, G.A., Schörken, U., Wiegert, T., Grolle, S., De Graaf, A.A., Taylor, S.V., Begley, T.P. *et al*. (1997) Identification of a thiamine-dependent synthase in *Escherichia coli* required for the formation of the 1-deoxy-D-xylulose 5-phosphate precursor to isoprenoids, thiamine and pyridoxol. *Proc. Natl. Acad. Sci. USA*, **94**, 12857–12862.

Stermer, B.A., Bianchini, G.M. and Korth, K.L. (1994) Regulation of HMG-CoA reductase activity in plants. *J. Lipid Res.* **35**, 1133–1140.

Stolze, A., Wanke, A., van Deenen, N., Geyer, R., Prűfer, D. and Gronover, C.S. (2017) Development of rubber-enriched dandelion varieties by metabolic engineering of the inulin pathway. *Plant Biotechnol. J.* **15**, 740–753.

Stonebloom, S.H. and Scheller, H.V. (2019) Transcriptome analysis of rubber biosynthesis in guayule (*Parthenium argentatum* Gray). *BMC Plant Biol.* **19**, 71.

Surmacz, L., Plochocka, D., Kania, M., Danikiewicz, W. and Swiezewska, E..(2015) cis-Prenyltransferase atCPT6 produces a family of very short-chain polyisoprenoids in planta. *Biochim Biophys. Acta.* **1841**, 240–250.

Suvachittanont, W. and Wititsuwannakul, R. (1995) 3-Hydroxy 3-methylglutaryl-CoA synthase in *Hevea brasiliensis*. *Phytochemistry*, **40**, 757–761.

Swanson, C.L., Buchanan, R.A. and Otey, F.H. (1979) Molecular weights of natural rubbers from selected temperate zone plants. *J. App. Poly. Sci.* **23**, 743–748.

Takahashi, S. and Koyama, T. (2006) Structure and function of *cis*-prenyl chain elongating enzymes. *Chem. Rec.* **6**, 194–205.

Takahashi, S., Lee, H.-J., Yamashita, S. and Koyama, T. (2012) Characterization of *cis*-prenyltransferases from the rubber producing plant *Hevea brasiliensis* heterologously expressed in yeast and plant cells. *Plant Biotechnol.* **29**, 411–417.

Tang, C., Yang, M., Fang, Y., Luo, Y., Gao, S., Xiao, X., An, Z. *et al*. (2016) The rubber tree genome reveals new insights into rubber production and species adaptation. *Nat. Plants*, **2**, 16073.

Tangpakdee, J. and Tanaka, Y. (1997) Characterization of sol and gel in *Hevea* natural rubber. *Rubber Chem. Technol.* **70**, 707–713.

Tangpakdee, J., Tanaka, Y., Wititsuwannakul, R. and Chareonthiphakorn, N. (1996) Possible mechanisms controlling molecular weight of rubbers in *Hevea brasiliensis*. *Phytochemistry*, **42**, 353–355.

Tata, S.T., Choi, J.Y., Jung, J.-Y., Lim, K.Y., Shin, J.S. and Ryu, S.B. (2012) Laticifer tissue-specific activation of the Hevea SRPP promoter in *Taraxacum brevicorniculatum* and its regulation by light, tapping and cold stress. *Ind. Crops Prod.* **40**, 219–224.

Tata, S.K., Jung, J.H., Kim, Y.H., Choi, J.Y., Jung, J.Y., Lee, I.J., Shin, J.S. *et al*. (2016) Heterologous expression of chloroplast-localized geranylgeranyl pyrophosphate synthase confers fast plant growth, early flowering and increased seed yield. *Plant Biotechnol. J.* **14**, 29–39.

Teng, K.-H. and Liang, P.-H. (2012) Structures, mechanisms and inhibitors of undecaprenyl diphosphate synthase: a *cis*-prenyltransferase for bacterial peptidoglycan biosynthesis. *Bioorganic Chem.* **43**, 51–57.

Tester, M. and Langridge, P. (2010) Breeding technologies to increase crop production in a changing world. *Science*, **327**, 818–822.

Tong, Z., Wang, D., Sun, Y., Yang, Q., Meng, X., Wang, L., Feng, W. *et al*. (2017) Comparative proteomics of rubber latex revealed multiple protein species of REF/SRPP family respond diversely to ethylene stimulation among different rubber tree clones. *Int. J. Mol. Sci.* **18**, 958–972.

Veatch, M.E., Ray, D.T., Mau, C.J.D. and Cornish, K. (2005) Growth, rubber and resin evaluation of two-year-old transgenic guayule. *Ind. Crops Prod.* **22**, 65–74.

Venkatachalam, P., Priya, P., Jayashree, R., Rekha, K. and Thulaseedharan, A. (2009) Molecular cloning and characterization of a 3-hydroxy-3-methylglutaryl coenzyme a reductase 1 (*hmgr1*) gene from rubber tree (*Hevea brasiliensis* Muell. Arg.): a key gene involved in isoprenoid biosynthesis. *Physiol. Mol. Biol. Plants*, **15**, 133–143.

Wadeesirisak, K., Castano, S., Berthelot, K., Vaysse, L., Bonfils, F., Peruch, F., Rattanaporn, K. *et al*. (2017) Rubber particle proteins REF1 and SRPP1 interact differently with native lipids extracted from *Hevea brasiliensis* latex. *Biochim. Biophys. Acta*, **1859**, 201–210.

Wahler, D., Colby, T., Kowalski, N.A., Harzen, A., Wotzka, S.Y., Hillebrand, A., Fischer, R. *et al*. (2012) Proteomic analysis of latex from the rubber-producing plant *Taraxacum brevicorniculatum*. *Proteomics*, **12**, 901–905.

Wallrapp, F.H., Pan, J.-J., Ramamoorthy, G., Almonacid, D.E., Hillerich, B.S., Seidel, R., Patskovsky, Y. *et al*. (2013) Prediction of function for the polyprenyl transferase subgroup in the isoprenoid synthase superfamily. *Proc. Natl. Acad. Sci.* **110**, E1196–E1202.

Wang, X.C., Shi, M.J., Wang, D., Chen, Y.Y., Cai, F.G., Zhang, S.X., Wang, L. *et al*. (2013) Comparative proteomics of primary and secondary lutoids reveals that chitinase and glucanase play a crucial combined role in rubber particle aggregation in *Hevea brasiliensis*. *J. Proteome Res.* **12**, 5146–5159.

Wang, X., Wang, D., Sun, Y., Yang, Q., Chang, L., Wang, L., Meng, X. *et al*. (2015) Comprehensive proteomics analysis of laticifer latex reveals new insights into ethylene stimulation of natural rubber production. *Sci. Rep.* **5**, 13778.

Warmke, H.E. (1943) Macrosporogenesis, fertilization, and early embryology of *Taraxacum kok-saghyz*. *Bull. Torrey Bot. Club*, **70**, 164–173.

Weber, D.J., Hess, W.M., Bhat, R.B. and Huang, J. (1993) Chrysothamnus: a rubber producing semi-arid shrub. In *New Crops* (Janick, J. and Simon, J.E., eds), pp. 355–357. New York: Wiley.

Whaley, W.G. and Bowen, J.S. (1947) *Russian Dandelion (kok-saghyz): An Emergency Source of Natural Rubber (US Department of Agriculture)*. Washington, D.C.: U.S. Dept. of Agriculture

Whited, G.H., Feher, F.J., Benko, D.A., Cervin, M.A., Chotani, G.K., McAuliffe, J.C., LaDuca, R.J. *et al*. (2010) Development of a gas-phase bioprocess for isoprene-monomer production using metabolic pathway engineering. *Ind. Biotechnol.* **6**, 152–163.

Wititsuwannakul, R., Pasitkul, P., Jewtragoon, P. and Wititsuwannakul, D. (2008) Hevea latex lectin binding protein in C-serum as an anti-latex coagulating factor and its role in a proposed new model for latex coagulation. *Phytochemistry*, **69**, 656–662.

Wood, D.F. and Cornish, K. (2000) Microstructure of purified rubber particles. *Int. J. Plant Sci.* **161**, 435–445.

Wright, L.P., Rohwer, J.M., Ghirardo, A., Hammerbacher, A., Ortiz-Alcaide, M., Raguschke, B., Schnitzler, J.P. *et al*. (2014) Deoxyxylulose 5-phosphate synthase controls flux through the methylerythritol 4-phosphate pathway in Arabidopsis. *Plant Physiol.* **165**, 1488–1504.

Yamashita, S., Yamaguchi, H., Waki, T., Aoki, Y., Mizuno, M., Yanbe, F., Ishii, T. *et al*. (2016) Identification and reconstitution of the rubber biosynthetic machinery on rubber particles from Hevea brasiliensis. *eLife*, **5**, e19022.

Yang, J.W. and Orihara, Y. (2002) Biosynthesis of abietane diterpenoids in cultured cells of *Torreya nucifera* var. *radicans*: biosynthetic inequality of the FPP part and the terminal IPP. *Tetrahedron*, **58**, 1265–1270.

Yang, Z.-P., Li, H.-P., Guo, D. and Peng, S.-Q..(2015) Identification and characterization of the EIN3/EIL gene family in Hevea brasiliensis. *Tree Genet. Genomes* **11**, 51.

Yeang, H.Y., Cheong, K.F., Sunderasan, E., Hamzah, S., Chew, N.P., Hamid, S., Hamilton, R.G. *et al*. (1996) The 14.6 kD rubber elongation factor (Hev b 1) and 24 kD (Hev b 3) rubber particle proteins are recognized by IgE from patients with spina bifida and latex allergy. *J. Allergy Clin. Immunol.* **98**, 628–639.

Zhang, H., Ohyama, K., Boudet, J., Chen, Z., Yang, J., Zhang, M., Muranaka, T. *et al*. (2008) Dolichol biosynthesis and its effects on the unfolded protein response and abiotic stress resistance in Arabidopsis. *Plant Cell*, **20**, 1879–1898.

