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Bistable RNA Pathways: (A Brief-Review)

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ABSTRACT

A Bistable RNA pairs have noteworthy importance in RNA ribo-switches, RNA thermometers and viroid replications, making this molecule particularly interesting to chemists and scientists working in field of biochemistry, virologists and computational chemistry. It adopts equilibrium between two distinct hairpin conformations. The two hairpin conformations adopt different functionality. In this review paper, we discuss the pathways used by bistable RNAs for switching confirmations and look into the future possibilities existing mechanisms can yield in RNA and retro-virus RNA studies.

Keywords: Bistable RNA, Unfolding-Refolding pathway, Base-pair-exchange pathway, Pseudo knot pathway, RNA dance pathway, Mutated RNA, 34-mer RNA.

INTRODUCTION

RNA is an important molecule in biology, as many kinds of RNA are involved in every aspect of gene expression, including m-RNA, t-RNA and in the ribosome. RNA plays a pivotal role in cellular functions as ribozymes and ribo-switches[1-4]. Knowing the three-dimensional structure is necessary to study the functionality of the molecule; hence RNA folding studies are of great importance to the scientific world. To investigate formation of secondary structures in RNA, temperature jump experiments have been reported, which concluded that folding begins from a completely unfolded state and attains a native state configuration according to the type of sequence[5-7]. The relation between sequence and native structure is attributed to the forces that initiate folding, this is because according to the sequence, the complementarity of base-pairing, the electrostatic interactions, and the Van der Waals forces acting on the molecule change. These forces are responsible for the RNA energy landscape being topologically frustrated[8]. Early experiments[9,10] suggest RNA folding to be hierarchical, as there is a rapid formation of secondary structures followed by a slower formation of tertiary and quaternary structures; this is the classical two state kinetic model. Laser-induced temperature jump,[11-14] and fluorescence correlation spectroscopy experiments[15-17] on random RNA systems, along with studies on tetrahyma ribozyme[18] by Wu and Tinoco, suggest that for relatively large RNAs, folding may not be hierarchical, meaning that secondary and tertiary mechanisms can at times be coupled; this is the multistate kinetic model.

The competition among forces that brings about folding, and the unpredictability of hierarchical folding, makes the RNA energy landscape relatively complex to study. These energy landscapes also exhibit kinetic traps that lead to misfolding. Kinetic traps are metastable states in the landscape where the molecule can exist in a disordered state. RNA structural transformation studies are of great interest, as RNAs serve as a regulator in many cellular functions[19]. An RNA that adopts equilibrium between two distinct hairpin conformations is termed a bistable RNA. The two conformations can have completely different functions.

Bistable RNAs: Functions and Importance

SV-11,[20] a 115 nucleotide RNA, which exists in two conformations; one is a metastable form and the other is a rod-like conformation[21]. The metastable conformation acts as a template for Qβ replicase, but the rod-like structure does not. The importance of bistable RNA relates to processes like RNA riboswitches, which structurally rearrange due to ligand binding that turns gene expression on and off. A system of this type is observed for bacterial m-RNAs, whose 5'-untranslated region consists of an aptamer domain, initiating conformational change upon ligand binding[22-24]. Replicative signals encoded from the leader sequence of HIV-1RNA genome also exhibit a conformational switch between bistable structures. This switching is induced when the leader sequence binds to a viral nucleocapsid protein (NCP) which acts as an RNA chaperone[25-29]. Many more studies suggest alternative folding to be involved in viroid replication processes[30-32]. In Escherichia oli and *Basillus subtillus*, the terminator and anti-terminator that regulate gene expression are bistable RNAs[33-35]. Wool *et al.,*[36] proposed that the protein biosynthesis cycle involves alternative conformations of 28S rRNA, to which elongation factors bind. Artificial RNA switches have been engineered to mimic the activity of the ribosomal-A site[37]. RNA thermometers change conformation in response to temperature[38].

Experimental studies on Bistable RNA Pathways

Metabolite binding to the aptamer domain of a riboswitch has been studied extensively, as it produces an on-off signal for gene expression. The SHAPE,[39,40] biochemical probing technique and small angle X-ray scattering (SAXS) studies[41-46] have impressively characterised ligand bound riboswitches, but characterisation of free riboswitches and bistable RNA systems without aptamer domains was not achieved until 2001. In 2001, Flamm *et al.,* introduced a method to design bistable RNA structures[47]. This work established conformational switching to occur in a class of naturally existing RNA, and not as an instance of unusual RNA behaviour. Small RNA molecules ranging from 25 to 34 nucleotides have been designed to exhibit conformational switching[48] and have been structurally probed using imino proton NMR spectroscopy by Hobartner and Micura in 2003. Ribose 2'-F labelling was proposed as an experimental tool to characterise RNA secondary structure equilibria, by 19F NMR spectroscopy in 2005.,[49] In 2007, NMR studies conducted on 34-mer RNA,[50] and subsequent smaller RNAs with 27 and 20 nucleotides that showcased bistability, proposed that these systems go through an unfolding-refolding pathway[51]. The pathway characterized folding as a two-step process, where in the first step fold-A transforms into a polynucleotide, which is a reversible rate limiting step. The second fast step constitutes the polynucleotide refolding into fold-B.

Computational studies on Bistable RNA Pathways

In 2012, Xiaojun and Shi-Jie used Kinetic Monte Carlo simulations on six different computationally modelled bistable RNA systems to suggest three possible pathways[52,53] for conformational switching, and possible competition conditions for a given sequence[51]. The mechanism that facilitates conformational change is always dependent on the sequence, as driving forces for folding depend on the arrangement of the nitrogenous bases. The three suggested paths were:

(i) **Unfolding-Refolding pathway:** a given RNA hairpin fold-A, completely unwinds into a straight chain polynucleotide, and then refolds into an alternative state, RNA hairpin fold-B. Here, unlike a straight chain polynucleotide folding to an RNA structure, there is disruption of the initial conformation and formation of new base pairs. The unfolding-refolding pathway (Fig. 1) has lower probability compared to the other two pathways, which have lower kinetic barriers. Hence competition between the base-pair exchange pathway and the pseudo-knot pathway dominates for a typical RNA. The unfolding-refolding pathway is favoured when

partial unfolding of either end of fold-A does not provide a favourable route to fold-B. The 34-mer bistable RNA[54-57] has been found to exhibit an unfolding-refolding pathway in NMR spectroscopic studies[58].

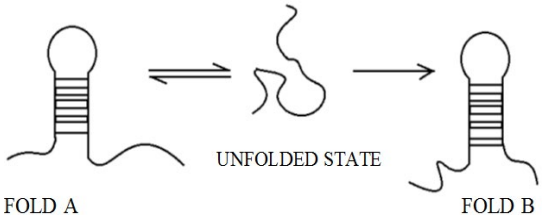

Fig.1. Schematic representation of the unfolding-refolding pathway, where the bistable RNA in the fold-A conformation unwinds and then rewinds into the fold-B conformation

(ii) **Pseudo-knot assisted pathway:** The pseudo-knot was first recognized in the turnip yellow mosaic virus[59]. The pathway involves formation of a knot-like structure in fold-A, followed by a rearrangement of base pairs to finally yield the alternative fold-B. Knot-like structure refers to the condition when the loop of a hairpin forms intermolecular pairs with bases outside the stem, which leads to formation of a second stem. The two-loop, two-stem system is termed a pseudo-knot[60]. Formation of a pseudo-knot structure is schematically represented in Fig. 2. Pseudo-knot pathways become more prominent when interaction of unfolding of fold-A from the 3' terminal end favours formation of a fold-A loop and the unfolded region leads to coexistence of fold-A and fold-B in the form of a pseudo-knot structure. It is considered a form of tertiary interaction[61]. The pseudo-knot pathway (Fig. 3.) is also favoured for short sequences and low temperature[51].

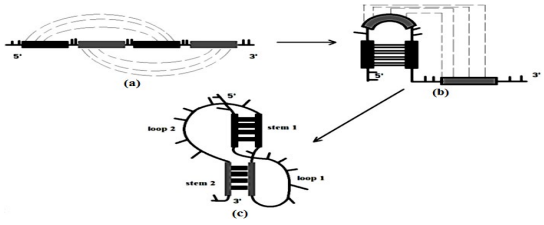

Fig. 2. Formation of an H-type pseudo-knot is represented schematically. In (a) the complementary base pairing regions of the sequence are shown in identical colours. The first hairpin stem is formed along with a loop in (b), where the loop consists of bases that can interact with bases outside the stem. In (c) the second hairpin stem is formed by interaction of the loop bases with the bases outside the stem. A second loop is also formed simultaneously and the structure obtained is called a pseudo-knot structure. The figure is adapted from reference [60] with minor changes

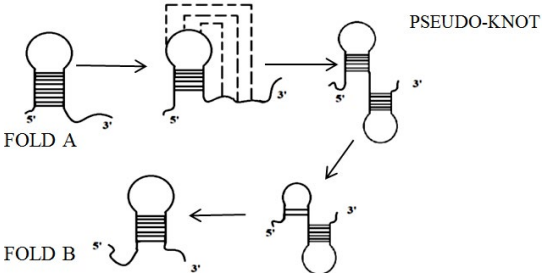

Fig. 3. Schematic representation of the pseudo-knot assisted pathway, where fold-A unwinds and the 3' terminal end is attracted to the fold-A loop, which eventually produces a state where two stems and two loops coexist: a pseudo-knot. The knot progresses to breaking base pairs in hairpin stem of fold-A, and forms new base pairs in the hairpin stem fold-B to complete the transformation

(iii) **Base-pair exchange pathway:** the given RNA, fold-A, changes by breaking existing base-pair connections, and at the same time simultaneously makes new base-pair contacts in an extending region, finally leading to formation of an alternative state, fold-B. In the base pair exchange pathway (Fig. 1.4), there is a strong G-C contact,[51] which favours zipping of terminal ends to transform into fold-B, but at the same time favours unzipping of base-pairs that form fold-A.

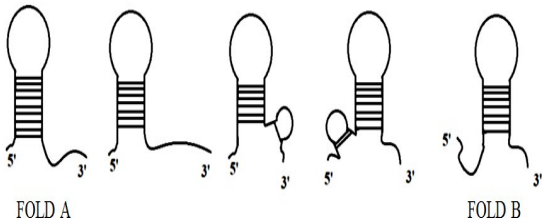

Fig. 4. Schematic representation of the base pair exchange pathway, where the dangling end of fold-A forms base pairs with the bases that break from the existing hairpin stem, to form a new stem loop system, which gradually grows, as the old stem loop unzips. The transformation gives fold-B

Other than these three pathways an alternative pathway has also been suggested for bistable RNA conformational switching called the RNA dance pathway. In 2013, Chen and Garcia[62] conducted molecular dynamics studies on hyper stable UUCG RNA tetra loop alternatives, which exhibited unusual loop structures. These structures and intermediates have found to be in agreement with spectroscopic experiments conducted on a UUCG tetra loop, by Gruebele and coworkers[63]. Certain intermediates closely represented bistable RNA pairs. The alternative pathway proposed resembles a dance

(Fig. 5.) which involves torsion angle twists and turns, sugar repuckering, while base stacking contributes to overall conformational flexibility.

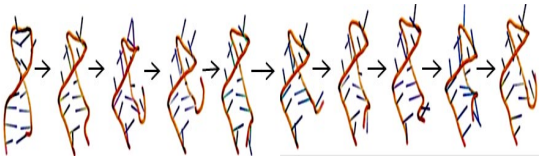

Fig. 5. The alternative pathway described as RNA 'dancing'. Adapted from reference[64]

Future Scope for Research in Bistable RNA pathways

34-mer RNA and subsequent smaller RNAs that underwent an experimental time resolved NMR spectroscopic study by Fürtig and co-workers[36], found the RNAs to exhibit an unfolding-refolding pathway during conformational switching in 2007, long before new pathways for conformational switching were proposed. There is a possibility that there are more pathways involved in the experiment, which can be found using simulation studies as RNA switching is a very fast and complex process for current characterising equipment's to detect. This could motivate experimentalists to modify their methods and increase efficiency of parameters in equipment's to study pathways of bistable RNAs.

Time resolved NMR spectroscopic studies[38] have confirmed that folding of a normal polynucleotide to an RNA native structure is a fast reaction, whereas the first step of a bistable RNA fold-A changing to fold-B via the unfolding-refolding pathway is slow and reversible (Fig. 1). If we can characterise the energy landscape[65] of an unfolding-refolding pathway for a bistable RNA pair, we have the opportunity to explore RNA folding patterns.

Folding from a normal polypeptide to RNA may have many kinetic traps, which gives us hope to further investigate paths that can lead to misfolding in mutated RNA and also develop methods to bring about mutation in retro virus RNAs to disable their harmful actions in the host system.

Finding systems that exhibit the Base pair exchange pathway and understanding its pathway is also of much importance as it is potentially relevant in transcription and translation[66].

CONCLUSION

An RNA, which adopts equilibrium between two distinct hairpin conformations, is called a bistable RNA. The two hairpin conformations adopt different functionality. They switch from one form, fold-A, to a lower energy form, fold-B, mainly through three types of mechanisms: unfolding- refolding pathway, base-pair exchange pathway, and pseudo-knot- pathway. The review suggests possibility of undiscovered pathways in experiment that can be found using simulation studies and there by learn more of RNA folding for application in studies of retro virus RNA's, transcription and translation.

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Conflict of Interest

I hereby declare there is no conflict of interest in publication of this article.

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