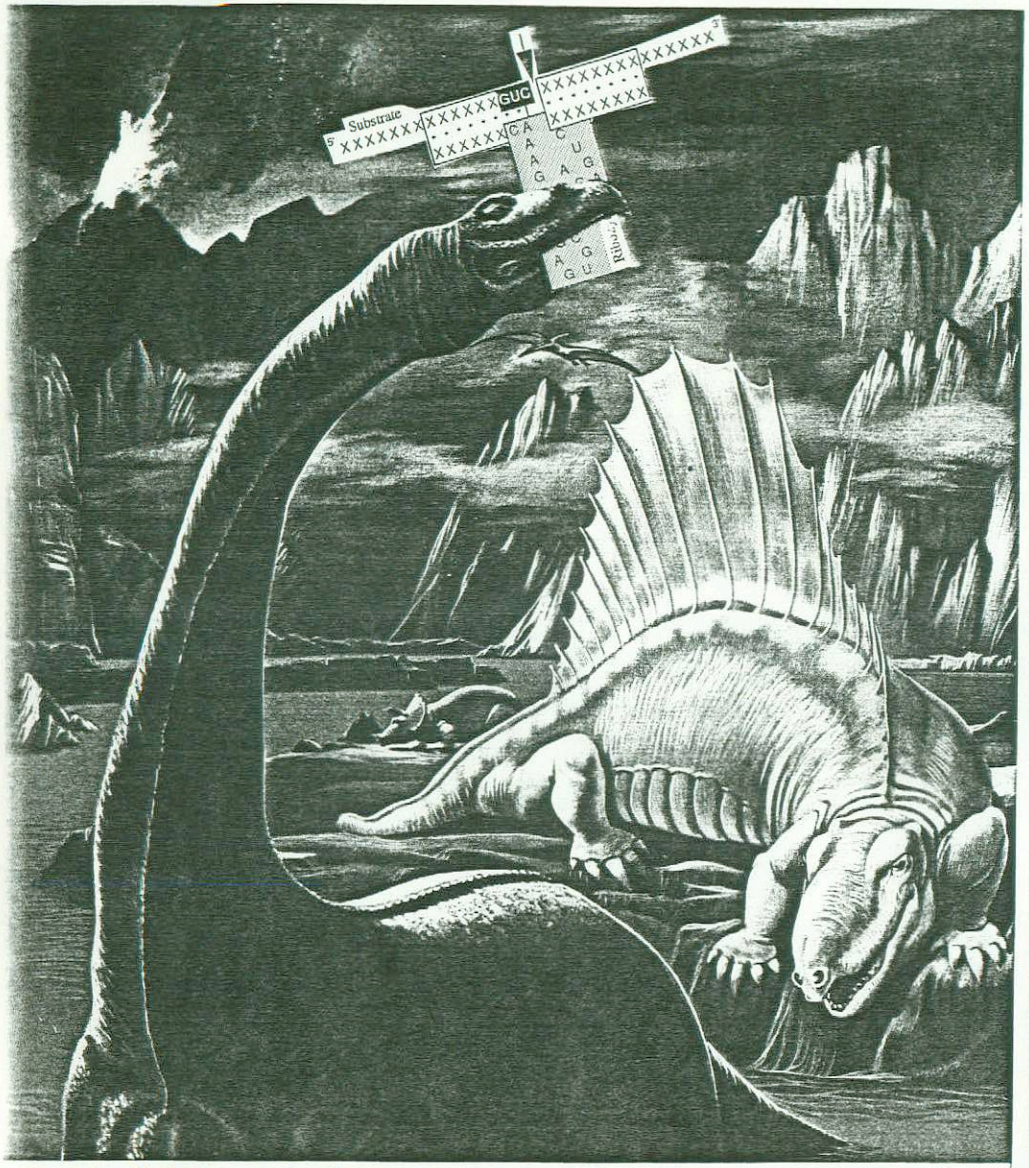
The
HOW
AND
WHY
wonder book
of

Trans-splicing ribozymes.

Jim Haseloff , Andrea Brand*, Norbert Perrimon* and Howard Goodman
(1). Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.
*(2). Department of Genetics, Harvard Medical School, Boston, MA 02115.



Synthetic RNA endoribonuclease activities can be designed to efficiently cleave chosen RNAs *in vitro*.

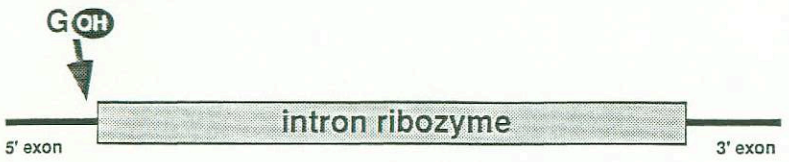
Application of such activities *in vivo* would result in disruption of gene expression. However, such ribozymes have given equivocal results *in vivo*.

In an alternate approach, ribozymes capable of self-catalyzed trans-splicing can be designed to insert new 3' exon sequences into a chosen target RNA in a precise manner.

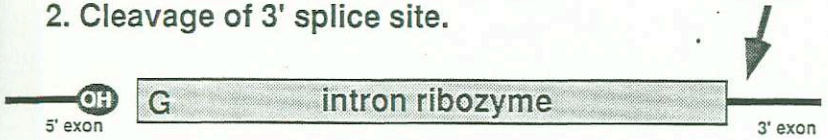
Whereas cleaving ribozymes must eliminate a chosen RNA to be effective *in vivo*, even inefficient trans-splicing might allow the useful expression of new gene activities, dependent on the presence of a target RNA.

Self-splicing of Group I introns.

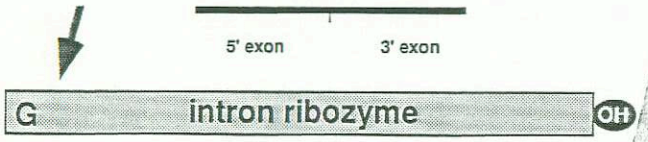
1. Guanosine-mediated cleavage of 5' splice site.



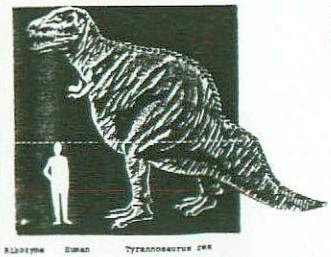
2. Cleavage of 3' splice site.



3. Ligation of exon segments.



4. Circularization of intron.



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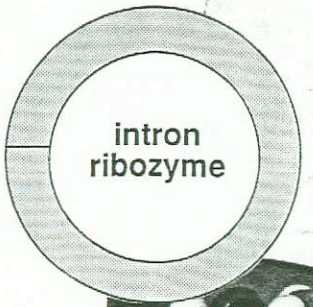
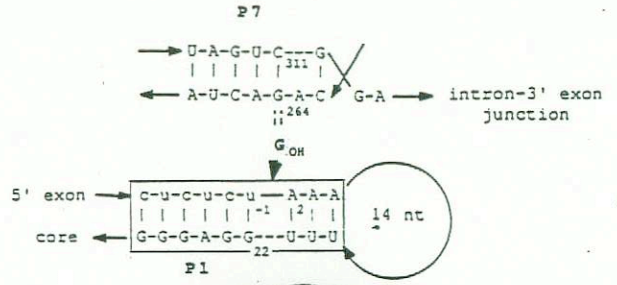
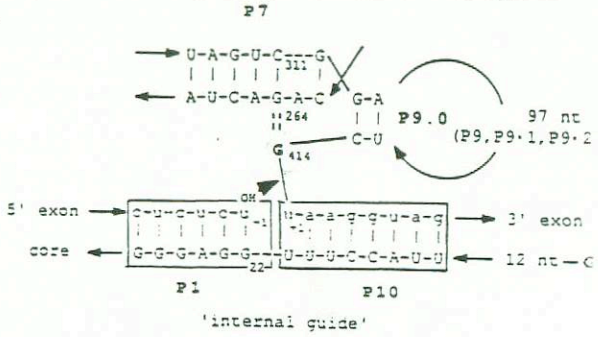
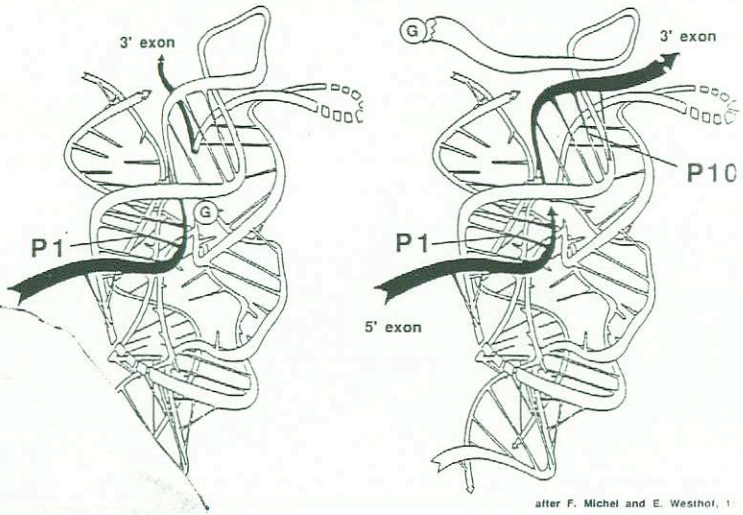






Self-splicing of Group I introns.



after F. Michel and E. Westhof, 1

Self-splicing of Group I introns

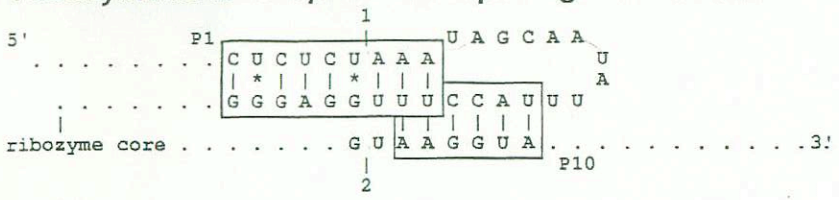
- Introns such as that of the *T. thermophila* rRNA pre-mRNA undergo RNA-catalysed excision.

- occurs through sequential trans-phosphorylations.

- relies on sequential formation of helices P1 and P10, to bring first 5' and then 3' exon sequences into close proximity to the active site.

Design of ribozymes for trans-splicing.

Tetrahymena thermophila self-splicing rRNA intron



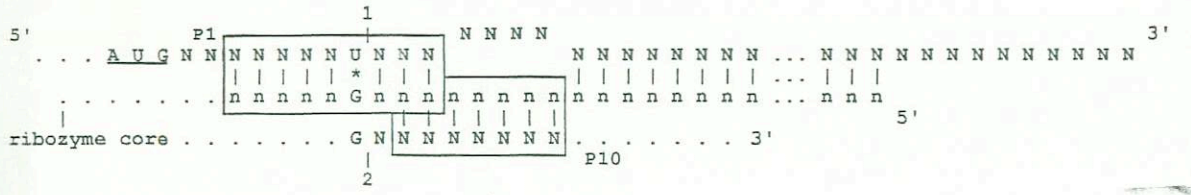
Trans-splicing ribozyme.

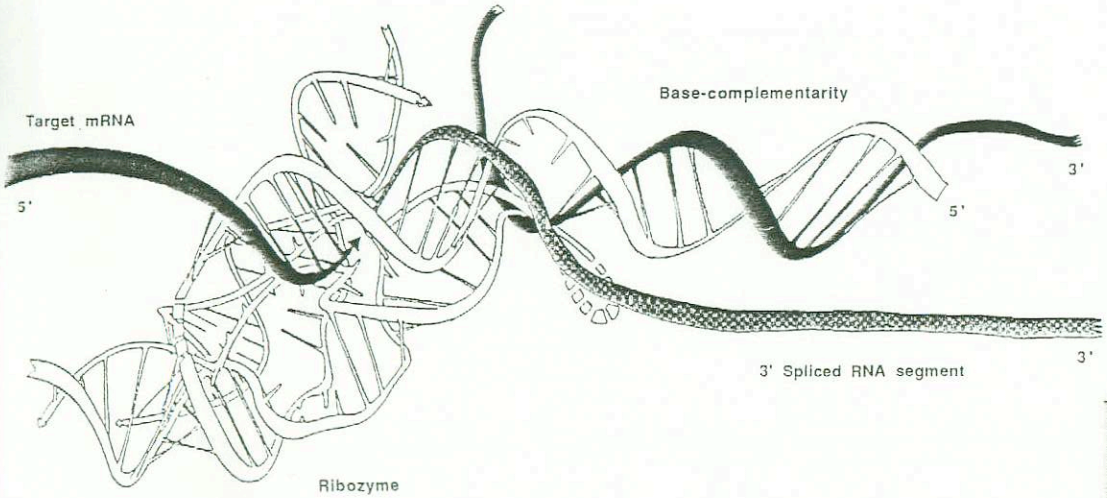
(1) A " 5' " site is chosen adjacent to a uracil residue within a chosen target RNA.

(2) Sequences complementary to the chosen RNA are fused to the 5' portion of a self-splicing Group I intron. Base-pairing between ribozyme and target RNA allow formation the helix P1.

(3) The chosen " 3' exon " sequences are fused to the 3' portion of the ribozyme, maintaining the conserved helix P10.

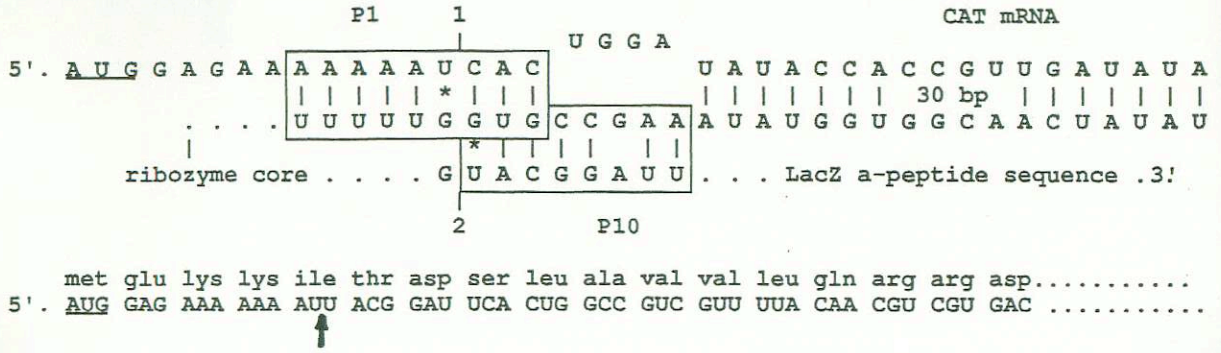
(4) To increase affinity for the target RNA, a section of extended sequence complementarity is fused to the 5' portion of the ribozyme - to allow the formation of 30-40 base-pairs.

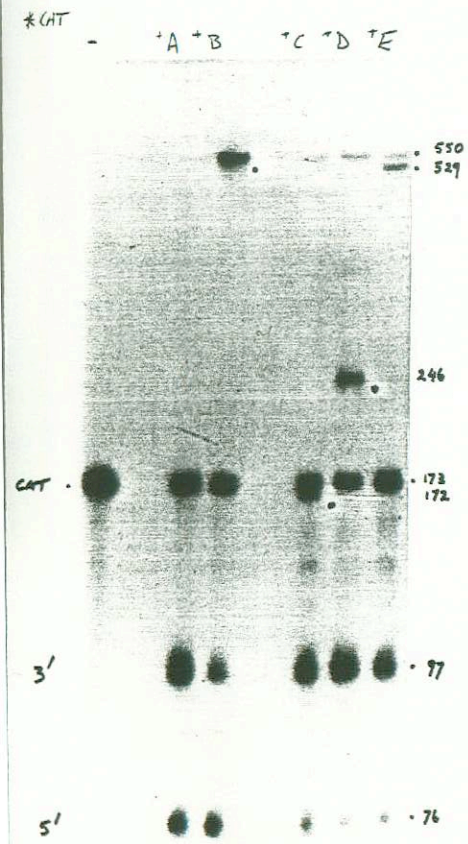




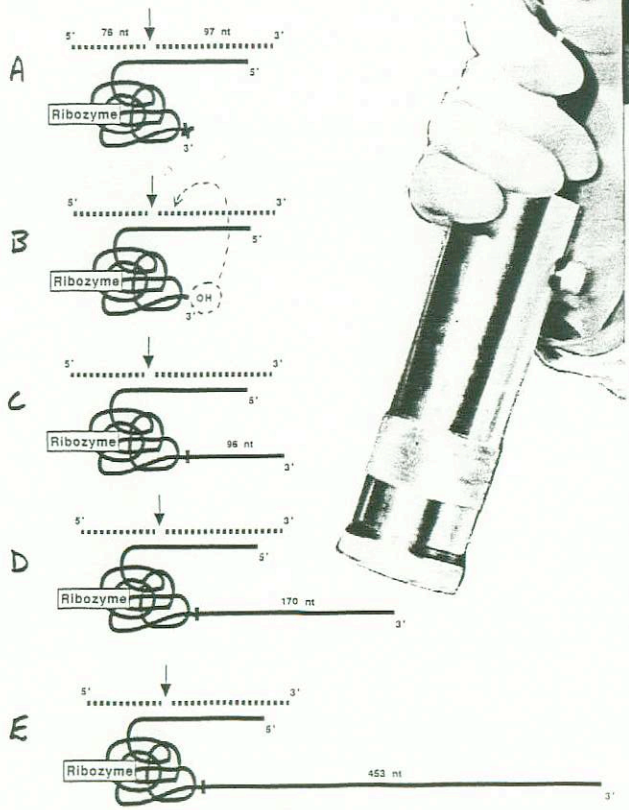
Design of CAT-LacZ α-peptide trans-splicing ribozyme.

CAT-LacZ trans-splicing ribozyme.





CAT - lacZ ribozyme-catalysed trans-splicing.



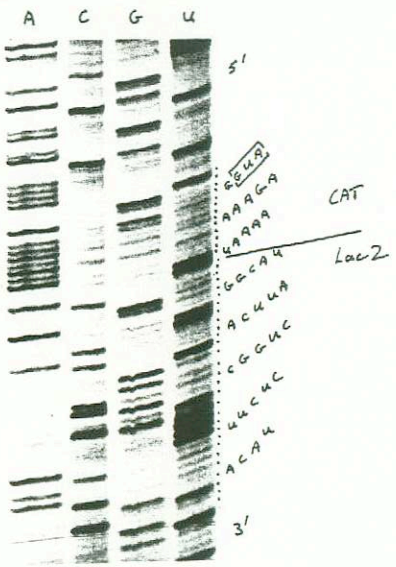
Design and testing of a chloramphenicol acetyl transferase (CAT) - lacZ a-peptide trans-splicing ribozyme.

The ribozyme gene was constructed using synthetic oligomers and PCR-mediated fusion with *T.thermophila* intron (ribozyme core) and lacZ gene sequences.

Variously truncated RNA transcripts were synthesized and incubated *in vitro* with radiolabelled CAT mRNAs to characterize spliced products.

Spliced products were cloned and sequenced to determine accuracy of trans-splicing.

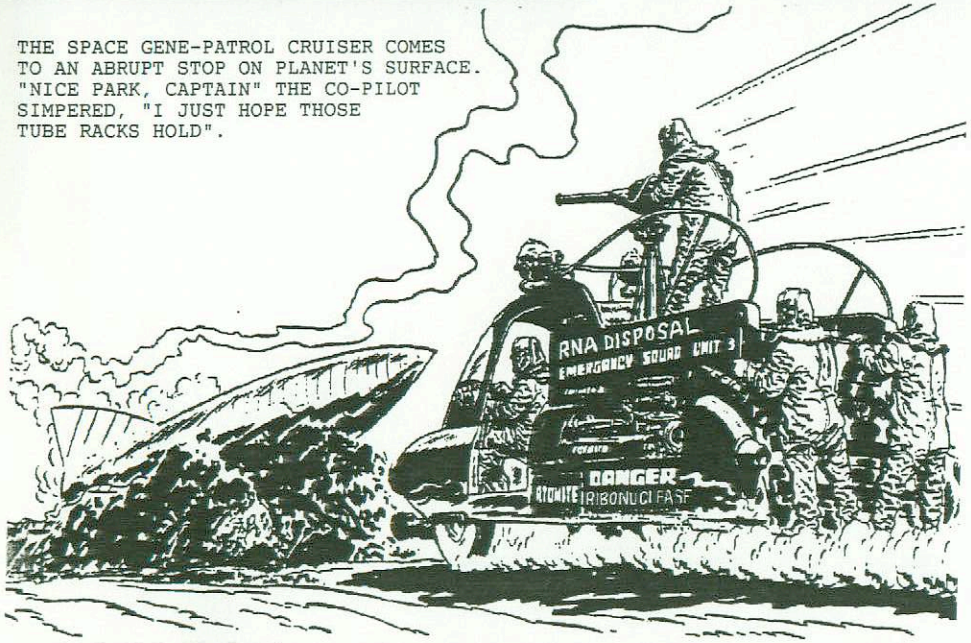
Sequence of spliced CAT - lacZ mRNA.



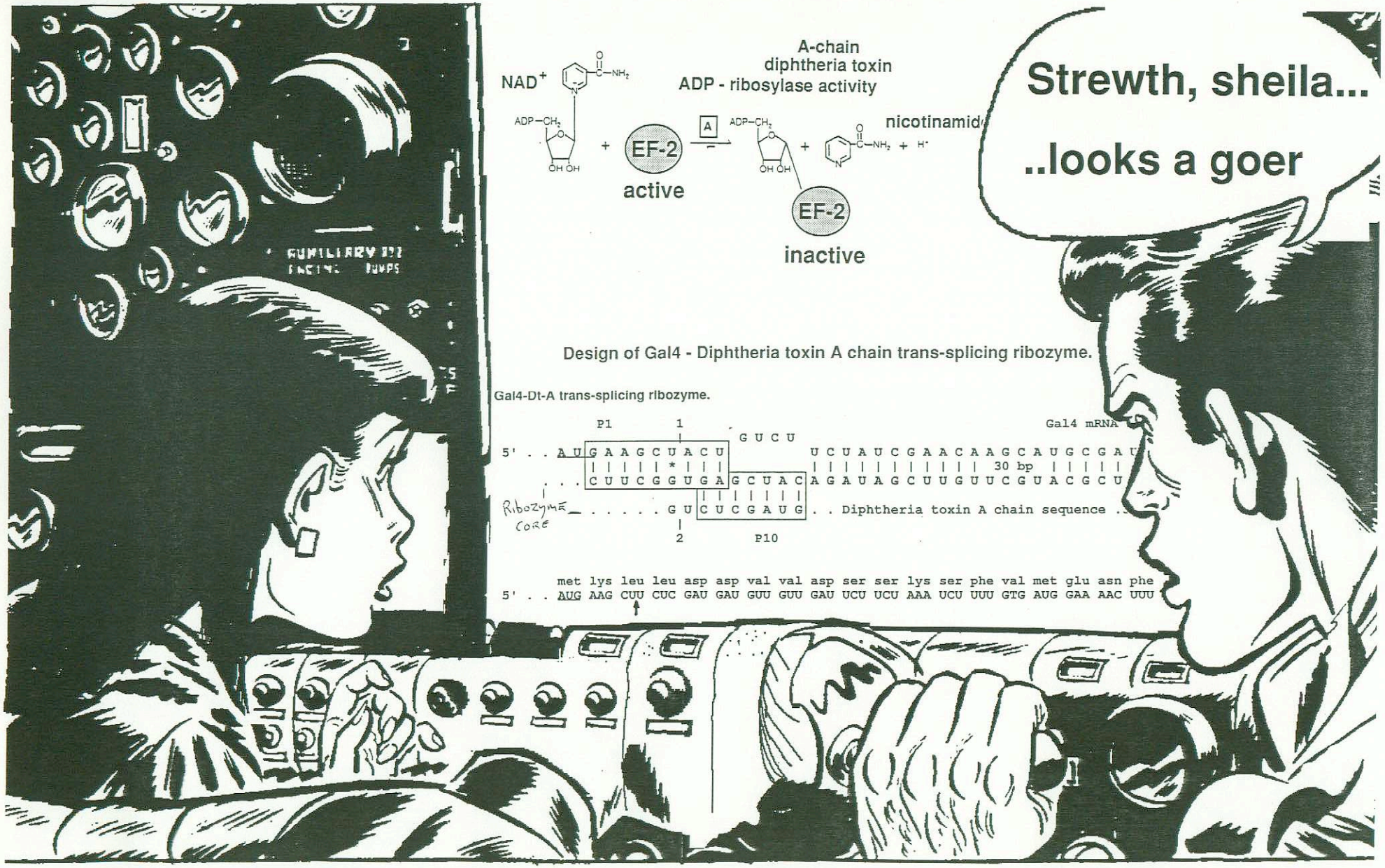
Hah - notarized lab-books indeed.

....and the ship slid through the vacuum of space, its load of experimental ribozymes seething within the hold. Suddenly....

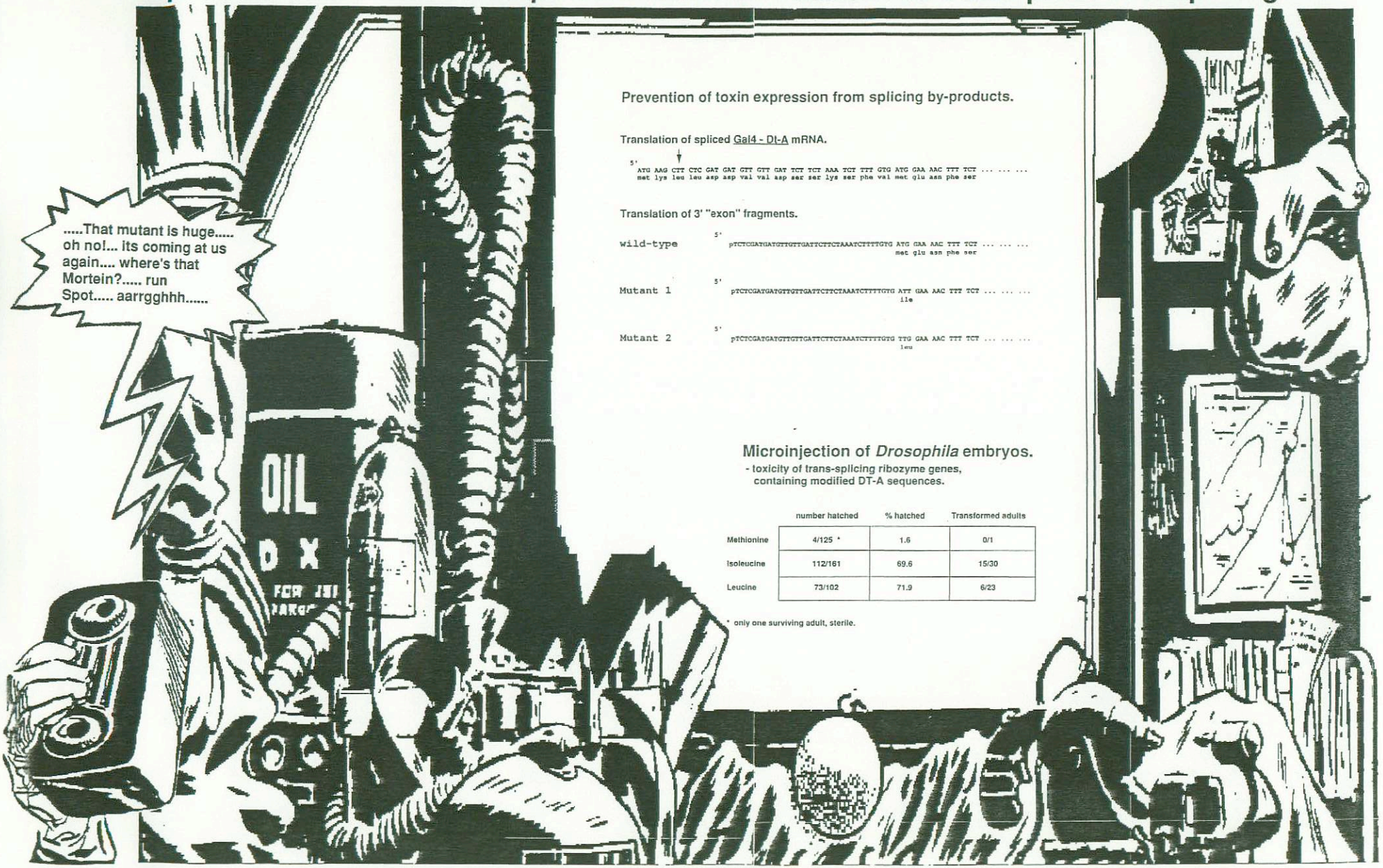
THE SPACE GENE-PATROL CRUISER COMES TO AN ABRUPT STOP ON PLANET'S SURFACE. "NICE PARK, CAPTAIN" THE CO-PILOT SIMPERED, "I JUST HOPE THOSE TUBE RACKS HOLD".



Something must be done! Could we use trans-splicing ribozymes to conditionally deliver a toxic activity into Drosophila cells? We could use Gal4 both to drive transcription of a ribozyme, and as a target for trans-splicing. The resultant hybrid mRNA would have the Gal4 initiation codon and diphtheria toxin A chain coding sequence.



We'll engineer the DT-A coding sequence to remove a 5' proximal AUG codon - to prevent expression from 3' "exon" sequences which are released as a side product of splicing.



Prevention of toxin expression from splicing by-products.

Translation of spliced Gal4 - Dt-A mRNA.

```
5'  ATG AAG CTT CTC GAT GAT GTT GTT GAT TCT TCT AAA TCT TTT GTG ATG GAA AAC TTT TCT ... ... ...
    met lys leu leu asp asp val val asp ser ser lys ser phe val met glu asn phe ser
```

Translation of 3' "exon" fragments.

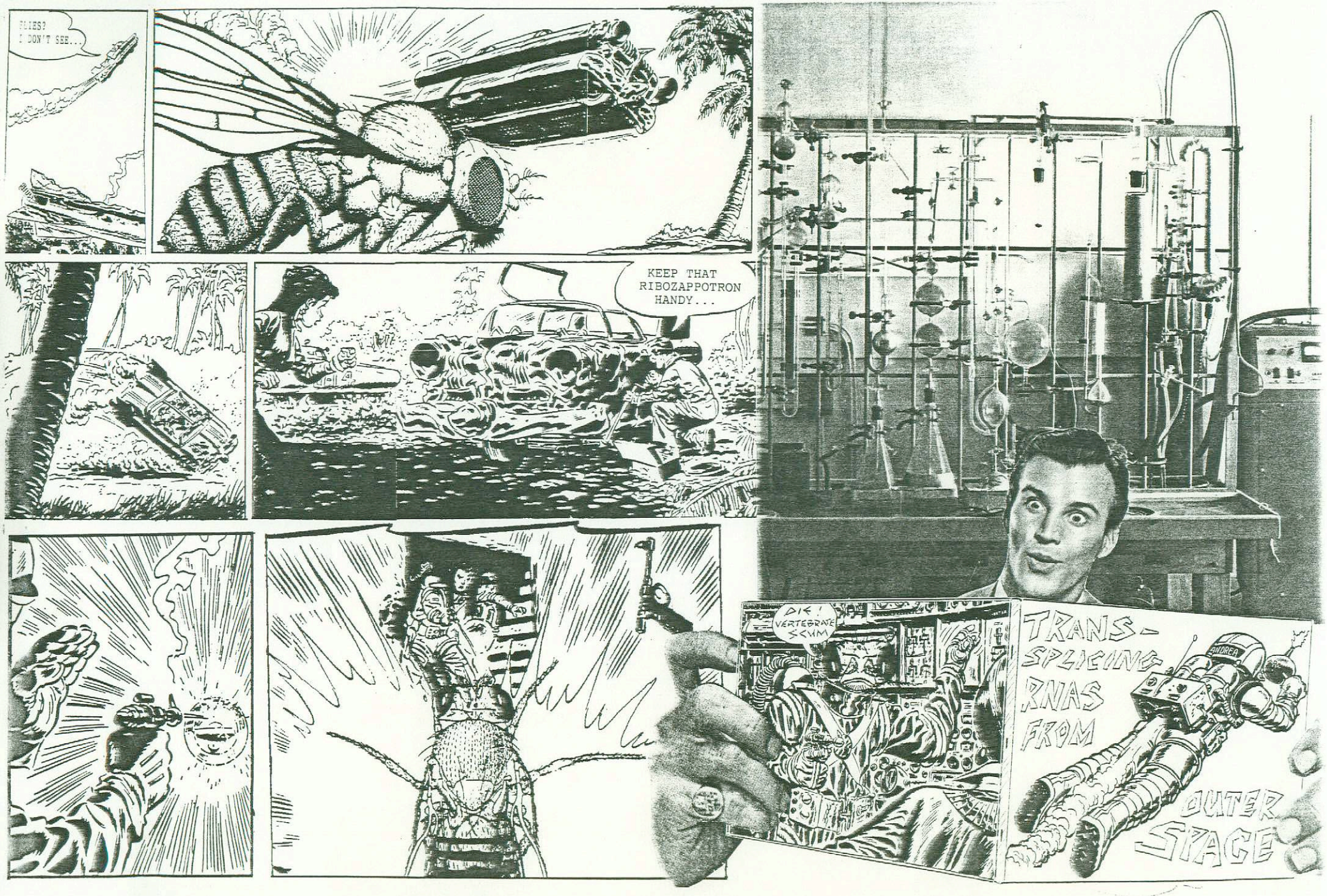
```
wild-type   5'  pTCTCGATGATGTTGTTGATTCTTCTAAATCTTTTGTG ATG GAA AAC TTT TCT ... ... ...
                                                    met glu asn phe ser

Mutant 1    5'  pTCTCGATGATGTTGTTGATTCTTCTAAATCTTTTGTG ATT GAA AAC TTT TCT ... ... ...
                                                    ile

Mutant 2    5'  pTCTCGATGATGTTGTTGATTCTTCTAAATCTTTTGTG TTG GAA AAC TTT TCT ... ... ...
                                                    leu
```

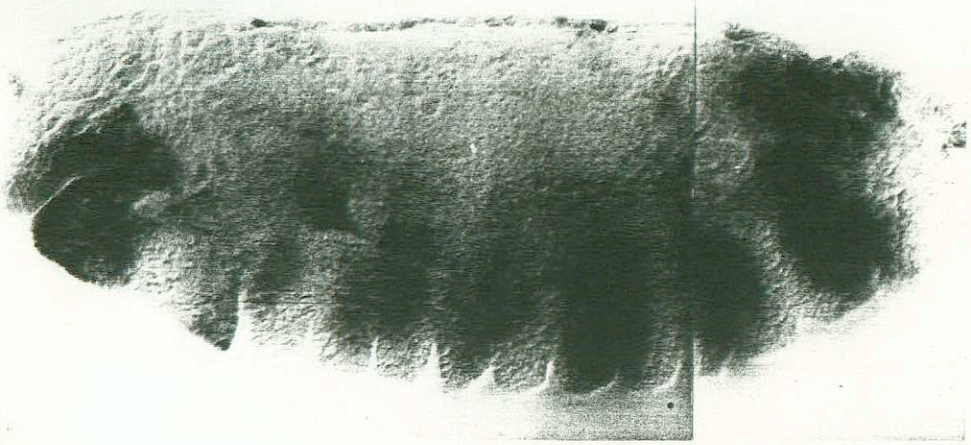
Microinjection of *Drosophila* embryos.

- toxicity of trans-splicing ribozyme genes, containing modified DT-A sequences.

	number hatched	% hatched	Transformed adults
Methionine	4/125 *	1.6	0/1
Isoleucine	112/161	69.6	15/30
Leucine	73/102	71.9	6/23

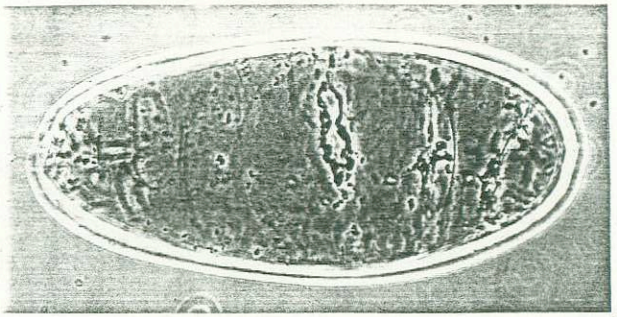
* only one surviving adult, sterile.

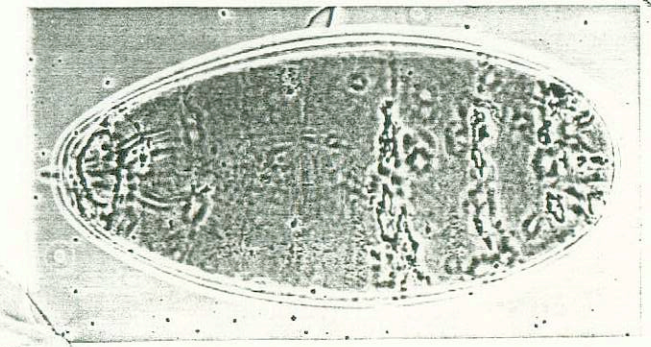

FLIES?
I DON'T SEE...
KEEP THAT RIBOZAPPOTRON HANDY...
DIE! VERTEBRATE SCUM
TRANS-
SPLICING
RNAS
FROM
OUTER
SPACE
ANDREA

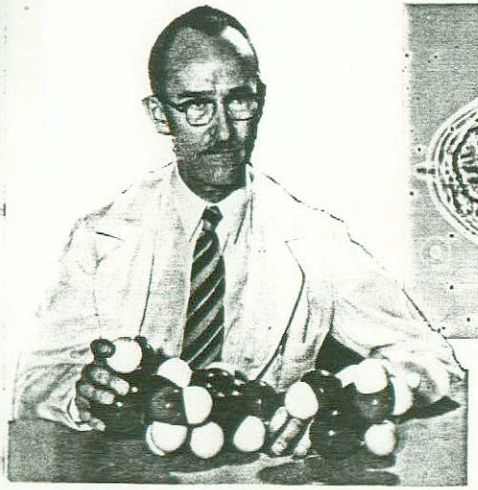


anti-lacZ stained embyo
(hairy-Gal4 x UAS-lacZ)

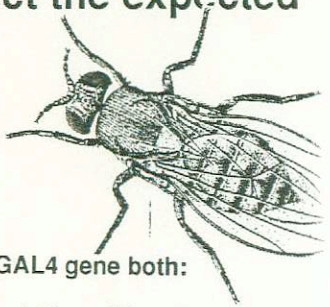
two cuticle preparations
from dead embryos
(hairy-Gal4 x UAS-Gal4/DT-A
trans-splicing ribozyme)



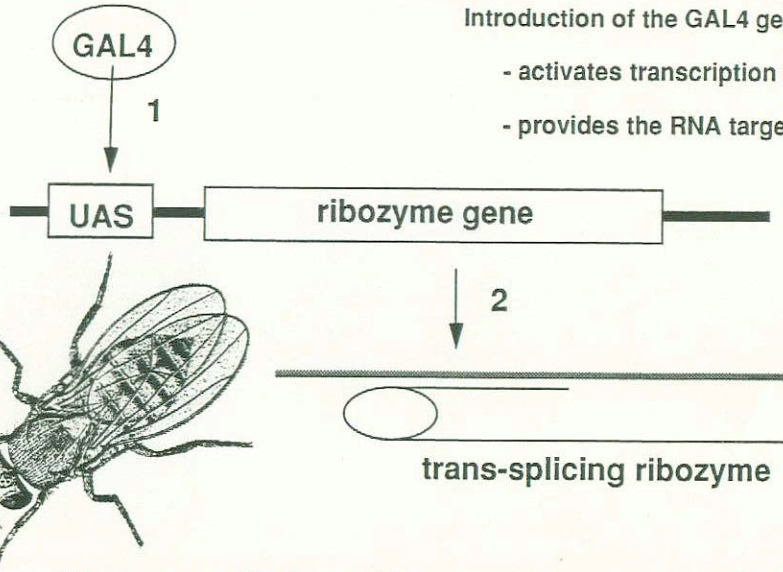




Gal4-mediated expression of the Gal4/DT-A trans-splicing ribozyme results in cell death. The patterns of cell death reflect the expected pattern of Gal4 expression.



Fidelity of trans-splicing?



Introduction of the GAL4 gene both:

- activates transcription of the ribozyme and.
- provides the RNA target for trans-splicing.

Trans-splicing ribozymes may be generally useful for the delivery of new gene activities - with delivery conditional for the presence of a specific target mRNA. The utility of this approach will be determined by both the *in vivo* efficiency and fidelity (or leakiness) of the ribozymes used.