CFAES

Comparative genomic analysis of putative rubber transferase subunits in rubber tree (*Hevea brasiliensis* Müll. Arg.), guayule (*Parthenium argentatum* Gray) and rubber dandelion (*Taraxacum kok-saghyz* Rodin)

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INTRODUCTION

Natural rubber (NR) is a high molecular weight polymer that cannot be replaced by synthetics in most applications. NR is currently harvested from the para rubber tree (*Hevea brasiliensis* Müll. Arg.) (Hevea), but is faced with serious challenges, such as supply shortages and pathogen threats [1]. Two plant species have gained considerable attention as potential alternative sources of natural rubber: guayule (*Parthenium argentatum* Gray) and rubber dandelion (*Taraxacum kok-saghyz* Rodin) [1, 2].

Rubber transferase (RT-ase) (EC 2.5.1.20) is a enzyme complex with catalyzes the polymerization of NR, which is made by over 2,500 plant species [3] in cytosolic rubber particles. RT-ase requires an allylic pyrophosphate molecule to initiate the reaction, isopentenyl pyrophosphate (IPP) as the monomer and a divalent cation Mg^{2+} cofactor or activator. RT-ase is bound to the proteo-phospho-lipid monolayer which surrounds the rubber particles and activity requires such an aqueous-organic interface. The identify and role of the members of the RT-ase complex are still unclear, but a schema is presented below.

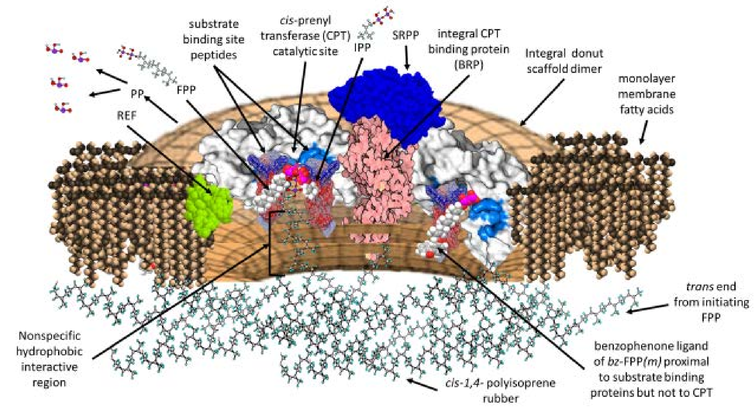

***Figure 1**. The schema is a hypothetical cross-section of the RT-ase complex with proteins believed to be involved in rubber biosynthesis in some way. The complex appears to contain a central channel through which the growing rubber polymers are transferred to the rubber particle interior. The nonspecific hydrophobic region interacts with the hydrocarbon chain of both the initial allylic pyrophosphate (APP) subtract used and the growing rubber polymer, and is C5 shorter in P. argentatum than in H. brasiliensis and F. elastics RT-ase. The proteins REF and SRPP play a role in molecular weight regulation but their exact position, conformation, and proximity to the growing rubber polymer, is not yet known. The structure of the small binding proteins are represented by the confirmation predicted by the amino acid sequence of the smaller of the two. The same structure is used for both small proteins because the complete amino acid sequence of the larger of the two small proteins, and therefore its predicted structure, is not yet known, and both bind the same APP substrates. The RT-ase CPT-type catalytic reaction is fostered by the substrate binding to the small subunits and the binding constants are quite distinct from other prenyl transferases. Although a putative CPT protein is labelled on the schema, the H. brasiliensis rubber particle bound CPT can be removed from the particles without a concomitant loss of RT-ase activity (see Fig. 2) so this is likely a different protein. The donut scaffold protein (a dimer) holds the small subunits in place, with three of each per scaffold monomer. The substrates in the binding site are indicated by space filled molecular models while the remaining substrates, polymers and released pyrophosphates are depicted as ball and stick models.*

In Hevea, two putative *HRT* genes have been isolated, and the HRT1 protein (but not HRT-2) restored rubber transferase activity in detergent-inactivated Hevea rubber particles, suggesting a role in rubber transferase (RT-ase) activity. Neither had RT-ase activity when reconstituted in micelles, demonstrating that HRT1 is probably a member of the RT-ase complex but is not RT-ase itself [4].

AIM

To identify homologous RT-ase genes from guayule and rubber dandelion and investigate if they are involved in rubber transferase activity.

METHODS

- ***Sequence retrieval***

The protein sequence of the *HRT1* gene was downloaded from NCBI Database (Accession No.: BAB71776). Local alignment search tool, BLASTP, was performed to identify homologous RT-ase genes from the guayule [5] and rubber dandelion (unpublished data) genomes

- ***Sequence analysis***

Sequence similarity analysis was executed by program Clustal2.1 [6].

- ***Phylogenetic tree construction***

A phylogenetic tree was constructed based on alignment of nine RT-ase protein sequences using MEGA7.0 [7] and the neighbor-joining (NJ) method, with 1,000 iterations for the bootstrap values, p-distance model, and pairwise deletion for gap treatment.

RESULTS and DISCUSSION

A BLASTP comparative genomic analysis search, using the Hevea HRT1 protein sequence as a query against the published guayule genome and our lab-assembled rubber dandelion genome, identified two guayule HRT homologs (*PaRT1* and *PaRT2*) and five rubber dandelion HRT homologs (*TkRT1-5*). Amino acid sequence analysis revealed that all 9 HRTs - from rubber tree, guayule, and rubber dandelion - belong to the *cis*-IPPS super family. The sequence similarity between *HRT* genes ranged from 52.04% to 82.78% (Fig. 2).

Figure 2. Percent identity matrix of nine Rubber Transferase (RTs) from Hevea (2), rubber dandelion (5), and guayule (2).

		1	2	3	4	5	6	7	8	9
1	HRT1_Hevea	**100**	91.49	54.68	56.37	54.45	53.31	52.3	56.55	56.55
2	HRT2_Hevea	91.49	**100**	52.04	54.41	53.45	52.84	52.86	54.65	54.65
3	PaRT2	54.68	52.04	**100**	80.15	54.34	53.31	53.14	54.1	54.1
4	TK_RT5	56.37	54.41	80.15	**100**	56.25	56	54.92	56.44	54.78
5	TK_RT3	54.45	53.45	54.34	56.25	**100**	98.34	76.24	76.4	74.18
6	TK_RT4	53.31	52.84	53.31	56	98.34	**100**	74.74	75.09	72.95
7	PaRT1	52.3	52.86	53.14	54.92	76.24	74.74	**100**	82.78	82.78
8	TK_RT1	56.55	54.65	54.1	56.44	76.4	75.09	82.78	**100**	100
9	TK_RT2	56.55	54.65	54.1	54.78	74.18	72.95	82.78	100	**100**

Phylogenetic analysis showed that *PaRT2* from guayule and *TkRT5* from rubber dandelion were classified into the same group with *HRT1* and *HRT2* according to their genetic distance; TkRT1 (273 aa) and TkRT2 (281 aa) exhibited 100% similarity on their matched area, indicating that they are probably pairs of duplicated genes (Fig 2).

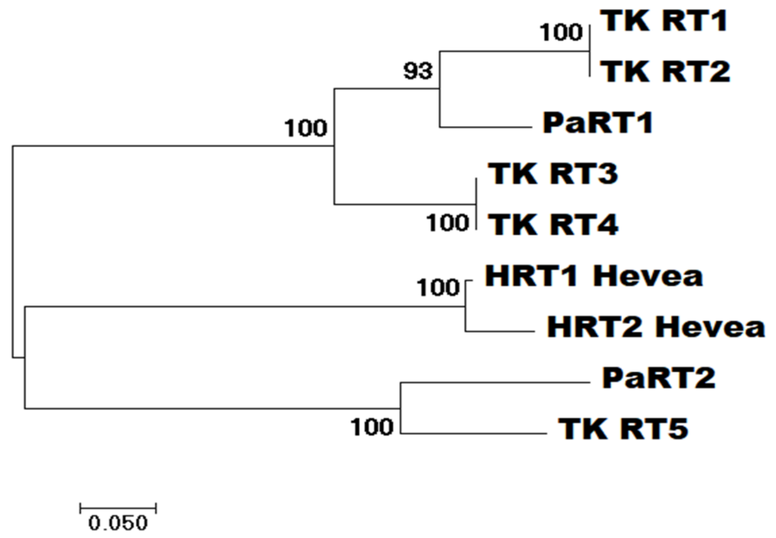

Figure 3. Molecular phylogenetic analysis of nine putative rubber transferase subunits (RTs) from Hevea (2), rubber dandelion (5), and guayule (2).

As noted in the Introduction, multiple proteins are involved in rubber formation. However, the homology among putative RT-ase proteins from multiple rubber-producing species, does support a key role for RT-1 like proteins in rubber biosynthesis.

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