

## SUPPLEMENTARY FIGURES AND LEGENDS

### **Bacterial noncoding Y RNAs are widespread and mimic tRNAs**

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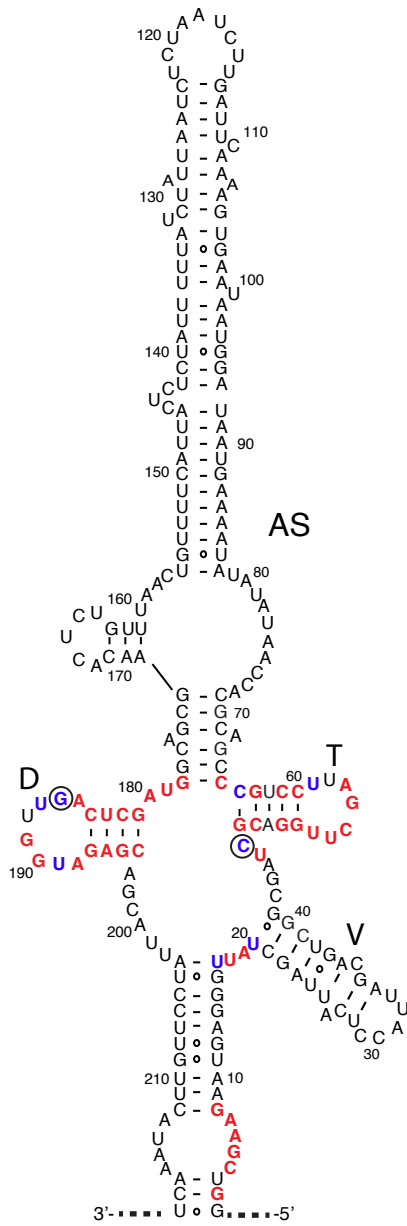
<sup>2</sup>Department of Molecular Biophysics and Biochemistry

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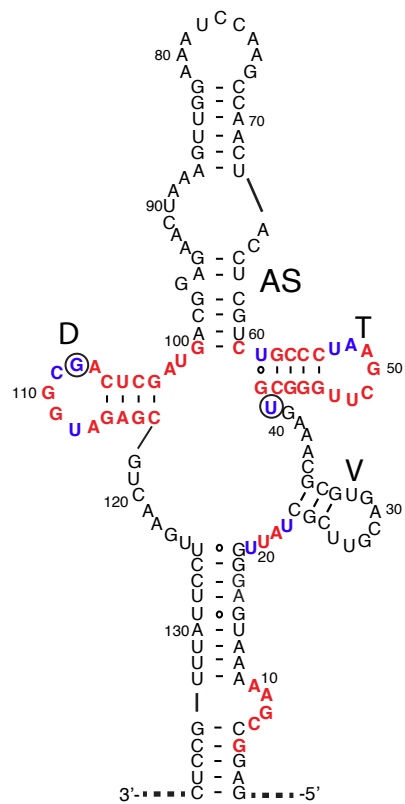
New Haven, CT 06510, USA

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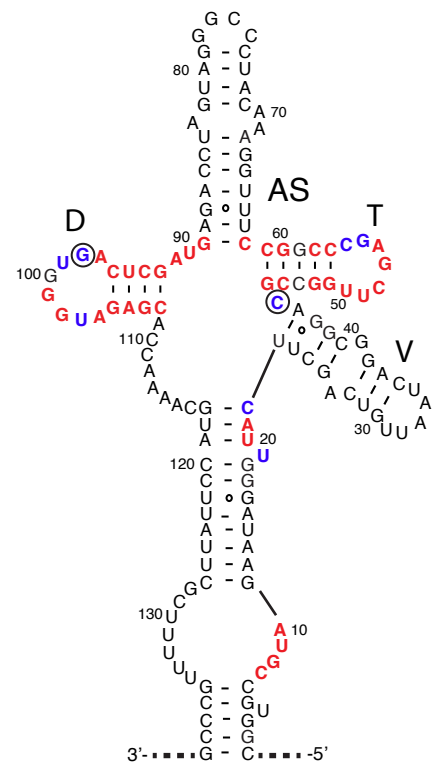
†These authors contributed equally to the work.



*Stanieria cyanosphaera*



*Gloeocapsa sp. PCC 73106*



*Fibrisoma limi BUZ 3*

Figure S1  
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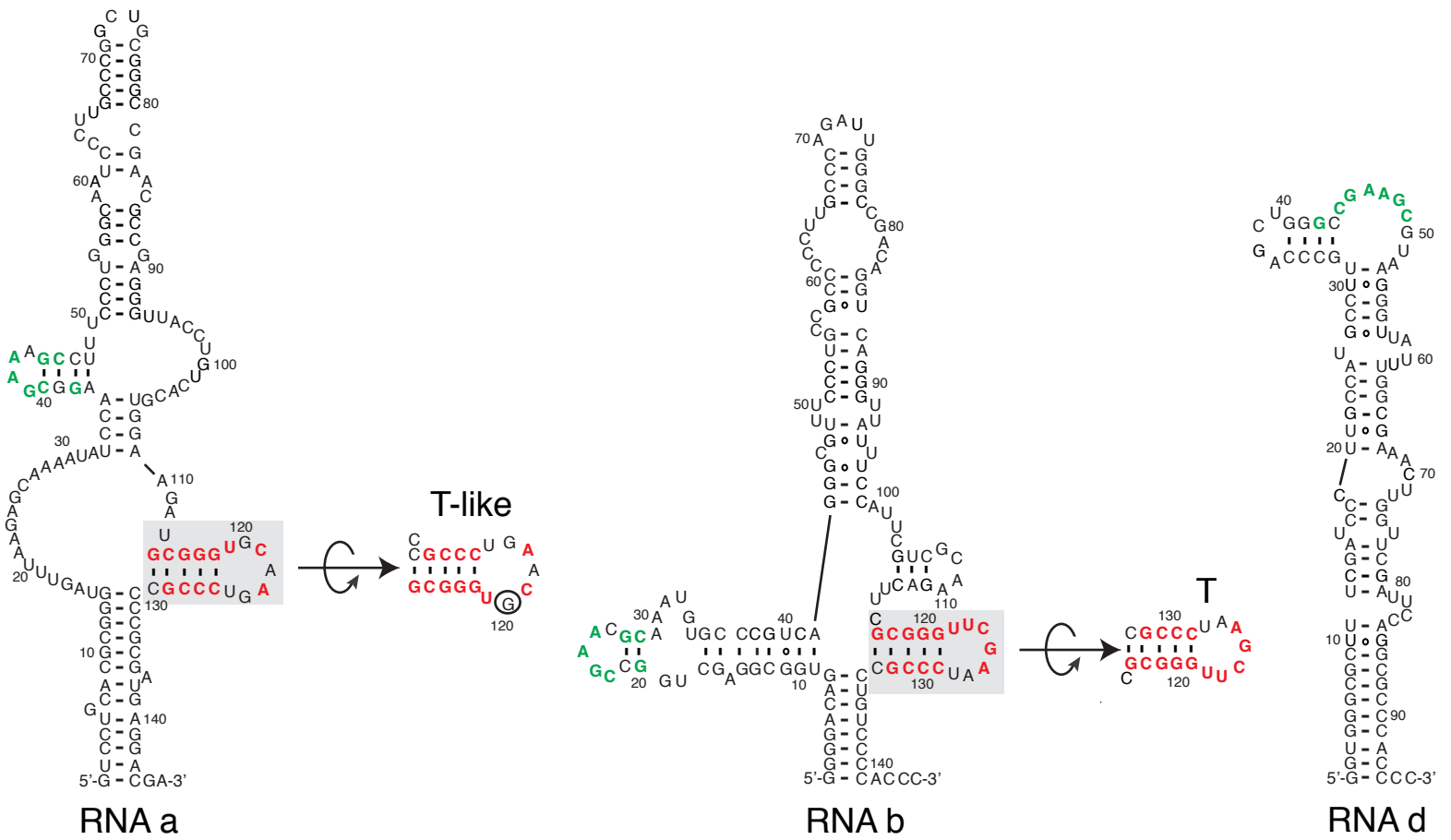
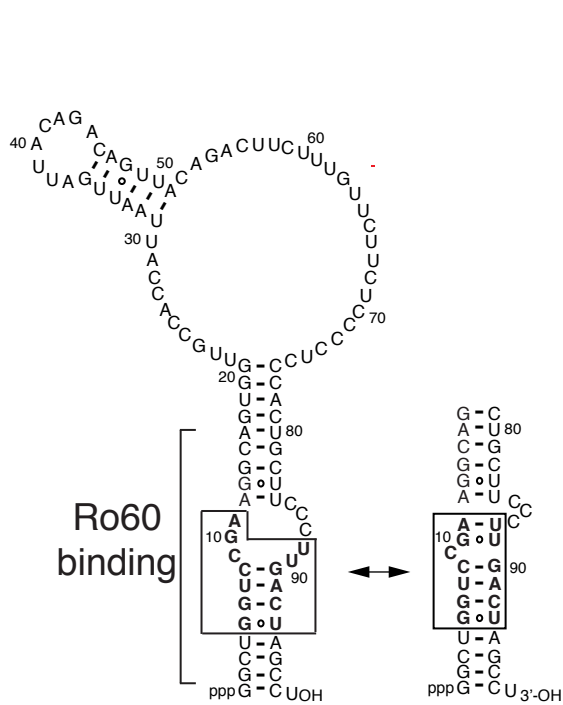
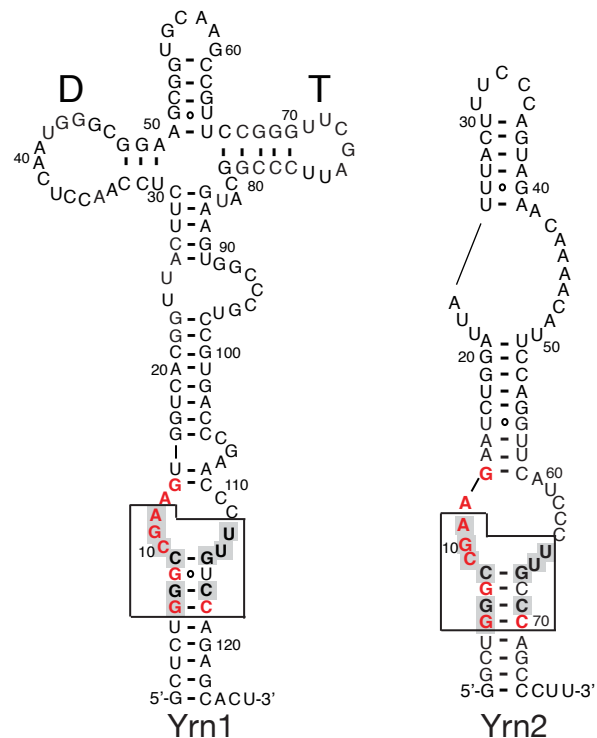


Figure S3  
Chen et al.

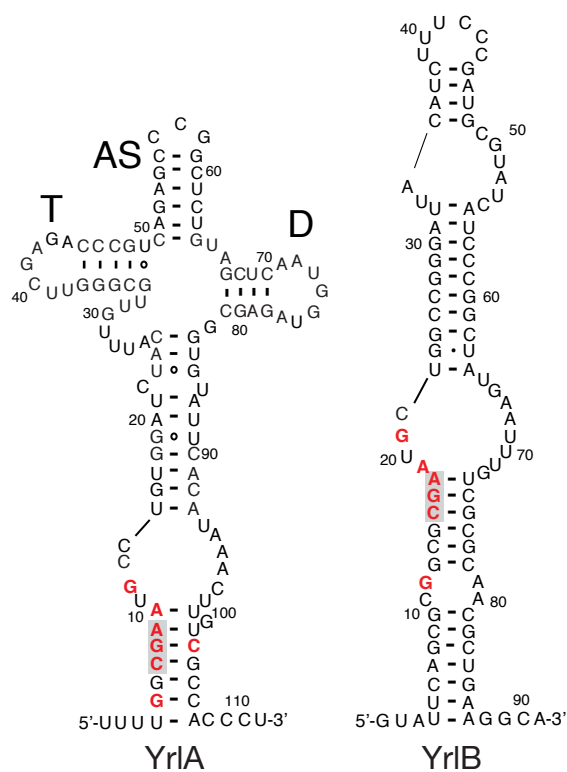
**A** *X. laevis* Y3



**B** *D. radiodurans*



**C** *S. Typhimurium*



**D** *M. smegmatis*

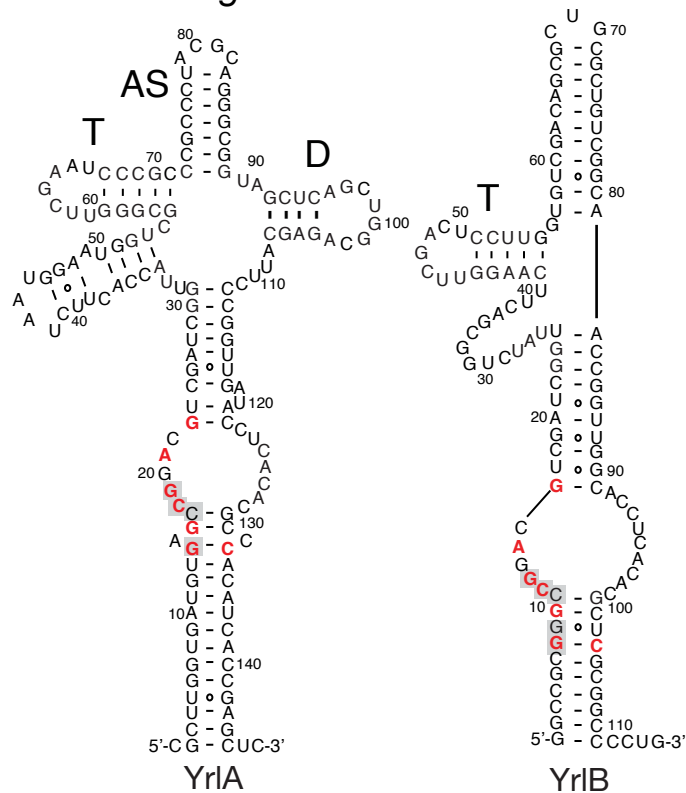


Figure S4  
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## Supplementary Figure Legends

### Figure S1. YrlA RNAs identified by Infernal also contain tRNA-like domains.

Three YrlA RNAs identified by Infernal from two cyanobacteria, *Stanieria cyanosphaera* and *Gloeocapsa sp.* PCC 73106, and *Fibrisoma limi* BUZ 3, a member of the *Bacteroidetes*. As they have not been experimentally determined, the 5' and 3' ends of the RNAs are indicated as dashed lines. Conserved nucleotides in the presumptive Ro60 binding site and tRNA-like domain are colored as in Fig. 2A,C. Structures were predicted using Mfold (Zuker 2003) and adjusted to maximize homology with other YrlA RNAs.

### Figure S2. Enzymatic probing of *S. Typhimurium* YrlA and *D. radiodurans* Yrn1.

A. 5'-labeled YrlA was incubated without (lane 3) or with the indicated RNases (lanes 4-6). For markers, denatured RNA was treated with alkali and T1 RNase (lanes 1 and 2).

B. Proposed secondary structure for YrlA. Sites of enzymatic cleavage are indicated.

C. 5'-labeled Yrn1 was incubated with the indicated enzymes in the absence (lanes 3,5,7) or presence (lanes 4,6,8) of *D. radiodurans* Rsr. Lanes 1 and 2, alkali and T1 ladders. Lines on the right indicate sites of protection from nucleases, while arrows indicate enhancements of V1 cleavage when Rsr is present.

D. Proposed Yrn1 secondary structure. Sites of cleavage are indicated, as are sites that show reduced or enhanced cleavage in the presence of Rsr.

### Figure S3. Possible structures of other ncRNAs encoded upstream of *D. radiodurans* Rsr

Structures predicted for RNA a, RNA b and RNA e by Mfold (Zuker 2003) and RNAfold (Gruber et al. 2008). RNA a is shown in the top Mfold-predicted structure, while RNAs b and d

are shown in structures predicted by both Mfold and RNAfold. Sequences that could potentially correspond to the GNCGAAN<sub>0-1</sub>GY motif are in green. A stemloop that resembles a T arm, but that lacks the U corresponding to tRNA Ψ55 is shaded in RNA a, as in a T arm in RNA b.

#### **Figure S4. Conserved features of Y RNAs**

A. *Xenopus laevis* Y3 RNA. The stem can exist in two conformers (arrow). Sequences important for Ro60 binding are bolded and boxed (Green et al. 1998).

B-D. Y RNAs from *D. radiodurans* (B), *S. Typhimurium* (C) and *M. smegmatis* (D). Mutations in the boxed region of Yrn1 disrupt Rsr binding (Chen et al. 2013). Yrn2 contains identical sequences. Nucleotides in this region that are conserved among YrlA RNAs (Fig. 1D) are in red, while nucleotides conserved between the vertebrate and bacterial Ro60 binding sites are shaded grey. Because the 3' strand of the Ro60 binding site is poorly conserved in *S. Typhimurium* and *M. smegmatis* Y RNAs, only nucleotides in the 5' strand are shaded.