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Expanding the application of RNA technology: potential for enhanced diagnostics

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Expanding the application of RNA technology: potential for enhanced diagnostics

Technical applications using RNA technology have been limited in scope due to the inability to selectively label specific nucleotides within an RNA molecule. The authors described a new methodology to label specific residues within an RNA molecule using either isotope or fluorescent labels, termed position-selective labeling of RNA (PLOR). This technique allows detection of RNA structure and dynamics using nuclear magnetic resonance spectroscopy or Förster resonance energy transfer, respectively, and could allow for the modification of RNA functionality. The technique attaches a solid-phase 5'-biotin–labeled duplex DNA template onto streptavidin-agarose or neutravidin-agarose beads and then initiates a pause-restart synthesis protocol in which labeled nucleotides can be incorporated into specific positions. This three-step protocol, consisting of initiation, elongation, and termination steps, can be performed using a robotic platform and can generate milligram quantities of site-specific–labeled RNAs using reusable bead-DNA templates and His-tagged T7 RNA polymerase (RNAP). This concept was tested using the 71-nucleotide aptamer domain of an adenine riboswitch, riboA71, from *Vibrio vulnificus*. RiboA71 undergoes a conformational change following adenine binding, which allowed the authors to test the PLOR technology under dynamic conditions. Selective incorporation of isotope and fluorescent labels was successful and demonstrated four distinct conformations of riboA71. Alternative methodologies for labeling RNA exist, but they are limited in their application. For example, solid-phase chemical synthesis is limited by reagent availability and restricted to use for RNAs of 60 nucleotides or less in length. Similarly, solution-phase transcription can generate larger RNAs using bacteriophage T7 or SP6 RNAPs but results in diffusely labeled samples that restrict the ability to detect small conformational changes in the background of high signal. The PLOR technique described herein holds potential for a number of applications of relevance to research and clinical diagnostics. Such examples include the improved ability to categorize complex RNA structure, alteration of cell function using selectively modified RNA, and enhanced clinical imaging using labeled RNA as a dynamic molecular cancer marker. The automation accompanying this technology has the potential to expand this new methodology into genomics and cell therapy laboratories in the future.

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