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## Nucleosides, Nucleotides and Nucleic Acids

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### Nucleoside Modifications Affect the Structure and Stability of the Anticodon of tRNA<sup>Lys,3</sup>

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NUCLEOSIDE MODIFICATIONS AFFECT THE STRUCTURE AND STABILITY OF  
THE ANTICODON OF tRNA<sup>Lys,3</sup>

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**Abstract:** NMR spectroscopy was used to determine the solution structures of RNA oligonucleotides comprising the anticodon domain of tRNA<sup>Lys,3</sup>. The structural effects of the pseudouridine modification at position 39 were investigated and are well correlated with changes in thermodynamic parameters. The loop conformation differs from that seen in tRNA<sup>Phe</sup> and provides an explanation of the critical role of modification in this tRNA.

Introduction

In addition to the normal RNA and protein interactions important for a tRNA molecule in protein synthesis, human tRNA<sup>Lys,3</sup>, is the primer for HIV reverse transcriptase and plays a fundamental role in the HIV-1 virus life cycle. The extensively modified anticodon of tRNA<sup>Lys,3</sup> has been shown to bind HIV-1 reverse transcriptase in a modification dependent manner<sup>1,2</sup>. In vivo and in vitro studies have shown that efficient elongation during reverse transcription requires a modification dependent interaction between an A-rich loop (bases 162-167) in the HIV-1 genome and the modified anticodon of tRNA<sup>Lys,3</sup><sup>3</sup>.

Unlike tRNA<sup>Phe</sup>, the unmodified anticodon stem-loop for tRNA<sup>Lys,3</sup> does not bind to programmed ribosomes<sup>4</sup> suggesting that partial or full modification is required for any biological activity and that modification in this tRNA may lead to a major structural reorientation. Our initial step toward understanding the structure-function relationships in tRNA<sup>Lys,3</sup> was to structurally characterize the unmodified anticodon and the effects of the simple modification, pseudouridine. We have used NMR to determine the high resolution structures of an unmodified 17-mer RNA oligonucleotide comprising the anticodon stem-loop sequence of human tRNA<sup>Lys,3</sup> and an 17-mer oligonucleotide that has the naturally occurring pseudouridine at position 39.

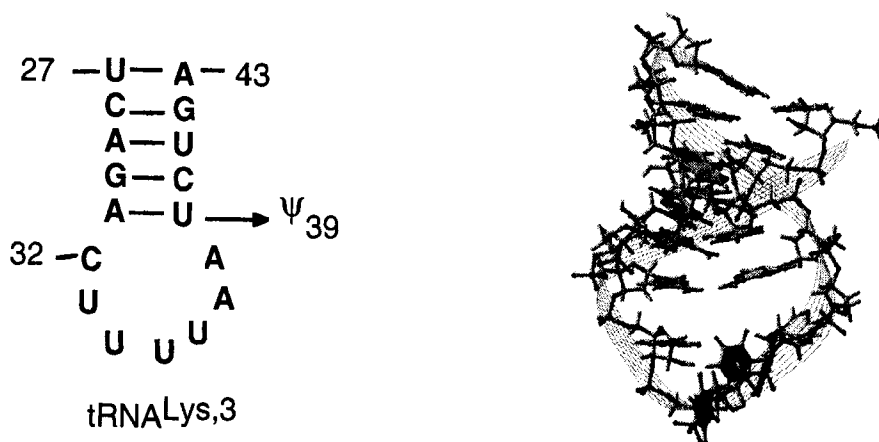


FIG. 1. **Left:** Secondary Structure of the tRNA<sup>Lys,3</sup> anticodon domain, and **Right:** NMR structure.

### Results and Discussion

<sup>1</sup>H-<sup>13</sup>C HMQC spectra for tRNA<sup>Lys,3</sup>- $\psi_{39}$  confirms formation of an A<sup>+</sup>-C base pair with protonation at N1 as evidenced by a 6 ppm change in <sup>13</sup>C chemical shift for the C2 of A38 upon decreasing the pH from 7.4 to 5.4<sup>5</sup>. The three anticodon bases (U34-U36) have significant 2'-endo character as indicated by large sugar coupling constants and strong intranucleotide H6-H2' relative to H6-H3' NOEs. In addition to the dynamic character of the anticodon residues, the NOE data indicates that there is no base stacking at the U34-U35 step. The <sup>31</sup>P NMR spectrum also provides evidence that the loop is dynamic rather than consisting of two rigid 5' and 3' domains as seen for tRNA<sup>Phe</sup>. All <sup>31</sup>P chemical shifts are within a narrow 0.88 ppm range that is typical of an RNA molecule with no sharp turns in the phosphodiester backbone. In contrast, the <sup>31</sup>P NMR spectrum of yeast tRNA<sup>Met</sup> has a single downfield resonance for the phosphate between residues 33 and 34 shifted 1 ppm downfield from the stem region phosphates<sup>6</sup>.

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