Supplementary Data

One RNA plays three roles to provide catalytic activity to a group I intron lacking an endogenous internal guide sequence

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Supplementary Figure S1. *Trans*-splicing assays of the IGS-less ribozyme **V•X•Y•Z**. In these reactions, 5′-end-labeled **V•X•Y•Z** was incubated with three versions of the *h*•**Z** fragment, which behaves both as a substrate for *trans*-splicing and also provides an exogenous IGS sequence to the ribozyme in *trans*. Reactions were run for the times indicated and then the products were electrophoresed through an 8% polyacrylamide / 8 M urea gel. Mutations of the IGS sequence from GUG to CUG at locations ④ and/or ⑤ (Fig. 1A) greatly diminish *trans*-splicing activity (to 35% and 22%, respectively, of the activity seen in the unmutated construct after 2 hr).

Supplementary Figure S2. Analysis of splice-site specificity during covalent self-assembly in the absence of a canonical internal guide sequence. In these reactions, the 5′ end of the head portion of the *h*•**Z** fragment was radiolabeled and reacted with the IGS-less fragment **V•X•Y** for the time indicated, and the products were electrophoresed through a 20% polyacrylamide / 8 M urea gel. Intermediates produced during covalent self-assembly of the product **V•X•Y•Z** and the remaining *h* fragment resulting from transesterification can be observed. If splicing occurs at the typical splice site following the CAU in the 5-nt head sequence (5′-GGCAU-3′), then a 5-nt product should result (indicated). Mis-splicing produces head fragments of varying lengths. The left-hand lanes on the gel depict results from self-assembly in which the **Y-Z** junction was designed to favor the two-step *R2F2* mechanism, while the right-hand lanes depict results from self-assembly in which the **Y-Z** junction was designed to favor the one-step *tF2* mechanism (19). Cloned RT-PCR **V•X•Y•Z** products from the *R2F2* reaction, if from a mis-splicing event, typically contained deletions of four nucleotides (see text), which would result in a nine-nucleotide head product (5 + 4 = 9) as a result of splicing events four nucleotides downstream of the CAU, and these can be clearly seen on the gel. Cloned RT-PCR **V•X•Y•Z** products from the *tF2* reaction, if from a mis-splicing event, typically contained deletions of three or four nucleotides (see text), which would result in eight- or nine-nucleotide head products, seen on the gel.

Figure S1

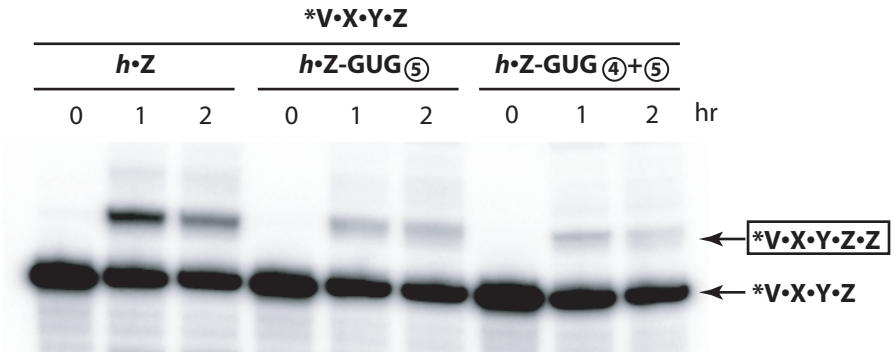
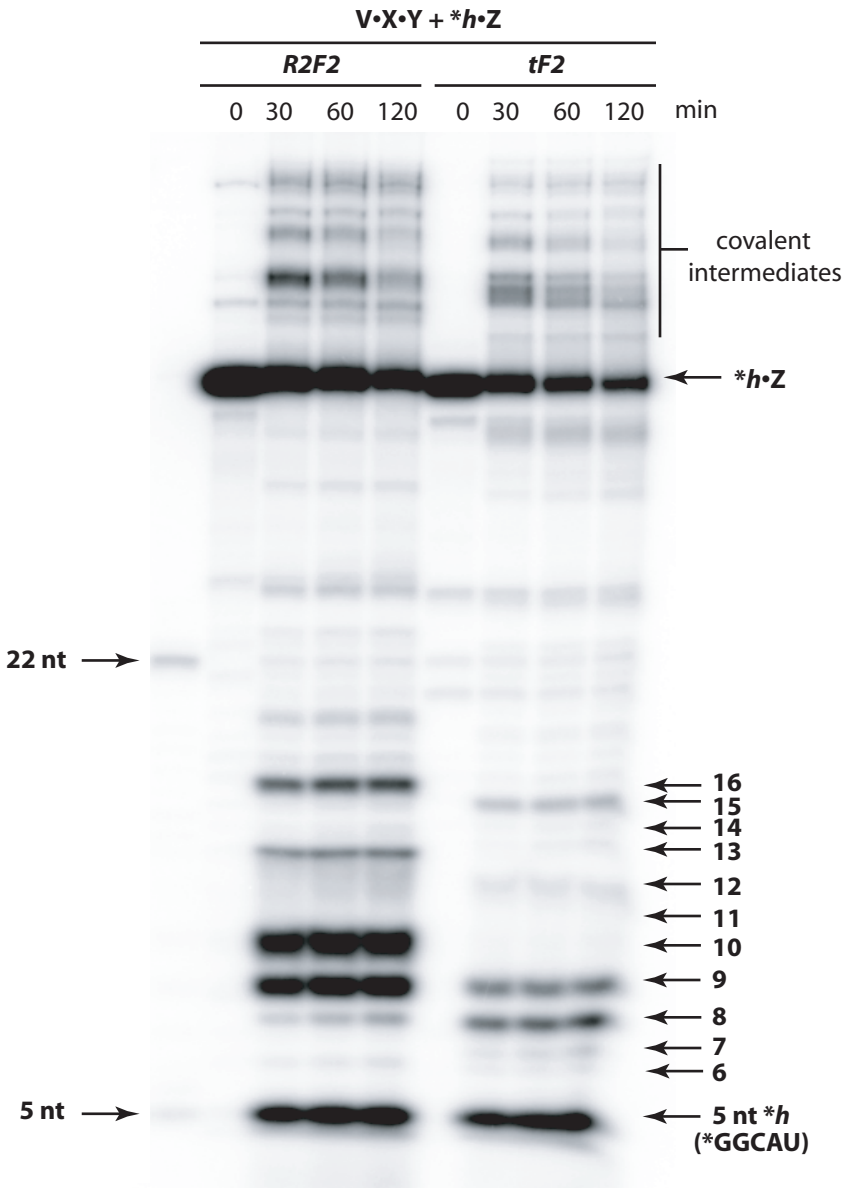


Figure S2


V•X•Y + *h•Z
R2F2
tF2
0 30 60 120 0 30 60 120 min
covalent intermediates
*h•Z
22 nt
16
15
14
13
12
11
10
9
8
7
6
5 nt
5 nt *h
(*GGCAU)