Latex and Laticifers

Secondary article

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Latex is a frequently milky plant exudate, often containing rubber, found in specialized cells called laticifers. Latex from *Hevea brasiliensis* is commercially important as the source of natural rubber.

Article Contents

Latex

The Latin word for a fluid, latex is a general term used to describe a frequently milky plant exudate (in which case the vessel containing it may be called lactiferous, from the Latin *lac*, meaning milk). Such milkiness arises from the difference in refractive indices of the dispersed particles and the dispersing medium. These particles frequently contain terpenoid hydrocarbons that may be of high molecular weight, such as rubber or gutta. Although latex is often milky, as in *Hevea* and *Ficus*, it may be yellow or orange (Papaveraceae), yellowish-brown (*Cannabis*), or it may even be clear (*Morus* and *Nerium oleander*). The capacity to form latex is found in plants growing in many different habitats, in herbs, shrubs, trees, fungi and saprophytic succulents. About 12 500 species, belonging to 900 genera from about 20 families, most of which are dicotyledons, form latex. In addition, latex occurs in the fungi, *Lactaria* and *Peziza*, in the fern *Regnellidium*, and in *Gnetum gnimon* (Gnetaceae). Latex is the exuded cytoplasmic sap of laticifers, the specialized cells in which it is contained (Metcalfe, 1967).

Laticifers

A laticifer has been defined as a 'specialized cell or row of cells containing latex' and was originally classified as articulated (jointed) or nonarticulated. Articulated laticifers consist of longitudinal chains of many cells in which the lateral walls separating the individual cells either remain intact, have become perforated or entirely eroded. Longitudinal walls may also be perforated to form lateral anastomoses with neighbouring chains or tubes to give a net-like reticulum. Nonarticulated laticifers (**Figure 1**) arise from a single cell and grow intrusively within intracellular air spaces, eventually ramifying throughout the plant tissue in a manner reminiscent of fungal hyphae. Nonarticulated laticifers may be branched or unbranched but typically they do not fuse with similar cells. Both types of laticifers may be found within the same family, e.g. Euphorbiaceae, and indeed within the same genus, e.g. *Jatropha*. The clear-cut distinction between articulated and nonarticulated laticifers becomes somewhat blurred in the leaves of *Hevea*, *Manihot* and *Cnidoscolus* where, after following the course of the veins, the articulated laticifers ramify throughout the mesophyll to resemble nonarticulated laticifers.

Latex may also be found in lumens similar to resin ducts. Thus in guayule, *Parthenium argentatum* (Compositae), rubber is initially found in the epithelial cells lining the resin ducts. Some Cactaceae (*Mammillaria*) have an apparently articulated laticifer, the development of which involves the complete lysis of masses of cells to give, at maturity, a lumen lined with a single or several cell-layered epithelium.

The distribution of articulated laticifers throughout the plant has been most thoroughly studied in the commercial rubber tree, *Hevea brasiliensis* (Euphorbiaceae), where they occur in all organs of the plant including the leaves, flowers, fruit and root. In the seed laticifers are numerous in the inner integument and in the cotyledons. The *H. brasiliensis* tree is exploited commercially by the systematic repeated excision of the bark of the trunk (tapping), the principal latex-bearing tissue. In a cross-section of the bark

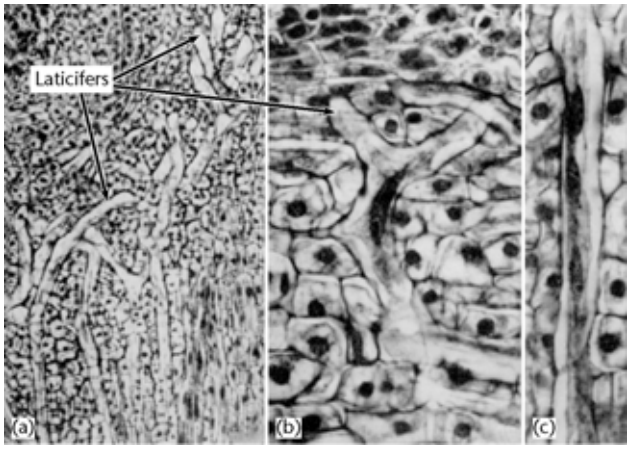


Figure 1 Nonarticulated laticifers of *Nerium oleander* seen by light microscopy. (a) Longitudinal section of stem near shoot apex showing branches of laticifers among parenchyma cells (× 200). (b) End of branching laticifer showing intrusive growth among parenchyma cells (× 530). (c) Part of a laticifer with several nuclei (× 530). Reproduced from Esau K (1965) *Plant Anatomy*, 2nd edn, p. 676. plate 46D. New York: J Wiley.

the circular or sometimes irregularly shaped laticifers are arranged in regular rows, almost parallel to the cambium, closely apposed to the neighbouring parenchyma. The several concentric rings of anastomizing laticifers, each resembling a sheet of expanded metalwork, are separated by rows of sieve elements and phloem parenchyma cells (**Figure 2**). It is this ring system of laticifers developing in the secondary phloem that makes *H. brasiliensis* such an outstanding rubber producer. The increase in numbers of these rings of laticifers achieved over about five breeding cycles has facilitated an approximately seven-fold increase in rubber yield.

In the tribe Lactuceae of the Compositae, the articulated laticifers accompany the vascular bundles and ramify into the mesophyll to reach the epidermis so that, as a result of lysis of the cell walls, the epidermal hairs of the floral involucres become directly connected with the laticifer. Thus latex is exuded on breaking the hairs.

The nonarticulated laticifers of the Moraceae are generally dispersed throughout the tissues while in the genus *Euphorbia* the main tubes of the branched non-articulated vessels are found in the outer part of the vascular cylinder.

The somewhat atypical laticifers of *Mammillaria* (see above) were found in the pith, cortex and tubercle of the vegetative body, but not in the roots, flowers or in young seedlings.

In the small shrub *Decaisnea fargesii* (Lardizabalaceae) latex occurs only in the fruit pericarp. Here it is found in the peripheral flask-shaped cavities, which have secreting cells at the base (Rudall, 1987).

Laticifer Organelles

For commercial reasons the laticifer organelles of *Hevea brasiliensis* (**Figure 3**) have been the most intensely studied. These coenocytic laticifers are multinucleate; nuclei and mitochondria are located parietally and are not normally exuded in tapped latex. Microtubules have been observed within, and plasmodesmata between, the walls of the laticifer and those of neighbouring cells. Chloroplasts are occasionally found together with proplastids in laticifers of green tissue. In addition to ribosomes and some fragments of endoplasmic reticulum, exuded *H. brasiliensis* latex contains two organelles characteristic of the laticifer. The yellow Frey-Wyssling particles, named after their eponymous discoverer, may be aggregated to form more elaborate Frey-Wyssling bodies. These more or less spherical organelles, ranging in diameter from 3 to 6 μm, are bounded by a double membrane and have a variable density dependent on their content of lipids and carotene. On centrifugation of latex the Frey-Wyssling particles may

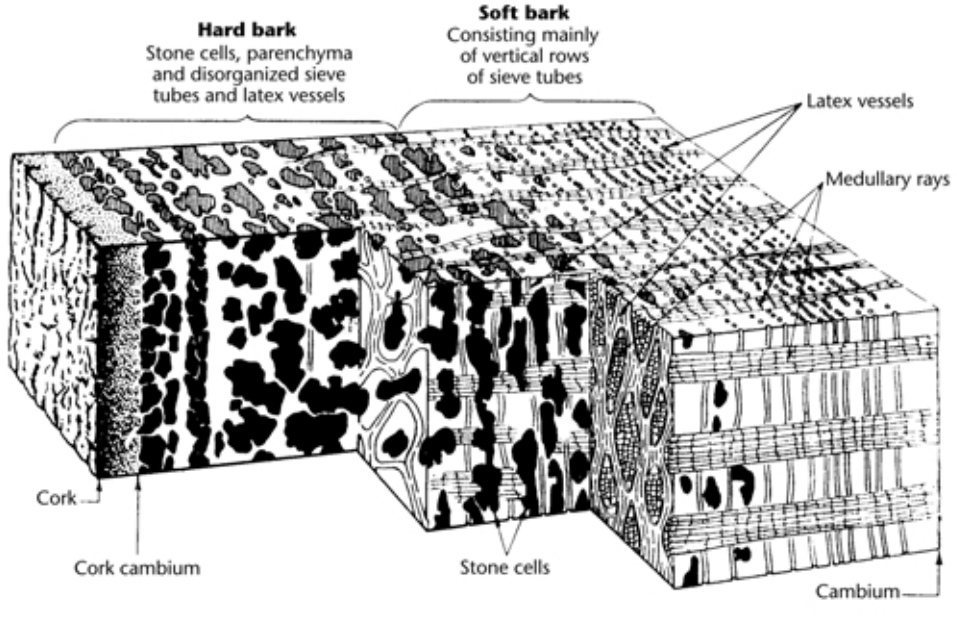


Figure 2 Three-dimensional diagram of the bark of *H. brasiliensis*. The sieve tubes that make up the bulk of the soft bark and parenchyma in both soft and hard bark are not shown. The black areas are clusters of stone cells. Reproduced from Riches JP and Gooding EGB (1952) Studies on the physiology of latex 1. Latex flow on tapping – theoretical considerations. *New Phytologist* **51**: 1–10.

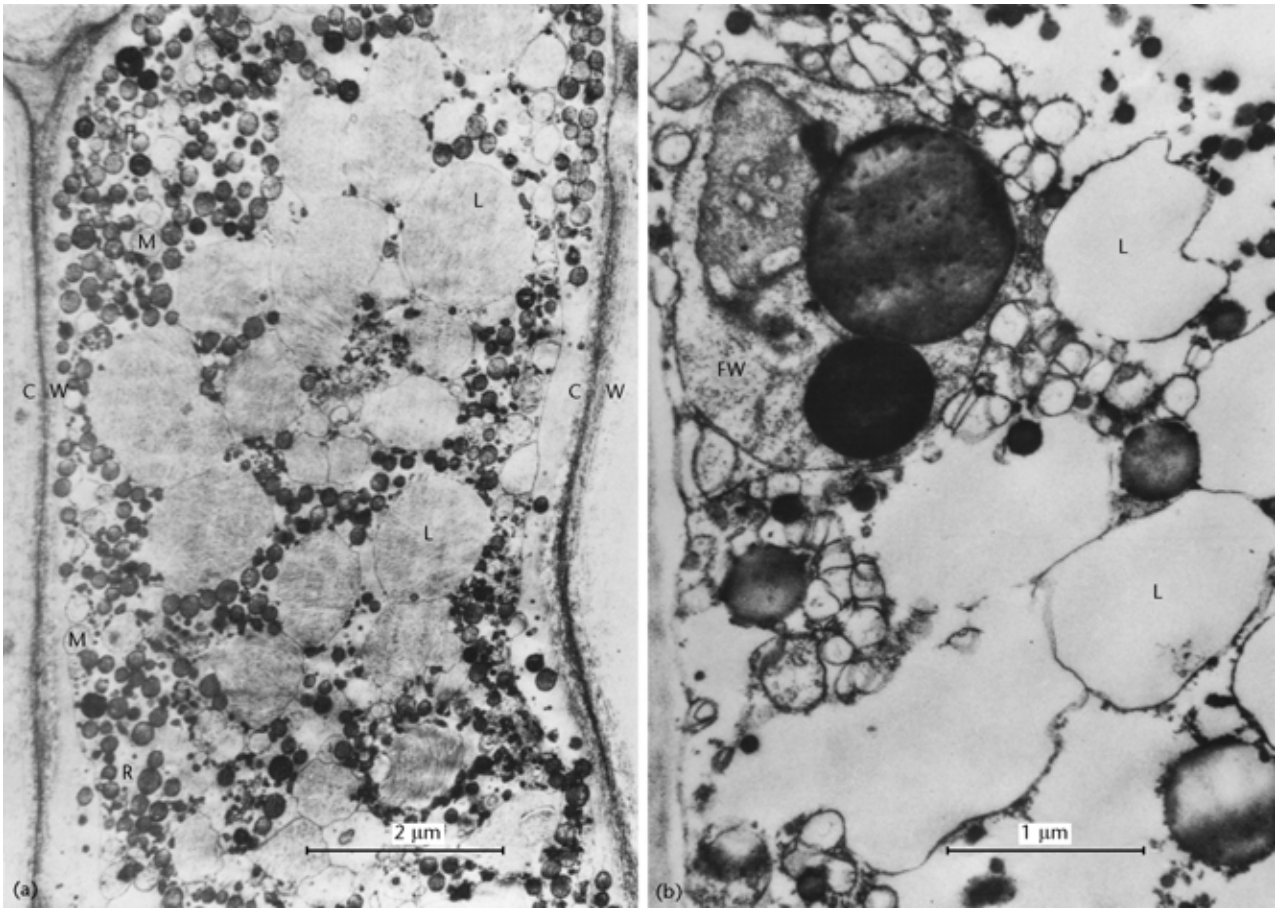


Figure 3 Electron micrographs of articulated laticifers of *H. brasiliensis*. (a) Young latex vessel in secondary phloem of green stem (× 9000). (b) Part of mature latex vessel in tapped bark (× 30 000). Key: L, lutoid particles; M, mitochondria; FW, Frey-Wyssling particles; R, rubber particle; CW, cell wall. Reproduced from Gomez JB and Moir GFJ (1979) *The Ultra Cytology of Latex Vessels in* Hevea brasiliensis. Monograph No. 4, pp. 9, 13, 28. Kuala Lumpur, Malaysia: Malaysian Rubber Research & Development Board.

sediment centrifugally or rise centripetally to form a zone beneath the rubber particles. It seems likely that the Frey-Wyssling particles are a form of plastid, although this has been disputed.

The lutoid particle (so called because of the incorrect original attribution of the yellow colour of the fraction) is the vacuolar tonoplast of the latex and is found in the sedimented or bottom fraction obtained on centrifuging latex, in addition to some Frey-Wyssling particles that actually cause the yellow colour. This highly osmosensitive organelle between 0.5 and 3 μm in diameter is bounded by an 8-nm-thick unit membrane and sometimes contains rubber particles. In addition to their vacuolar function, lutoid particles have the characteristics of lysosomes (see below). Release of protein components from lutoid particles of exuded latex lysed as a result of contact with rainwater can cause cessation of latex flow, due to coagulation, during tapping of *H. brasiliensis* trees.

The alkaloidal vesicles of *Papaver somniferum* (Papaveraceae), which are active in morphine synthesis, have a single bounding membrane and resemble *H. brasiliensis* lutoid particles. The association of lysosomal character and alkaloid accumulation has also been reported for the vacuoles of *Chelidonium* latex. *P. somniferum* latex also contains an organelle having a double boundary membrane that resembles the Frey-Wyssling particle of *Hevea*.

Amyloplasts containing starch granules are a feature of the nonarticulated laticifers of some *Euphorbia* species. Poinsettia (*E. pulcherrima*) laticifers contain rod-shaped long grains. Although there is some evidence for mobilization of poinsettia starch grains, those of other *Euphorbia* species seem to be metabolically inert.

Laticifer Biochemistry

The detailed understanding of laticifer biochemistry relates almost entirely to metabolic studies of *H. brasiliensis* latex.

However, it is recognized, as noted above, that this latex does not represent the entire laticifer contents, there being no nuclei and few mitochondria in the exudate. Notwithstanding this caveat, *H. brasiliensis* latex shows great biochemical complexity. Two-dimensional electrophoresis reveals the presence of upwards of 200 polypeptides and the approximately 1% protein content of this latex accounts for nearly 100 described individual enzyme activities. In addition to the discrete enzymes studied, there are those whose presence must be inferred from the presence of entire metabolic pathways such as those of fatty acid and of protein synthesis. Indeed, *H. brasiliensis* latex may be metabolically more active than that of, for example, *Ficus elastica* or *P. somniferum*, both of which have a lower protein content.

Unlike some other members of the Euphorbiaceae, *H. brasiliensis* latex contains no starch. However, it is relatively rich in sucrose (1–50 mmol L^{-1}), the degradation of which is the probable source of acetyl–coenzyme A used for the formation of the isoprenoid monomer isopentenyl diphosphate (IDP). Glucose produced from sucrose by the highly pH-sensitive latex invertase is subject to degradation by the enzymes of the glycolytic sequence. The conversion of [C^{14}] glucose into the rubber monomer, IDP, via acetyl–coenzyme A, requires two molecules of reduced nicotinamide adenine dinucleotide phosphate (NADPH) for the reduction of the intermediate 3-hydroxy-3-methylglutaryl–coenzyme A (**Figure 4**). This reductive requirement may be generated by the pentose phosphate pathway. However, although the presence of this pathway in latex has been claimed, its presence has not been clearly demonstrated and the source of the reducing power required for rubber biosynthesis remains obscure.

While the enzymes of carbohydrate breakdown are found in the laticifer cytoplasm, the so-called C serum of centrifuged latex, other metabolic pathways are found in latex organelles. Isolated latex contains a fatty acid synthetase that may be associated with the Frey-Wyssling particles and can also incorporate exogenous amino acids into a wide range of latex proteins using the ribosomes of the laticifer cytosol. Latex mRNA has been used as a template for the generation of latex cDNA libraries.

The highly osmosensitive lutoid tonoplast of the bottom fraction of centrifuged latex has the pH-regulating function characteristic of vacuoles. The single bounding membrane has an inward H^+ pumping ATPase and an outward H^+ translocating activity mediated by NADH cytochrome *c* reductase. In addition the lutoid particles accumulate Ca^{2+} and Mg^{2+}, apparently at the expense of H^+ and citrate, by a seemingly irreversible mechanism. Lutoid particles also resemble lysosomes, being the site of most of the hydrolytic activities of latex. Exceptions to this are the phospholipases C and D, which are found in the soluble cytoplasm.

Other latices in which biochemical activities have been studied include *P. somniferum,* the alkaloidal vesicles of which synthesize morphine, and the latices of various Euphorbiaceae, which contain α amylase. The latex of papaya, *Carica papaya* (Caricaceae), contains a protease, papain, used for meat tenderizing.

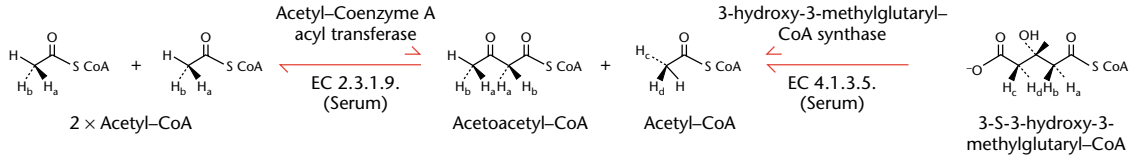
Biological Function of Laticifers

A number of functions have been ascribed to laticifers. It has been suggested that they form a secretory tissue, but the advantage to the plant of the latex stored is doubtful. The enormous amount of energy involved in the synthesis of rubber or of other terpenes does not seem to be recoverable. Isoprenoids including terpenes and steroids seem to be particularly resistant to total biological degradation and there is no evidence for the mobilization of starch in the latex amyloplast of some *Euphorbia* species. It was first suggested nearly 100 years ago that, since latex is frequently found in plants of arid terrains, laticifers may offer a reserve water supply, but laticiferous plants are by no means confined to dry regions. However, it is the considerable diurnal variation of turgor pressure (of about 4 atm, or approximately 4×10^5 Pa) in *H. brasiliensis* laticifers that necessitates early-morning tapping to achieve maximum latex yield.

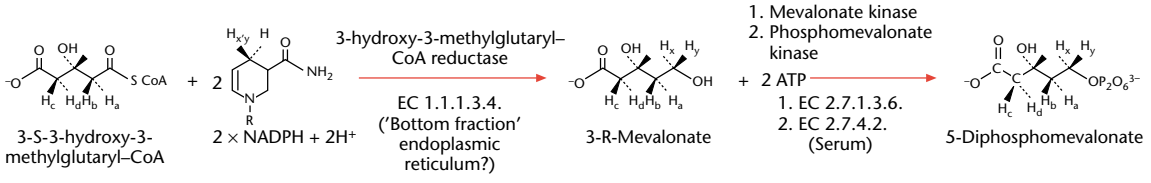
Possibly the most popular justification of laticifer presence has been a presumed protective role. The credibility of the proposal that latex offers protection from attacks by fungi and insect pests has been increased by the characterization in *H. brasiliensis* latex of the chitin-binding protein, hevein, and of chitinase activity, both of which are found in the lutoid organelle. However, such protective proteins are not peculiar to latices (hevein shows considerable homology to wheat germ agglutinin) and even the presence of rubber does not seem to offer protection by deterring herbivore feeding, insect attack or the incidence of fungal disease.

On the other hand some plant latices contain substances toxic both to vertebrates and to insects; thus the drainage of latex from the leaves of some Euphorbia species can render them edible to slugs. Some insects (e.g. *Labidomera clivicolis*) consuming the leaves of milkweed (Ascelepiadaceae) circumvent the toxic effects of the cardenolides contained therein by severing the latex-bearing leaf veins prior to the consumption of the distal tissue. Other insects such as the monarch butterfly (*Daneaus plexipus*) and the milkweed bug (*Oncopeltus fasciatus*) sequester these chemicals for subsequent use in defence against vertebrate predators. In conclusion, it must be admitted that during the twentieth century little advance has been achieved in our understanding of the biological functions or advantages of laticifers to the plant.

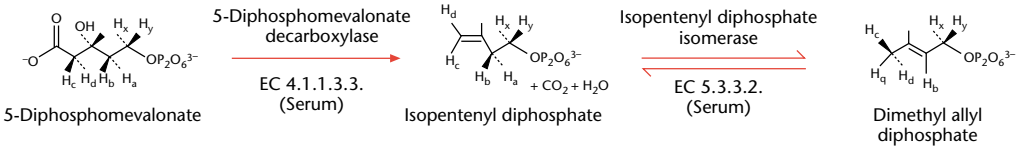
Stage 1 The formation of 3-hydroxy-3-methylglutaryl–CoA



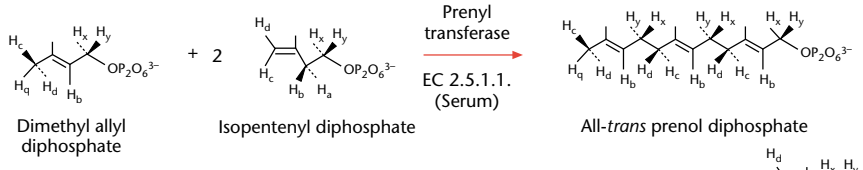
Stage 2 The reduction of 3-hydroxy-3-methylglutaryl–CoA and the formation of 5-diphosphomevalonate



Stage 3 The formation of the isoprenoid monomer isopentenyl diphosphate and the primer dimethyl allyl diphosphate



Stage 4 The formation of the all-*trans* prenol diphosphate primer



Stage 5 The formation of *cis*-polyisoprene

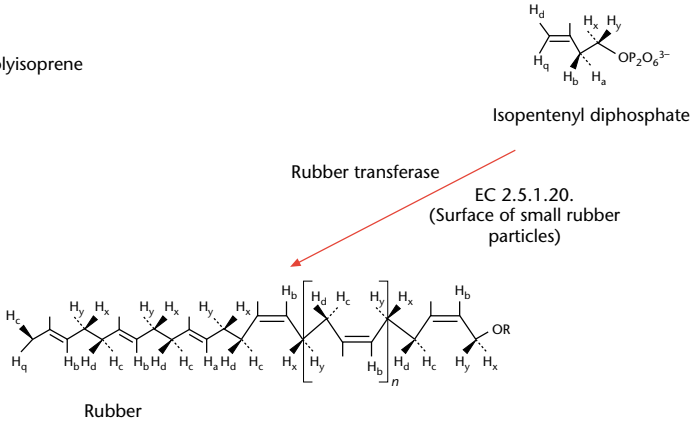


Figure 4 The biosynthesis of rubber from acetyl–coenzyme A.

Rubber and Other Polyisoprenoids

Rubber

Occurrence

Rubber, *cis*-1,4 polyisoprene, has been found in about 1800 plant species distributed among about 300 genera in eight families of higher plants; the Aceraceae, Apocynaceae, Ascelepiadaciae, Compositae, Euphorbiaceae, Loranthaceae, Moraceae and Sapotaceae in Gnetum; and in the basidiomycete *Lactarius* and the ascomycete sp. *Peziza*. Rubber may be present in laticifers, as in *H. brasiliensis*, or in the parenchyma, as in guayule, *P. argentatum*. Rubber particles may be occasionally found associated with

chloroplasts or vacuoles within the cell. Rubber molecules are aggregated to form particles that, in *Hevea*, range in diameter from 50 to 3000 nm and are surrounded by a half-unit membrane composed largely of phosphatidylcholine.

Structure

Rubber molecules have molecular weights (M_n) ranging from 10^5 to 2×10^6 Da, although low molecular weight rubbers of M_n about 10^4 Da have been found in species such as *Lactaria volemus*, *Helianthus annuus*, *Lactuca serrulata* and *Euphorbia antisyphilitica*. Rubber of the commercial rubber tree, *H. brasiliensis*, shows a bimodal distribution of M_n with maxima at about 1.5×10^5 Da and 2×10^6 Da; the proportion of high molecular weight molecules increases with the age of the tree.

Rubber from all species so far examined has an ω-terminal *trans* oligoprenol sequence followed by possibly 10^4 *cis* isoprene units. While *H. brasiliensis* rubber has been shown to have 2 ω-terminal *trans* isoprene units, that from the leaves of sunflower (*H. annuus*) or golden rod (*Solidago altissima*) may have two or three such *trans* units. The predicted ω-terminal dimethylallyl group has not been detected in *H. brasiliensis* rubber, and while evidence for such groups has been obtained for *Lactaria* rubber, their molar proportion has been reported to decrease during the ageing of the fungal sporophores. The expected free α-terminal hydroxyl group has not been detected either in *H. brasiliensis* or in *Lactaria* rubber; in both instances there appears to be esterification of the α-terminal OH by a long-chain fatty acid, frequently stearic.

Biosynthesis

As with other isoprenoids, the monomer from which rubber is formed is the five-carbon isopentenyl diphosphate (IDP), itself arising from the simultaneous decarboxylation and dehydration of the six-carbon mevalonate 5-diphosphate (MVADP) (**Figure 4**). The enzymic formation of mevalonate (MVA) from exogenous acetate and its subsequent conversion to IDP has been demonstrated in serum of *H. brasiliensis* latex. The route-determining and possibly the rate-determining step in this process, as with the formation of other isoprenoids, is the reduction of 3-hydroxy-3-methylglutaryl–coenzyme A (HMGCoA) to MVA, a step involving the oxidation of two molecules of NADPH. The incorporation of IDP, formed from MVADP, into rubber by washed rubber particles requires the addition of either an oligoprenol diphosphate primer, which may be of either the *cis* or *trans* configuration, or of an enzyme system with the capacity to form such an oligoprenol diphosphate from IDP. The utilization of an initiating *trans*-oligoprenol is in accord with the structural studies of rubber. The *cis*-polyprenol phosphate-forming enzyme, the so-called rubber transferase, appears to be an integral part of the half-unit membrane bounding the rubber particle (Cornish, 1993; Backhaus, 1985; Archer and Audley, 1987).

On the basis of experiments demonstrating the incorporation of IDP into rubber by preparations of the bottom fraction of latex obtained by centrifuging latex at 49 000***g***, it has been presumed that membrane-bound particles of this subcellular fraction have an important role in the synthesis of new rubber molecules (Tangpakdee *et al*., 1997).

In addition to the stereospecific steps involved in the formation of IDP, the synthesis of *cis*-polyisoprene characteristically involves the elimination by rubber transferase of the 2 pro-S hydrogen of IDP, while the formation of a *trans*-initiating oligoprenol diphosphate by a prenyl transferase results in the elimination of the 2 pro-R hydrogen of IDP.

Other polyisoprenoids

Trans-polyisoprene is found in gutta-percha, a nonelastic thermoplastic substance obtained from the latices of *Palaquin gutta* and *P. oblongifolia* (Sapotaceae), trees of the Malay peninsula. Chicle resin, the coagulated latex of sapodilla or naseberry (*Sapota acjias*), found in Guatemala, which is the basic ingredient of chewing gum, contains both *cis*- and *trans*-polyisoprenes of M_n about 1.5×10^5 Da and 10^4 Da, respectively.

The remarkable durability of the *cis*-polyisoprene molecule and its early evolution has been shown by the demonstration, using ^{13}C nuclear magnetic resonance, of its presence in fossil nonarticulated laticifers, the sole cells remaining from angiosperms laid down in the brown coal deposits of the Eocene Period 50 million years ago.

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