

Update section

Sequence

Molecular cloning and nucleotide sequencing of the rubber elongation factor gene from *Hevea brasiliensis*

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About 30% of the world demand for elastomers is satisfied by the high-molecular-weight (500–2000 kDa) *cis*-polyisoprene formed in the specialized laticifers of *Hevea brasiliensis* (Euphorbiaceae) and which constitutes about 90% of the dry weight or about 30% of the total weight of the latex exuded on severing the anastomosing laticifers by tapping [1]. The larger rubber particles of latex of diameter 0.1 μ m which are active in rubber biosynthesis *in vitro* are surrounded by a half-unit lipid membrane [2, 3], which in the case of these particles contains a predominant 14.5 kDa polypeptide [4] termed by Dennis and Light [4] the rubber elongation factor (REF). To this polypeptide these workers have attributed the function of interacting with the soluble cytoplasmic farnesyl diphosphate-forming prenyl transferase to give a complex forming a *cis*-polyisoprenoid chain commencing with a *trans* oligomer at the ω terminus [5, 6, 7]. The amino acid sequence of the REF has been determined by Dennis *et al.* [8]. The present report concerns the cloning and sequencing of the gene encoding the REF found in a cDNA library derived from the mRNA of the polysomes exuded in *Hevea* latex [9, 10].

Polysomes were prepared from freshly tapped latex and mRNA isolated by oligo-dT-cellulose

chromatography. This was then used to prepare cDNA which, following the addition of *Eco* RI adapters, was cloned into λ gt11. Approximately 20000 clones from this library were screened using an REF-specific antibody that had been raised against purified REF (Fig. 1). A single clone with an insert size of about 730 bp was identified. The cloned fragment was amplified by PCR using primers derived from the λ gt11

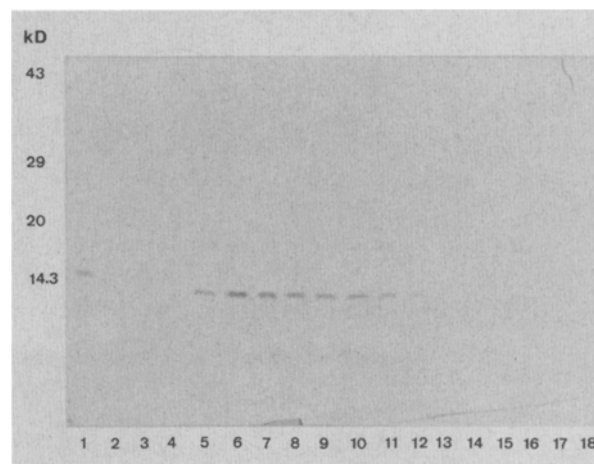


Fig. 1. Immunoblot of rubber particle membrane polypeptides separated in 15% polyacrylamide. Track 1: total latex proteins. Tracks 2–18: 14.5 kDa REF polypeptide from rubber particles eluted in sequential fractions after application of latex to a Sephacryl S-500 column.

sequence adjacent to the cloning site. The primers also contained a *Bam* HI recognition site which facilitated the cloning of the PCR product into the *Bam* HI site of pBluescript SK⁺ to produce pREF1.

Nucleotide sequencing of pREF1 revealed the

cDNA insert to be 681 bp in length (Fig. 2). Comparison of the translated sequence with the published REF amino acid sequence [8] confirmed that pREF1 does indeed encode REF. The clone encodes the entire coding region of the mature REF. An ATG initiation codon is located

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      10              30              50
      ttttgatcttccatttttgcaaaaggaaatcttcgattatggctgaagacgaagacaacc
                                M A E D E D N Q

      70              90              110
      aacaagggcagggggaggggttaaaatatttgggttttgcaagacgggcaacttatg
      Q G Q G E G L K Y L G F V Q D A A T Y A

      130             150             170
      ctgtgactaccttctcaaacgtctatcttttggccaaagacaaatctggtccactgcagc
      V T T F S N V Y L F A K D K S G P L Q P

      190             210             230
      ctgggtgtgatatcattgaggggtccggtgaagaacgtggctgtacctctctataataggt
      G V D I I E G P V K N V A V P L Y N R F

      250             270             290
      tcagttatattcccaatggagctctcaagttttagacagcacgggtgttgcatctgtca
      S Y I P N G A L K F V D S T V V A S V T

      310             330             350
      ctattatagatcgctctcttccccaattgtcaaggacgcacatctatccaagttgtttcag
      I I D R S L P P I V K D A S I Q V V S A

      370             390             410
      caattcgagctgccccagaagctgctcgttctctggttcttctttgcctgggcagacca
      I R A A P E A A R S L A S S L P G Q T K

      430             450             470
      agatacttgctaagggtgttttatggagagaattgagccccaatttgcaccaattgcttcc
      I L A K V F Y G E N

      490             510             530
      aactaagcaagttaatgatatgctcaagaatatatatctattgtgagctttttttatggt
      550             570             590
      ctcacctgagtggttgagactatgttttcgtttgaatattatactgtgttttattatgtg
      610             630             650
      ttttgaatattcataatgagaataaagggcccaattgaattattggccaatatgtaatgat
      670
      acataaatttcgtgattgagt
  
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Fig. 2. DNA and protein sequence of the rubber elongation factor (REF) gene cloned in pREF1. A putative polyadenylation signal element is underlined.

immediately upstream of the GCT, which encodes the amino terminal Ala of the mature peptide. A further 38 nucleotides are located upstream of the ATG. The coding region is followed by a 3' untranslated region of 221 bp. A putative polyadenylation signal (AATAAA) is located 170 nucleotides downstream of the stop codon.

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