to be identical between S. UTMP and H. SCCA. The tripeptide sequence Pro–Phe–Leu happens to be a part of the SERPIN signature site of both, S. UTMP and H. SCCA. On the other hand, the sequence of P. UFAP, which is known to be similar to S. UTMP, was found to have only 9.35% identity with H. PZP even though both are pregnancy-associated proteins having SERPIN activity.

In the present study, the overall identity of sequences of pregnancy-associated proteins with SERPINs and of a pregnancy-associated protein with a carcinoma antigen were quite significant. An unrelated protein c-myb oncoprotein used as a control, when compared with the pregnancy-associated proteins, was found to have identities of only 3.96%, 4.32% and 5.30% with sheep uterine milk protein, pig uteroferrin-associated protein and human pregnancy zone protein, respectively. This relationship is not only statistically significant but also important biologically.

Similarities between pairs of protein sequences are potentially important because they may indicate some functional, structural or evolutionary relationship between the proteins. Biological significance of this kind does not necessarily imply a strong statistical significance.

A low statistical significance does not imply that a similarity is not biologically important; on the other hand, a similarity which is very improbable does imply that sequences are related, even if this relationship is not yet understood. The main value of assessment of the statistical significance of a similarity is therefore to provide additional evidence that a biological relationship exists[1].

The immunoglobulin superfamily includes certain receptor proteins like the PDGF receptor. The sequence resemblances in members of this family are very low, often amounting to as few as 10 or 12 residues per 100, but they are consistent enough across a large number of comparisons that the statistical likelihood is compelling.

In the case of G-protein (transducer)-linked receptor proteins, the per cent identity of the most distant pairs is well below 25, however, all these proteins appear to have seven segments rich in hydrophobic amino acids. In case of the most distant members of the group, the major evidence for divergence is not statistical, it is physiological[1].

The present findings support the hypothesis proposed above as well as the possibility of a common mechanism of evasion of host immune response in tumour and pregnancy since the immune-mediated lysis of target cells in both involves serine protease-based lytic systems.

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HARI MOHAN SAXENA

Department of Veterinary Microbiology,
College of Veterinary Science,
Punjab Agricultural University,
Ludhiana 141 004, India

A rapid and efficient method for isolation of RNA from bark tissues of *Hevea brasiliensis*

Efficient extraction of high quality RNA from a variety of plant tissues including bark is very important and is the first step in many molecular biology techniques, such as analysis of gene expression, cDNA library construction, *in vitro* translation, etc. Preparation of high quality RNA from tissues containing high levels of polyphenols such as bark of *Hevea brasiliensis* is difficult because of RNA degradation mediated by secondary plant products like phenolics, terpenoids and tannins which may bind to RNA after cell lysis. In the recent past, different protocols have been described for the isolation of RNA from various tissues[1-4] except bark tissues. When we tried to apply these methods for the isolation of RNA from *Hevea* bark tissues, they failed either due to degradation or very low yield of the RNA. RNA degradation may be due to the increase of RNAses and RNA degrading compounds (like polyphenols) in wounded slices[2,5]. The low yield of RNA might be due to the fact that after wounding, insoluble starch is converted into soluble polysaccharides which contaminate the RNA as they display very similar physicochemical properties[6].

Hevea brasiliensis is the commercial source of natural rubber, *cis*-1,4 polyisoprene, present in latex, the milky cytoplasm of specialized cells called laticifers located adjacent to phloem vessels. The latex is collected by the controlled wounding of the bark of the main trunk. Upon wounding, the cytoplasmic contents of these cells are expelled and sealing of wound sites occurs by coagulation of flowing latex. The extensive tapping of latex causes certain disorders in the bark characterized by browning and drying followed by cessation of latex flow, a physiological disorder called tapping panel dryness (TPD). There is an urgent need to study the molecular mechanism of wound healing, the stability of bark tissues during latex tapping, control of tapping panel dryness, etc. in the bark of *H. brasiliensis*. A pre-requisite for successful use of the molecular biology techniques to study the function of bark is a simple and reliable method for the isolation of RNA from bark tissues. There were a few reports on RNA isolation and cDNA library construction in *Hevea* in the past[7-10]. However, in all these reports RNAs were isolated either from latex or leaf. To our knowledge there are no reports on RNA isolation from bark tissues in general and *Hevea* in particular. We, therefore, attempted to develop a simple and efficient method for the isolation of large quantities of good quality and undegraded RNA from wounded *Hevea* bark tissues.

Initially, four protocols published earlier were tried for the isolation of RNA from *Hevea* bark tissues. First, the method of Palmiter[1] was tried followed by the methods of Logemann *et al.*[2], Verwoerd *et al.*[3] and Shirzadegan *et al.*[4]. None of these protocols could yield good

quality RNA from *Hevea* bark tissues (Figure 1 *a*). So a modified protocol has been developed by combining certain steps from protocols developed by others. The method outlined in this report is a modification of protocols developed by Logemann *et al.*[2] and Shirzadegan *et al.*[4]. Our procedure is specifically designed to reduce the amount of insoluble polysaccharides and remove polyphenols by addition of polyvinyl polypyrrolidone. An efficient yield of RNA suitable for poly (A)$^+$ enrichment and analysis of less-abundant transcripts in *Hevea* bark tissues were also maintained. When compared with earlier published protocols, this is a simple and rapid method for isolating total cellular RNA especially from bark tissues. The other advantage is that most of the reagents and equipment required are found in labs performing even the most preliminary molecular biology experiments.

Fresh *Hevea* (var. RRII-105) bark tissues were collected from 15-year-old-trees at the Rubber Research Institute of India, Kerala and immediately washed with sterile diethyl pyrocarbonate (DEPC)-treated water and quickly frozen in liquid nitrogen before transportation to the laboratory. Bark tissues (1 g) were ground in liquid nitrogen to a fine powder using mortar and pestle and transferred into a polypropylene centrifuge tube containing 1 : 1 volume (20 ml) of extraction buffer (EB) and phenol (EB saturated). The extraction buffer contains 0.2 M NaCl, 0.1 M Tris-HCl (pH 7.0), 0.01 M EDTA, 1.5% SDS and 2% β-mercaptoethanol (added immediately before use). Solid polyvinyl polypyrrolidone (PVPP) was added to a final concentration of 1.5%. The homogenate was thoroughly mixed and centrifuged at 10,000 rpm for 15 min. The upper aqueous phase was transferred to a new tube and re-extracted once again with equal volume of chloroform and centrifuged for 15 min at 10,000 rpm. The aqueous phase is then mixed with 1/3 volume of 8 M LiCl and RNA was precipitated for 4 h at -20°C. Subsequently, RNA was pelleted by centrifugation for 20 min at 10,000 rpm. At this point the RNA pellet appears impure and requires at least one additional wash in 2 M LiCl and centrifugation for 15 min at 10,000 rpm. The soluble polysaccharides can be simply removed by the addition of equal volume of 100% distilled ethanol. After centrifugation (10,000 rpm; 15 min, 4°C), the supernatant was discarded completely. The pellet was dried and dissolved in 500 µl sterile water. The RNAs were concentrated by precipitation with 0.1 volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of 100% distilled ethanol. The precipitated RNA was pelleted at 10,000 rpm for 10 min and washed twice with 70% ethanol and dried. The RNA pellet was subsequently dissolved in sterile water. RNA samples (2 µl) were loaded in a 1% formaldehyde denaturing agarose gel and electrophoresis was carried out at 25 V for 2 h in 0.5 × TBE buffer. The gel was stained with ethidium bromide and viewed under UV transilluminator. The transfer of RNA to Hybond-N$^+$ nylon membrane (Amersham, London, UK) and conditions of prehybridization, and hybridization of labelled DNA (non-radioactive labelling) to RNA blots were performed as per manufacturer's protocol. After hybridization, blots were washed and exposed to X-ray film as recommended by the manufacturer (Amersham, London, UK).

In the present method, the highest quality of RNA results from the extraction of *Hevea* bark samples with extraction buffer containing a high concentration of sodium chloride. As described above, this isolation procedure has the advantage of disrupting whole tissue samples rapidly and completely, while simultaneously inactivating RNAse, even when it is present in greater abundance. The nuclear and organelle

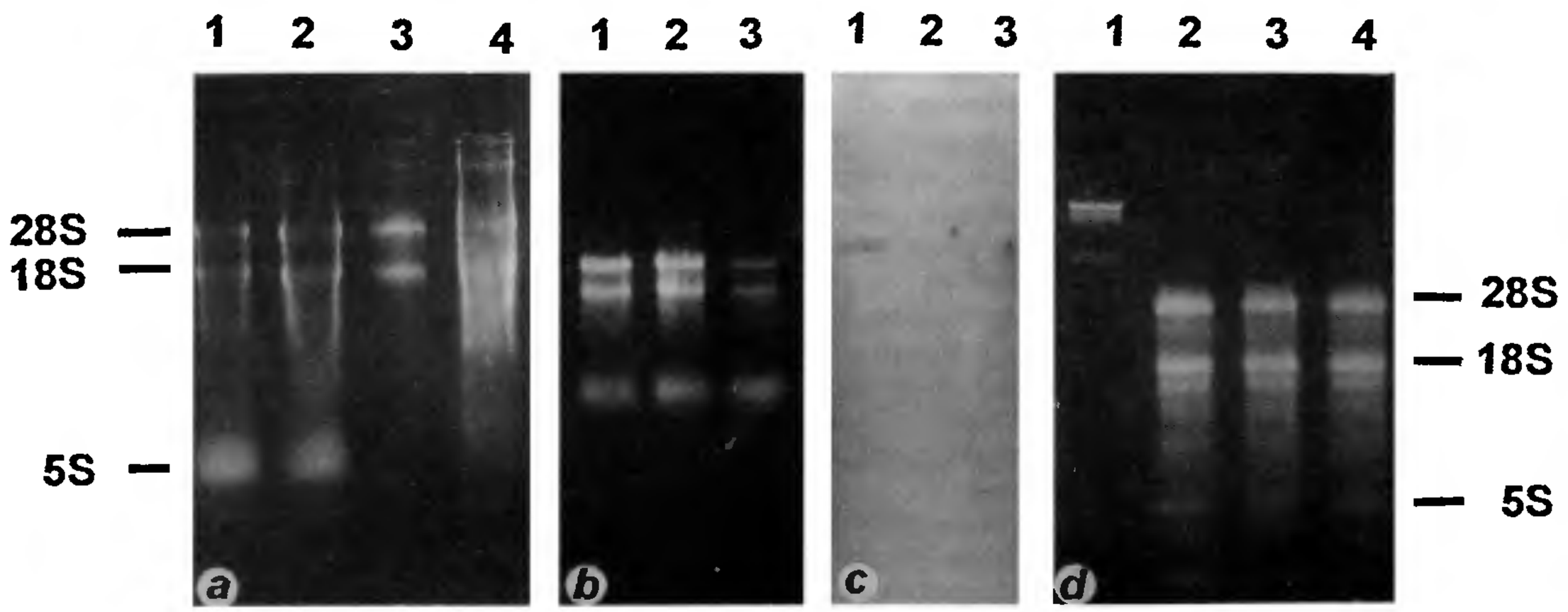

Figure 1. *a*, Agarose (1%) formaldehyde denaturing gel containing RNA using previously published protocols. Lane 1, Palmiter[1]; lane 2, Logemann *et al.*[2]; lane 3, Verwoerd *et al.*[3]; lane 4, Shirzadegan *et al.*[4]; *b*, Agarose (1%) formaldehyde denaturing gel containing RNA isolated from bark tissues using the modified method. Lane 1, Normal bark tissues; lane 2, Bark tissues partially affected by TPD; and lane 3, Bark tissues completely affected by TPD; *c*, Northern blot hybridization with *Hevea* specific probe. Signal present in lane 1 and no signal observed in lanes 2 and 3. *d*, Agarose (1%) formaldehyde denaturing gel electrophoresis of RNA extracted from latex using the modified method. Lane 1, Molecular marker; and lanes 2–4, RNA from latex of different trees.

disruptions that accompany membrane solubilization liberates nuclear RNA and genomic DNA, both of which copurify with cytoplasmic RNA species. Isolating intact plant RNA is not possible with high levels of endogenous active RNAses in *Hevea* tissues[11], which leads to RNA degradation during isolation. We have overcome this problem by using RNAse inhibitors including chelators such as EDTA and ionic detergents such as sodium dodecyl sulfate (SDS) in the EB. The efficiency of protein denaturation (including disruption of RNAses) may be enhanced by the inclusion of β-mercaptoethanol, a reducing agent which breaks intramolecular protein disulfide bonds. The addition of exogenous RNAse inhibitors such as guanidinium thiocyanate and vanadyl ribonucleoside complexes are not required in the present protocol. SDS is a key component used to liberate the nucleic acids, because of its ability to inhibit RNAse and deoxyribonuclease (DNAse) activity. Incorporation of an extraction buffer prepared by mixing EB-saturated phenol with chloroform will cause a partitioning of RNA into the aqueous phase. Phenol and chloroform are organic solvents that very efficiently denature and cause the precipitation of proteins. Chloroform stabilizes the phenol, imparts a greater density to this organic extracting material, improves the efficiency of deproteinization of the sample and facilitates removal of lipids from the RNA preparation. Large RNAs were efficiently precipitated with LiCl. The contaminated polysaccharides were removed by addition of one volume of ethanol. Then RNA was concentrated by precipitation with sodium acetate (pH 5.2) and ethanol and collected after centrifugation. The salt was removed by a final wash with 70% ethanol and the RNA pellet was dissolved in sterile water (DEPC-treated). The optical density of the RNA obtained using this method was around 1.8 (A_{260}) (55 µg/100 mg bark tissue). The quality of RNA and its contamination by DNA were checked by gel electrophoresis in denaturating formaldehyde gels. Figure 1 *b* shows the presence of two narrow bands due to the 28S and 18S RNA, indicating good quality of intact RNA. The 5S RNA comigrate at the leading edge of the gel and usually appear as an indiscrete splotch. The intactness of specific RNA species was further examined by Northern blot hybridization using a specific probe developed in *Hevea* (Figure 1 *c*). RNAs isolated by our method gives discrete signal in Northern blot, indicating that they are not degraded.

We have described a simple, quick and relatively inexpensive method for plant RNA isolation from *Hevea* bark tissues. Adequate volumes of EB and EB-saturated phenol should be made up fresh before use because oxidation of phenol can greatly compromise the quality of RNA preparation. The concentrations of ingredients present in the EB lead to significant improvements in yield and quality of RNA. In conclusion, the modified method presented here can be used for the isolation of intact RNA with high yield not only from bark samples but also from other tissues and latex of *Hevea* in a short period (Figure 1 *d*). It is well suited for obtaining RNA from tissues with high polyphenol content.

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P. VENKATACHALAM
I. THANSEEM
A. THULASEEDHARAN

Biotechnology Division,
Rubber Research Institute of India,
Kottayam 686 009, India

Molt-related limb loss in *Macrobrachium nobilii*

In crustaceans, intraspecific predation in communal culture under high stocking densities and among field population is quite typical[1,2]. Unsuccessful predation leads to reflex severance of one or more limbs during aggressive and agonistic interactions between conspecifics[3]. Factors such as predator–prey encounters, interactions while competing for limited resources like food, shelter and mate[4–6], season and density[7] contribute to limb loss. However, there is a paucity of literature on autotomy induced during the process of molt. In freshwater prawn, *Macrobrachium rosenbergii*[1] and the crayfish, *Procambarus clarkii*[7,8] the loss of appendages in freshly molted individuals is quite common since they are more vulnerable in any encounter. In cultured *Homarus* spp., when fed with exclusive purified diet a molt death syndrome was prevalent due to partial molting and the individuals died either during a molt or subsequently with deformed appendages[9]. Limb loss may also be resorted as a growth strategy before an ensuing molt as reported in blue claw males of *M. rosenbergii*[10]. Such incidence also occurs when individuals cannot postpone an obligatory molt further[11], which may even result in the total loss of reproductive effort in incubating *M. nobilii*[12]. Here we report the unusual incidence of limb autotomy associated with partially shed exuvium in *M. nobilii* even when reared individually under ideal conditions.