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Structure-based mechanistic insights into catalysis by small self-cleaving ribozymes

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Small self-cleaving ribozymes are widely distributed in nature and are essential for rolling-circle-based replication of satellite and pathogenic RNAs. Earlier structure–function studies on the hammerhead, hairpin, *glmS*, hepatitis delta virus and Varkud satellite ribozymes have provided insights into their overall architecture, their catalytic active site organization, and the role of nearby nucleobases and hydrated divalent cations in facilitating general acid-base and electrostatic-mediated catalysis. This review focuses on recent structure–function research on active site alignments and catalytic mechanisms of the Rzb hammerhead ribozyme, as well as newly-identified pistol, twister and twister–sister ribozymes. In contrast to an agreed upon mechanistic understanding of self-cleavage by Rzb hammerhead and pistol ribozymes, there exists a divergence of views as to the cleavage site alignments and catalytic mechanisms adopted by twister and twister–sister ribozymes. One approach to resolving this conundrum would be to extend the structural studies from currently available pre-catalytic conformations to their transition state mimic vanadate counterparts for both ribozymes.

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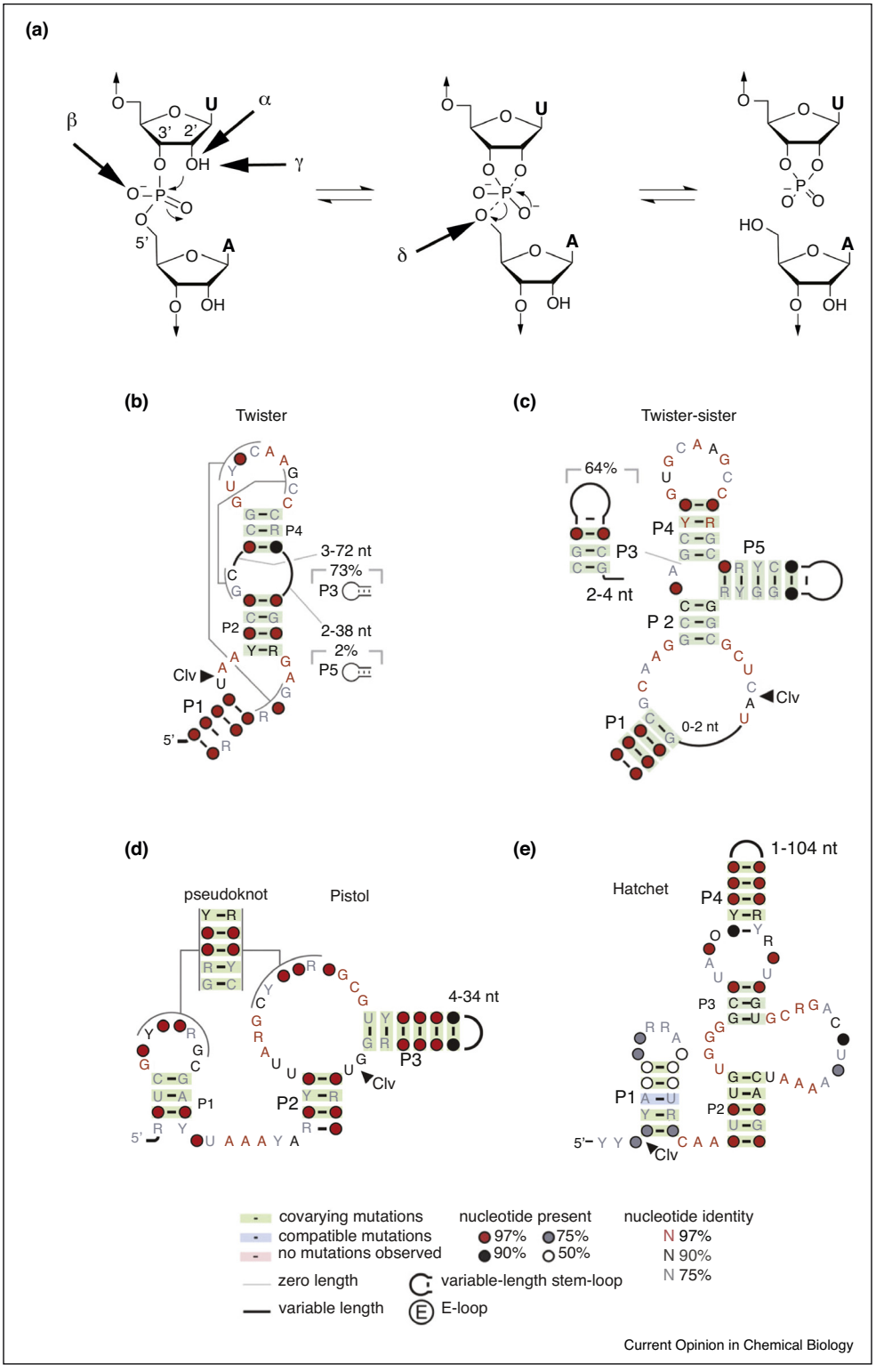
Introduction

RNA has been postulated to play a central role in early life (the RNA world hypothesis), given that both genomic information and enzymatic function reside in the same biological macromolecule. RNA enzymes (ribozymes) are composed of only four nucleotides of a common chemical nature projecting from a negatively-charged ribose-phosphate backbone with the capacity to accelerate cleavage of the phosphodiester backbone by up to 10^6-fold. This raises the issue as to how ribozyme catalysis occurs at neutral pH, given that ionization of nucleobases and riboses only occur under pronounced basic/acidic conditions. The focus of this review will be on recent studies of natural self-cleaving ribozymes, due to their small size and catalytic versatility, thereby offering unprecedented opportunities to understand chemistry in the prebiotic world and its evolution over time to the modern era.

Small self-cleaving ribozymes are widely distributed in nature [1••] and are essential for rolling-circle-based replication of satellite and pathogenic RNAs [2,3] and processing of repetitive RNA species [4]. Current understanding of the various contributions to small self-cleaving ribozyme-mediated rate enhancement has emerged from earlier structure–function studies of the hammerhead [5–7], hairpin [8–10], *glmS* [11–13], hepatitis delta virus (HDV) [14–16] and Varkud satellite (VS) [4,17,18] ribozymes. Structural studies of these ribozymes in their pre-catalytic, and in some cases in vanadate transition state mimic conformations, have provided insights into their overall architecture, the topology of their catalytic active site organization, and the role of nearby nucleobases and hydrated divalent cations in facilitating general acid-base and electrostatic-mediated catalysis [19,20••,21–25].

A working hypothesis regarding natural self-cleaving ribozymes is that they utilize a network of defined hydrogen bonds, ionic and hydrophobic interactions to generate catalytic pockets, which capitalize on steric constraints to generate in-line cleavage alignments and general acid-base chemistry to catalyze site-specific cleavage of the phosphodiester backbone. There is general agreement that there are four factors that can contribute to RNA phosphodiester cleavage involving targeting by the 2′-OH group of the adjacent P-O5′ bond at the scissile phosphate associated with a pentacoordinated phosphorane transition state intermediate (Figure 1a) [19]. These include contributions from in-line alignment of the 2′-O atom and the to-be-cleaved P-O5′ bond (α factor); those associated with electrostatic compensation resulting from the enhanced negative charge on the non-bridging scissile phosphate oxygens in the transition state (β factor); those of general base in activating the 2′-OH for nucleophilic attack on the scissile phosphate (γ factor); and those of general acid in donating a proton to the developing negative charge on the O-5′ leaving group (δ factor). For a given ribozyme, revealing which combination of factors contribute and predominate, remains a challenge,

Figure 1



Catalytic strategies for cleavage of the phosphodiester backbone by self-cleaving ribozymes, as well as consensus sequences and secondary structures of newly-identified self-cleaving twister, twister–sister, pistol and hatchet ribozymes. **(a)** Four factors that can contribute to catalysis during internal phosphodiester transfer reaction. α factor: in-line alignment of the 2′-O, phosphorus and O-5′ at the scissile phosphate; β factor: neutralization of the developing negative charge on the nonbridging phosphate oxygen; γ factor: general base-mediated deprotonation of the 2′-O; δ factor: general acid-based neutralization of the developing negative charge in the O-5′ position. (Adopted from [19]). **(b–e)** Consensus sequence