Temperature and Mutation Switches in the Secondary Structure of Small RNAs

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Abstract

Conformational switching in the secondary structure of RNAs has recently attracted considerable attention, fostered by the discovery of 'riboswitches' in living organisms. These are genetic control elements that were found in bacteria and offer a unique regulation mechanism based on switching between two highly stable states, separated by an energy barrier between them. In riboswitches, the energy barrier is crossed by direct metabolite binding, which facilitates regulation by allosteric means. However, other event triggers can cause switching to occur, such as single-point mutations and slight variations in temperature. Examples of switches with these event triggers have already been reported experimentally in the past. Here, our goal is to computationally design small RNA switches that rely on these triggers. Towards this end, our computer simulations utilize a variety of different similarity measures to assess the distances between an initial state and triggered states, based on the topology of the secondary structure itself. We describe these combined similarity measures that rely on both coarse-grained and fine-grained graph representations of the RNA secondary structure. As a result of our simulations, we provide some candidate sequences of approximately 30-50 nt, along with the exact triggers that drive the switching. The event triggers under consideration can be modelled by mfold or the Vienna package. To begin with, we concentrate on designing small temperature and mutation switches.

1. Introduction

Recent discoveries have demonstrated the peculiar possibilities of an RNA molecule to control fundamental processes in living cells. Although the functional role of RNAs are often related to their three-dimensional structure, the RNA secondary structure is experimentally accessible and contains much information to shed light on the relationship between structure and function. In general, RNA folding is thought to be hierarchical in nature, where a stable secondary structure forms first and subsequently there is a refinement to the tertiary fold. Thus, phenomena that utilize the rugged energy landscape of an RNA molecule containing conformational traps can be understood at the level of RNA secondary structure [2]. For example, in the newly discovered genetic control elements called 'riboswitches' [13, 8], a mechanism for bacterial gene regulation can be observed by examining the secondary structure alone. A switch between two highly stable states (e.g., a transcription terminator and an anti-terminator in *Bacillus subtilis* [8]) occurs as a consequence of direct metabolite binding that allows to cross the energy barrier between these states. Conformational switching in the secondary structure can also be achieved by other event triggers. For example, it was noted in [10] that there is some probability that even a single mutation can substantially alter the RNA secondary structure. Experimentally, this was obeserved in the spliced leader of *Leptomonas collosoma* [6]. Reviews and valuable information about RNA switches with event triggers other than metabolite binding are available in [7, 9].

Given an RNA sequence, an algorithm has been developed to evaluate the predictability of conformational switching in that sequence [12] without simulating temperature and mutations. When attempting to design new artificial switches, the seminal work of [3] can be useful, since a multistable RNA is effectively a switch when the exact event triggers to cross the energy barrier between the stable states are found. Here, we attempt to construct small RNA switches by starting from initial sequences and simulating the specific event triggers that we would like to investigate. The initial sequences are derived from cuts and pieces of larger sequences found in nature. By further division and assembly, we construct small sequences of about 30-50 nt, consequently running a folding prediction on these sequences with *mfold* [14] or the Vienna package [5]. For each candidate sequence, we predict the wildtype secondary structure as well as all triggered combinations according to the drives we specify in advance. Here, we chose the event

triggers to either be a difference of 1 or 2 degrees Celsius from the reference at room temperature, or single point mutations [1] performed on the wildtype reference. Thus, the overall amount of calculation in this procedure is not expensive, noting that we are considering small RNAs. The challenging step is to automatically assess the differences between the secondary structure of the wildtype state and the triggered states as accurately as possible, to ensure we do not miss attractive candidates. Towards this end, we use a combination of diversed similarity measures, all sharing in common that the distance estimation is based on the topology of the secondary structure itself. This strategy is based on the premise that no single similarity measure is immune against false positives, but by examining several that operate at various resolutions we can reach the specified goal.

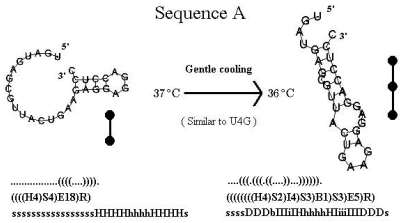

Figure 1. Small RNA Artificial Temperature Switch (A). Secondary Structure of Wildtype and Triggered State with Accompanied Representations: Coarse-Grain Tree-Graph, Dot-Bracket Notation, Coarse-Grain String, Hybrid (HCT) String.

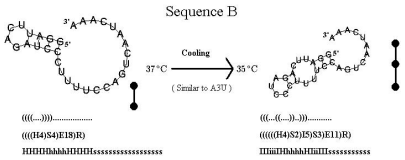

Figure 2. Small RNA Artificial Temperature Switch (B).

2. Future Work

Our goal is to design small RNA switches by a computer, collecting candidates that can further be tested experimentally. This line of research opens the door to assist in genetically engineering RNA elements that can be used for detection and control purposes.

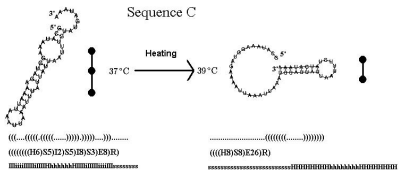

Figure 3. Small RNA Artificial Temperature Switch (C).

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