Paul Agris Research Statement Details

Structure-function relationships of nucleic acids, such as that of tRNA in protein synthesis, are fundamental to all cell and molecular biology. In order to probe the structure-function relationships of RNAs as potential targets or tools, we have developed methods for the introduction of native, non-natural, and stable isotope labeled nucleosides. We have found that modified nucleosides in tRNA play an important structural and functional role both within the tRNA molecules and in tRNA anticodon recognition of select codons at the wobble position. Modified nucleosides alter codon "wobble", enhance ribosome binding, explain programmed translational frameshifting, are determinants for aminoacyl-tRNA synthetase recognition, and are involved in human immunodeficiency virus selection of a specific human tRNA to prime reverse transcription.

The introduction of modified nucleosides into RNA and DNA produces tight metal ion binding sites. The sites are formed either directly by the modification providing ligands for the metal, or indirectly by the modification inducing a structural transition in the nucleic acid, thereby exposing ligands not previously available. Mg2+ is the metal of choice, in vivo. However, we have found that heavy, toxic metal compete effectively for the same binding site as Mg2+.

Chemical synthesis of oligonucleotides with phosphoramidite chemistries is used for the site-specific placement of these RNA or DNA nucleosides. The chemical synthesis of oligonucleotides also permits the design of new nucleic acids by introduction of uncommon, modified and non-natural nucleosides for investigations of new functions. Transfer RNA is actually a large set of molecules which we tend to speak of as the generic tRNA structure, and for which one of the most common features are the modified nucleosides. The biophysical aspects of tRNA function are relatively unknown. Our studies of tRNA and other RNAs utilize molecular genetic, microbiological, biochemical and chemical and biophysical (nuclear magnetic resonance, NMR) methods to more clearly and precisely define the site-specific structure-function relationships and design new nucleic acids. For instance, many aspects of tRNA structure have now been found to exist in DNA. Our technologies have permitted us to design a DNA analog to an RNA, and for that DNA to have the same function as the RNA in protein synthesis. The newly designed DNA prevents native tRNA from binding the ribosome. Other DNAs we have designed block aminoacylation of tRNA and posttranslational modification of tRNA.