Computational Analysis of Influenza RNA Structures: A New Hypotheses for Old Problems

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Increasing experimental and computational evidence points to the existence of extensive RNA structures in the coding regions of mRNA molecules. Secondary structure elements in RNA play important roles in different biological processes. Predicting exact RNA structures from a sequence remains a challenging problem, primarily because a single RNA molecule can fold into different co-existing conformations — thereby representing the entire ensemble of RNA structures. Nevertheless, mathematical models exist that can predict the probability of each nucleotide being paired in a double-stranded conformation, instead of predicting a particular structure (1). We applied this approach to investigate mRNA secondary structures of the influenza virus.

In a recent study, we investigated if mRNA structures can be associated with the cold-adapted, temperature-sensitive (ca/ts) phenotype of the influenza A virus which has been used as live attenuated vaccines for decades (2). We hypothesized that a relatively minor change in temperature (32 – 39 °C) causes perturbations in mRNA secondary structures, and that RNA structures in wild type (wt) and ca/ts strains react differently to the temperature change (3,4). We identified those areas within an RNA chain where dissimilarities of RNA secondary structures at two different temperatures are particularly pronounced. However, these areas are not identical for the wt sequences and their ca/ts counterparts. Such areas were identified in the NS2, PA, PB2 and NP mRNAs. Differences in temperature-induced structural changes of wt and ca/ts mRNA structures may constitute a yet unappreciated

molecular mechanism of the temperature-sensitivity and/or cold-adaptation phenomena. Additionally, we created a publicly available web service, RNAtips (http://RNAtips.org), to enable the access of scientific community to the analysis of temperature-induced perturbations in RNA structures.

The data presented above demonstrates that preserving particular RNA secondary structures is necessary to maintain the expression level of viral proteins. This observation motivated us to test if influenza mRNAs have specific patterns of structured regions and whether these patterns of RNA structurization influence mutation rates at the particular positions. To approach this problem, first of all, it was necessary to define "mutability" and "structurization". After these terms were formalized, we compared the profile of mutable/conserved positions along the sequence to the profile of regions possessing conserved RNA secondary structures. Our results have demonstrated that: i) the most mutable and/or the most conservative positions are evenly distributed within each mRNA, and do not form clusters; ii) influenza mRNAs contain apparent clusters of structured RNA possessing conserved RNA secondary structures, while the rest of the RNA molecule is much less structured. Thus, mRNAs of influenza virus may contain areas of secondary structure with previously unknown functional elements, conservation of which could be very important for the biology of influenza virus.

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