GNRA LOOPS IN RIBOSOMAL RNAs MAY PROVIDE TIMING INFORMATION REGARDING THE RELATIVE AGE OF VAROUS RNA REGIONS. Q. Tran, G. E. Fox (fox@uh.edu)

Much of the development of the modern ribosome occurred prior to the last common ancestor making usual phylogenetic methods for determining the age of various components difficult as large numbers of them are universally shared by members of three Domains of life. In order to make progress, it has been observed that one can utilize timing events (1) which allow one to determine the relative age of various features. In the case of the SSU and LSU RNAs, it is widely recognized that all parts of these very large RNAs can not be equally old.

Initial efforts to determine which regions are in fact the oldest examined connectivity as an indicator of timing. The underlying idea was that relatively new regions of the RNAs would have had less opportunity to be integrated into the overall structure and hence would be recognizable by the presence of only a small number of interactions with other regions. The LSU RNA was broken into local regions and using data from crystallographic studies, the number of interactions between various regions was determined. Domain V, which contains the peptidyl transferase center, was deduced to be the oldest region followed by parts of Domain 2 and Domain 4 (2).

Recently, this approach has been improved (3). A common structural feature of the rRNAs known as the A-minor motif exists, in which a stack of unpaired bases pack with a distant helical region in the RNA primary sequence. This interaction can not occur until both components are present in the RNA and therefore represents a possible timing event. However, which portion would come first? It was observed that in the case of the A-minor interactions involving the peptidyl transferase region of Domain V that the helical region was always in Domain V. Since the PTC is thought to be the oldest region of the RNA, it was argued that in each case the helical region should be considered to be older than the associated base stack. Using this argument, the authors were able to deduce a hierarchical model for the relative age of the various helical elements in the 23S rRNA. The resulting model is in good agreement with the earlier studies using connectivity. However, the ability to place the data in a hierarchical structure is an important advance because it allows for the possibility of relating other ribosomal features to the various branches in the model.

In the work presented here, we examine another common structural motif, the GNRA tetraloop. There are approximately 11 occurrences of this feature in the 23S rRNA. In most cases, at least one of the bases in the GNRA loop forms a base-base interaction with a distant region in the RNA primary sequence. Thus, we have another possible timing interaction as such an interaction can not form until both regions are present. Which comes first? Again, one can make a deduction based on interactions involving the PTC. It is deduced that the GNRA loop is newer than the interacting region. This hypothesis was subsequently tested against the hierarchy proposed by Bokov and Steinberg. In every case, the GNRA loop occurs in a region that is later in the hierarchy than the interacting region. Because they are of the same general nature, these additional timing interactions can be readily added to those based on the A-minor motif. In addition, similar timing events are found in the small subunit RNA and can be used to study the relative age of various features therein.

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