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Recognition Highlights and Commentaries

A tale in molecular recognition: the hammerhead ribozyme

Eric Westhof*

Architecture et Réactivité de l'ARN, Institut de Biologie Moléculaire et Cellulaire du CNRS, Strasbourg, France

A new crystal structure of the hammerhead ribozyme demonstrates the influence of peripheral tertiary contacts on the local conformations around the active site. This structure resolves many conflicting results obtained on reduced systems. Copyright © 2006 John Wiley & Sons, Ltd.

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INTRODUCTION

Ribozymes are catalytically active RNA molecules. In the early 1980s, the discovery of catalytic activity in RNA molecules (Grabowski *et al*., 1981; Guerrier-Takada *et al*., 1983) led to a change in paradigm in molecular biology that is still ongoing. A couple of years later, small self-cleaving RNAs were discovered in viroids and satellite RNAs of plant viruses (Buzayan *et al*., 1986; Forster and Symons, 1987). The hammerhead ribozyme belongs to those small nucleolytic ribozymes. The hammerhead ribozyme is characterized by a minimal catalytic core consisting of a three-way junction containing some 15 invariant nucleotides (Haseloff and Gerlach, 1989; Symons, 1997; Figure 1). The basic proton transfer steps (Slim and Gait, 1991; Uhlenbeck, 1987) necessary for chain cleavage were soon established (Figure 2). The small size and the well-identified conserved elements of the hammerhead ribozyme led to a flurry of chemical and biophysical studies that attempted to understand the structure–function relationships of the molecule (Hammann and Lilley, 2002; Wedekind and McKay, 1998). New chemical and biophysical techniques were devised or extended to study RNA molecules. A model of the hammerhead architecture with two co-axially stacked helices and a third helix at an angle was deduced from fluorescence data (Tuschl *et al*., 1994). Following the first crystal structures (Pley *et al*., 1994; Scott *et al*., 1995) which showed the proper orientation of helix I and displayed the non-Watson–Crick base pairs involving the conserved residues (Figure 3 right), further kinetic and chemical studies were performed. However, it was soon realized that it was extremely difficult to reconcile all the data obtained by various laboratories around the world (McKay, 1996; Scott, 1999). Although single molecule studies (Lilley, 2005) and elegant kinetic experiments using modified ribozymes (Wang *et al*., 1999) underscored the importance of molecular dynamics and the ensuing conformational changes, no definite molecular mechanisms emerged from these studies.

In the August issue of *Cell*, Martick and Scott (2006) reported on the much awaited structure of the hammerhead ribozyme close to the transition state. The structure solves so many riddles and controversies about the catalytic mechanism of the hammerhead ribozyme that this paper is destined to become a classic for many years to come. The main architecture of the hammerhead fold is preserved, but a couple of key changes in torsion angles lead to a pre-transition state (Figure 3 left), in complete agreement with all accumulated data and the known chemistry of phosphodiester cleavage. Three years earlier, a major insight had been achieved when it became clear that not much catalytic activity remained when the hammerhead ribozyme was reduced to its core of conserved residues maintained by the helices of the three-way junction (De La Pena *et al*., 2003; Khvorova *et al*., 2003; Uhlenbeck, 2003). This showed that the naturally occurring peripheral elements

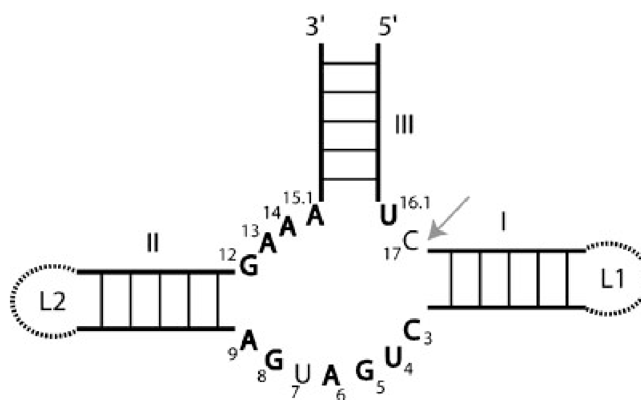

Figure 1. The basic secondary structure of the hammerhead ribozyme consists of a three-way junction and 11 conserved nucleotides (in bold and numbered).

*Correspondence to: E. Westhof, Institut de biologie moléculaire et cellulaire, Centre National de la recherche scientifique, UPR9002, Architecture et Réactivité de l'ARN, Université Louis Pasteur, 15 rue René Descartes, F-67084 Strasbourg, France.
E-mail: E.Westhof@ibmc.u-strasbg.fr

Abbreviations used: RNA, ribonucleic acid.

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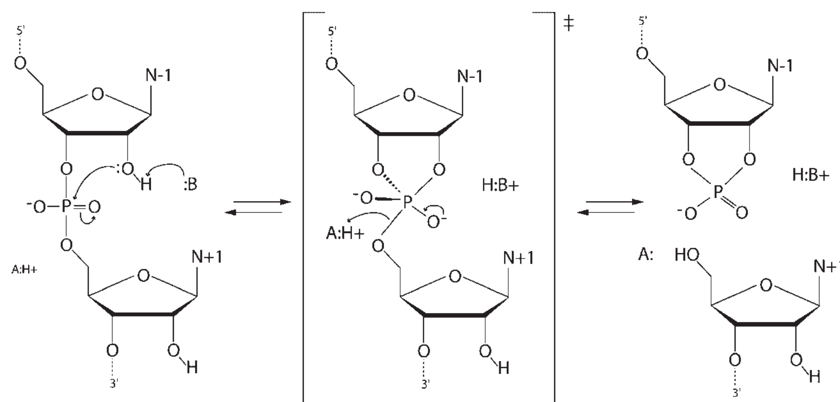

Figure 2. The proton transfer steps occurring during cleavage of the phosphodiester bond. The adjacent 2′-hydroxyl group is activated for nucleophilic attack. In a concomitant way, a proton is given to the leaving 5′-oxygen group.

play a crucial biological role and cannot be ignored when trying to understand the structure–function relationships of the molecule. The new structure based on the hammerhead ribozyme of the parasite *Schistosoma mansoni* shows how the presence of tertiary contacts between loops far removed from the catalytic site induces conformational changes in the core that lead to the active state of the ribozyme. Besides the relative positions and orientations of conserved nucleotides in the single stranded region linking helix I and II, the local conformational changes involve the exchange between two base pairs. The cleavable residue C17 instead of forming a loose contact with C3 forms a non-Watson–Crick pair with A13, while C3 forms a Watson–Crick pair with G8 (which was previously forming a non-Watson-Crick pair with A13; Figure 3 left).

For many years, the hammerhead ribozyme was therefore studied in a reduced and minimal form. The existence of numerous conformations in dynamic exchange, most of them inactive, required the presence of high concentrations of divalent ions and resulted in only weak activity. However,

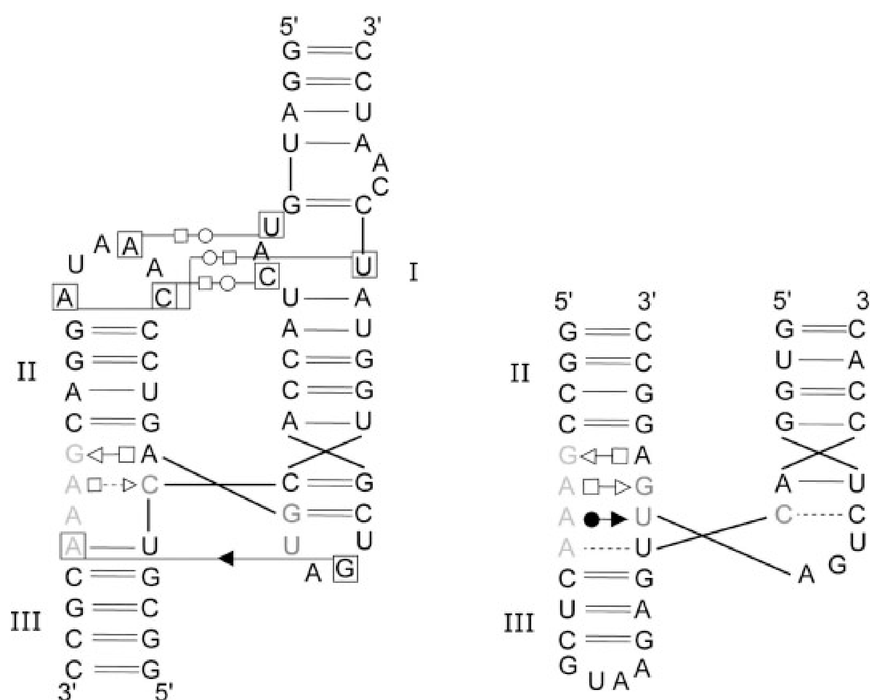

Figure 3. The catalytic core of the hammerhead ribozyme. Helices I, II and III are indicated. The core is composed of thirteen nucleotides of which 11 are strictly preserved. Pairs and interactions are represented according to the nomenclature of (Leontis and Westhof, 2001). Schematic diagrams of the three-dimensional structures of the *S. mansoni* hammerhead with peripheral elements (at left) (Martick and Scott, 2006) and of the minimal hammerhead (at right) (Scott *et al.*, 1995).

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the native peripheral regions (De La Pena *et al*., 2003; Khvorova *et al*., 2003; Martick and Scott, 2006), by interacting with each other, facilitate and stabilize the folding into a single active structure. These regions are necessary for optimal activity in physiological conditions, although they are not directly involved in the catalysis.

Our new understanding of ribozyme structure and activity raises the question of the relevance of earlier conclusions drawn from data obtained with highly simplified molecular systems (Uhlenbeck, 2003; Nelson and Uhlenbeck, 2006). Historically, proper understanding of these molecules was delayed by two main factors. First, the focus on the regions around conserved nucleotides leads to a purely chemical analysis of the reacting atoms. Secondly, this analysis was seriously hampered by a static view of RNA molecules, unaffected by mutations and nucleotide changes. It was not recognized initially that global kinetic parameters were derived from mixtures of conformers, only some of which were active. The influence of nucleotide changes on these equilibria cannot be predicted or properly assessed. Finally, although the important role of interactions between peripheral elements was fully appreciated in the case of large ribozymes, this knowledge was not extrapolated to the small nucleolytic ribozymes.

One could argue that the new insight, which has now been gained, should serve as a reminder of the need to be very careful when attempting to explain biological activity on the basis of crystallographic structures. The hammerhead tale is not necessarily a warning for those looking at a structure as the starting point for understanding its function (Nelson and Uhlenbeck, 2006). The hammerhead lesson stems from the fact that, soon after the discovery of catalytic activity (Buzayan *et al*., 1986; Forster and Symons, 1987), the sequences of the hammerhead ribozyme were reduced to a 'consensus' set of conserved residues which led the field astray for a long time. It is thus a reminder for those who wish to reduce sequences to a 'consensus' region and ignore the multiplicity of sequences that have arisen during biological evolution. It also warns against the tendency to regard crystallographic structures as rigid and static and ignore the molecular dynamic processes inherent to any molecule. The newly determined crystal structure (Martick and Scott, 2006) underlines the fundamental relationships between the folding pathway, the selection and stabilization of a single native state by tertiary interactions between peripheral elements and the catalytic activity of ribozymes. However, a full understanding of the chemical and biological actions of nucleolytic ribozymes has certainly not yet been achieved and surprises are still lurking.

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