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Characterization of *cis*-prenyl transferase activity localised in a buoyant fraction of rubber particles from *Ficus elastica* latex

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Abstract We investigated rubber biosynthesis in *Ficus elastica*, a latex-producing plant species. Rubber particles were purified and enzyme activity was assayed by following the incorporation of radiolabel, from ^{14}C-isopentenyl diphosphate, into newly synthesised rubber (*cis*-1,4-polyisoprene). We characterised the rubber particle-bound *F. elastica cis*-prenyl transferase (rubber transferase), and describe the association of the active enzyme with a buoyant fraction of latex rubber particles. Also, we provide experimental data that may explain how isolated *F. elastica* rubber particles were mistakenly believed to be enzymatically inert.

Key words *cis*-1,4-polyisoprene, *cis*-prenyl transferase, natural rubber, rubber particles, rubber transferase, *Ficus elastica*, *Hevea brasiliensis*.

Abbreviations DTT, dithiothreitol; FPP, farnesyl diphosphate; IPP, isopentenyl diphosphate; SDS-PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate; THF, tetrahydrofuran.

INTRODUCTION

About 2,500 plant species produce the polymeric, secondary product, natural rubber (*cis*-1,4-polyisoprene) from isoprene monomers (C_5H_8) derived from isopentenyl diphosphate (IPP) (Bonner, 1991). The rubber polymers are packaged in subcellular rubber particles. One rubber-producing species, *Ficus elastica* Roxb. (the Indian rubber tree), is a rapidly-growing plant popularised in temperate regions as an ornamental. *F. elastica* produces rubber-containing latex in laticifers, and was one of the first plants of tropical Asia to be grown for rubber production (Polhamus, 1962). However, *F. elastica* rubber particles contain predominantly short-chain, poor quality rubber not valued commercially. All natural rubber currently used is obtained from a single tropical species, *Hevea brasiliensis* Muell. Arg (the Brazilian rubber tree) (d'Auzac *et al.*, 1989). Nonetheless, *F. elastica* provides a useful, comparative biochemical system for use in studies of the regulation of rubber polymer length.

Rubber particles have been used in biochemical assays of proteins extracted from rubber-producing species in attempts to isolate *cis*-prenyl transferase (rubber transferase, EC 2.5.1.20) from *H. brasiliensis* (Archer *et al.*, 1963; McMullen and McSweeney, 1966; Archer and Cockbain, 1969; Light and Dennis, 1989) and *Parthenium argentatum* Gray (guayule) (Madhavan and Benedict, 1984). The rubber particles, *F. elastica* often being the preferred source, were thought to provide a source of rubber diphosphate necessary for the reaction, since the protein extracts, in the presence of IPP, apparently could not synthesise rubber in the absence of rubber particles.

Rubber particle-bound rubber transferase activity has been identified (Berndt, 1963) and characterised (Archer and Audley, 1987; Cornish, 1993) in *H. brasiliensis* and in *P. argentatum* (Madhavan *et al.*, 1989; Cornish and Backhaus, 1990). Rubber transferase activity *in vitro* is IPP-dependent and requires divalent cations (Mg^{2+}) and allylic diphosphate, such as farnesyl diphosphate (FPP), to initiate the biosynthesis of new rubber molecules (Archer and Audley, 1987; Cornish and Backhaus, 1990). Rubber

particles of evolutionarily-divergent rubber-producing species share many commonalities (Cornish *et al.*, 1993) but contain a variety of species-specific proteins (Cornish and Backhaus, 1990; Backhaus *et al.*, 1991; Siler and Cornish, 1992, 1993; Cornish *et al.*, 1993). As was the case in *H. brasiliensis* and *P. argentatum*, the *F. elastica* rubber transferase has proved to be associated with rubber particles (Siler and Cornish, 1993, 1994; Cornish *et al.*, 1994) although, until now, it had not been kinetically characterised.

In this paper, we provide experimental data that may explain how *F. elastica* rubber particles were mistakenly assumed to be inert. Also, we characterise the rubber particle-bound *F. elastica* rubber transferase, and describe the restriction of the active enzyme to a buoyant fraction of rubber particles in latex.

RESULTS

Ficus elastica rubber particles

A series of experiments, determining IPP-incorporation rates by non-fractionated rubber particles purified from *F. elastica* latex, showed indications of enzymatic activity against a large background of non-enzymatic binding of IPP. For example, the IPP-incorporation rate was affected by temperature (*fig.* 1) which suggests the presence of enzymatic activity. However, the large amount of IPP incorporated at cool temperatures, even at 0°C, suggests that most of the [^{14}C]IPP used to follow incorporation had been passively trapped by the particles in the rubber transferase assay. Denaturation of all particle-bound enzymatic activity, by heating at 60 or 100°C before assay, eliminated the temperature-dependent rise (*fig.* 1) in IPP-incorporation rate but not the high background (data not shown). Hence, we proceeded to investigate the source of the apparent enzymatic activity.

Fractionated *Ficus elastica* rubber particles

Rubber particles from *F. elastica* latex were separated into buoyant and heavy fractions using centrifugation (see Methods) and then assayed for rubber transferase activity. This rubber transferase assay was developed to study *in vitro* rubber biosynthesis in isolated particles of two other rubber-producing species, *P. argentatum* (Cornish and Backhaus, 1990), and *H. brasiliensis* (Cornish, 1993). Both of these species demonstrated *in vitro* rubber transferase activity that was enhanced by the addition of the

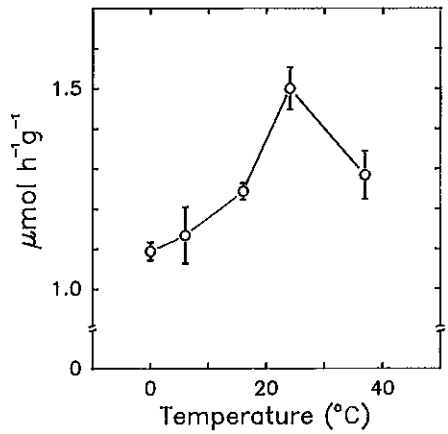

Figure 1. *Temperature dependence of IPP incorporation by* F. elastica *purified, non-fractionated, rubber particles.* Particles (14.4 ± 0.4 mg in 500 μl) were incubated at different temperatures for 1 h in the presence of 80 μM IPP and 20 μM FPP. Each value is the mean of three ± s.e. Experimental details in Methods.

rubber molecule initiator, FPP, and that was Mg^{2+}-dependent (activity completely inhibitable by EDTA). The buoyant *F. elastica* rubber particles also showed FPP-enhancible, EDTA-inhibitable rubber transferase activity (*fig.* 2). However, IPP-incorporation by the heavy rubber particles was not fully EDTA-inhibitable (*fig.* 2) and so could not be interpreted as rubber transferase activity. Rubber particle preparations containing a large proportion of heavy particles, relative to buoyant ones, exhibit high levels of nonspecific IPP incorporation, such as seen in figure 1, which can obscure any true rubber transferase activity.

Gel permeation chromatography demonstrated that the molecular mass profiles of the rubber contained in the heavy and buoyant particles were very similar (*fig.* 3). The results agree with the molecular mass analysis reported for nonfractionated *F. elastica* rubber particles (Cornish *et al.*, 1993). Both particle fractions contained long chain rubber as well as low molecular mass material (*fig.* 3). Also, the size distribution of the particles in both fractions was similar to that reported previously for *F. elastica* rubber particles (Cornish *et al.*, 1993) and scanning electron microscopy demonstrated similar spherical rubber particles in both fractions. Furthermore, SDS-PAGE analysis (Laemmli, 1970) showed identical protein profiles in each rubber particle fraction.

Rubber transferase activity in buoyant particles

Since preliminary evidence (*fig.* 2) indicated the presence of rubber transferase activity in buoyant

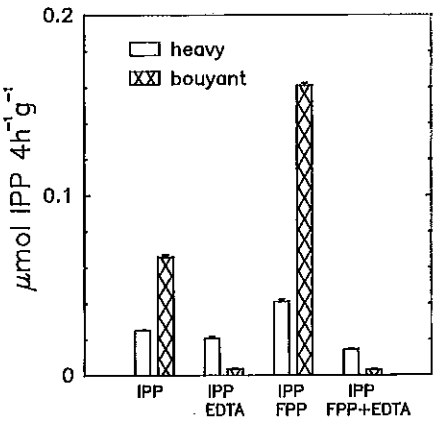

Figure 2. *The effect of FPP and EDTA on IPP incorporation by two fractions of* F. elastica *purified rubber particles which differ by specific gravity (heavy and buoyant).* The particles were incubated for 4 h at 16°C in 80 μM IPP ± 20 μM FPP ± 20 mM EDTA. Buoyant particles and heavy particles were used at concentrations of 8.9 ± 1.4 and 13.9 ± 1.0 mg in 500 μl, respectively. Each value is the mean of four ± s.e. Experimental details in Methods.

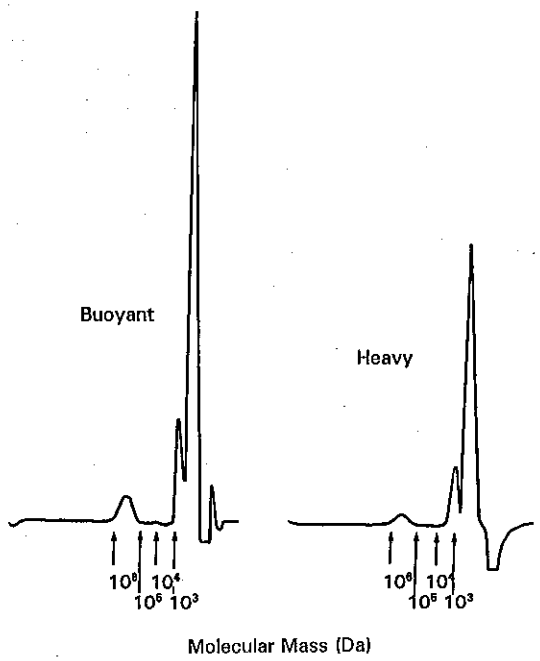

Figure 3. *Molecular mass analysis of two fractions of rubber particles purified from* F. elastica *latex which differ according to specific gravity (buoyant and heavy), using gel permeation chromatography.* Experimental details in Methods.

rubber particles, we performed additional experiments to characterise the observed activity. We have observed that the proportion of heavy and buoyant particles purified from *F. elastica* latex varies according to the condition of the plant (*e.g.* age, water status, anatomical location of latex collection) and upon the chemical composition of the purification and assay media (*e.g.* the concentration of different salts) and the method of preparation (*e.g.* type and concentration of osmotica, or centrifugal force employed) (unpublished data and Siler and Cornish, 1993). Buoyant particles were purified from two different ages of *F. elastica* greenhouse-grown plants and assayed. Particles from both young and old plants had similar FPP-enhancible, EDTA-inhibitable activity (*fig.* 4) characteristic of rubber particle-bound rubber transferases. Complete inhibition, in an *in vitro* assay containing 4 mM exogenous Mg^{2+} cofactor, was fully effected at 5 mM EDTA. We found that enzymatically-active buoyant rubber particles may be isolated from any *F. elastica* plant large enough for latex collection, irrespective of age (data not shown).

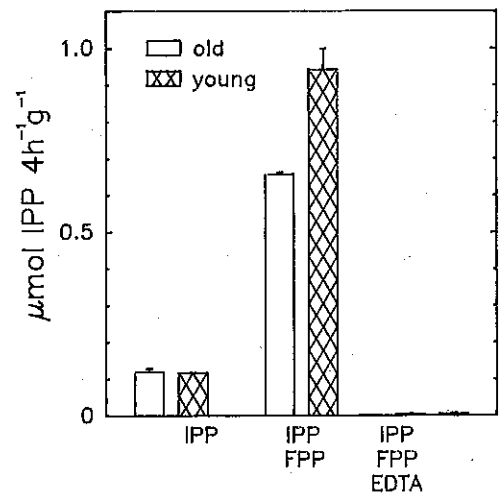

Figure 4. *Rubber transferase activity in buoyant fraction rubber particles purified from latex collected from old* (ca. *3 years) and young* (ca. *6 months*) F. elastica *plants.* The particles (at concentrations of 0.46 ± 0.01 and 1.39 ± 0.07 mg in 50 μl, from the old and young plants, respectively) were incubated for 4 h at 25°C in 5 mM IPP ± 20 μM FPP ± 20 mM EDTA. Each value is the mean of three ± s.e. Experimental details in Methods.

The rate of IPP incorporation by buoyant particles increased linearly over time at three different temperatures tested (*fig.* 5). Enzyme activity was stable for at least 8 h (*fig.* 5). When the temperature dependence of IPP-incorporation was investigated (8.03 ± 0.44 mg particles in 500 μl, incubated for 4 h in 200 μM IPP ± 20 μM FPP, temperature range

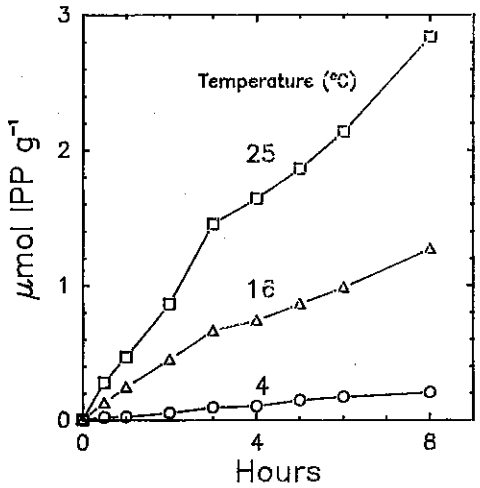

Figure 5. *Time course of IPP incorporation at three different temperatures by buoyant fraction rubber particles purified from* F. elastica *latex.* The particles (7.04 ± 0.53 mg in 500 μl) were incubated at 4, 16 or 25°C for 4 h in 200 μM IPP ± 20 μM FPP. Each value is a single data point. Experimental details in Methods.

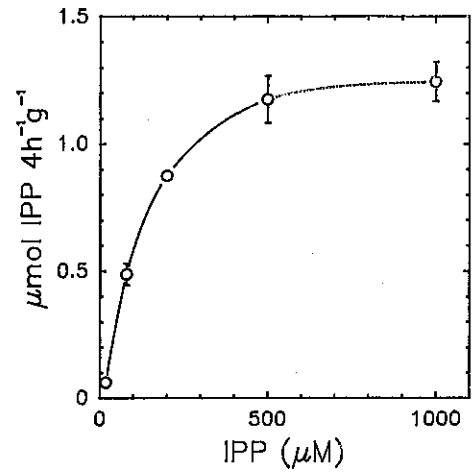

Figure 6. *IPP concentration dependence of rubber transferase activity in buoyant fraction rubber particles purified from* F. elastica *latex.* Particles (0.85 ± 0.17 mg in 50 μl) were incubated for 4 h at 25°C in 20 μM FPP. Each value indicated by the solid line is the mean of three ± s.e. Experimental details in Methods.

4 to 37°C, means of four) the linear Arrhenius plot (log rate against 1/T) indicative of an enzymatic reaction was observed (d.f. = 5, r = 0.958). Particles placed at 100°C for 5 min to denature proteins, before being incubated for 4 h in the reaction mixtures and incubated over the same temperature range, did not incorporate IPP. These results for buoyant particles are very different from the apparent incorporation rates observed in nonfractionated particles (*fig.* 1).

Rubber transferase activity could not be removed by repeated washing of the buoyant particles (data not shown) demonstrating that the enzyme is firmly associated with the rubber particles, as has been shown for rubber transferases in other plant species (Cornish and Backhaus, 1990; Cornish, 1993).

The kinetics of the *F. elastica* buoyant particle-bound rubber transferase were then investigated by following the incorporation of radiolabelled isoprene, from [^{14}C]IPP, into newly synthesised rubber molecules. The IPP concentration dependence (*fig.* 6), when subjected to an Eadie-Hofstee analysis (rate plotted against rate/[substrate]), indicated that the apparent K_m for IPP was 160 μM. The apparent K_m for FPP, similarly derived from the FPP concentration dependence (*fig.* 7) was about 0.3 μM.

The pH optimum for the rubber transferase was determined to be 7.5 (*fig.* 8). IPP incorporation rates were inhibited at both low and high pH. The enzyme proved more stable at low pH than at high pH, at which the enzyme was irreversibly denatured. Approximately 25% of the inhibition observed at pH 5 and 6 could be reversed upon the return of the particles to pH 7.5 (*fig.* 8). The pH range of rubber transferase activity is quite broad (*fig.* 8) with 50% of maximum activity being observed at pH 6.1 and 9.3. In a similar experiment with purified rubber particles of *H. brasiliensis*, a pH optimum of 7.2 was observed, with 50% activity remaining at pH 6.3 and 8.6 (unpublished results).

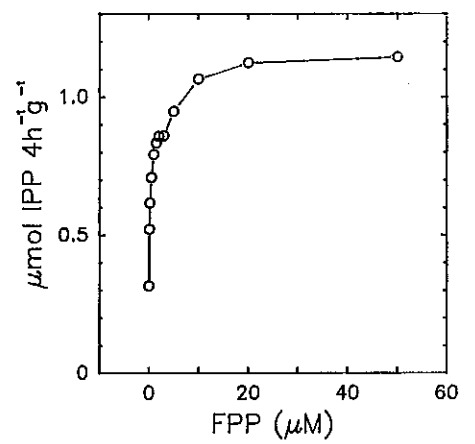

Figure 7. *FPP concentration dependence of rubber transferase activity in buoyant fraction rubber particles purified from* F. elastica *latex.* Particles (1.79 ± 0.06 mg in 50 μl) were incubated for 4 h at 25°C in 1 mM IPP. Each value is a single data point. Experimental details in Methods.

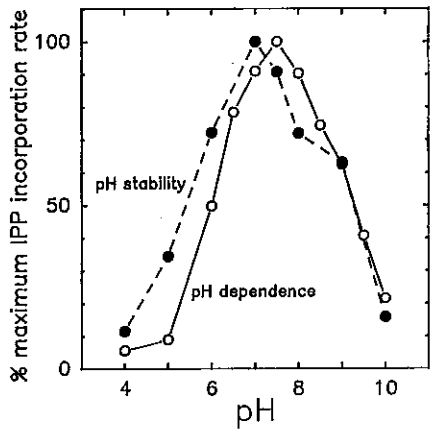

Figure 8. *Effect of pH on rubber transferase activity and on the stability of rubber transferase activity in buoyant fraction rubber particles purified from* F. elastica *latex.* Some particles (indicated by the dashed line) were pretreated by incubation for 2 h at 25°C, in a universal buffer adjusted to different pH, then readjusted to pH 7.5 before assaying for rubber transferase activity by incubation (particle concentration = 7.10 ± 0.82 mg in 500 μl) for 4 h at 25°C in 1 mM IPP and 20 μM FPP. Each value is the mean of two. Other particles (10.10 ± 0.76 mg in 500 μl) were assayed without pretreatment (indicated by the solid line). Each value is the mean of four. Experimental details in Methods.

DISCUSSION

Buoyant and heavy *F. elastica* rubber particles differ in various ways. Heavy particles have no detectable rubber transferase activity and bind radiolabelled IPP nonspecifically. Buoyant particles are enzymatically-active and do not passively bind radiolabelled IPP. The cause(s) of these striking differences remain obscure since the two particle fractions both contain rubber particles of similar size, content and protein complement. *F. elastica* rubber particles have other unusual properties not present in particles from *H. brasiliensis* or *P. argentatum.* For example, the particles are sticky, and even the enzymatically-active buoyant particles were found to bind proteins nonspecifically (Cornish *et al.*, 1994). Also, the relative density of the particles can be altered by various salt treatments (Siler and Cornish, 1993). The relative proportions of the buoyant and heavy particles vary but all latex samples harvested and examined to date, have contained enzymatically-active buoyant particles.

In vitro assays measure predominantly *de novo* rubber biosynthesis (Archer and Audley, 1987; Cornish and Backhaus, 1990; Cornish, 1993), in which a source of allylic diphosphate initiator molecules is required before rubber can be made. Protein extracts previously reported to have rubber transferase activity (Archer *et al.*, 1963; McMullen and McSweeney, 1966; Archer and Cockbain, 1969; Light and Dennis, 1989) were probably part of the rubber molecule initiation system, synthesizing allylic diphosphates but not rubber itself (Cornish, 1993). The confusion caused by this misconception was compounded when *F. elastica* was used as a source of the supposedly-inert rubber particles. We have shown here that *F. elastica* rubber particles not only contain rubber transferase, but also passively bind the radioalabelled IPP required by the assay. This means that experiments performed in IPP alone, or in IPP and allylic diphosphate, give results difficult to separate from the high background caused by the presence of heavy *F. elastica* particles (*e.g.* see *fig.* 2). The characterisation, reported here, of the *F. elastica* rubber transferase demonstrates that it is an enzyme very similar to the ones characterised in *H. brasiliensis* and *P. argentatum.* All three rubber transferases have much higher binding affinity for the FPP initiator than for IPP (the apparent K_m's, determined for IPP in the presence of 20 μM FPP and for FPP in the presence of 5 mM IPP, are: *H. brasiliensis*, IPP = 400 μM, FPP = 0.8 μM (Cornish, 1993); *P. argentatum,* IPP = 300 μM, FPP = 3 μM (Cornish and Backhaus, 1990); *F. elastica*, IPP = 160 μM, FPP = 0.3 μM (this report)).

The pH optimum of 7.5 for the *F. elastica* rubber transferase is similar to the pH optimum of 7.2 we observed and that of near 8 reported for *H. brasiliensis* (Lynen, 1969). These rubber transferase optima also are similar to those reported for a variety of other prenyl transferases (Poulter and Rilling, 1981; Croteau and Purkett, 1989), which also share, with rubber transferase, similar substrate (allylic and nonallylic diphosphate) and cofactor (Mg^{2+}) requirements.

Comparison of the pH stability with the pH dependence (*fig.* 8) indicates that most of the decline in activity at high pH ($\geq$ 9) is due to irreversible inactivation of the enzyme. However, part of the activity decline between pH 7.5 and 9, and part of the decline in activity observed at low pH is reversible and results from the formation of an improper ionic form of the enzyme and/or substrate (Segel, 1993). The reversible declining activity at acidic pH is consistent with the protonation of aspartate residues in the active site, such as have been characterised in the *trans*-prenyl transferase, FPP-synthase (Tarshis

et al., 1994). In this enzyme, it appears that aspartate residues bind diphosphate groups of the substrates through Mg^{2+} bridges. Also, two aspartate-rich regions are highly conserved in plant, microorganismal and animal prenyl transferases (Chen *et al.*, 1994). Since the substrates and cofactor requirements of rubber transferase are similar to FPP-synthase it seems probable that a similar aspartate/Mg^{2+}-mediated mechanism exists in its active site, although this has not yet been proven. Confirmation awaits successful purification and sequencing of a rubber transferase.

Furthermore, it is interesting to note that, despite their strong similarities, the soluble FPP-synthase and the rubber particle-bound rubber transferase make the very different reaction products of FPP (a *trans*-C-15 allylic diphosphate) and rubber (a large indeterminant *cis*-polyisoprene).

We had earlier shown, through a series of cross-specific immunoinhibition experiments, that rubber transferase activity in *F. elastica* seems to be associated with a 375 kDa protein (Siler and Cornish, 1993, 1994; Cornish *et al.*, 1994). We have now demonstrated that this enzyme is kinetically comparable to the rubber transferases of *H. brasiliensis* and *P. argentatum*. However, the average size of rubber polymers produced *in vivo* is considerably larger in *H. brasiliensis* and *P. argentatum* (and therefore of higher quality from a manufacturing stand-point) than in *F. elastica*. We are now in a position to investigate how polymer length is regulated in these contrasting rubber-producing species, and to determine whether final polymer length is a property of the rubber transferase itself or of other endogenous factors, such as substrate availability.

In conclusion, we have characterised the *F. elastica* rubber particle-bound rubber transferase (a *cis*-prenyl transferase). The active enzyme is kinetically similar to the rubber transferase of *H. brasiliensis* and *P. argentatum*, but is uniquely localised on a buoyant subset of the rubber particles.

METHODS

Materials. *Ficus elastica* plants were purchased from a local nursery and grown in a greenhouse. Unlabelled IPP and [1-^{14}C]IPP (2.035×10^9 and 2.22×10^9 Bq.mmol^{-1}), and unlabelled FPP were purchased from American Radiolabeled Chemical Inc. (Bowling Green, Missouri). Latex was tapped from stems and petioles of plants by removing the apical portion with an angled cut using a razor blade. Latex was collected in tubes and stored on ice until used.

Purification of rubber particles. Non-fractionated particles were purified by a centrifugation procedure previously described (Siler and Cornish, 1993).

Purification of buoyant and heavy rubber particles. *F. elastica* latex was suspended in ice-cold buffer containing 100 mM Tris-HCl (pH 8), 2.5 mM $MgSO_4$, 5 mM KF, 0.1 mM phenylmethylsulphonyl fluoride, 10 mM DTT and 12% glycerol. The suspension was centrifuged at 2,500 g_n, for 10 min, at 4°C using a bucket rotor. The fraction of rubber particles that floated to the top (the buoyant particles) were collected, the supernatents were decanted and the sedimented particles (the heavy particles) also were collected. The buoyant particles were resuspended in the same buffer and recentrifuged. This washing procedure was again repeated. The buoyant particles were collected and suspended in wash buffer (100 mM Tris-HCl at pH 8, 2 mM $MgSO_4$) containing 5 mM dithiothreitol (DTT) and stored on ice until used. The heavy particles collected from the first centrifugation step were resuspended in wash buffer (without glycerol) and recentrifuged. This procedure was repeated twice. The heavy washed rubber particles were resuspended in wash buffer and stored on ice until used.

Rubber molecular mass analysis. Thrice-washed samples of buoyant and heavy rubber particles were dried, dissolved in tetrahydrofuran (THF) and analysed on a Phenogel column (Phenomenex, Torrence, California) using THF as the carrier solvent and a refractive index detector. The column was calibrated against polyisoprene standards (Polymer Laboratories, Inc., Foster City, California).

Assay of rubber transferase activity. Rubber transferase (EC 2.5.1.20) activity was determined by the method previously described for *P. argentatum* (Cornish and Backhaus, 1990) by following the incorporation of radiolabelled isoprene monomers from [^{14}C]IPP into rubber. In the experiments reported here, 14 to 36 μg dw of rubber particles per μl of reaction mixture were assayed, depending upon the experiment, in siliconised 1.5 ml microfuge tubes (USA/Scientific Plastics, Ocala, Florida). Reactions were incubated at the temperatures and times described in the figure legends. The relatively low assay temperature of 16°C was used since the rubber transferase activity in rubber particles isolated from *P. argentatum* is temperature labile (Cornish and Backhaus, 1990). It seemed possible that the apparent lack of activity in *F. elastica* isolated rubber particles also might be a consequence of temperature instability. Reaction mixtures were either 50 or 500 μl and included either 110,000 or 220,000 dpm of [^{14}C]IPP. Reactions contained IPP concentrations of 0.08, 0.2, 1 or 5 mM IPP (*ca.* 0.5, 1.3, 6 and 31x K_m, respectively), depending upon the experiment. Except when stated otherwise, FPP was used at a concentration of 20 μM (*ca.* 70x K_m) in the reactions and EDTA was used at 20 μM. Reactions also included 5 mM DTT, 100 mM Tris-HCl,

and 4 mM $MgSO_4$ (which preliminary experiments indicated was sufficient to maintain maximal activity without being inhibitory (Note: K_m was not determined for *F. elastica*; the Mg^{2+} K_m for *H. brasiliensis* is about 70 μM, unpublished data)). All reactions, except where otherwise stated, were incubated at pH 7.5. Boiling and 60°C treatments were effected by immersing tubes of rubber particles into water baths for 5 min. Boiling had no visible effect on the rubber particle suspensions. Rubber transferase reactions were halted by the addition of 20 mM EDTA. The rubber particles were harvested by filtration, the filters washed to remove unincorporated radiolabelled IPP, and the radioactivity on the filters determined by liquid scintillation spectroscopy, as described (Cornish and Backhaus, 1990).

pH-dependent rubber transferase experiments. A universal buffer was prepared with 50 mM each of sodium acetate, BisTris, Tris-HCl and glycine, providing an overall buffering range of pH 4 to 10. Buoyant rubber particles from *F. elastica* were diluted in universal buffer adjusted to pH 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0 with HCl or NaOH. Rubber transferase activity was measured by incubating the particles at the different pH's for 4 h at 25°C in 1 mM IPP and 20 μM FPP, and then following the harvesting procedure described above. A titration curve prepared with rubber particles in universal buffer was identical to one prepared in the absence of particles, indicating no significant buffering by the particles themselves. The pH did not change detectably during the course of the assay. Stability of rubber transferase with respect to pH was determined by incubating particles for 2 h at 25°C in universal buffer adjusted to pH 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0 with HCl or NaOH. The pH was adjusted to 7.5 with HCl or NaOH, and rubber transferase activity was measured as described above.

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REFERENCES

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

Archer B. L. and Cockbain E. G., 1969. Rubber transferase from *Hevea brasiliensis* latex. *Methods Enzymol.*, **15**, 476-480.

Archer B. L., Audley B. G., Cockbain E. G. and McSweeney G. P., 1963. The biosynthesis of rubber. *Biochem. J.*, **89**, 565-574.

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.

Berndt J., 1963. The biosynthesis of rubber. *U.S. Government Res. Rep.*, AD-**601**, 729.

Bonner J., 1991. The history of rubber. In *Guayule natural rubber: a technical publication with emphasis on recent findings.* Whitworth J. W. and Whitehead E. E., ed., Guayule Administrative Management Committee and USDA Cooperative State Research Service, Office of Arid Lands Studies, The University of Arizona, Tucson, 1-16.

Chen A., Kroon P. A. and Poulter C. D., 1994. Isoprenyl diphosphate synthases-protein sequence comparisons, a phylogenic tree, and predictions of secondary structure. *Protein Sci.*, **3**, 600-607.

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. and Backhaus R. A., 1990. Rubber transferase activity in rubber particles of guayule. *Phytochemistry*, **29**, 3809-3813.

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 Rubb. 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*. *Phytochemistry*, **35**, 1425-1428.

Croteau R. and Purkett P. T., 1989. Geranyl pyrophosphate synthase: characterization of the enzyme and evidence that this chain-length specific prenyltransferase is associated with monoterpene biosynthesis in sage (*Salvia* officinalis). *Arch. Biochem. Biophys.*, **271**, 524-535.

d'Auzac J., Jacob J-L. and Chrestin H. 1989. *Physiology of Rubber Tree Latex.* CRC Press, Boca Raton, Florida.

Laemmli U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680-685.

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

Lynen F. 1969. Biochemical problems of rubber synthesis. *J. Rubb. Res. Inst. Malaya*, **21**, 389-406.

Madhavan S. and Benedict C. R., 1984. Isopentenyl pyrophosphate *cis*-1,4-polyisoprenyl transferase from guayule (*Parthenium argentatum* Gray). *Plant Physiol.*, **75**, 908-913.

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.

McMullen A. J. and McSweeney G. P., 1966. Incorporation of isopentenyl pyrophosphate into purified

rubber particles by a soluble latex-serum enzyme. *Biochem. J.*, **101**, 42-47.

Polhamus L. G., 1962. *Rubber: Botany, production, and utilization*, World Crops Series, Leonard Hill, London.

Poulter C. D. and Rilling H. C., 1981. Prenyl transferases and isomerase. In *Biosynthesis of isoprenoid compounds*, Porter J. W. and Spurgeon S. L., ed. **1**, 161-224.

Segel I. H. 1993. *Enzyme kinetics.* John Wiley and Sons.

Siler D. J. and Cornish K., 1992. Potential for allergy amelioration by rubber particle purification from *Hevea brasiliensis* latex, and for allergy circumvention by use of alternate rubber-producing plants. *Proceedings of the International Latex Conference: Sensitivity to Latex in Medical Devices*, 34, FDA, Washington, D.C.

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.

Tarshis L. C., Yan M., Poulter C. D. and Sacchettini J. C., 1994. Crystal structure of recombinant farnesyl diphosphate synthase at 2.6-A resolution. *Biochemistry*, **33**, 10871-10877.