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LETTER TO THE EDITOR

Identification of the universally conserved core of ribonuclease P RNA

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Ribonuclease P (RNase P), the endonuclease that produces the mature 5′-end of tRNAs, is essential in all organisms and unique in that it is composed of both RNA and protein subunits. The bacterial versions of RNase P RNA are catalytically active in vitro without the protein cofactor (Guerrier-Takada et al., 1983; reviewed by Pace & Brown, 1995). In contrast, no eucaryal or archaeal RNase P RNA has yet proved to be active in the absence of protein in vitro. All these RNAs are homologues, have common ancestry, so it is expected that a common foundation of structure underlies all modern RNase P RNAs. The secondary structures of bacterial and archaeal RNase P RNAs are now well-established by phylogenetic–comparative studies. Some homologies between the eucaryal RNAs and the bacterial and archaeal versions have been identified (Forster & Altman, 1990; Tranguch & Engelke, 1993), but no general structure for eucaryal RNase P RNA has been identified. Consequently, the universally conserved core of RNase P RNA, the structural basis for its catalytic mechanism, could not be articulated. We report here the identification of that common core and its implications for a general model of the eucaryotic version of the RNA.

To identify the universally conserved core, we aligned 10 RNase P RNA sequences obtained from GenBank, 3 from each of the primary phylogenetic domains, Archaea, Bacteria, and Eucarya, and 1 mitochondrial RNA, as shown in Figure 1. Despite the overall limited sequence conservation, each of the RNAs contains landmark helical elements and five distinct conserved regions (CR): CR I, **GNAANNUC**; CR II, **AGNRA**; CR III, **UGNRA**; CR IV, **AGNNNNAU**; CR V, **ACNNR ANNNNGNNUA**. We propose that these regions have specific homologues in all cellular RNase P RNAs. Because these highly conserved nucleotides occur in RNase P RNAs from all organisms, they are expected to occupy the structural core and functional heart of the RNA.

By keying on the CRs and the conserved positions of elements of secondary structure, we found that the eucaryal sequences concur in potential structure with minimum-consensus structure of the bacterial version of the RNA (Fig. 2). The commonalities in all the RNAs, the constituents of the universally conserved structural core of all RNase P RNAs, consist of all five sequence-conserved regions, CR-I–CR-V, and several helical elements, P1–4 and P7–10/11, that are variable in sequence, but occur at the same positions in the different RNAs (Fig. 2). Because these commonalities occur universally, they are predicted to have functional relevance and also are expected to outline the structural core of the RNA.

The CR elements comprise discrete substructures within the consensus core of secondary structure. CR-I and CR-V together comprise helix P4 and the immediately adjacent sequences, the most highly conserved structural element in all RNase P RNAs (Fig. 2). This element is thought to be the catalytic center of the ribozyme (Harris & Pace, 1995). CR-IV is adjacent to the active site (Burgin & Pace, 1990) and possibly participates with helix P4 in formation of the catalytic structure. CR-II and CR-III are located in the "universal internal loop" between helices P10/11 and P12 (Fig. 2). This universal internal loop had not been detected in previously proposed vertebrate RNase P RNA models (Altman et al., 1993; Eder et al., 1996), but had been seen in the fungal, bacterial, and archaeal RNAs (Tranguch & Engelke, 1993; Haas et al., 1994, 1996). Because of the apparently extended helix P10/11, the universal internal loop of eucaryal RNAs is slightly smaller than that of bacterial and archaeal RNAs. However, P12 elements occur in the universal internal loop of all the different RNAs at the same position.

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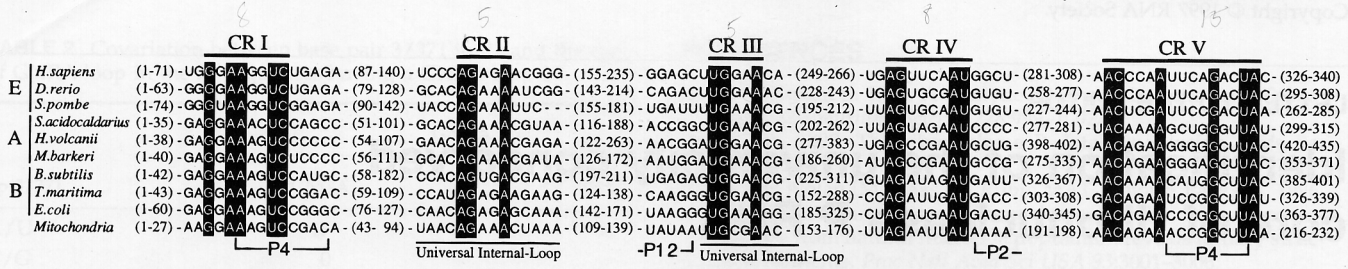

FIGURE 1. Alignment of universally conserved regions of RNase P RNAs. The alignment includes three eucaryal (denoted E), three archaeal (denoted A), three bacterial (denoted B), and one mitochondrial RNase P RNAs, as follows, with GenBank accession numbers. *Homo sapiens* (X15624, Baer et al., 1990); *Danio rerio* (U50408; Eder et al., 1996); *Schizosaccharomyces pombe* (X04013; Krupp et al., 1986); *Sulfolobus acidocaldarius* (L13597; LaGrandeur et al., 1993); *Haloferax volcanii* (M61003; Nieuwlandt et al., 1991); *Methanosarcina barkeri* (U42984; Haas et al., 1996); *Bacillus subtilis* (M13175; Reich et al., 1986); *Thermotoga maritima* (M64709; Haas et al., 1991); *Escherichia coli* (M17569; Reed et al., 1982); the mitochondrial RNA of *Aspergillus nidulans* (X93307; Lee et al., 1996). Due to their degenerate nature, mitochondrial RNase P RNAs display dramatic size and sequence variation (Wise & Martin, 1991). Therefore, we include in the alignment only the mitochondrial RNA from *Aspergillus nidulans*, which contains all CR sequences. Highlighted nucleotides indicate identities between all 10 RNAs. CR sequences are indicated with bars above the alignment. Numbers between each CR are the numbers of omitted nucleotides in this alignment. Secondary structures associated with CRs are indicated by brackets below the alignment. The complete alignment used in this study is available at the URL: http://crab2.berkeley.edu/pacelab/publications.html.

An intriguing correlation between the eucaryal and bacterial RNase P RNA models is the disappearance of the bacterial P15 helix/loop from the eucaryal RNA and the acquisition of a eucaryote-specific internal loop in helix P3. Important functions of the loop of P15 in the bacterial RNA are in binding the 3′-CCA of pre-tRNA and in coordinating catalytic metal ions (Kirsebom & Svärd, 1994; Oh & Pace, 1994). Loss of these important functions through loss of the P15 helix/loop presumably must be compensated either by other RNA structural elements or by the protein component of RNase P. In the case of the bacterial RNA, elements P3 and P15 crosslink to one another, so must be neighbors in the global structure (J.-L. Chen & N.R. Pace, unpubl. data). Consequently, it seems possible that the acquisition of the internal loop in the eucaryotic version of helix P3 compensates structurally or functionally for the loss of the P15 helix/loop from the eucaryotic RNA. Based on the currently known structure of the bacterial RNA, deletion of the P15 helix/loop and re-linkage of the remaining sequence would be expected to impart little distortion on global structure; the sequences flanking P15 are adjacent to one another (Burgin & Pace, 1990; Harris et al., 1994).

Although the eucaryal and archaeal RNAs are not catalytically active in the absence of protein, they have retained the essence of the ancestral catalytic RNA. Protein-independent catalytic activity of the RNA presumably was lost in the evolutionary lineage that led to Archaea and Eucarya. Because the core structure that we have identified is strictly conserved in a background of such extensive evolutionary remodeling, we believe that it contains the essential catalytic elements of RNase P in all organisms.

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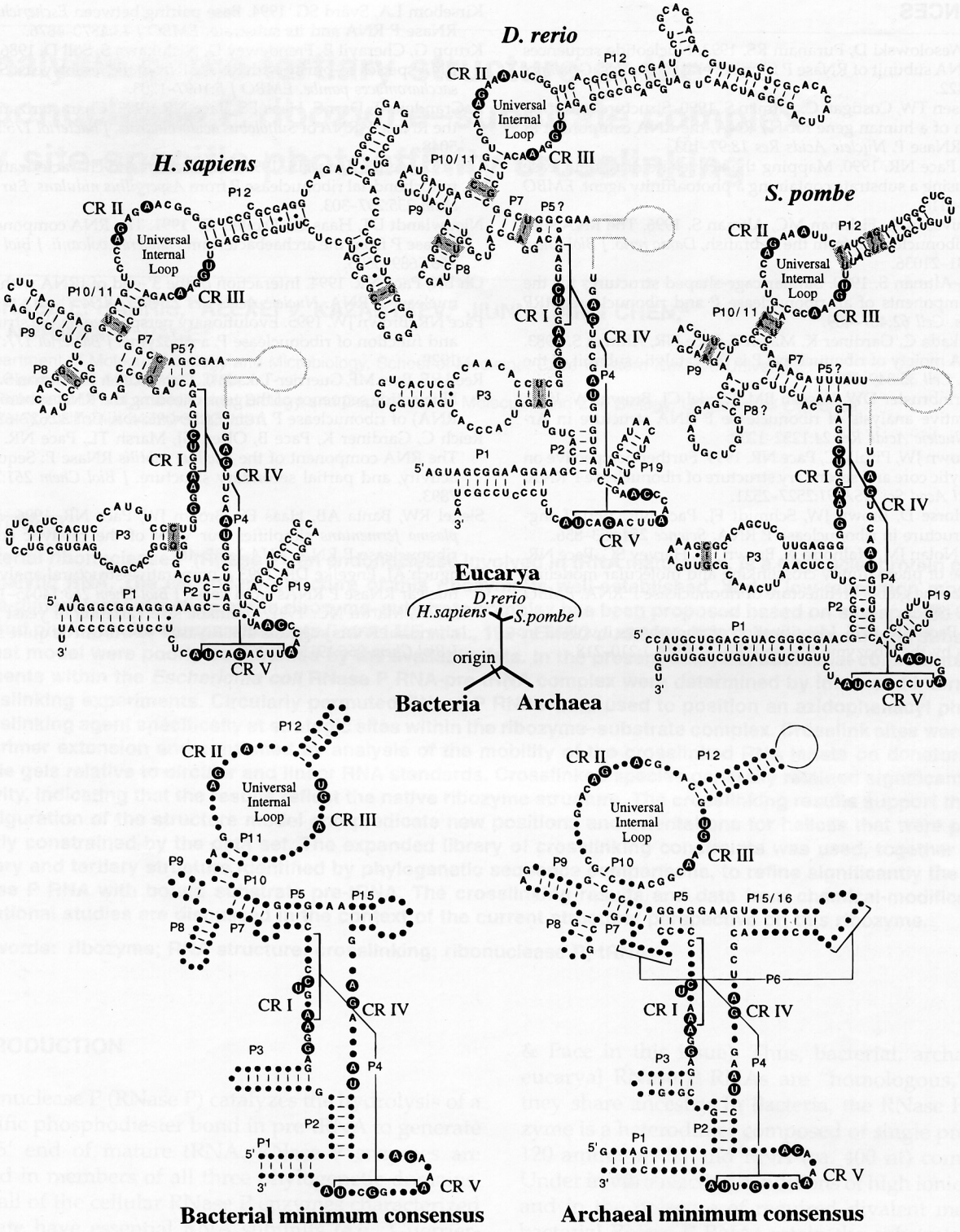

FIGURE 2. Locations of CRs in eucaryal, archaeal, and bacterial RNase P RNAs. The consensus bacterial and archaeal RNase P RNAs are based on Siegel et al. (1996) and Haas et al. (1996), in which invariant nucleotides are indicated by letter (G, A, U, or C); nucleotides that are universally present but vary in identity are indicated by filled circles. Proposed secondary structures for *H. sapiens*, *D. rerio*, and *S. pombe* RNAs are supported by several scattered covariations, indicated by shaded boxes. The *S. pombe* model is based on the structure proposed by Tranguch and Engelke (1993). Highlighted nucleotides are universally conserved in identity among ca. 150 known RNase P RNAs from all three phylogenetic domains. Nomenclature of the eucaryal structures is based on putatively homologous bacterial counterparts (Haas et al., 1994). Helices with question marks are questionable as to homology with the corresponding bacterial helix because of sequence variation. Filled dots indicate proposed G/U base pairs and open dots indicate noncanonical, non-G/U base pairs. The grey line indicates the corresponding position of the bacterial P15 helix/loop that is absent in the eucaryal secondary structures. Phylogenetic relationships of the organisms corresponding to the RNase P RNAs are shown in the central rooted three-domain phylogenetic tree.

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