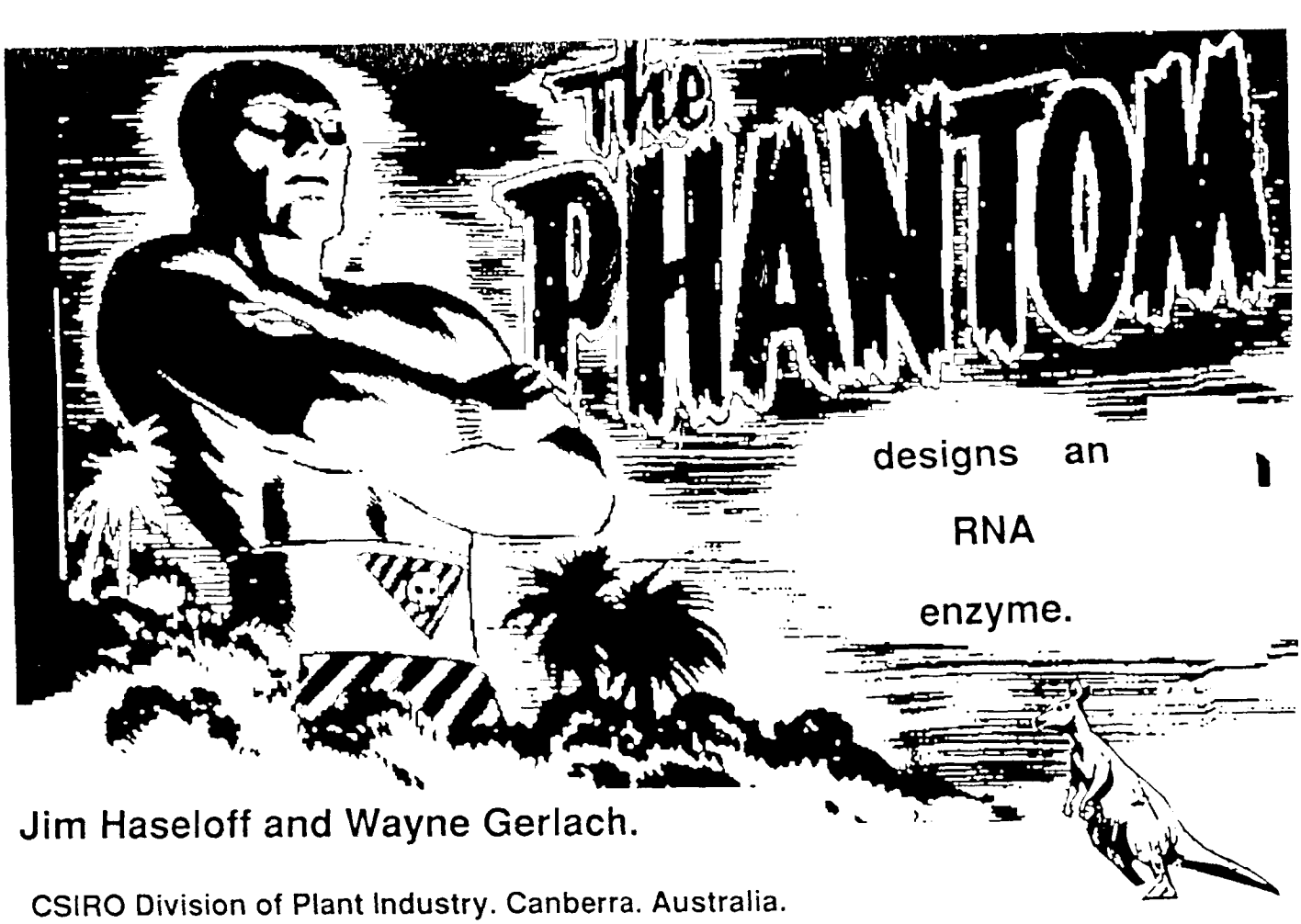
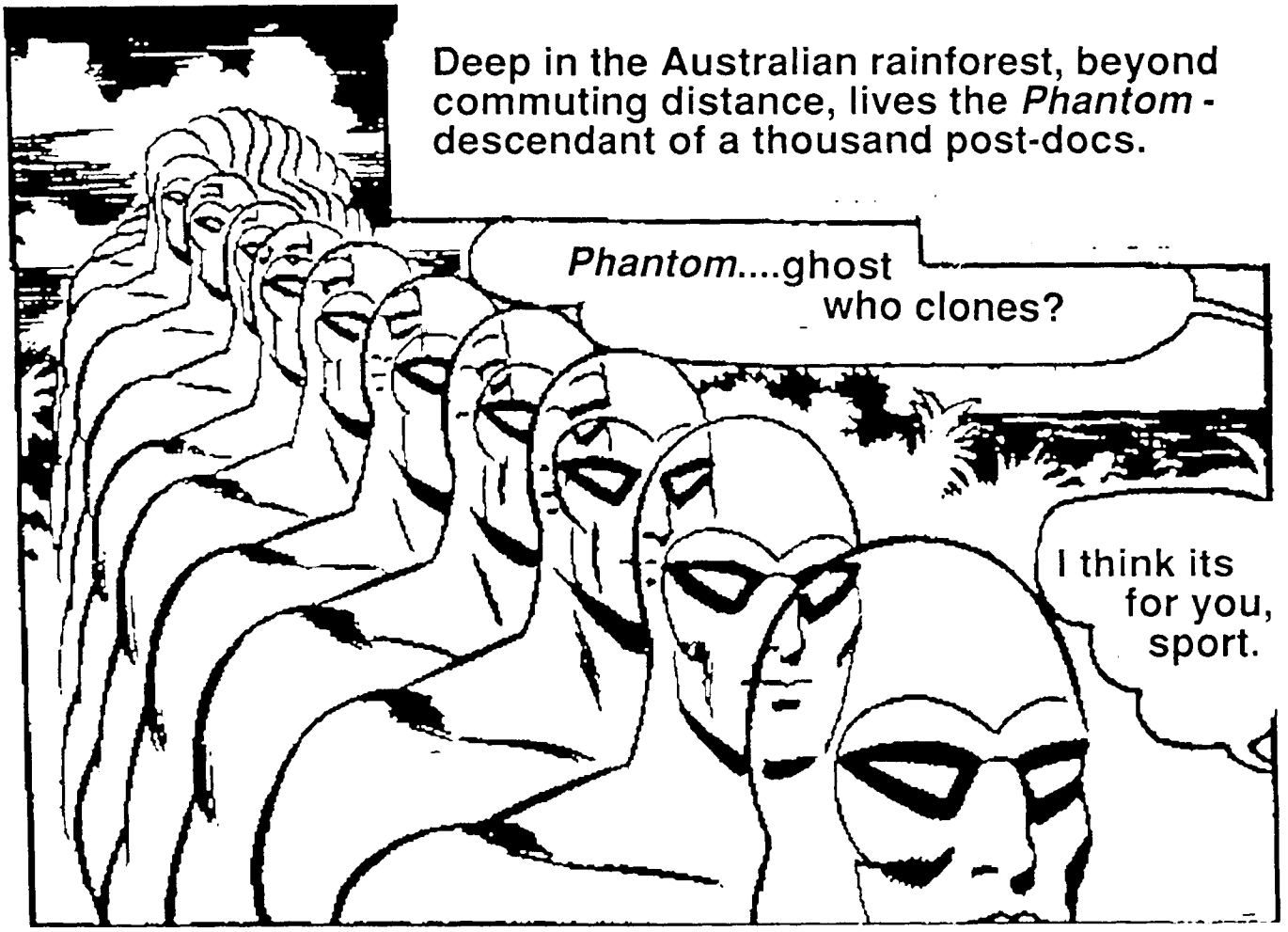
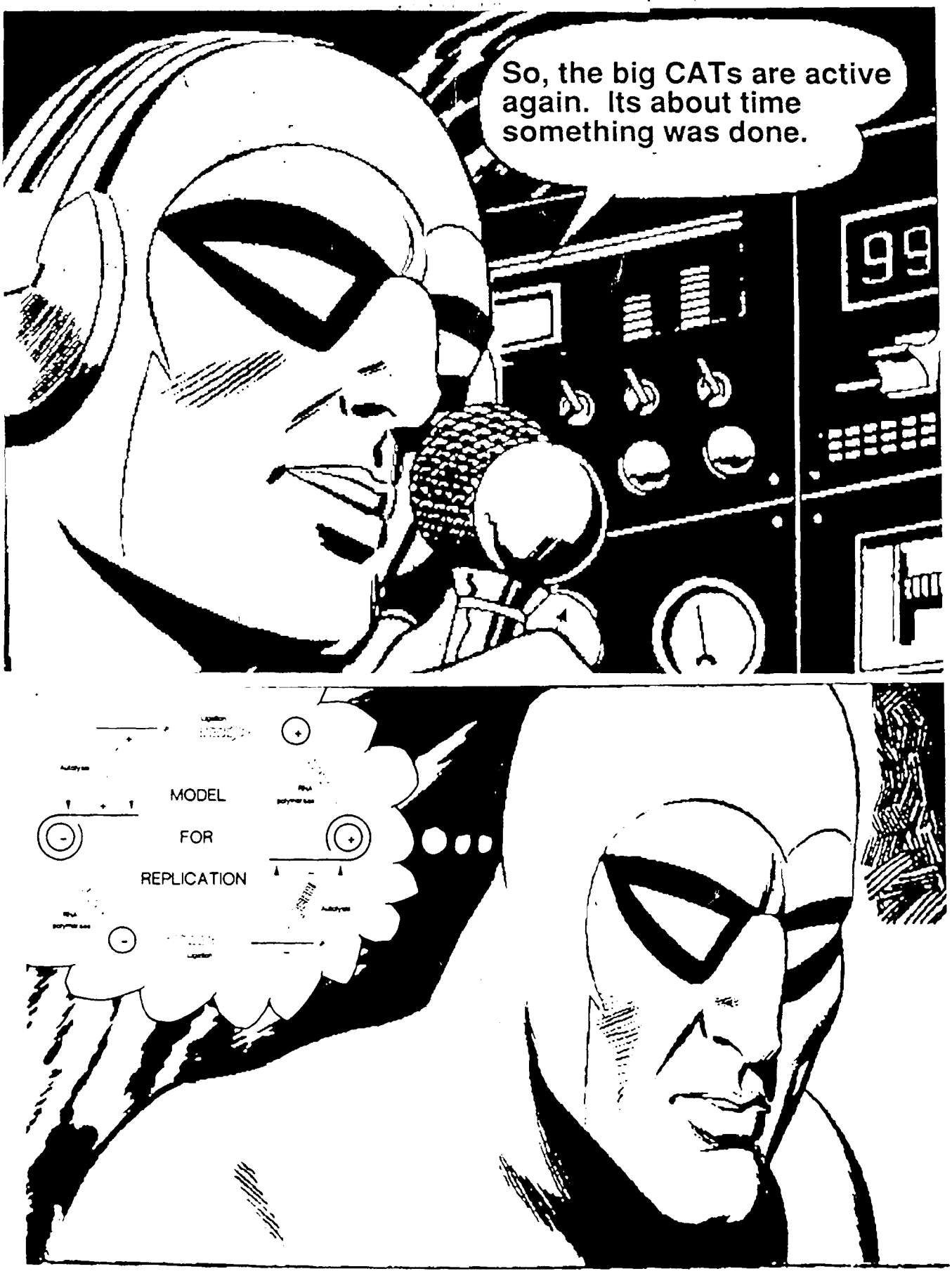


Jim Haseloff and Wayne Gerlach.

CSIRO Division of Plant Industry. Canberra. Australia.



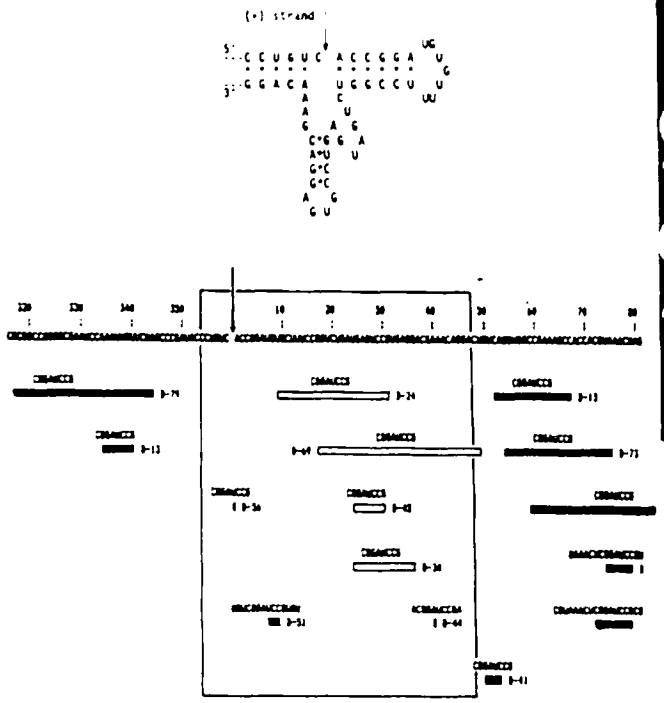
The *Phantom* receives an urgent call.....



The *Phantom* realizes that spontaneous RNA cleavage is a naturally occuring part of plant virus RNA replication. He wonders whether this mechanism might prove useful against the CAT menace.

Effect of *in vitro* mutations on self-cleavage.

■ : ✓ ▭ : ×

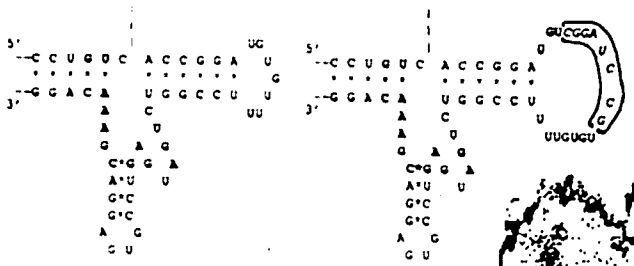


In vitro mutagenesis of the satellite RNA of tobacco ringspot virus revealed a secondary structure associated with RNA self-cleavage.

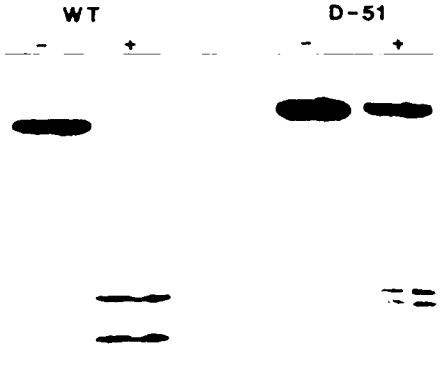
This secondary structure is conserved in other RNAs which undergo self-cleavage.

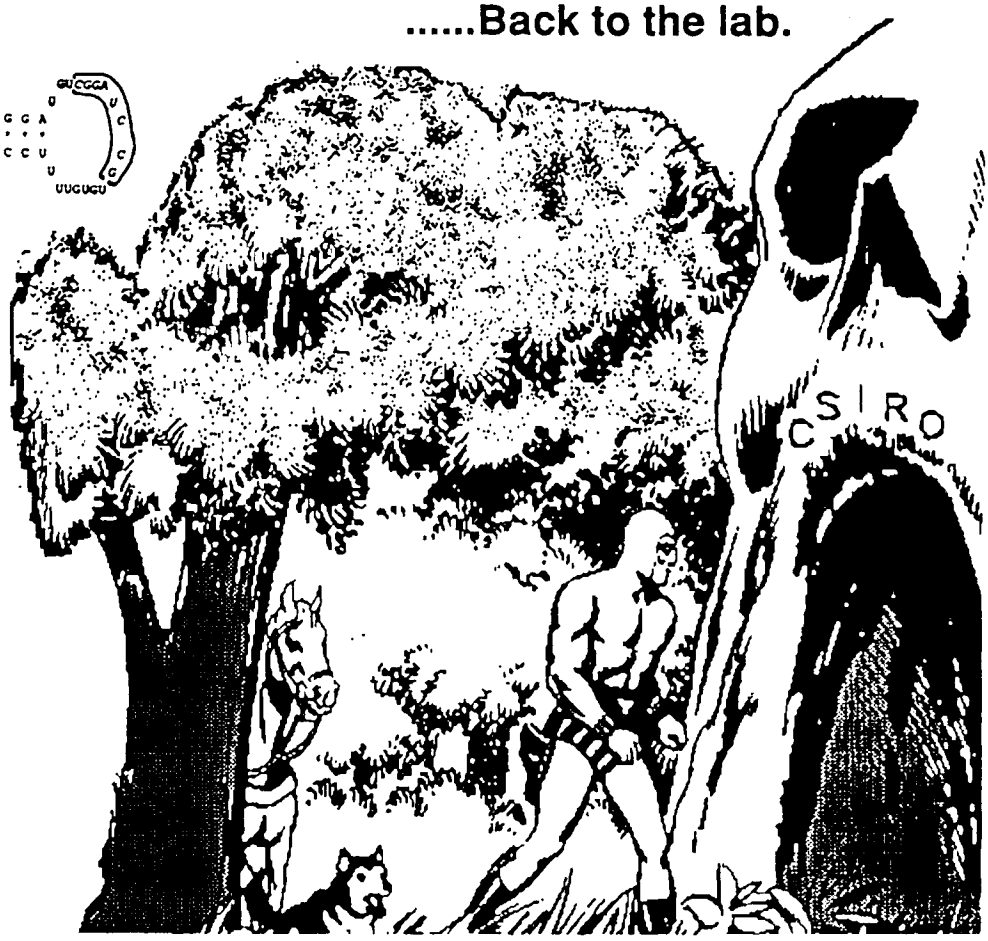
Yet....One of the *in vitro* mutants containing an alteration to this conserved structure still cleaves.

.....Back to the lab.



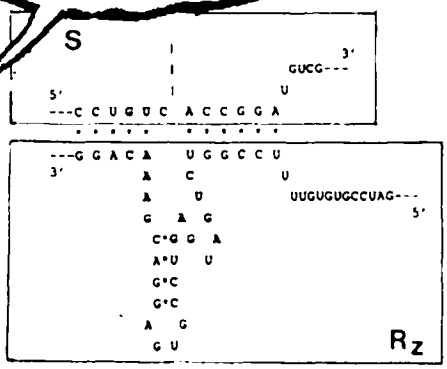
Self-catalysed cleavage of wild-type and D-51 mutant (+) strand tobacco ringspot virus satellite RNA.

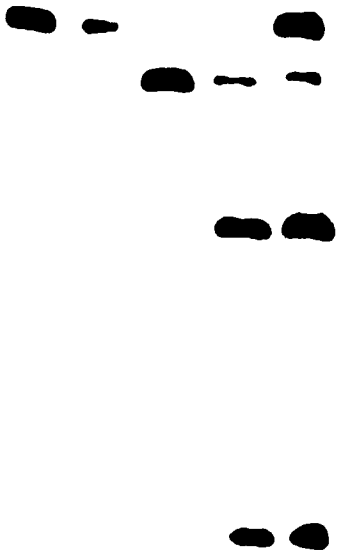


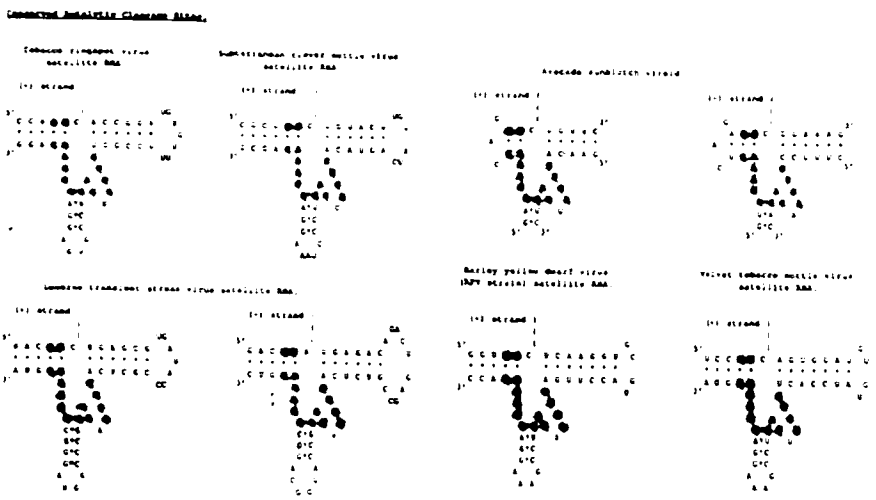


The phantom realizes that the mutated loop may not be involved in cleavage. He uses the D-51 mutant to obtain a separate RNA fragment containing the site for cleavage. When this is mixed with a fragment containing the remaining sequences, cleavage occurs.

R_z	R_z	-	R_z	R_z
-	S	S	S	S

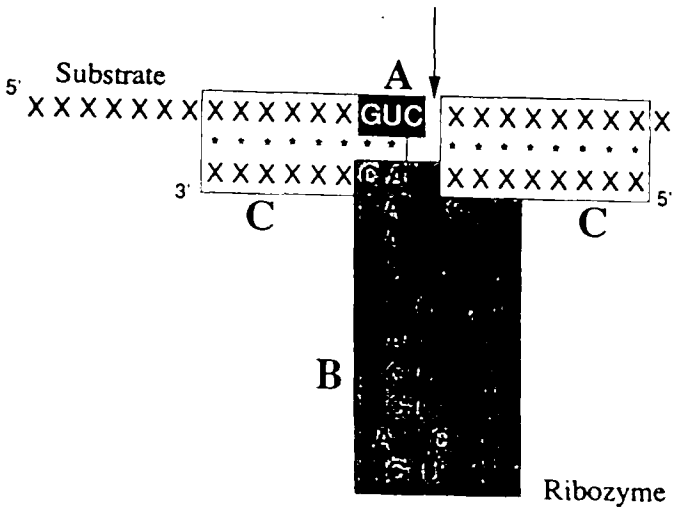


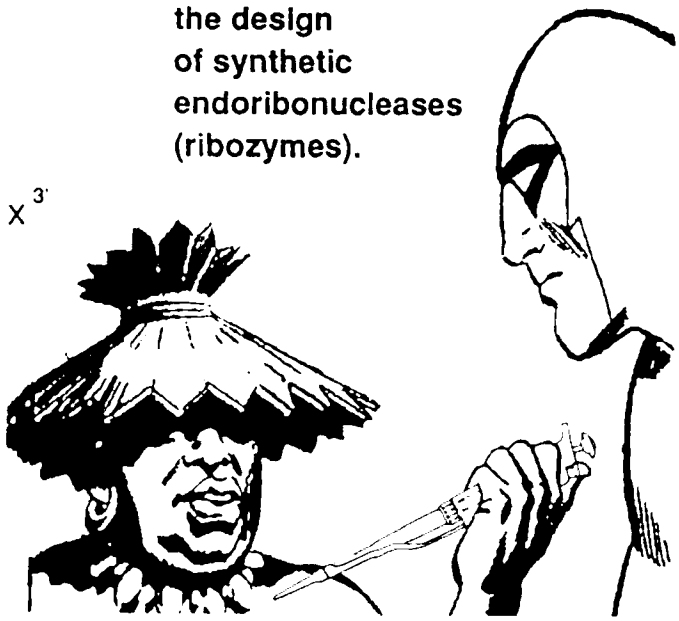




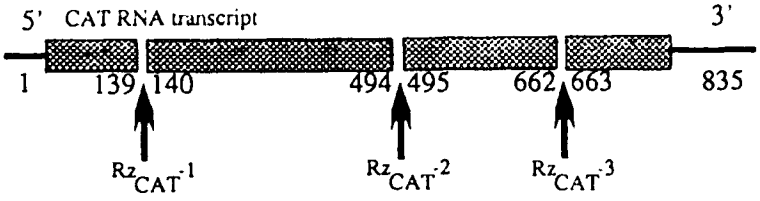
Similar highly conserved secondary structures are found associated with self-cleavage in other satellite and viroid RNAs. Some nucleotide sequences are highly conserved. However, only GU(C) is conserved at the site of cleavage. We can use this consensus as a rule..

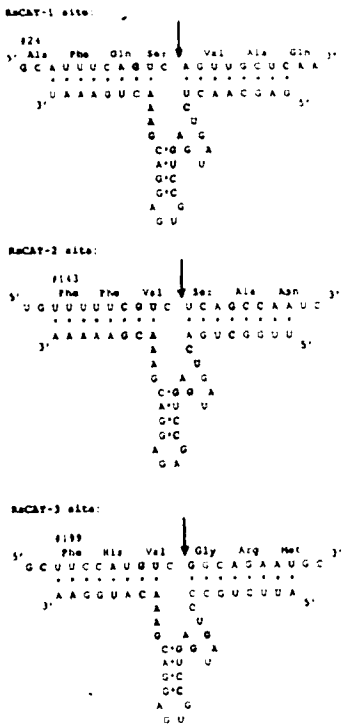
..for the design of synthetic endoribonucleases (ribozymes).





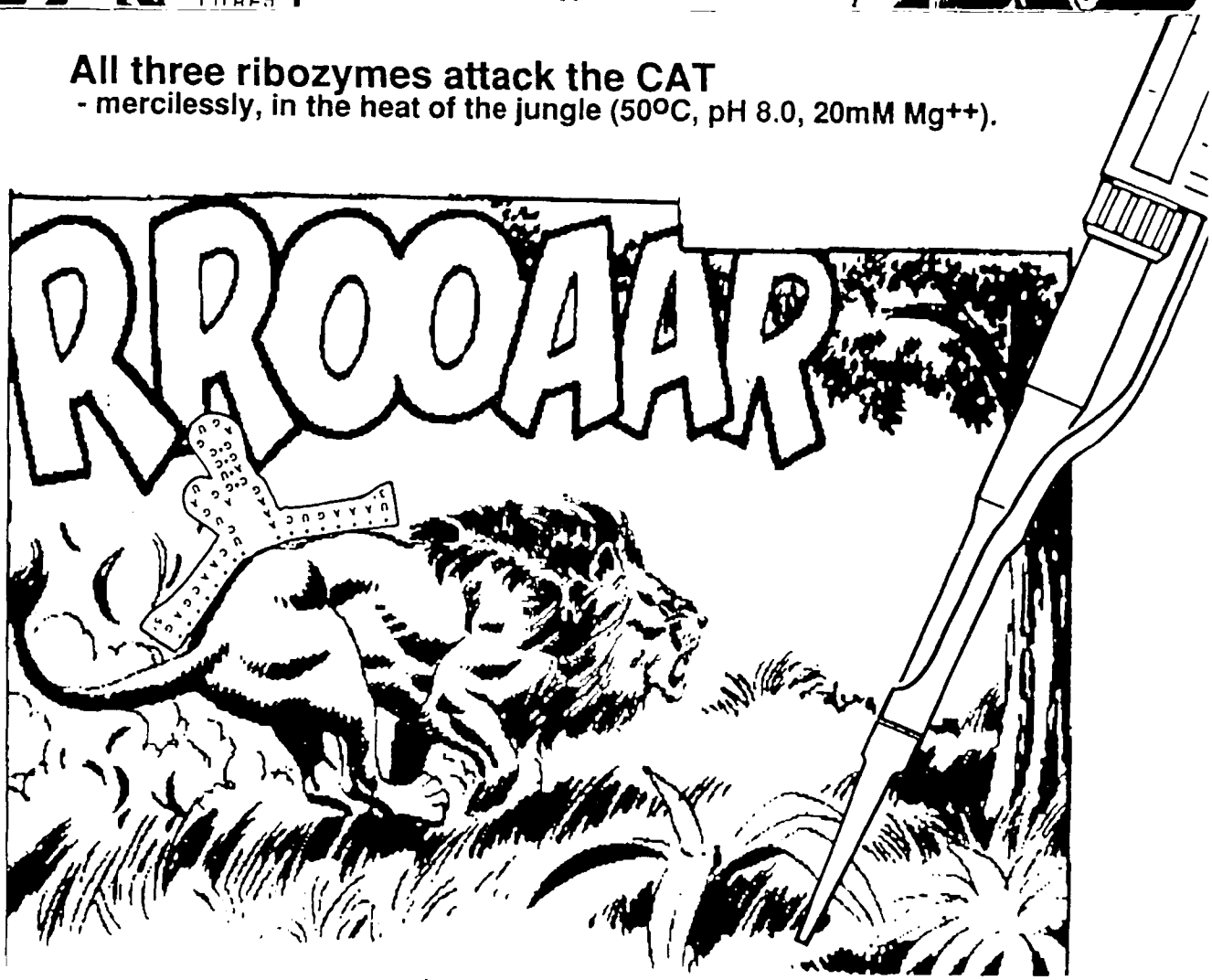
The phantom outlines his plans to inactivate the CAT gene mRNA



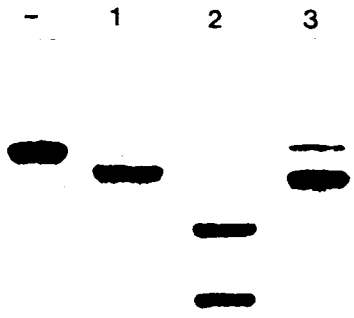


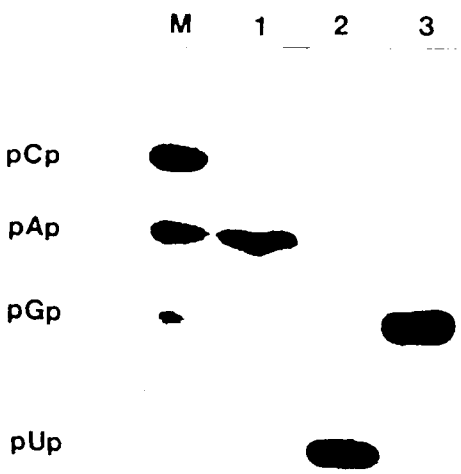
- designing three synthetic ribozymes to cut the transcript.

All three ribozymes attack the CAT
- mercilessly, in the heat of the jungle (50°C, pH 8.0, 20mM Mg++).



Cleavage of CAT transcripts by synthetic ribozymes.

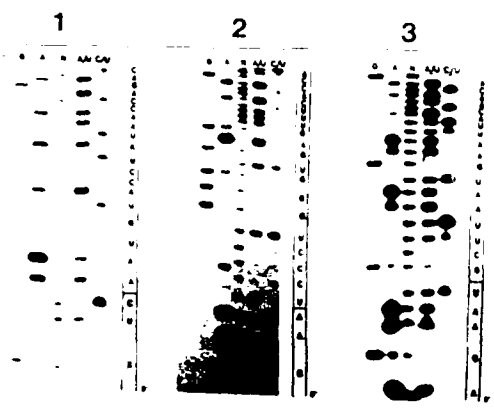




Checking the inactivated CATs - shows that all of the ribozymes have worked precisely as expected.

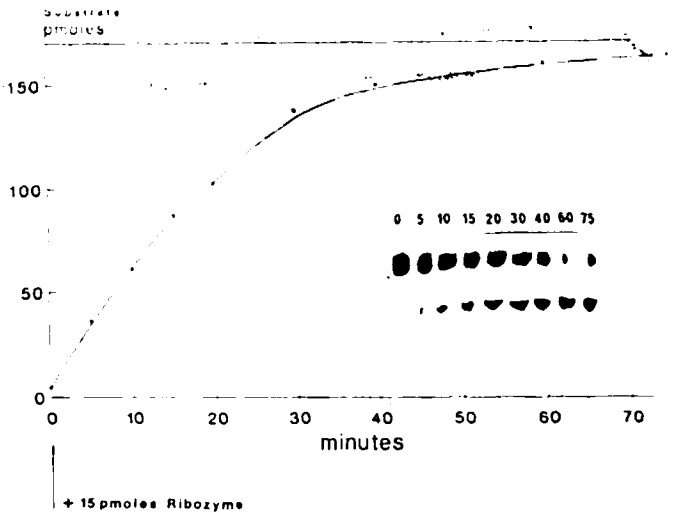
a. Terminal base analysis.

b. Terminal sequence analysis.

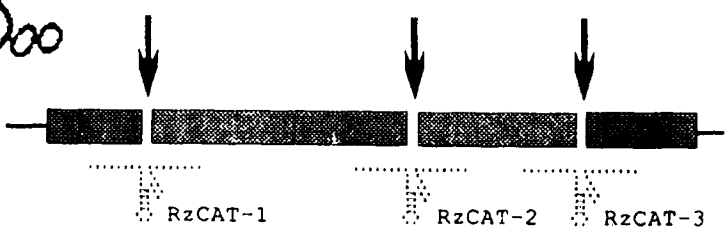


The ribozymes act as true RNA enzymes.

- catalyzing cleavage of multiple CAT substrates.



Rates of Cleavage are slower at lower temperature.

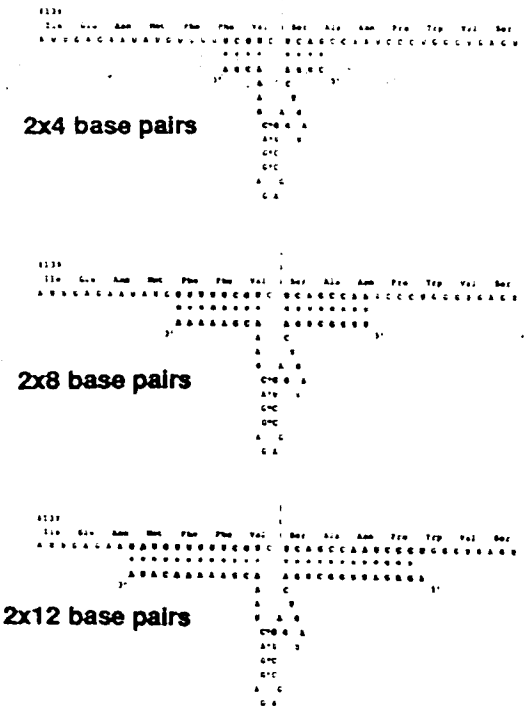


	$t_{1/2}$ (minutes		
50°C	3.5	3.5	2.5
37°C	55	70	65

But....

Increasing the length of base-pairing between ribozyme and substrate markedly increases the rate of cleavage.

EXTENT OF RIBOZYME-SUBSTRATE COMPLEMENTARITY.



$T_{1/2}$ (2x4) = no cleavage.

$T_{1/2}$ (2x8) = 70 mins @ 37°C

$T_{1/2}$ (2x12) = 10 mins @ 37°C

RzCAT-2

Trudging wearily home....the *Phantom* realizes that highly sequence-specific endoribonucleases can be made for use against any targetted host or virus RNA sequence. ....He ponders the potential of such ribozymes for *in vivo* inactivation of host or viral gene activities.