IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

BirdLife International/Paul Jepson

Burning forest, Tanimbar, Indonesia

tries in the top priority quadrat (sector I — high endemism and high deforestation) include threatened hotspots identified by earlier analyses[9–11], but our approach also highlights additional priority nations in central America and the Caribbean. In particular, we propose greatest importance should be given to countries like the Philippines and Haiti, which combine pronounced endemism and deforestation with very poorly developed networks of protected areas.

We stress that the present results are subject to several significant caveats. Key sites for one group of species do not always match those for other taxa[12]. Moreover, using national averages for deforestation and endemism may yield misleading priority ratings where large, heterogeneous countries contain smaller hotspots facing acute threats (see *b* in the figure for the example of Brazil). Our analyses also ignore the effects of forest fragmentation, which generally increases with overall loss[1]. Nevertheless, we believe our findings are of broad significance, both in revealing a worrying link between forest loss and biological value, and in helping to construct an agenda for remedial conservation action.

Andrew Balmford
Institute of Zoology,
Zoological Society of London,
Regent's Park,
London NW1 4RY, UK
Adrian Long
BirdLife International,
Wellbrook Court, Girton Road,
Cambridge CB3 0NA, UK

Unexpected activity of saporins

SIR — A group of plant proteins with antiviral activity inhibits protein synthesis by irreversibly damaging ribosomes, and hence are provisionally called ribosome-inactivating proteins (RIPs, reviewed in ref. 1). These proteins can be divided into type 1 RIPs, single-chain proteins and type 2 RIPs (ricin and related toxins), consisting of an active A chain bound to a B chain with the properties of a galactose-specific lectin. Type 1 RIPs, as well as the A chain of type 2 RIP, are *N*-glycosidases which depurinate ribosomal RNA by cleaving the *N*-glycosidic bond of a single adenine in a specific position of rRNA (A_{4324} of rat liver 28S rRNA)[2], although some RIPs cleave more than one adenine from rat liver rRNA[3]. We report here that saporin-L1, a RIP from the leaves of *Saponaria officinalis*[4], also releases adenine residues from various RNAs, from DNA and from poly(A), but not from ATP, adenosine or dATP.

EFFECT OF SAPORIN L-1 ON VARIOUS ADENINE CONTAINING SUBSTRATES

Substrate	Adenine released (pmol)
Poly(A)	2,038
Globin mRNA (rabbit reticulocytes)	1,587
DNA (herring sperm)	747
Bryonia dioica poly (A) – RNA	524
Escherichia coli rRNA (16S + 23S)	435
Saccharomyces cerevisiae $tRNA^{phe}$	425
Tobacco mosaic virus (TMV) genomic RNA	371
Bacteriophage MS 2 genomic RNA	336

Reaction mixtures were contained in a final volume of 50 ml 20 mM Tris–HCl buffer, pH 7.8, 100 mM ammonium chloride, 10 mM magnesium acetate, 0.07 μM saporin-L1 and substrate (10 μg RNA or poly (A), 12 μg DNA). Incubation was at 25 °C for 40 min. Adenine released was determined as in ref. 3. Ricin A chain under the present experimental conditions has no effect on *E. coli* rRNA, in agreement with Endo[2], who showed the release of one adenine residue per mol of each species of RNA (equivalent to 11.4 pmol in our experimental conditions) at much higher reactant concentrations than we used in our experimental system.

When assayed with bacteriophage MS 2 genomic RNA as substrate, 0.2 μM saporin-L1 released more than 130 mol adenine per mol MS 2 RNA. Saporin-L2 and saporin-R2 are half as active, whereas all other RIPs assayed (49 type 1 and the A chains of 4 type 2 RIPs) are inactive or release only traces of adenine from MS 2 RNA.

Saporin-L1 releases several hundred pmol adenine from various other RNAs, poly (A) and DNA (see table). The amount of adenine released from MS 2 and TMV RNA, and from poly(A), is proportional to the concentration of the enzyme. No bases are liberated from poly(C), poly(G) and poly(U) (as assayed spectrophotometrically, results not shown).

Thus our results demonstrate that the action of at least some saporins is not limited to rRNA, but is exerted on all tested ribo- and deoxyribopolynucleotides. Obviously, these RIPs do not release adenine only from the GAGA minimal sequence[5], and actually seem not to have specificity for any nucleotide sequence, although they do not act on adenosine or ATP. The effect of these proteins on globin messenger RNA and on transfer RNA suggests that they may inhibit protein synthesis by altering not only ribosomes, but also mRNA and tRNAs. These proteins would be more appropriately called polynucleotide: adenosine nucleosidases and the question arising from this is what is their natural substrate?

It has previously been supposed that RIPs can more easily enter cells damaged by viral infection, thus inactivating ribosomes, killing the infected cells, and arresting infection[6–9]. But our results show that saporins, at least, acting on substrates other than rRNA, could directly inhibit the replication of viruses by damaging their genomic or messenger RNA. Because these saporins depurinate TMV genomic RNA at concentrations similar to those present in plants, it is possible that this is a mechanism whereby these RIPs exert their antiviral activity in nature. It remains to be seen whether this is the only function of RIPs, or whether they have a role in normal plant metabolism.

Because of their effect on all types of RNA and DNA, these proteins would kill any cell, including bacteria. Thus in plant cells these RIPs would presumably either be segregated within the cytoplasm, or exist in an inactive form, or be kept inactive by an inhibitor.

Luigi Barbieri
Paola Gorini
Paola Valbonesi
Paola Castiglioni
Fiorenzo Stirpe
Dipartimento di Patologia sperimentale,
Università di Bologna,
I-40126 Bologna, Italy

1. Barbieri, L., Battelli, M. G. & Stirpe, F. *Biochim. biophys. Acta* **1154**, 237–282 (1993).
2. Endo, Y. in *Immunotoxins* (ed. Frankel, A. E.) 75–89 (Kluver, Boston, 1988).
3. Barbieri, L., Ferreras, J. M., Barraco, A., Ricci, P. & Stirpe, F. *Biochem. J.* **286**, 1–4 (1992).
4. Ferreras, J.M. *et al. Biochim. biophys. Acta* **1216**, 31–42 (1993)
5. Glück, A., Endo, Y. & Wool, I. G. *J. molec. Biol.* **226**, 411–424 (1992).
6. Ussery, M. A., Irvin, J. D. & Hardesty, B. *Ann. N. Y. Acad. Sci.* **284**, 431–440 (1977).
7. Foà-Tomasi, L., Campadelli-Fiume, G., Barbieri, L. & Stirpe, F. *Archs. Virol.* **71**, 323–332 (1982).
8. Ready, M., Brown, D. T. & Robertus, J. D. *Proc. natn. Acad. Sci.* U.S.A. **83**, 5053–5056 (1986).
9. Taylor, B. E. & Irvin, J. D. *FEBS Lett.* **273**, 144–146 (1990).