NUS National University of Singapore

Department of Biological Sciences
Seminar Announcement

Assessing RNA conformations in living cells: Implications for translational control of BACE1 mRNA in Alzheimer's disease

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Cell-free methods have been used successfully to probe the conformation of various short structural RNAs; however, such approaches have limitations, particularly for mRNAs because the test tube environment cannot accurately replicate the cellular experience of the RNA. To overcome limitations of existing methods, we adapted a lead(II) acetate cleavage method used in bacteria to probe RNA conformations in mammalian cells. $Pb2+$ is ideal for this application as it is cell permeable and induces RNA cleavage that is essentially sequence non-specific at or near magnesium binding sites as well as at bulged, looped, or single stranded nucleotides. In this study, the feasibility of this method was demonstrated by probing the endogenous RNase P RNA of rat neuroblastoma cells. The results were largely consistent with universally conserved features of the RNase P RNA, validating the use of this method in mammalian cells. The method was then extended to a cellular mRNA – the β-site APP cleaving enzyme 1 (BACE1) mRNA. In Alzheimer's disease, increased translation of this mRNA has been suggested to underlie elevated BACE1 enzyme levels, which are responsible for the production of neuritic plaques. We previously showed that translation of this mRNA was cap-dependent and that four AUG-codons in the 5' leader were bypassed. $Pb2+$-probing of the endogenous BACE1 mRNA in living rat cells suggested that the 5' leader was largely inaccessible but that nucleotides immediately upstream of the initiation site and coding sequences were accessible. These findings have important implications for our understanding of translation initiation, which will be discussed. It is noteworthy that for both RNase P and BACE1 RNAs, the results obtained in cells bore little resemblance to those obtained using in vitro transcripts, even when these transcripts were subjected to a denaturation-renaturation protocol. These data highlight the limitations of non-physiological approaches and the importance of using *in vivo* approaches to evaluate RNA conformations.

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Artwork by Yong Ann Nee, DBS, NUS