Structural Biology of RNA Folding Using Synchrotron Footprinting

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Synchrotron hydroxyl radical "footprinting" is a new technique that provides time-resolved analysis of conformational changes of nucleic acids. Passage of an x-ray beam through water produces hydroxyl radicals that cleave the phosphodiester backbone of nucleic acids in regions not protected by protein binding or folding. As with the classical Fe-EDTA footprinting method, this technique permits the solvent-accessible surface of a nucleic acid to be mapped with single base resolution. However, the technique of x-ray footprinting is also capable of resolving folding events ranging from milliseconds to minutes. The white light beamline X9A at the National Synchrotron Light Source at Brookhaven National Laboratory produces 10^{15} photons/sec, a high enough flux to allow exposures on the order of milliseconds that result in enough cleavage of nucleic acids for footprinting analysis. We have used x-ray footprinting to study the folding of the 400 base catalytic RNA from the *Tetrahymena thermophila* group I intron. Kinetic rates are determined for 23 separate regions within all the major domains of the RNA. This analysis has defined one specific structure of a member of structural-kinetic intermediates in the folding reaction and provides a model for the entire tertiary folding of the macromolecule. The technique is entirely general and can be applied to analyze the conformational changes of both DNA and RNA alone and in their interactions with proteins.