Nobel Prize for Medicine or Physiology

A long due Nobel Prize for the discovery of splicing of eukaryotic mRNAs was finally awarded this year to Phillip A. Sharp of Massachusetts Institute of Technology, Cambridge, USA, and Richard J. Roberts of the Cold Spring Harbor Laboratories, New York, USA (currently at the New England Biolabs, Beverly, USA, where his research interests are in the field of restriction/modification systems). In 1977, research groups of these scientists analysed hybrids of late mRNAs of adenovirus 2 with its genome by R-looping[1,2]. The electron microscopic images of the hybrids revealed an unusual phenomenon in that the adjacent sequences of a mRNA hybridized to discontinuous stretches of DNA, leaving an intervening region of DNA which did not hybridize. This observation was fundamentally different from what was known for prokaryotic systems and gave rise to the concept of splicing. Splicing involves breaking and making of the phosphodiester bonds within the mRNA precursors to remove intervening sequences (intron) and joining of the two flanking regions (exons) present in the mature *m*RNA. In the same year research groups of D. S. Hogness, I. B. David and N. Davidson found that the longer genes of *Drosophila* 28 S rRNA also contained interruptions which were not present in rRNA. However, because of the multiplicity of the rRNA genes it was not clear if the longer genes were expressed. The presence of introns in β-globin, ovalbumin genes and tRNA genes was also discovered in 1977 by P. Chambon, P. Leder, R. A. Flavell and B. D. Hall and their colleagues. Soon it became clear that eukaryotic genes in general possessed introns. Chambon's group compiled sequences of the boundaries of introns from a large number of protein coding eukaryotic genes which revealed the presence of consensus sequences at the intron exon junctions. Of these, GT was always found at the 5′ side of the intron (left splice junction) and AG at the 3′ (right splice junction). This became popularly known as GT–AG or Chambons's rule. These sequences are so important that many of the genetic diseases such as thalassemias could be readily attributed to point mutations which created or abolished these splice junctions. Sharp's laboratory (along with other groups) has since then played a pivotal role in elucidating the machanism of the splicing reaction. Splicing reaction is energy-dependent and occurs within a complex called spliceosome. Spliceosomes are constituted of many proteins and small nuclear RNAs (snRNAs) which play a significant role in recognition of the splice junctions and a pyrimidine-rich conserved region present just upstream of the right splice junction (branch site or TACTAAC box). As shown in Figure 1, the splicing process starts with breaking of the phosphodiester bond 5′ to the conserved G of the intron at left junction (as a result of nucleophilic attack from 2′ –OH of the conserved A residue within the branch site) and formation of an unusual phosphodiester bond (5′–2′) between the G and the A residues. This is then followed by a nucleophilic attack from the 3′ -OH of the last residue of the left exon onto the phosphorus centre of the phosphodiester bond between the last residue (G) of the intron and the first residue of the right exon. As a result, the intron is released in the form of a circle with a tail (lariat structure). Splicing in *C. elegans* and trypanosomes has revealed another very interesting phenomenon of trans–splicing where mature mRNA is obtained by splicing of sequences from two separate transcipts, one contributing to upstream

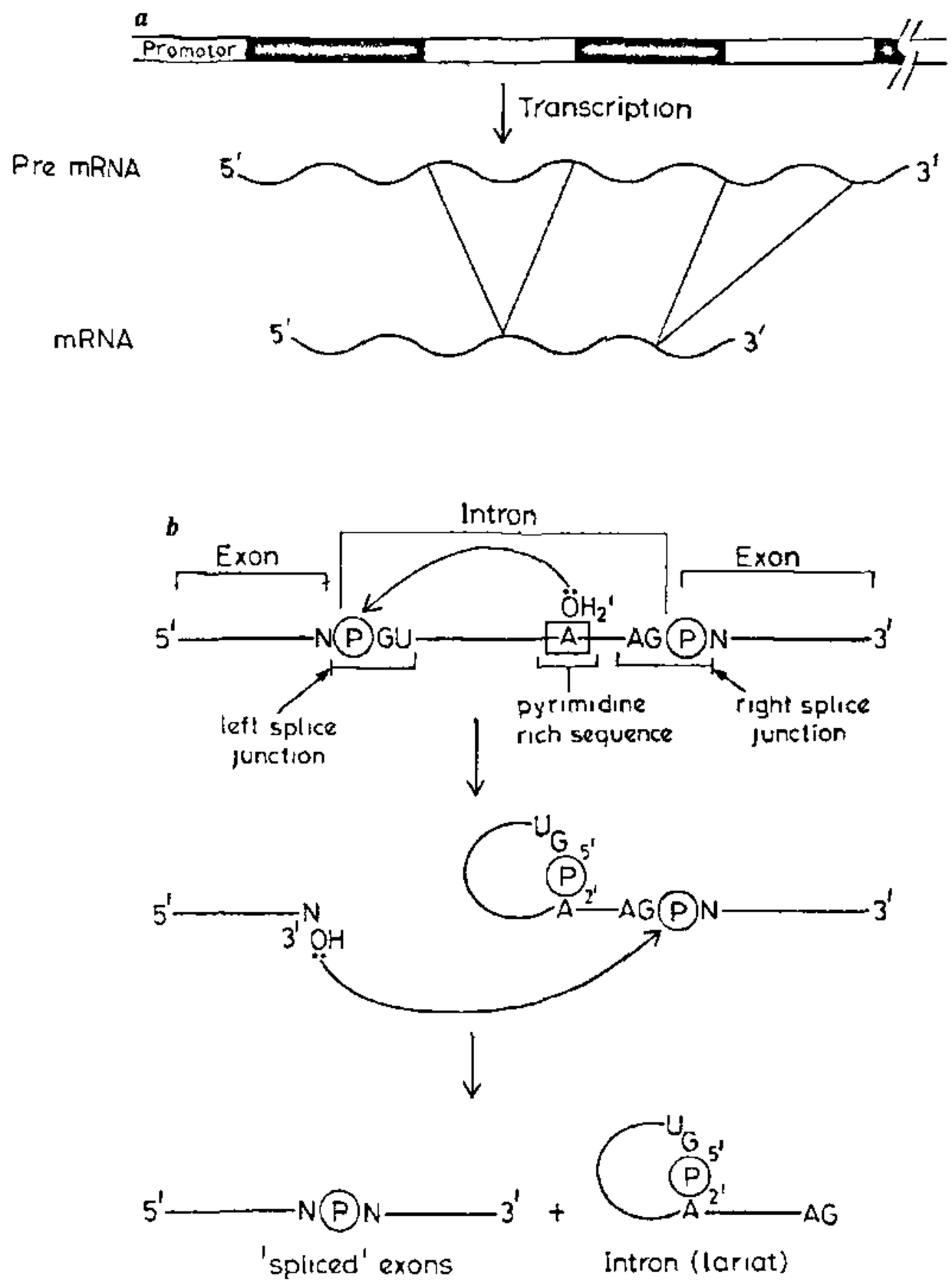

Figure 1. ***a***, Biogenesis of eukaryotic mRNA by splicing. Processing at the 5′- and the 3′-untranslated regions is not shown. Introns from a transcript are not necessarily removed in the 5′ to 3′ order. ***b***, Mechanism of splicing. As shown, during cis-splicing the introns are removed as a lariat. During trans-splicing, as the intron sequences are contained in two separate transcripts, the circle of the lariat is not covalently closed resulting in their excision as a Y-shaped molecule (*not shown*).

exon and the left half of the intron and the other to the right half of the intron and the downstream exon. A Y-shaped molecule representing intron can be detected in splicing reactions *in vitro*, which suggests commonalties of the steps in cis- and trans-splicing. Differential splicing of the mRNA precursors containing multiple exons has been observed during different stages of development or in a tissue-specific manner in several systems. For example, membrane-bound and secreted forms of immunoglobulins are generated as a result of differential splicing of exons. Thus the process of splicing is vital for eukaryotic gene expression. Further, the phenomenon of splicing is widespread in that not only the other classes of eukaryotic RNAs (rRNA and tRNA) but also the prokaryotic genes contain introns. The presence of introns in the genes may be a consequence of evolution. Different domains of the existing genes may have been put together in different combinations and the transcripts spliced to generate mature RNA coding for a new function.

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2. Chow, L. T., Gelinas, R. E. Broker, T. R. and Roberts, R. J., *Cell*, 1977, **12**, 1–8.

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SCIENTIFIC CORRESPONDENCE

Comparative chemical analysis of oleogum resin of *Commiphora wightii* Arnott. Bhandari

Commiphora wightii (Arnott). Bhandari (Syn. *Commiphora mukul*) of the family of Burseraceae is an important medicinal plant. The plant is generally distributed in arid regions, particularly on the Indian side of the Thar desert[1]. The present communication describes analytical data of guggal (the oleogum resin) of different agroclimatic zones of Rajasthan.

The petroleum ether extractive values were determined as mentioned in *British Pharmacoepia*[2]. Nitrogen was estimated by the Kjheldahl method using a Khel autonitrogen analyser. Essential oils were determined by the method described by Indian Standards Institute[3]. To estimate guggalipids, one gram of guggal was macerated in 25 ml of ethyl acetate in a mortar and filtered. The process was repeated several times. The pooled ethyl acetate fractions were then concentrated under vaccum and weighed to obtain guggalipids (ethyl acetate soluble material). The separation of E- and Z-guggalsterones was carried out according to Sukhdev (a personal communication) with slight modifications by HPLC on μ bond C_{18} (RCSS) (10 mm × 8 cm column) using 35% water/methanol as mobile phase, at a flow rate of 1 ml min^{-1}. Sterones were monitored at 254 nm. For quantitative analysis, E & Z-guggalsterones were used as standards.

Analytical data are presented in Table 1. Essential oils, petroleum ether extractive values and nitrogen exhibited variation depending on the place of collection. The minor amount of nitrogen has been established for gum exudates of several genera[4]. In the present study, highest guggalipids (47.55%); E-guggalsterone (0.99%), Z-guggalsterone (2.45%) and combined E- and Z-guggalsterones (3.47%) were in guggal from Udaipurwati. Because of the non-availability of standards, other steroids could not be quantified. Guggalipids have been reported as hypolipidemic agent. Ketonic fraction of guggalipids has significant lipid-lowering activity and in this fraction E- and Z-guggalsterones are mainly responsible for hypolipidemic activity. An earlier reported[5] concentration of E- and Z-guggalsterones was 2%.

The results reveal that the guggal from Udaipurwati contained highest content of guggalipids and active ingredients mainly responsible for hypolipidemic activity. Hence, it seems to be superior in quality as compared to others. According to results the composition of the oleogum resin varies with geographic location, cli-

Table 1. Composition of oleogum resin of *Commiphora wightii* (Arnott) Bhandari collected from various places in Rajasthan

% of oleogum resin	Guggal herbal farm Mangliwas (Ajmer)	Forest of Nimkathana (Sikar)	Forest of Udaipurwati (Jhunjhunu)	Forest of Jaisalmer	Forest of Balotra (Barmer)
Ethyl acetate soluble material (guggalipids)	37.14	36.00	47.55	31.61	19.55
Petroleum ether extractive value	10.18	12.74	10.26	13.67	7.05
Essential oils	0.50	0.45	0.60	0.60	0.40
Nitrogen	0.25	0.24	0.22	0.30	0.25
E-guggalsterone	0.65	0.64	0.99	0.41	0.15
Z-guggalsterone	1.70	1.49	2.48	0.97	0.60
E + Z-guggalsterone	2.35	2.13	3.47	1.38	0.75