Appl Microbiol Biotechnol (2000) 53: 355–365

MINI REVIEW

H. Mooibroek · K. Cornish

Alternative sources of natural rubber

Received: 9 August 1999 / Received revision: 15 October 1999 / Accepted: 16 October 1999

Abstract Rubber (*cis*-1,4-polyisoprene) is one of the most important polymers naturally produced by plants because it is a strategic raw material used in more than 40,000 products, including more than 400 medical devices. The sole commercial source, at present, is natural rubber harvested from the Brazilian rubber tree, *Hevea brasiliensis*. Primarily due to its molecular structure and high molecular weight (>1 million daltons) this rubber has high performance properties that cannot easily be mimicked by artificially produced polymers, such as those derived from, e.g., bacterial poly-hydroxyalkanoates (PHAs). These high performance properties include resilience, elasticity, abrasion resistance, efficient heat dispersion (minimizing heat build-up under friction), and impact resistance. Medical rubber gloves need to fit well, be break-resistant, allow the wearer to retain fine tactile sensation, and provide an effective barrier against pathogens. The sum of all these characteristics cannot yet be achieved using synthetic gloves. The lack of biodiversity in natural rubber production renders continuity of supply insecure, because of the risk of crop failure, diminishing acreage, and other disadvantages outlined below. A search for alternative sources of natural rubber production has already resulted in a large number of interesting plants and prospects for immediate industrial exploitation of guayule (*Parthenium argentatum*) as a source of high quality latex. Metabolic engineering will permit the production of new crops designed to accumulate new types of valued isoprenoid metabolites, such as rubber and carotenoids, and new combinations extractable from the same crop. Currently, experiments are underway to genetically improve guayule rubber production strains in both quantitative and qualitative respects. Since the choice for gene activities to be introduced or changed is under debate, we have set up a complementary approach to guayule with yeast species, which may more quickly show the applicability and relevance of genes selected. Although economic considerations may prevent commercial exploitation of new rubber-producing microorganisms, transgenic yeasts and bacteria may yield intermediate or alternative (poly-)isoprenes suitable for specific applications.

H. Mooibroek (✉) · K. Cornish
Agrotechnological Research Institute (ATO-DLO),
P.O. Box 17, NL-6700 AA Wageningen, The Netherlands
e-mail: a.mooibroek@ato.dlo.nl
Fax: +31-317-475347

Permanent address:
K. Cornish
United States Department of Agriculture,
Agricultural Research Service (USDA-ARS),
Western Regional Research Center,
Albany, CA 94710, USA

Introduction

History

Ancient Mesoamerican peoples used a variety of processed rubber products as early as 1600 BC (Hosler et al. 1999), primarily from *Castilla elastica* (a fig tree relative) latex mixed with juice from *Ipomoea alba* (a morning glory vine). Also, Indians from South and Central America were the first to produce rubber from the latex of a number of plants, including *H. brasiliensis* (Schurer 1957). In 1736, Charles de la Condamine was the first European scientist to recognize the potential importance of the so-called wild rubber (caoutchouc) (Coates 1987). However, Francois Fresneau made the first systematic observations on rubber, and the whereabouts of the *H. brasiliensis* tree, and provided descriptions of the tree and methods of latex tapping and rubber preparation (Schurer 1957). Samples of rubber were sent to Europe in 1751 for evaluation by the French Academy of Science (Sethuraj and Mathew 1992).

Significant commercial use of rubber did not occur until the nineteenth century when, in 1818, James Syme discovered that coal tar naphtha was an efficient solvent (Schurer 1952) and Thomas Hancock discovered mastication (Schurer 1962). However, unvulcanized rubber proved unsuitable for the climatic extremes of North

America, which caused rubber products to become sticky in the heat and rigid in the cold. This performance failure was overcome, in 1838, by Charles Goodyear, who discovered that a mixture of latex and sulfur, after vulcanization, yields a rubber that retains its firmness at normal and higher temperatures, and flexibility at cold temperatures (Barker 1938; Simmons 1939). Latex is defined as a colloidal suspension of a polymeric material in a liquid system mostly aqueous in nature. In the case of *H. brasiliensis* latex, the colloidal suspension consists of rubber particles, each containing many rubber polymers, suspended in aqueous cytoplasm. Vulcanization is defined as a process in which rubber, through a change in its chemical structure (e.g., crosslinking) is converted to a condition in which the elastic properties are conferred or re-established or improved or extended over a greater range of temperatures (Mausser 1987).

Originally, wild *Hevea* trees in the Amazon region were exploited – the taxonomy of *Hevea* has been reviewed by Richard Schultes (1970). The anticipated overexploitation resulted in the first plantations of *H. brasiliensis* (the Brazilian rubber tree), first in South America, followed by South-East Asia and, more recently, by Africa (Sethuraj and Mathew 1992). Demand for natural rubber has continued to increase throughout the twentieth century (Fig. 1) despite competition from synthetic rubber. Asia now produces about 90% of the world demand for *H. brasiliensis* natural rubber, which, in 1998, was 6.61 million metric tonnes (40% of the total rubber demand; the remaining 60% is synthetic rubber produced from petroleum) (Table 1, from data supplied by the International Rubber Study Group). The United States, with no natural rubber production of its own, is the largest single consumer of this material. In 1998, the United States imported nearly 1.2 million metric tonnes of natural rubber for its own product manufacture, at a cost approaching US$ 2000 million (US$ 1.67/kg). In addition, the United States imports a considerable quantity of finished goods: in 1998, these contained 349,000 metric tonnes of natural rubber and cost US$ 8,060 million (data from US Department of Commerce, Office of Trade and Economic Analysis).

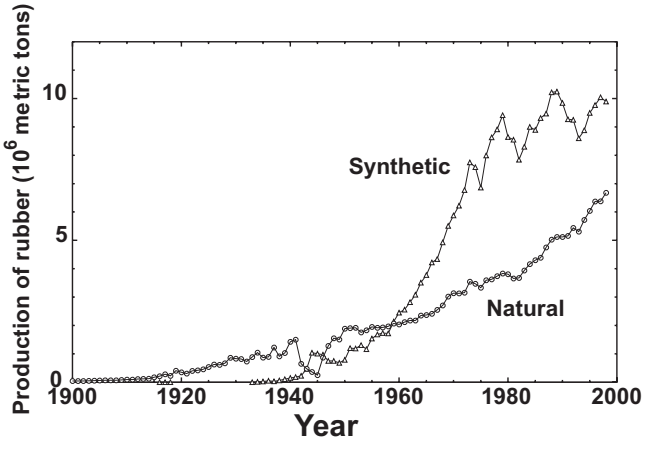

Fig. 1 Global production of natural (–○–) and synthetic (–△–) rubber from 1900. The drop in natural rubber production and rise in synthetic rubber production during World War II is clear

Threats

Latex tapped from the Brazilian rubber tree *H. brasiliensis* is currently the sole commercial source of natural rubber (*cis*-1,4-polyisoprene). Global dependence on this single species for natural rubber is risky because *H. brasiliensis* cultivars have very little genetic variability, leaving the rubber plantations at risk of serious pathogenic attack (Davis 1997). Moreover, *H. brasiliensis* has strict climatic requirements, limiting its cultivation to specific tropical regions. Natural rubber prices also are directly tied to labor costs, which increase as the rubber-producing countries develop; latex production is labor-intensive since it is tapped by hand following incisions in the bark of the trees. A shortage of natural rubber is expected in the near future. Another fundamental problem with *H. brasiliensis* rubber concerns the world-wide occurrence of life-threatening, IgE-mediated, latex allergy caused by the proteins in the latex. Complete protein removal is neither cheap nor easy, and, when it is achieved, negatively impacts latex performance.

Other species naturally producing rubber

H. brasiliensis belongs to the family of *Euphorbiaceae* with species which are well known for the milky substance (latex) they release upon wounding. For reasons specified above, a search for alternative sources of natural rubber from other plant species was initiated some 40 years ago, increasing the number of known *cis*-1,4-polyisoprene-producing plant species to about 2500. Table 2 provides an overview of the most prominent plant species, the country of their natural habitat, and some essential characteristics. *H. brasiliensis* still occupies its leading position by virtue of its rubber yield and high molecular weight.

Among the alternative species, *P. argentatum* has received the most attention, most recently as a source of high-quality, hypoallergenic latex. Cultivation trials are underway in several countries. However, guayule, a native of the Chihuahuan desert of the United States and Mexico, is restricted to semi-arid regions, and highly specific winter temperatures are required for good rubber production (Whitworth and Whitehead 1991). Just before and during World War II, *P. argentatum*, *Solidago altissima* (goldenrod), and some dandelion species (e.g., *Taraxacum kok-saghyz*) were used successfully for the production of rubber for army vehicle tires. Due to their relatively poor agronomic performance these species were not further exploited, but these trials showed that rubber production does not have to be restricted to equatorial plant species. In addition, a number of

Table 1 Production and consumption of natural and synthetic rubber in 1998. All values are in 10^3 metric tonnes. Data supplied by the International Rubber Study Group

Country/region	Natural rubber		Synthetic rubber	
	Production	Consumption	Production	Consumption
USA	0	1157	2610	2354
Canada	0	148	191	238
Mexico	0	101	167	180
Brazil	60	168	340	315
Other Latin America	59	135	54	215
European Union	0	1078	2253	2168
Other Europe	0	261	924	996
Africa	322	127	63	130
Australia	0	50	38	58
China	450	839	589	1000
India	591	580	66	155
Japan	0	707	1520	1116
Thailand	2206	186	45	100
Indonesia	1750	100	17	85
Malaysia	886	334	0	79
Other Asia	456	641	1020	668
World total	6780	6607	9897	9857

Euphorbia species have been selected for oil (e.g., *E. lathyrus*, gopher plant) and rubber (e.g., *E. lactiflua* and *E. characias*) production. *E. lactiflua* and *E. characias* come from Mediterranean countries in contrast to most of the other *Euphorbia* species, which are native to African countries, as are most rubber-producing species. The rubber yield (weight of *cis*-1,4-units per volume of latex) from *E. characias* (30%) competes well with yields from *H. brasiliensis* (44.3%), *Ficus* spp. (15–30%), *Alstonia boonei* (15.5%), and *P. argentatum* (8%), but rubber molecular weight (a prime determinant of rubber quality) is greatest in *H. brasiliensis* and *P. argentatum* (> 1 million daltons). To date, about 60 *Euphorbia* species have been identified in Europe, most of which have not yet been screened for their possible rubber production.

Alternative rubber production systems, employing cell or tissue cultures, are outside the scope of this review and will not be considered in detail. Examples include attempts with *H. brasiliensis*, *F. elastica*, *F. benjamina*,

Table 2 Prominent plant species investigated for polyisoprene rubber production. Literature searches on prominent rubber-producing plant species. Searches were performed in February/March 1998 using TelNet (*stn.fiz-karlsruhe.de*), *http://beal.cpp.msu.edu/bot336/chp11.htm* (hits indicated with *) and WebSpirs (*http://www.agralin.org/cgi-bin/webspirs.cgi*). Search statements included: rubber, polyisopren?, *cis*-polyisopren?, *trans*-polyisopren?, hevea, parthenium or guayule, euphorbia, asclepias or milkweed, solidago or goldenrod, ficus, taraxacum or dandelion, castilla, landolphia, manihot, and lactarius

Species	Common name	Native or cultivation area	Comment
Asclepias spp.*	Milkweeds		
A. linaria	Milkweed	Arizona	ND
A. speciosa	Showy milkweed	Utah	*Cis*-1,4-units: 2.2% of hexane extract (1% of plant); M_w: 5.2×10^4 Da
A. syriaca L	Common milkweed		*Cis*-1,4-units
Castilla spp.*	Panama rubber	South America	10 species, latex bleed usually kills the tree
Euphorbia spp.*	Spurge		13 species
Ficus spp.*			
F. elastica	Indian rubber tree	Nigeria	Yield 1,4-polyisoprene (w/v of latex): 24.8%
F. ovata		Nigeria	Yield 1,4-polyisoprene (w/v of latex): 20.4%
F. pumila		Nigeria	Yield 1,4-polyisoprene (w/v of latex): 14.7%
F. volgelii		Nigeria	Yield 1,4-polyisoprene (w/v of latex): 28.1%
*Hevea brasiliensis**	Brazilian rubber tree	South-East Asia	M_w: $5 \times 10^5 - 2 \times 10^6$ Da; yield 1,4-polyisoprene (w/v of latex): 44.3%
Landolphia spp.*			First species to yield commercial latex for rubber
L. dulcis		Nigeria	ND
L. owariensis		Nigeria	Yield 1,4-polyisoprene (w/v of latex): 24.2%
*Manihot glaziovii**	Ceara rubber tree	Brazil	ND
Manilkara zapota	Chicle	Mexico	ND
*Parthenium argentatum**	Guayule	Chihuahuan desert	M_w: $> 10^6$ Da; rubber content: 0.7–5.5 mg/g DW from August–December
Solidago spp.*	Goldenrod		Successfully applied during WWII
S. altissima	Goldenrod		ND
S. riddellii		Ohio	ND
*Taraxacum kok-saghyz**	Russian dandelion	Russia	Successfully applied during WWII, M_w: 3×10^5 Da

F. macrophylla, and *P. argentatum*, although none have led to commercially competitive systems. Immobilized algae *Botryococcus braunii*, *Chlamydomonas mexicana*, and *Porphyridium cruentum* have also been considered (Gudin et al. 1984). Fungal species show more promise and some naturally produce rubber. Within the group of ectomycorrhizal fungi belonging to the genus *Lactarius*, which also produce a milky type of latex upon wounding, *Lactarius volemus* produces up to 7% (DW) of relatively short-chain *cis*-1,4-polyisoprenes (Ohya et al. 1998), as many alternative plant species do. Although high-molecular-weight rubber is essential for conventional applications, the low-molecular-weight fractions that predominate in many alternative rubber-bearing species may find specific applications, such as in wood impregnation (Bultman et al. 1998), low-viscosity analogues of epoxidized natural rubber (Schloman 1992), or in films, coatings, and adhesives.

No bacterial/yeast species are currently known to produce natural rubber, although some do, of course, possess the isoprenoid pathway that underlies natural rubber biosynthesis and synthesize the rubber precursors isopentenyl pyrophosphate, farnesyl pyrophosphate, and geranyl geranyl pyrophosphate.

Conclusions can be drawn from the literature searches detailed in Table 2 as follows:

- Many plant species, not only those belonging to the *Euphorbiaceae*, are known to produce polyisoprene, and some exclusively make rubber (*cis*-1,4-polyisoprene) as opposed to *trans*-1,4-polyisoprene.
- Polyisoprene yields, which cannot be compared easily due to inconsistencies in terminology, vary greatly between species, tissues, growth areas and time of harvest.
- Highest rubber yields were recorded for *H. brasiliensis*, *Ficus* spp., *Euphorbia* spp., *Landolphia owariensis*, *Funtumia* spp., *Chrysophyllum albidum*, *Alstonia boonei*, and *P. argentatum*.
- Molecular weights of polyisoprenes may differ considerably between 10^3 and 2×10^6 Da.
- Only three European species have been considered: *E. characias*, *Helianthus annuus*, and *E. tirucalli*, which were tested in Southern Europe. A selection of European natives has not yet been made from the *cis*-1,4-polyisoprene-producing species (approximately 50 genera) found in our literature search.

Rubber particles

Natural rubber is compartmentalized within subcellular rubber particles, located in the cytosol of cells, whether these be specialized laticifers (pipe-like anastomized cell systems which produce latex), as in *H. brasiliensis* (d'Auzac et al. 1989) and *F. elastica* (Heinrich 1970), or generalized cells, such as the bark parenchyma of *P. argentatum* (Backhaus 1985). The average size of the particles varies from species to species [e.g., *F. elastica* 3.8 μm, *E. lactiflua* 0.42 μm (Siler et al. 1997; Wood and Cornish 2000)], and differently sized subsets of particles can even be found in the same plant. *H. brasiliensis*, for example, has two distinct subsets of particles with mean diameters of 1.0 and 0.2 μm (Cornish et al. 1993; Wood and Cornish 2000). The overall structure of rubber particles is similar in all species examined so far, in that they contain a homogeneous rubber core surrounded by an intact monolayer membrane (Cornish et al. 1999; Irving and Cornish 1997; Wood and Cornish 2000). The membrane is made up of a highly species-specific complement of lipids and proteins (Cornish et al. 1993; Siler et al. 1997), although very large proteins or protein complexes appear to be present (Cornish et al. 1993; Siler and Cornish 1993, 1994a). The glycosylated moieties of some of the particle-bound proteins and the hydrophilic head groups of the phospholipids enable the particles to interface with the aqueous cytosol. Although soluble rubber transferases have been reported (Archer et al. 1963; McMullen and Sweeney 1966; and see literature cited in Cornish 1993 and Cornish and Siler 1996), rubber polymerization is actually catalyzed by a rubber particle-bound enzyme ("rubber transferase" EC 2.5.1.20) (Berndt 1963; Archer and Audley 1987; Madhavan et al. 1989; Cornish and Backhaus 1990; Cornish 1993; Cornish and Siler 1996); the earlier reports can be explained by the role of the soluble enzymes in the synthesis of allylic diphosphate initiator molecules (Cornish 1993, 1996). Immunoinhibition and immunoprecipitation studies have demonstrated that some structural similarities probably exist between the rubber transferases of *F. elastica*, *H. brasiliensis*, and *P. argentatum* (Siler and Cornish 1993; Cornish et al. 1994).

Rubber biosynthesis, initiation, and genes involved

Acetyl CoA is the essential metabolite used for fatty acid biosynthesis involving acetyl CoA carboxylase (ACC) and also the metabolite that enters the isoprenoid pathway via the action of 3-hydroxy-3-methyl-glutaryl-CoA synthase (HMGS) and reductase (HMGR) enzymes (Fig. 2). Currently, a large variety of isoprenoids and their derivatives ($>22{,}000$) is known. Unfortunately, a complete overview on these metabolites and the organisms that produce them is not available in any of the American, English, and Dutch searchable literature databases. However, fragmentary information is available in a number of review articles. Isoprenoid biosynthesis, with emphasis on rubber production by *H. brasiliensis*, has been reviewed by Kush (1994). A general description on the biochemistry and molecular biology of isoprenoid biosynthesis is described by Chappell (1995), and information on cloned plant genes and 30 enzymes involved in isoprenoid biosynthesis is given by Scolnik and Bartley (1996). Bach (1995) reviewed some new aspects, including the so-called Rohmer pathway, of the multibranched isoprenoid pathway in plants, branched-chain amino acids as precursors of

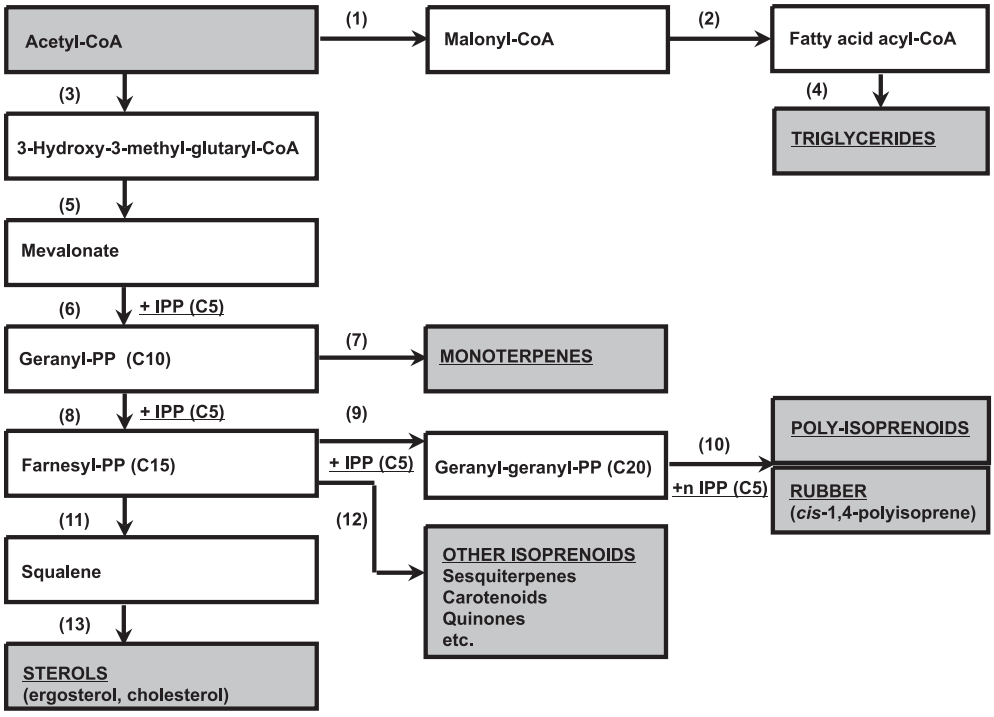

Fig. 2 Interrelationship between fatty acid, monoterpene, sterol, and (poly)isoprenoid pathways. Enzymes: *1* acetyl CoA carboxylase, ACC; *2* fatty acid synthase, FAS; *3* 3-hydroxy-3-methyl-glutaryl CoA synthase, HMGS; *4* enzymes of the triglyceride synthetic pathway; *5* 3-hydroxy-3-methyl-glutaryl CoA reductase, HMGR; *6* geranyl diphosphate synthase (prenyl transferase), GPPS; *7* enzymes of the limonene pathway; *8* farnesyl diphosphate synthase (prenyl transferase), FPPS; *9* geranyl geranyl diphosphate synthase (prenyl transferase), GGPS; *10* rubber transferase, RTR; *11* squalene synthase, SQS (ERG9); *12* isoprenoid pathways; *13* ergosterol or cholesterol synthetic pathway

isoprenoids, and isoprene emission by plants. The role of plastids in isoprenoid biosynthesis, including the reactions leading from phytoene to colored carotenoids, has been reviewed by Kleinig (1989). Lichtenthaler (1999) reviewed current knowledge on the novel 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway for IPP biosynthesis in plastids. This pathway starts from D-glyceraldehyde-3-phosphate (GA-3-P) and pyruvate with DOXP synthase as the starting enzyme.

Structural properties of specific metabolites such as prenylated isoflavonoids (Tahara and Ibrahim 1995) and isoprenylated flavonoids (Barron and Ibrahim 1996) have been reviewed. The latter includes more than 700 prenylated metabolites and tabulated data provide the structure, common name, and plant source of these compounds.

Rubber molecules are an end product and so provide a solid sink for the isoprenoid precursors *iso*-pentenyl diphosphate (IPP, C5), and the allylic diphosphates farnesyl diphosphate (FPP, C15) and geranyl geranyl diphosphate (GGPP, C20) (Cornish 1993; Kush 1994; Chappell 1995). The allylic diphosphates are condensed by the action of prenyltransferases and are essential initiators of rubber formation: the rubber transferase must first bind a single allylic diphosphate molecule before it can catalyze the formation of the *cis*-1,4-polyisoprene polymer (Fig. 3). A model for rubber biosynthesis from IPP has been described by Cornish (1993). According to Tanaka (1989) and Kush (1994) the first three monomers are added in *trans* (Fig. 3). Downstream of the branch to natural rubber biosynthesis, another important biosynthetic pathway leading to sterol biosynthesis branches off from the isoprenoid pathway via the key regulatory enzyme squalene synthase (SQS, in *Saccharomyces cerevisiae* denoted ERG9) (see Fig. 2). Regulation of this enzyme could affect the availability of the substrates used in rubber biosynthesis. Table 3 lists a number of genes that are considered essential for the biosynthesis of rubber and its precursors, although this list will undoubtedly expand as research progresses. The importance of SQS (ERG9) as a regulatory enzyme in the isoprenoid pathway has been revealed over recent years (Table 4). Specific chemical inhibitors have been identified, like squalestatin and zaragosic acid (Bergstrom et al. 1993) and schizostatin from the basidiomycetous fungus *Schizophyllum commune* (Tanimoto et al. 1996), which completely and specifically block SQS (ERG9) activities at picomolar concentrations when administered to rat or yeast cells. These compounds mimic mutations within the

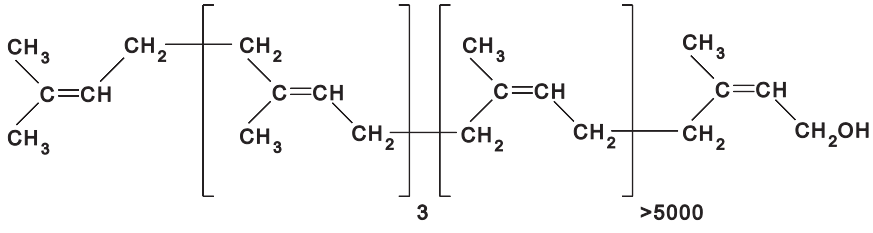

Fig. 3 Structural formula of *cis*-1,4-polyisoprene in natural rubber. The rubber biosynthesis starts with the addition of three IPP monomers in *trans*, followed with the addition of up to 5000 units in *cis* (according to Kush 1994, with modifications)

Table 3 Genes involved in (poly-)isoprenoid (precursor) biosynthesis

Organism	Gene	Function	Reference
Yeast	*ERG9*	Squalene synthase (farnesyl-diphosphate:farnesyl-diphosphate farnesyl transferase) regulates flux of isoprenoids through sterol pathway.	Fegueur et al. 1991
Rat	*SQS*	Squalene synthase, inhibition of ergosterol synthetic pathway may cause accumulation of isoprenoids and increase of *HMGR* mRNA.	Ness et al. 1994
H. brasiliensis	*HMGR*	3-Hydroxy-3-methylglutaryl-coenzyme A reductase, three differentially expressed genes: *hmg1* (ethylene-induced), *hmg2* (high expression in laticifers), *hmg3* (constitutive isoprenoid biosynthesis), key step in isoprenoid synthesis.	Chey et al. 1991, 1992
P. argentatum	*HMGR*	3-Hydroxy-3-methylglutaryl-coenzyme A reductase, mRNA levels (seasonal changes) increase with rubber synthesis.	Ji et al. 1993
H. brasiliensis	*FPPS*	Farnesyl diphosphate synthase (prenyl transferase), 47 kDa in latex-producing cells, expression increased after tapping of latex.	Adiwilaga and Kush 1996
Capsicum annuum	*PRT*	GGPPS (geranyl geranyl pyrophosphate synthase) condensation of GPP > FPP > GGPP.	Romer et al. 1992
H. brasiliensis	*REF*	Rubber elongation factor, 14 kDa cytosolic protein.	Goyvaerts et al. 1991
H. brasiliensis	*RTR*	Rubber transferase, elongation of rubber precursor molecule with IPPs.	–

corresponding gene with possible relevance to the accumulation of isoprenoid precursors (Keller 1996), the co-ordinated regulation of the *HMGR* gene (Peffley and Gayen 1997), and the biosynthesis of rubber.

Scope and results

Within the framework of a search for alternative sources of natural rubber, we have initiated a collaborative research project aimed at the improvement of the current guayule rubber production and quality, and at testing the relevance of genes that have been implicated in the biosynthesis of (poly-)isoprenes and their precursors in yeasts.

Guayule

The best lines out of plant breeding programs produce about 10% (dry weight) of high-molecular-weight, high-quality natural rubber, but further gains do not seem readily obtainable, probably due to the facultative apomictic nature of guayule's seed production. Effective tissue culture and transformation protocols have been developed for guayule (Pan et al. 1996; Castillón and Cornish 2000) and attempts are now underway (in the Cornish laboratory) to increase rubber production through overexpression of genes for substrate synthesis. In vitro experiments have shown that the rubber biosynthetic rate is dependent upon both substrate concentration and on the size and stereochemistry of the allylic diphosphate used to initiate new rubber molecules (Cornish and Siler 1995; Cornish et al. 1998). However, increasing yield will only be beneficial if the rubber produced still consists of high-molecular-weight polymers. Although the regulation of rubber molecular weight in vivo is not yet fully understood, it is known that, like biosynthetic rate, polymer molecular weights in vitro are dependent upon substrate concentration, the ratio of IPP and allylic-PP, and the size of the allylic-PP

Table 4 Observations on the *ERG9*-mutation in *Saccaromyces cerevisiae*

Year	Observations	Reference
1991	SQS (farnesyl-diphosphate:farnesyl-diphosphate farnesyltransferase, EC. 2.5.1.21) regulates flux of isoprene intermediates through sterol pathway; *SQS* (*ERG9*) gene complements the *S. cerevisiae erg9* mutation; *ERG9* (Genbank M63979) is a single copy gene, essential for cell growth; PEST (proline, glutamic acid, serine, threonine-rich) consensus motif; one or two membrane-spanning domains.	Jennings et al. 1991
1991	*ERG9* gene localized on 2.5-kb DNA fragment; functional expression in *E. coli*, protein 444 AA (51,600 Da), 1–4 transmembrane domains, localization in membrane of ER.	Fegueur et al. 1991
1993	Structural and functional conservation between human, budding and fission yeast SQS, especially in regions involved in interaction with prenyl substrates (= 2 × FPP); complementation of *erg9* mutation; inhibition of sterol synthesis by HMGR-specific lovastatin increases levels of *SQS* mRNA.	Robinson et al. 1993
1993	C-terminus-truncated protein expressed in *E. coli*; soluble enzyme is monomeric and catalyzes two-step conversion of FPP > presqualene diphosphate > squalene using Mg^{2+} and NADPH.	Zhang et al. 1993
1995	Nine of 11 genes involved in ergosterol pathway cloned, the first three genes *ERG9* (squalene synthase), *ERG1* (squalene epoxidase), *ERG7* (lanosterol synthase), being essential for aerobic growth.	Lees et al. 1995
1996	Functional complementation of *erg9* mutation by *Nicotiana benthaminia* and *N. tabacum* cDNAs (1600 nt).	Hanley et al. 1996

initiator (Castillón and Cornish 1999). In summary, the higher the IPP concentration, the higher the biosynthetic rate, the greater the number of rubber molecules produced and, when allylic-PP levels are limiting, the larger the polymer molecular weight produced. In contrast, the higher the allylic-PP concentration, the higher the overall biosynthetic rate, the greater the number of rubber molecules, but the smaller the polymer molecular weight produced. Also, the larger the allylic-PP initiator, the greater the overall rubber biosynthetic rate, the faster the rate of rubber molecule initiation, but the shorter the rubber polymer length. Thus, strategies to enhance the yield of high molecular weight rubber in *P. argentatum*, or to alter rubber yield or quality in other systems, through genetic engineering of endogenous substrate concentrations, must be carefully crafted, and will be absolutely dependent upon in vivo testing of regenerated transformants. However, this research does suggest that it may be possible to increase the molecular weight of the poor quality rubber produced by most species and lead to high quality rubber production.

Yeasts

Oleaginous yeasts, such *Yarrowia lipolytica* or *Cryptococcus curvatus*, have the capacity to accumulate up to about 50% (DW) of storage carbohydrates in oil bodies. Rubber particles are analogous to oil bodies, although any possible evolutionary relationship has yet to be determined. Attempts are now underway to re-direct the metabolic flux from acetyl CoA in favor of the formation of (poly)-isoprenes, using multiple transgenesis. Target genes include acetyl CoA carboxylase (*ACC*, disruption), hydroxy-methyl-glutaryl CoA reductase (*HMGR*, over-expression), squalene synthase (*SQS*, disruption) for the increase of precursor supplies, and rubber polymerase genes from *H. brasiliensis* or *P. argentatum*. As a first step for metabolic engineering, the isolation of the *Y. lipolytica SQS* gene is reported here. Figure 4 shows the amino acid sequence alignment of the *Y. lipolytica SQS* gene PCR fragment and the high degree of homology and similarity with other known *SQS* (*ERG*9) genes. The N- and C-termini of the fragment correspond with the primers used. This is the first example of a partial *SQS* gene from an oleaginous yeast. The full-length *Y. lipolytica SQS* sequence and its possible disruption in vivo will be published elsewhere (Merkulov et al. in press).

Possibilities for metabolic engineering and fermenter-mediated polyisoprene production

Relatively short polyisoprene chains occur in bacteria, yeasts, and mammalian cells. For example, deca- and undecaprenol, C50 and C55, respectively, are present in a number of complex polyisoprenoid compounds, such as lipid carrier (Rick et al. 1998), sugar side-chains (Schutzbach 1994; Zhu and Laine 1996), glycoproteins (Roos et al. 1994), proteins (Orlean 1990; Fujiyama et al. 1991; Ashby et al. 1992; Giannakouros et al. 1992) and coenzyme Q (Ashby and Edwards 1990). The occurrence of these compounds indicates that these organisms and cells also have a limited isoprenoid polymerase activity.

Transformation of bacteria or yeasts with the *H. brasiliensis*, *P. argentatum*, or other rubber transferase genes should reveal whether this is the only limiting step for rubber biosynthesis in these organisms and

```
                                 210        220        230        240        250
YL-SQS       201   ---------------SMGL-LQKVNI-RDY-EXIDVN---RAFWPREIWH 250
X59959       201   FANESLYSNE-QLYESMGLFLQKTNIIRDYNEDLVDG---RSFWPKEIWS 250
D89610       201   FGDKTLTENNFAKADSMGLFLQKTNIIRDYHEDLQDG---RSFWPREIWS 250
L06071       201   LEDPDLAHSQ-AISNSLGLFLQKVNIIRDYREDFDDN---RHFWPREIWS 250
U60057       201   -GKEDLASD--SLSNSMGLFLQKTNIIRDYLEDINEVPKCRMFWPREIWS 250
                                   *.** *** ** *** * .      * ***.***

                                 260        270        280        290        300
YL-SQS       251   KYAEEMRDFKDPKYSKK---ALHCTSDLVANALGHATDCLDYLDNVTDPS 300
X59959       251   QYAPQLKDFMK---PENEQLGLDCINHLVLNALSHVIDVLTYLAGIHEQS 300
D89610       251   KYTENLQDFHKVKTPAKEFAGVSCINELVLNALGHVTDCLDYLSLVKDPS 300
L06071       251   KYTSSFGDLCLPDNSEK---ALECLSDMTANALTHATDALVYLSQLKTQE 300
U60057       251   KYVNKLEEL---KYEDNSAKAVQCLNDMVTNALSHVEDCLTYMSALRDPS 300
                   .*     ..       .   .. * ...  *** *  * * *.  .

                                 310        320        330        340        350
YL-SQS       301   TFTFCAIPQVMAI------------------------------------ 350
X59959       301   TFQFCAIPQVMAIATLALVFNNREVLHGDVKIRKGTTCCLILKSRTLRGC 350
D89610       301   SFSFCAIPQVMAVATLAEVYNNPKVLHGVVKIRKGTTCRLILESRTLPGV 350
L06071       301   IFNFCAIPQVMAIATLAAVFRNPDVFQTNVKIRKGQAVQIILHSVNLKNV 350
U60057       301   IFRFCAIPQVMAIGTLAMCYDNIEVFRGVVKMRRGLTAKVIDQTRTIADV 350
                    * *********.
```

Fig. 4 Amino acid sequence alignment (Clustal V) between our *Y. lipolytica*, *Saccharomyces cerevisiae* (X59959), *Candida albicans* (D89610), *Schizosaccharomyces pombe* (L06071), and *Nicotiana tabacum* (U60057) *SQS* gene segments (GeneBank accession numbers). Myers/Miller multiple alignment parameters were as follows: fixed gap penalty, 10; floating gap penalty, 10; toggle transitions, weighed; protein weight matrix, PAM 250. * Identical amino acids, • similar amino acids

whether the normal cytoplasmic concentrations of different rubber precursors would be compatible with optimal rubber transferase enzyme activity. To date there are no reports on attempts to produce highly polymeric rubber molecules in bacteria or yeasts. However, in rubber-producing organisms (plants or transformed microorganisms), overexpression of HMGR (and/or the DOXP synthase gene, when available) and prenyl transferase genes, perhaps coupled with disruption of the SQS gene, might result in a considerable increase in the flow of substrates to rubber biosynthesis (Fig. 2). A similar metabolic engineering strategy, i.e., overexpression of a cytosolic equivalent of HMGR, disruption of the *SQS* gene, in addition to the introduction of bacterial carotenoid (*crt*) synthetic genes, has resulted in the accumulation of the carotenoid lycopene in *Candida utilis* (Shimada et al. 1998).

However, given the theoretical option that rubber could be produced by metabolic engineering of microorganisms, like yeasts, it can be calculated that the bulk production process probably cannot compete economically with its agricultural production. Assuming that in yeasts per liter culture medium 150 g of cell dry matter can be produced with 10% rubber (per cell dry weight) at the end of the fermentation run, the total need for rubber production in the USA would require 8×10^{10} l fermentation capacity, which is unrealistic. However, with a glucose price of US$ 0.15/kg (*Financial Times*, 4 September 1999) and a final glucose concentration of 20 g/l, the production alone, without, for example, equipment purchase and depreciation, other medium requirements, downstream processing, and waste disposal, would cost US$ 0.24×10^9 against US$ 1.7×10^9 for imported rubber. This implies that microbial production of polyisoprenes can be competitive only if these additional costs can be sufficiently controlled (which will need a detailed cost analysis of the entire chain), or if specialty products, like carotenoids or nonconventional polyisoprenes, can be produced with a much higher market price.

In this respect, Gerngross (1999) provided a detailed cost analysis of different bulk commodity production chains employing microbial fermentation. These also included biodegradable poly-(3-hydroxy-alkanoates) (PHAs) that can be utilized for a number of applications such as films, (cheese) coatings, paint binder, and elastomers (Van der Walle et al. 1999). It was concluded that the microbial fermentation systems cannot compete with mineral oil-based systems, neither from cost nor from environmental perspectives. Therefore, in the ATO laboratory an EU-sponsored project has been initiated with other European laboratories and is now in progress aiming at the large-scale production of PHAs in starch-storing crop plants (Eggink et al. 1998).

Prospects and bottlenecks

The prospects for the introduction of new sources of natural rubber are bright because, for the first time since the 1920s, non-*H. brasiliensis* natural rubber can be profitably produced. This opportunity has arisen with the emergence of an important market for *P. argentatum* natural rubber latex in which *H. brasiliensis* cannot safely be used, i.e., hypoallergenic latex products. Increased use of *H. brasiliensis* latex products, coupled with manufacturing short-cuts, have led to serious and widespread health problems (Kekwick 1993). Repeated exposure to the proteins present in these latex products can induce immediate, allergenic (Type I) hypersensitivity which, in its most serious manifestation, leads to life-threatening anaphylaxis (Morales et al. 1989; Slater 1989; Tomazic at al. 1992; Kekwick 1993; Pailhories 1993; Ownby et al. 1994). Clinical trials on humans have shown that even severely *H. brasiliensis* latex-hypersensitive patients have no reaction to *P. argentatum* latex (Carey et al. 1995; Siler at al. 1996). Large-scale production of this latex is feasible and its high quality makes it suitable for hypoallergenic product manufacture (Cornish 1996, 1998). Of course, *P. argentatum* latex products must be produced with low protein levels so that allergy problems do not develop with the new material. *P. argentatum* rubber particles have much less protein than those of *H. brasiliensis* (Cornish et al. 1993; Siler and Cornish 1994b) so the latex can be produced with lower protein levels than even highly purified *H. brasiliensis* latex. Several additional trials have demonstrated that *P. argentatum* latex can be compounded using similar procedures to *H. brasiliensis* latex (Schloman et al. 1996), and can be used to produce latex medical products with effective viral barrier properties (Cornish and Lytle 1999). As far as the United States is concerned, a large-scale domestic natural rubber crop would guarantee sufficient natural rubber to supply existing strategic demands as well as the new and major, health-related, hypoallergenic rubber market. A sustainable domestic rubber industrial crop also would enhance rural economies, utilize semi-arid lands, and bring retired farmlands back into profitable production. Cultivation trials with guayule are underway in the Mediterranean region of Europe, but the current lines are not suited to more temperate climates. Continuing research into the regulation of natural rubber biosynthesis should lead to the introduction of genetically engineered guayule lines or, for example, *Euphorbia* spp. with enhanced rubber yield and extended growing range, as well as the eventual introduction of profitable rubber-producing annual crops, or bioreactors. The acceptance of *P. argentatum* hypoallergenic latex by agribusiness also will greatly facilitate the subsequent introduction of additional natural rubber-producing crops, since their product would be entering an established market place.

Acknowledgements The authors gratefully acknowledge the contributions to the present line of research of Dr. Ruxton Villet (USDA) and Prof. Dr. Hans Derksen (ATO-DLO), and Ms. Katja Moors-Pollaart, Ms. Annet Bijlsma, and Dr. Sergey Merkulov for isolating and sequencing the *SQS* gene fragment from *Y. lipolytica*.

References

Adiwilaga K, Kush A (1996) Cloning and characterization of cDNA encoding farnesyl diphosphate synthase from rubber tree (*Hevea brasiliensis*). Plant Mol Biol 30: 935–946

Archer BL, Audley BG (1987) New aspects of rubber biosynthesis. Bot J Linn Soc 94: 181–196

Archer BL, Audley BG, Cockbain EG, McSweeney GP (1963) The biosynthesis of rubber. Biochem J 89: 565–574

Ashby MN, Edwards PA (1990) Elucidation of the deficiency in two yeast coenzyme Q mutants: characterization of the structural gene encoding hexaprenyl pyrophosphate synthetase. J Biol Chem 265: 13157–13164

Ashby MN, Kutsunai SY, Ackerman S, Tzagoloff A, Edwards PA (1992) COQ2 is a candidate for the structural gene encoding *p*-hydroxybenzoate: polyprenyltransferase. J Biol Chem 267: 4128–4136

Bach TJ (1995) Some new aspects of isoprenoid biosynthesis in plants – a review. Lipids 30: 191–202

Backhaus (1985) Rubber formation in plants – a mini-review. Isr J Bot 34: 283–293

Barker PW (1938) Charles Goodyear. Rubber Age 43: 229–230; 294–296; 307–309; 44: 29–31; 88–90

Barron D, Ibrahim RK (1996) Isoprenylated flavonoids – a survey. Phytochemistry 43: 921–982

Bergstrom JP, Kurtz MM, Rew DJ, Amend AM, Karkas JD, Bostedor RG, Bansal VS, Dufresne C, Van Middlesworth FL, Hensens OD (1993) Zaragosic acids: a family of fungal metabolites that are picomolar competitive inhibitors of squalene synthase. Proc Natl Acad Sci USA 90: 80–84

Berndt J (1963) The biosynthesis of rubber. US Government Research Report AD-601729, pp 1–22

Bultman JD, Chen-Shihlieh, Schloman WW Jr, Shen SL (1998) Antitermitic efficacy of the resin and rubber in fractionator overheads from a guayule extraction process. Ind Crops Prod 8: 133–143

Carey AB, Cornish K, Schrank PJ, Ward B, Simon RA (1995) Cross reactivity of alternate plant sources of latex in subjects with systemic IgE mediated sensitivity to *Hevea brasiliensis* latex. Ann Allergy Asthma Immunol 74: 317–320

Castillón J, Cornish K (1999) Regulation of initiation and polymer molecular weight of *cis*-1,4-polyisoprene synthesized in vitro by particles isolated from *Parthenium argentatum* (Gray). Phytochemistry 51: 43–51

Castillón J, Cornish K (2000) A simplified protocol for micropropagation of guayule (*Parthenium argentatum*, Gray). In Vitro Cell Dev Biol Plant 36 (in press)

Chappell J (1995) Biochemistry and molecular biology of the isoprenoid biosynthetic pathway in plants. Annu Rev Plant Physiol Plant Mol Biol 46: 521–547

Chey ML, Kush A, Tan CT, Chua NH (1991) Characterization of complementary DNA and genomic clones encoding 3-hydroxy-3-methylglutaryl-coenzyme A reductase from *Hevea brasiliensis*. Plant Mol Biol 16: 567–578

Chey ML, Tan CT, Chua NH (1992) Three genes encode 3-hydroxy-3-methylglutaryl-coenzyme A reductase in *Hevea brasiliensis*: *hmg1* and *hmg3* are differentially expressed. Plant Mol Biol 19: 473–484

Coates A (1987) The commerce in rubber – the first 250 years. Oxford University Press, Singapore

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 no 5580942

Cornish K (1998) Hypoallergenic natural rubber products from *Parthenium argentatum* (Gray) and other non-*Hevea brasiliensis* species. US Patent no 5717050

Cornish K, Backhaus RA (1990) Rubber transferase activity in rubber particles of guayule. Phytochemistry 29: 3809–3813

Cornish K, Lytle CD (1999) Viral impermeability of hypoallergenic, low protein, guayule latex films. J Biomed Mat Res 47: 434–437

Cornish K, Siler DJ (1995) Effect of different allylic diphosphates on the initiation of new rubber molecules and on *cis*-1,4-polyisoprene biosynthesis in guayule (*Parthenium argentatum* Gray). J Plant Physiol 147: 301–305

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

Cornish K, Castillón J, Chapman MH (1998) Membrane-bound *cis*-prenyl transferase activity: regulation and substrate specificity. In: Steinbüchel A (ed) Biochemical principles and mechanisms of biosynthesis and biodegradation of polymers. Wiley-VCH, Weinheim, pp 316–323

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

D'Auzac J, Jacob J-L, Chrestin H (eds) (1989) Physiology of rubber tree latex. CRC Press, Boca Raton, pp 470

Davis W (1997) The rubber industry's biological nightmare. Fortune, 4 August 1997

Eggink G, Kessler B, Mooibroek H, Steinbüchel A, Wang T, Witholt B (1998) >PHAstics: sustainable production of biodegradable polyesters in starch-storing crop plants. In: Ciarán Mangan (ed) Renewable biomaterials, European Commission Fourth framework program project catalogue. Office for official publications of the European Communities, Luxemburg, pp 117–119 (ISBN 92-828-6116-3)

Fegueur M, Richard L, Charles AD, Karst F (1991) Isolation and primary structure of the *ERG9* gene of *Saccharomyces cerevisiae* encoding squalene synthase. Curr Genet 20: 365–372

Fujiyama A, Tsunasawa S, Tamanoi F, Sakiyama F (1991) S-Farnesylation and methyl esterification of carboxyl-terminal domain of yeast RAS2 protein prior to fatty acid acylation. J Biol Chem 266: 17926–17931

Gerngross TU (1999) Can biotechnology move us toward a sustainable society? Nat Biotechnol 17: 541–544

Giannakouros T, Armstrong J, Magee AI (1992) Protein prenylation in *Schizosaccharomyces pombe*. FEBS Lett 297: 103–106

Goyvaerts E, Dennis M, Light D, Chua NH (1991) Cloning and sequencing of the cDNA encoding the rubber elongation factor of *Hevea brasiliensis*. Plant Physiol 97: 317–321

Gudin C, Bernard A, Chaumont D, Thepenier C, Hardy T (1984) Direct bioconversion of solar energy into organic chemicals. World Biotech Rep 1: 541–559

Hanley KM, Nicolas O, Donaldson TB, Smith-Monroy C, Robinson GW, Hellmann GM (1996) Molecular cloning, in vitro expression and characterization of a plant squalene synthase cDNA. Plant Mol Biol 30: 1139–1151

Heinrich G (1970) Elektronenmikroskopische Untersuchung der Milchrören von *Ficus elastica*. Protoplasma 70: 317–323

Hosler D, Burkett SL, Tarkanian MJ (1999) Prehistoric polymers: rubber processing in ancient Mesoamerica. Science 284: 1988–1991

Irving DW, Cornish K (1997) Microstructure of rubber particles using cryo and conventional high-resolution scanning electron microscopy. Proc SCANNING 97: 169–179

Jennings SM, Tsay YH, Fischt TM, Robinson GW (1991) Molecular cloning and characterization of the yeast gene for squalene synthase. Proc Natl Acad Sci USA 88: 6038–6042

Ji W, Benedict CR, Foster MA (1993) Seasonal variations 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

Kekwick RGO (1993) Origin and source of latex protein allergy. In: Latex protein allergy: the present position. Crain Commun. Ltd, Rubber Consultants, Brickendonbury, Hertford, UK, pp 21–24

Keller PK (1996) Squalene synthase inhibition alters metabolism of nonsterol in rat liver. Biochim Biophys Acta Lipids Lipid Metab 1303: 169–179

Kleinig H (1989) The role of plastids in isoprenoid biosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40: 39–59

Kush A (1994) Isoprenoid biosynthesis: the *Hevea* factory. Plant Physiol Biochem 32: 761–767

Lees ND, Skaggs B, Kirsch DR, Bard M (1995) Cloning of the late genes in the ergosterol biosynthetic pathway of *Saccharomyces cerevisiae* – a review. Lipids 30: 221–226

Lichtenthaler HK (1999) The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. Annu Rev Plant Physiol Plant Mol Biol 50: 47–65

Madhavan S, Greenblatt GA, Foster MA, Benedict CR (1989) Stimulation of isopentenyl pyrophosphate incorporation into polyisoprene in extracts from guayule plants (*Parthenium argentatum* Gray) by low temperature and 2-(3,4-dichlorophenoxy)triethylamine. Plant Physiol 89: 506–511

Mausser RS (ed) (1987) The Vanderbilt latex handbook, 3rd edn. Vanderbilt Co, Norwalk, Conn, p 253

McMullen AJ, McSweeney GP (1966) Incorporation of isopentenyl pyrophosphate into purified rubber particles by a soluble latex-serum enzyme. Biochem J 101: 42–47

Merkulov S, Van Assema F, Springer J, Fernandez del Carmen A, Mooibroek H (2000) Cloning and characterization of the *Yarrowia lipolytica* squalene synthase (*SQS*) gene and functional complementation of the *Saccharomyces cerevisiae erg*9 mutation. Yeast 16 (in press)

Morales C, Bascomba A, Carrieira J, Sastre A (1989) Anaphylaxis produced by rubber gloves contact: case reports and immunological identification of the antigens involved. Clin Exp Allergy 19: 425–430

Ness GC, Zhao Z, Keller RK (1994) Effect of squalene synthase inhibition on the expression of the hepatic cholesterol biosynthetic enzymes, LDL receptor, and cholesterol 7-alpha hydroxylase. Arch Biochem Biophys 311: 277–285

Ohya N, Takizawa J, Kawahara S, Tanaka Y (1998) Molecular weight distribution of polyisoprene from *Lactarius volemus*. Phytochemistry 48: 781–786

Orlean P (1990) Dolichol phosphate mannose synthase is required in vivo for glycosyl phosphatidylinositol membrane anchoring, O mannosylation, and N glycosylation of protein in *Saccharomyces cerevisiae*. Mol Cell Biol 10: 5796–5805

Ownby DR, Ownby HE, McCullough JA, Shafer AW (1994) The prevalence of anti-latex IgE antibodies in 1000 volunteer blood donors. J Allergy Clin Immunol 93: 282

Pailhories G (1993) Reducing proteins in latex gloves. Clin Rev Allergy 11: 391–402

Pan Z, Ho J, Fang Q, Huang D-S, Backhaus RA (1996) *Agrobacterium*-mediated transformation and regeneration of guayule. Plant Cell Tissue Organ Cult 46: 143–150

Peffley DM, Gayen AK (1997) Inhibition of squalene synthase but not squalene cyclase prevents mevalonate mediated suppression of 3-HMGR synthesis at postranscriptional level. Arch Biochem Biophys 337: 251–260

Rick PD, Hubbard GL, Kitaoka M, Nagaki H, Kinoshita T, Dowd S, Simplaceanu V, Ho C (1998) Characterization of the lipid-carrier involved in the synthesis of enterobacterial common antigen (ECA) and identification of a novel phosphoglyceride in a mutant of *Salmonella typhimurium* defective in ECA synthesis. Glycobiology 8: 557–567

Robinson GW, Tsay YH, Kienzle BK, Smith-Monroy CA, Bishop RW (1993) Conservation between human and fungal squalene synthases: similarities in structure, function and regulation. Mol Cell Biol 13: 2706–2717

Romer S, Saint-Guily A, Montrichard F, Schantz ML, Weil JH, Schantz R, Kuntz M, Camara B, Argyroudi-Akoyunoglou JH (1992) Characterization of cDNAs which encode enzymes involved in chromoplast differentiation and carotenoid biosynthesis in *Capsicum annuum*. In: Argyroudi-Akoyunoglou JH (ed) Regulation of chloroplast biogenesis. (NATO ASI series A: Life sciences, vol 226) Plenum, New York, pp 63–69

Roos J, Sternglanz R, Lennarz WJ (1994) A screen for yeast mutants with defects in the dolichol-mediated pathway for N-glycosylation. Proc Natl Acad Sci USA 91: 1485–1489

Schloman WW Jr (1992) Low-molecular-weight guayule natural rubber as a feedstock for epoxidized natural rubber. Ind Crops Prod 1: 35–39

Schloman WW Jr, Wyzgoski F, McIntyre D, Cornish K, Siler DJ (1996) Characterization and performance testing of guayule latex. Rubber Chem Technol 69: 215–222

Schultes RE (1970) History of taxonomic studies in *Hevea*. Bot Rev 36: 197–276

Schurer H (1952) The macintosh. India Rubb J 122: 632–637

Schurer H (1957) Rubber – a magic substance of ancient America. Rubber J 132: 543–549

Schurer H (1962) The trials of Thomas Hancock. Part I. The "macintosh" problem. Rubber Plast Weekly 143: 943–949

Schutzbach JS (1994) Is there a "dolichol recognition sequence" in enzymes that interact with dolichols and other polyisoprenoid substrates? Acta Biochim Pol 41: 269–274

Scolnik PA, Bartley GE (1996) A table of some cloned plant genes involved in isoprenoid biosynthesis. Plant Mol Biol Rep 14: 305–319

Sethuraj MR, Mathew NM (eds) (1992) Natural rubber: biology, cultivation and technology. (Developments in Crop Science 23) Elsevier, Amsterdam, p 610

Shimada H, Kondo K, Fraser PD, Miura Y, Saito T, Misawa N (1998) Increased carotenoid production by the food yeast *Candida utilis* through metabolic engineering of the isoprenoid pathway. Appl Env Microbiol 64: 2676–2680

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

Siler DJ, Cornish K (1994b) Hypoallergenicity of guayule rubber particle proteins compared to *Hevea* latex proteins. Ind Crops Prod 2: 307–313

Siler DJ, Cornish K, Hamilton RG (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 DJ, Goodrich-Tanrikulu M, Cornish K, Stafford AE, Mckeon TA (1997) Composition of rubber particles of *Hevea brasiliensis*, *Parthenium argentatum*, *Ficus elastica* and *Euphorbia lactiflua* indicates unconventional surface structure. Plant Physiol Biochem 35: 281–290

Simmons HE (1939) Charles Goodyear, the persistent researcher. India Rubber Wld 101: 48–49

Slater JE (1989) Rubber anaphylaxis. N Engl J Med 320: 1126–1130

Tahara S, Ibrahim RK (1995) Prenylated isoflavonoids: an update. Phytochemistry 38: 1073–1094

Tanaka Y (1989) Structure and biosynthesis mechanism of natural polyisoprene. Prog Polym Sci 14: 339–371

Tanimoto T, Onodera K, Hosoya T, Takamatsu Y, Kinoshita T, Tago K, Kogen H, Fujioka T, Hamano K, Tsujita Y (1996) Schizostatin, a novel squalene synthase inhibitor produced by the mushroom *Schizophyllum commune*. 1. Taxonomy, fermentation, isolation, physico-chemical properties and biological activities. J Antibiot (Tokyo) 49: 617–623

Tomazic VJ, Withrow TJ, Fisher BR, Dillard SF (1992) Latex-associated allergies and anaphylactic reactions. Clin Immunol Immunopathol 64: 89–97

Van der Walle GAM, Buisman GJH, Weusthuis RA, Eggink G (1999) Development of environmentally friendly coatings and paints using medium-chain-length poly(3-hydroxyalkanoates) as the polymer binder. Int J Biol Macromol 25: 123–128

Whitworth JW, Whitehead EE (eds) (1991) Guayule natural rubber: a technical publication with emphasis on recent findings. Guayule Administrative Management Committee and USDA Cooperative State Research Service, Office of Arid Lands Studies, The University of Arizona, Tucson, Ariz

Wood DF, Cornish K (2000) Microstructure of purified rubber particles. Int J Plant Sci (in press)

Zhang D, Jennings SM, Robinson GW, Poulter CD (1993) Yeast squalene synthase: Expression, purification and characterization of soluble recombinant enzyme. Arch Biochem Biophys 304: 133–143

Zhu BCR, Laine RA (1996) Dolichyl-phosphomannose synthase from the Archae *Thermoplasma acidophilum*. Glycobiology 6: 811–816